



Université catholique de Louvain
Institut de la matière condensée et des nanosciences
Bio- and Soft Matter
Laboratoire de Chimie Macromoléculaire

Use of light to control the formation of functional nanostructures in solution

Olivier Bertrand

Promoteurs:

Pr. Jean-François Gohy
Pr. Charles-André Fustin

Dissertation présentée en vue de l'obtention
du grade de Docteur en Sciences

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*"Goodbye, young human. I hope you make it. But if you don't - well,
we shall yet succeed."*

The Reverend Mother Gaius Helen Mohiam to Paul Atreide

Dune, Frank Herbert

ACKNOWLEDGMENTS

Etant assez friand de littérature *fantasy* et *heroic fantasy*, j'aime me représenter ces quatre années de thèse comme une aventure épique. L'aventure contée dans cette chronique se déroule dans un monde peu connu du commun des mortels : le monde de la Science et plus particulièrement dans les terres de la Chimie. Dans ces terres, les héros sont reconnaissables grâce à leurs tabliers de labo et leurs quêtes sont des projets de recherche. Lors d'une thèse, comme toute aventure, le héros est confronté à différentes problématiques tant scientifiques que sociales. Du point de vue scientifique, cette thèse m'a permis de mettre en pratique les connaissances acquises durant le temps passé sur les bancs du LAVO 51 et aussi de développer ces connaissances pour la réalisation de ce projet de longue haleine. Du point de vue social, elle m'a permis de rencontrer et de côtoyer des personnes fantastiques, dont l'aide a été précieuse pour la réalisation et la rédaction de mon épopée. Il me faut donc remercier ces personnages (principaux ou secondaires) dont la contribution vous permet, en cet instant, de lire l'histoire relatant mon aventure.

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présenter les résultats de mes travaux au sein de différents congrès Belges et internationaux (Espagne et USA).

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ABBREVIATIONS AND SYMBOLS

α	degree of neutralization
χ	Flory-Huggins interaction parameter
ΔG_M	free energy of mixing
ΔH_M	enthalpy of mixing
ΔS_M	entropy of mixing
γ	interfacial tension
λ	wavelength
$\lambda_{\max}$	maximum of absorbance
ν	frequency
a_o	area of the hydrophilic group
c	speed of light
f_A	volume fraction of the block A
l_c	length of the hydrophobic domain
p	packing parameter
R_g	radius of gyration
R_h	hydrodynamic radius
R_c	radius of the micellar core
R_m	radius of the micelle
5Pr-2NB-BiB	5-propargylether-2-nitrobenzyl bromoisobutyrate
Et ₃ N	triethylamine
E_a	activation energy
AFM	atomic force microscopy
ATRP	atom transfer radical polymerization
BCC	body-centred cubic
BIEE	bis(2-iodoethoxy)ethane
C343	coumarin 343
CGC	critical gel concentration

CMC	critical micellization concentration
CMT	critical micellization temperature
CRP	controlled radical polymerization
CuAAC	copper assisted azide-alkyne cycloaddition
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DLS	dynamic light scattering
DMF	N,N-dimethylformamide
DMNNBr	4,5-dimethoxy-2-nitrobenzyl bromide
dNbipy	4,4'-dinonyl-2,2'-dipyridyl
EBiB	ethyl 2-bromoisobutyrate
EDCHCl	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
EDTA	ethylenediaminetetraacetic acid disodium salt dehydrate
FCC	face-centred cubic
FRET	Förster resonance energy transfer
FRP	free radical polymerization
h	Plank's constant
HEX	hexagonally ordered cylinders
IPEC	interpolyelectrolyte complex
LAM	lamellae
lbl	layer by layer
LCST	lower critical solution temperature
LIM	light induced micellization
N_{agg}	aggregation number
NBBr	2-nitrobenzyl bromide
NIR	near-infrared
NMP	nitroxide mediated polymerization
NMR	Nuclear magnetic resonance
NPPMI	N-(n-propyl)-2-pyridylmethanimine
P2VP	poly(2-vinyl pyridine)
P4VP	poly(4-vinyl pyridine)
PAA	poly(acrylic acid)
PAH	poly(allylamine hydrochloride)
PAP	poly(N-acryloyl-L-phenylalanine)
PAPBA	poly(3-acrylamidophenylboronic acid)
PCL	poly(ϵ -caprolactone)
PDEA	poly[(diethylamino)ethyl methacrylate]
PDMAEMA	poly[2-(dimethylamino)ethyl methacrylate]
PDVB	Poly(divinylbenzene)

PEA	poly(ethyl acrylate)
PEO	poly(ethylene oxide)
PGA	poly(glutamic acid)
PI	poly(isoprene)
PLys	poly(lysine)
PMAA	poly(methacrylic acid)
PMDETA	N,N,N',N'',N''-pentamethyldiethylenetriamine
PMEMA	poly[(N-morpholino)ethyl methacrylate]
PMF	poly(melamine formaldehyde)
PMMA	poly(methyl methacrylate)
PNAGA	poly(N-acryloylglycinamide)
PNEAm	poly(N-ethylacrylamide)
PNIPAm	poly(N-isopropyl acrylamide)
PNPAm	poly(N-propylacrylamide)
POEGMA	poly[oligo(ethylene glycol) methyl ether methacrylate]
PPO	poly(propylene oxide)
PS	polystyrene
PSPP	poly((3-[N-(3-methacrylamidopropyl)-N,N-dimethyl] am- moniopropane sulfonate)
PSS	poly(styrene sulfonate)
PtBA	poly(<i>tert</i> -butyl acrylate)
PVP	poly(vinylpyrrolidone)
R_p	propagation rate
R_{ter}	termination rate
R_{trans}	transfer rate
RAFT	reversible addition-fragmentation transfer
ROP	ring opening polymerization
SANS	small-angle neutron scattering
SAXS	small-angle X-ray scattering
SLS	static light scattering
T_g	glass transition temperature
T_{cp}	cloud point temperature
TEM	transmission electron microscopy
TFA	trifluoroacetic acid
TsCl	tosyl chloride
UCNP	upconverting nanoparticle
UCST	upper critical solution temperature
v	volume of the hydrophobic chains

CHAPTER ONE

INTRODUCTION

Abstract

Over the last decades, block copolymers have demonstrated their potential as building blocks of high value materials. Their ability to self-assemble into well-organized structures at the nanoscale, either in the bulk or in solution, is the key parameter explaining the success story of block copolymers in (nano)technology and life sciences. Since this thesis focuses on the synthesis and the self-assembly of photo-responsive block copolymers, this introduction will set the basic concepts needed for the understanding of the project. The chemical nature of (co)polymers, as well as some synthetic tools to obtain them are developed here. Afterwards, the principles of self-assembly in bulk and in solution are detailed. In addition, some elements of photochemistry are also discussed since this thesis deals with photo-responsive block copolymers.

1.1 A few words about block copolymers

Polymers are formed by a set of macromolecules constituted of tens to thousands of repeating units called *monomers* linked by covalent bonds. While homopolymers are composed of monomers of the same chemical nature, copolymers are built from chemically different monomers. Block copolymers can adopt a large variety of compositions depending on the relative arrangement of the constituting monomers. Random, alternating, gradient and block copolymers are formed when the constituting monomers are respectively disposed randomly, alternatively, with a gradient and in several separated blocks [1].

Block copolymers occupy a special place in the copolymers family. Indeed, depending on the number and relative arrangement of the constituting blocks, several architectures (or topologies) can be formed (Figure 1.1). Moreover, block copolymers, when they self-assemble, generally undergo a phase separation in which each phase adopts the characteristics of the constituting polymer (Section 1.3) [2].

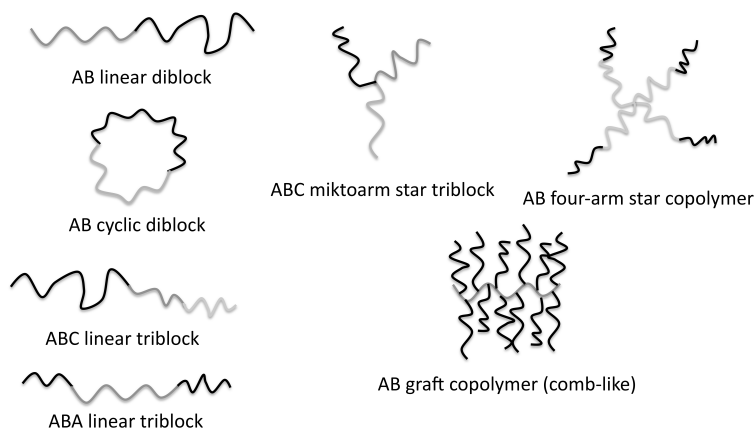


Figure 1.1: Typical architectures of block copolymers. [3]

The growing interest in block copolymers for high value applications is explained by the two aforementioned characteristics. Indeed, the ability of block copolymers to self-assemble enables the formation of materials with a nano-scale organization, which have numerous potential

applications. As typical examples, they are used as thermoplastic elastomers in bulk [4]. When they are self-assembled in thin films, they can act as lithographic masks, filtration devices and nano-templates [5–9]. Finally, their dissolution in a selective solvent promotes the formation of micelles or vesicles. These structures are particularly sought for the biomedical and pharmaceutical fields due to their ability to carry hydrophilic or hydrophobic drugs [10–21]. Micelles and vesicles have also great potential as nanoreactors in chemistry [22–24]. The variation of the copolymer topology, in conjunction to the copolymer composition changes, drastically influences the materials properties. It is then possible to finely tune the materials characteristics by the modification of the building blocks architecture and chemical nature.

1.2 Toolbox for the synthesis of block copolymers

The discovery of new synthetic techniques in the mid 90s for the preparation of complex polymeric systems, has greatly promoted the development of new functional materials. Alongside with the development of polymerization techniques, the emergence of new functionalization procedures has allowed the synthesis of new functional polymers and thus the creation of new materials.

1.2.1 Polymerization techniques

Before the birth of the *controlled radical polymerization* (CRP) techniques, in the mid 90's, the method of choice to synthesize copolymers were anionic and cationic polymerizations [25–27]. The living feature of this polymerization is particularly interesting for the synthesis of well-defined homo and copolymers. However, one of the major drawback of this technique is the high reactivity of the propagating anion toward electrophiles. The polymerization should then be carried out with extra-pure chemical reagents (solvent, monomer and special glassware). Moreover, the polymerization of monomers bearing electrophilic functions is almost impossible.

Radical polymerizations, on the other hand, are particularly tolerant toward functional monomers. However, radicals are highly reactive species that can anarchically react during the polymerization. Transfer processes compete with the propagation during the time of live of the radicals (around 1 second) then the termination occurs. All these process leads to the broadening of the molar masses distribution. To limit these undesired processes, CRPs were developed. These polymerization techniques rely on the establishment of a dynamic equilibrium between growing free radicals and dormant species (Figure 1.2).

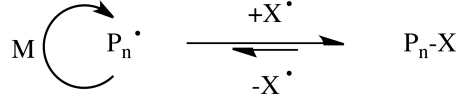


Figure 1.2: Principle of the CRP: equilibrium between active ($\text{P}_n^\bullet$) and dormant ($\text{P}_n\text{-X}$) species.

The dynamic equilibrium is largely displaced toward the dormant species and thus the concentration of radical species is drastically reduced. As revealed by the kinetic equations, the propagation rate (R_p in equation 1.1) and the transfer rate (R_{trans} in equation 1.3) are proportional to the concentration of radicals in the medium ($[\text{P}^\bullet]$). On the other hand the termination rate is proportional to the square of the radicals concentration (R_{ter} in equation 1.2). The decrease in concentration of radicals will thus decrease the overall probability of termination and transfer during the polymerization.

$$R_p = k_p[M][\text{P}^\bullet] \quad (1.1)$$

$$R_{ter} = k_{ter}[\text{P}^\bullet][\text{P}^\bullet] \quad (1.2)$$

$$R_{trans} = k_{trans}[X][\text{P}^\bullet] \quad (1.3)$$

Depending on the type of dormant species, three main type of CRP have been developed: *nitroxide-mediated polymerization* (NMP) [28], *reversible addition-fragmentation transfer* (RAFT) [29–31] and *atom transfer radical polymerization* (ATRP) [32–34].

Atom transfer radical polymerization

Developed simultaneously by Matyjaszewski [35] and Sawamoto [36], the *atom transfer radical polymerization* (ATRP) reveals itself to be a powerful tool for polymer chemists. The versatility of this polymerization technique enables the synthesis of a wide range of homo and copolymers including a wide variety of styrenic, (meth)acrylate, (meth)acrylamide and acrylonitrile monomers. Moreover, ATRP allows the synthesis of various polymeric architectures. [32]

This polymerization technique is based on a redox equilibrium between an active radical species and a dormant chain, often an halide terminated polymer chain (Figure 1.3). This redox equilibrium is catalyzed by a metal/ligand complex. The metal should have at least two readily accessible oxidation states separated by one electron. Several transition metals are able to catalyze the ATRP equilibrium, however the copper(I) halides (CuBr and CuCl) are often used with bidentate and tridentate nitrogen ligands. In addition of nitrogen ligands, phosphorous ligands are used in ATRP [32]. The first role of the ligand is to solubilize the transition metal salt in the polymerization medium but the ligand also plays an important role in the polymerization process. Indeed the control is largely influenced by the equilibrium between the active and the dormant species and thus by the redox potential of the metal/ligand complex, which is dependent of the nature of the ligand [37].

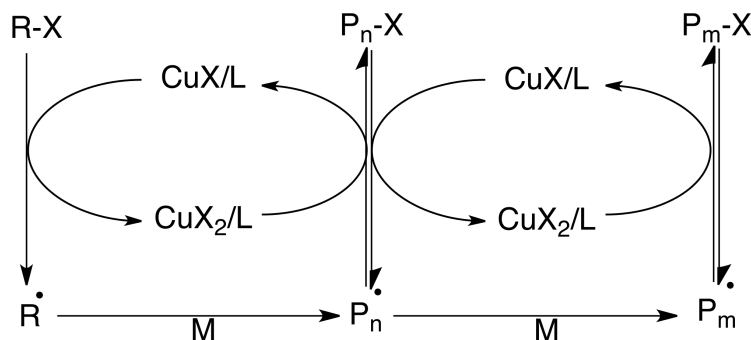


Figure 1.3: Mechanism of the ATRP catalysed by copper halides.

Figure 1.3 presents the mechanism of ATRP catalysed by copper halides (CuX). The first step is the initiation, where the halogenated initiator (R-X) is reduced by the metal/ligand complex (CuX/L) via a halogen transfer. The active initiator ($\text{R}^\bullet$) can thus react on the monomer to form an active polymer chain ($\text{P}_n^\bullet$). During the propagation step, the copper(II) halide/ligand complex can transfer an halide atom to the growing polymer chain which will thus adopt its dormant form. This dormant chain may be reactivated by a second reduction step to reform an active polymer chain which can thus continue the propagation. These cycles of oxidation and reduction are successively repeated during the polymerization and then ensure the control of the polymerization. As the overall propagation rate is decreased by the successive redox cycles, the initiation rate proportionally appears much faster. In addition, since the equilibrium is shifted towards the dormant species, the termination and transfer reaction are limited because of the reduced concentration of radicals in the polymerization medium. These two characteristics are essential prerequisites for a controlled polymerization to be observed.

This section presents the basic characteristic of the ATRP process. Interested readers are encouraged to take a closer look in the literature to understand all the factors that can influence the control in ATRP and the potential of this polymerization technique for the synthesis of well-defined functional polymers [32, 38–41].

1.2.2 Functionalization techniques

Although the development of anionic and controlled radical polymerizations have increased the library of available synthetic polymers, the synthesis of well-defined functional polymers can still be harsh using those techniques. Indeed, some functional groups may completely prevent controlled polymerization [42, 43]. Protected monomers may be used for the formation of functional polymers, however the deprotection step may not necessarily be quantitative and may also affect the structural integrity of the polymer backbone [44].

Fortunately, the chemist toolbox is composed of a wide range of reactions that allow the attachment of functional moieties onto a polymer chain (Figure 1.4). As typical examples, homo and copolymers were successfully functionalized by nucleophilic substitutions [45, 46], reactions with activated esters [47–49], click reactions [50–54] and other approaches [44].

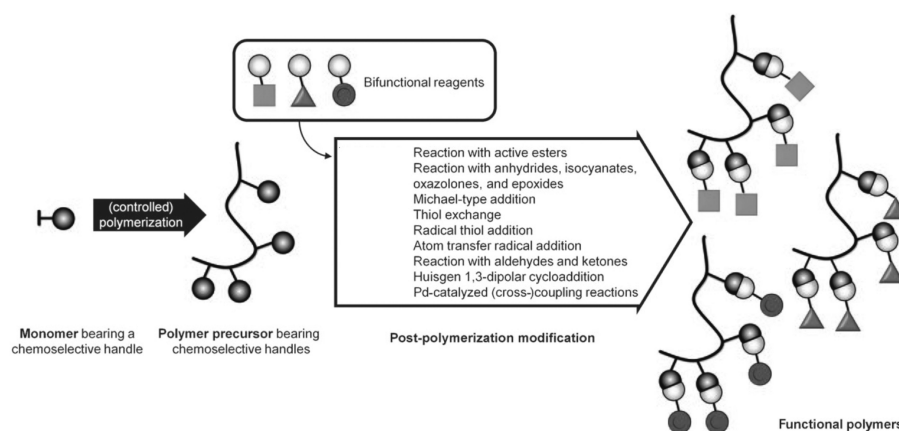


Figure 1.4: Synthesis of functional polymers via post polymerization modifications [44].

Introduced in 2001 by Sharpless and coworkers, the concept of "click chemistry" quickly attracted the chemist's attention. The click chemistry concept regroups numbers of fast and reliable reactions that are useful for the synthesis of new substances. These reactions have to fulfill a certain set of criteria to be qualified as *click*. They have to be *modular*, *wide in scope*, *to give very high yields*, *to generate only inoffensive byproducts that can be removed by non-chromatographic methods* and *to be stereospecific* [55].

When the concept of click chemistry is applied for the polymer synthesis, the criteria defined by Sharpless were not sufficient. Indeed, when the click reactions were applied to the coupling of polymer chains, the criterion of equimolarity of the reactions was added to the other indispensable criteria (Figure 1.5) [52]. Nevertheless, click chemistry is a

powerful and almost indispensable tool for the synthesis of functional polymers as attested by the number of reviews dedicated to this subject [50–54, 56].

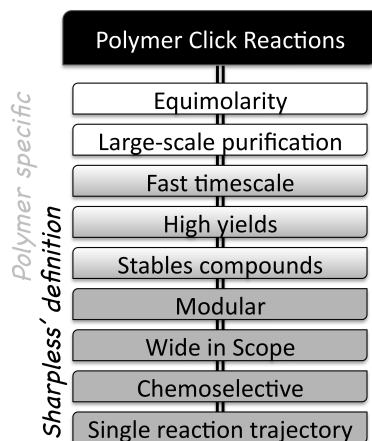


Figure 1.5: Requirement for the click concept applied to polymer synthesis [52].

Copper assisted azide-alkyne cycloaddition

The success story of the click chemistry was build by the *copper assisted azide-alkyne cycloaddition* (CuAAC). This reaction is an improvement of the Huisgen 1,3-dipolar cycloaddition, which enables the coupling of two molecules bearing, respectively, an alkyne and an azide function leading to a triazole function. The major drawbacks of this reaction are the elevated temperature at which it is performed and the non-regioselectivity of the cycloadditions, the 1,4 and 1,5 regioisomers being obtained.

When performed with copper(I) as the catalyst, the 1,3-dipolar cycloaddition proved to be highly efficient at room temperature. Moreover, only the 1,4 regioisomer is produced when the cycloaddition is catalysed by Cu(I) [55,57]. These two characteristics promoted the success of these reactions in organic chemistry, for the synthesis of new substances, and in polymer science.

Figure 1.6 presents the commonly approved mechanism for the CuAAC. The real mechanism remains unknown until now, the hypothesis of a mechanism involving more than one atom of Cu(I) is also postulated [58]. The first step of the catalytic cycle is the formation of a Cu^I-acetylide (**1**) from the alkyne and the Cu^I complex. The azide can then react with this Cu^I-acetylide, by a complexation of the Cu^I moiety. The triazolidine (**3**) is obtained after the presumed formation of a six membered metallacycle (**2**). After the elimination of the Cu(I) species, the final triazole product is formed (**4**).

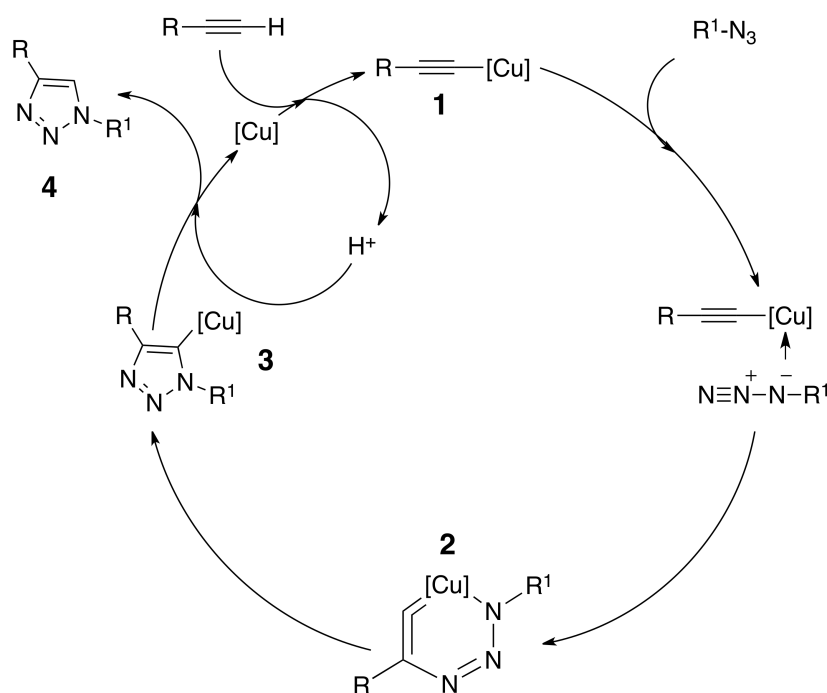


Figure 1.6: Simplified CuAAC mechanism.

Since the introduction of the CuAAC-click reaction, this technique has been extensively combined with different polymerization methods for the synthesis of functional homo and copolymers [59–65]. The ATRP is especially interesting in combination with CuAAC. Indeed, the introduction of the alkyne or the azide moieties on the polymer chain-ends or as side groups is easily realized by post-modification. Moreover, the cat-

alyst of these reactions is the same, *i.e.* copper(I)/ligand complex. It is thus possible to perform the two reactions in a "one pot" process [53,56]. In the "one pot" process, two approaches can be denoted, the "one pot" asynchronous (or tandem) and the "one pot" simultaneous. In the first process the fact that the click reaction occurs at lower temperature than the ATRP is used to first graft the functional group then the temperature is increased to initiate the polymerization. [62] On the other hand, in the simultaneous approach, both reactions are performed in the same time [59,66–68].

1.3 Self-assembly of block copolymers

As previously presented, one of the features that explained the success of block copolymers is their ability to self-assemble into periodic structures with characteristic size laying in the range of the nanometer. The self-assembly is "*the spontaneous and reversible process that allows the association of two or more components to form larger, non covalently bound aggregates*". [69] In the case of block copolymers, the self-assembly process leads generally to regularly arranged nano-scale structures. This is the consequence of the general immiscibility of polymer blocks and the existence of a covalent bond between them. The first parameter will force a phase separation between the different sequences and the second parameter will impose a limitation in space for the phase separation process.

The observed morphologies for the arranged nano-scale structures are strongly influenced by the state in which the block copolymer are self-assembled. In solution spherical micelles, cylindrical micelles and vesicles are commonly observed. In bulk a large variety of self-assembled nanostructures were monitored, including lamellae, hexagonally ordered cylinders, arrays of spherical microdomains and more complex arrangements such the gyroid morphology. When self-assembled in thin films, the microdomains of thin films are arranged in an array of spheres, cylinder or lamellae (Figure 1.7).

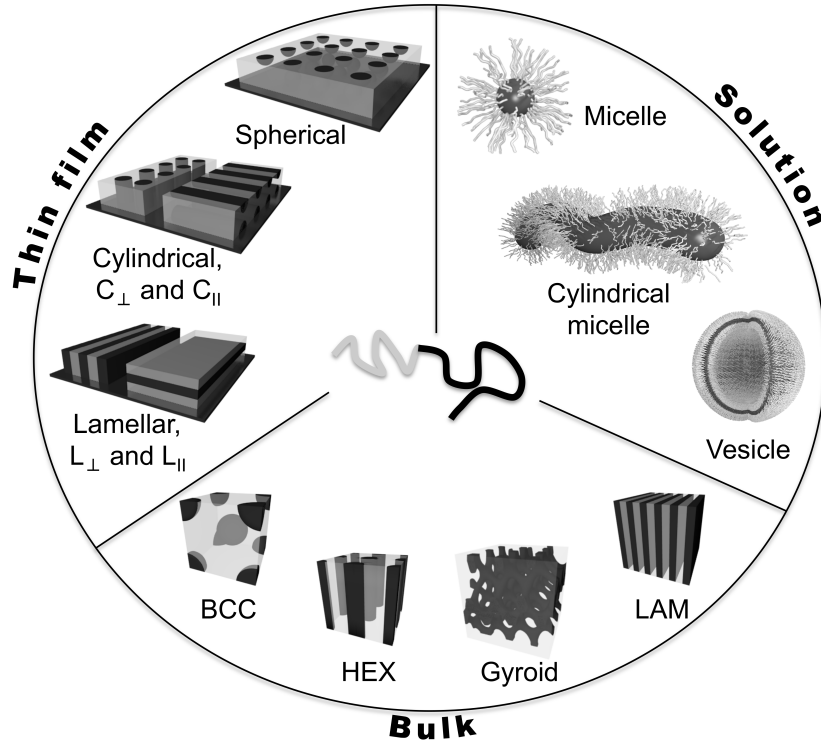


Figure 1.7: Typical morphologies observed by the self-assembly of diblock copolymers in solution, bulk and thin film.

1.3.1 Self-assembly in bulk

In bulk, block copolymers are generally observed in a microphase separated phase. The phase behavior of diblock copolymers was extensively studied over the years from an experimental and theoretical point of views. [2, 70]. A large variety of copolymer morphologies including lamellar (LAM), hexagonally ordered cylinders (HEX), arrays of spherical microdomains (BCC, FCC) and gyroid morphologies are commonly observed in bulk (Figure 1.8)

The phase separation of block copolymers is dictated by the enthalpy of demixing of the blocks. This enthalpy is proportional to the Flory-Huggins interaction parameter (χ). The demixing entropy is influenced by the degree of polymerization (N) of the block copolymers. The product χN thus expresses the enthalpic/entropic balance of the process [2]. The composition of the block copolymers strongly controls the morphology of the self-assembled materials. Indeed, the different morphologies observed are geometrical forms that maximize the contact between the blocks of the same nature and minimized the contact surfaces between the dissimilar blocks. The composition of the polymer is reflected by the volume fraction of one of the block (f_A or f_B , with $f_A = 1 - f_B$).

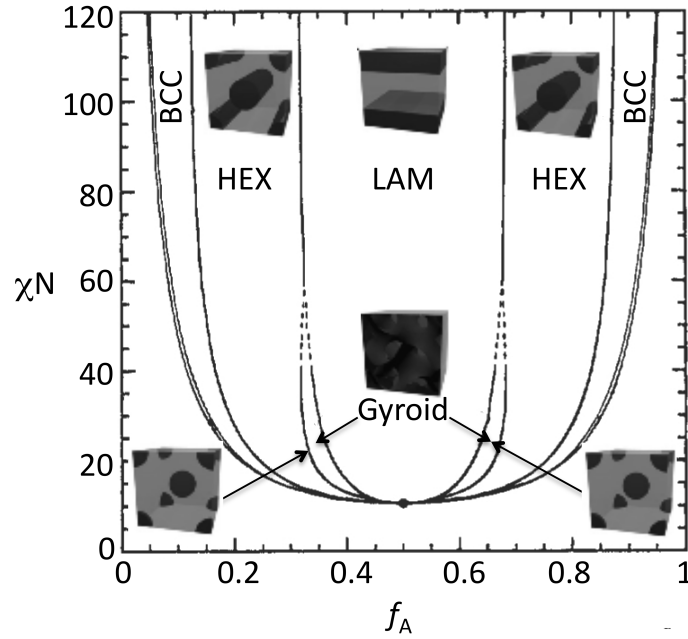


Figure 1.8: Theoretical phase diagram for the PS-*b*-PI copolymer. Adapted from [7, 71] .

The bulk morphologies can be mapped in a phase diagram depending on these factors (χN and f_A). Figure 1.8 presents the theoretical phase diagram of PS-*b*-PI diblock copolymers in bulk. In this figure, the strong impact of the block copolymer composition on the microphase morphology is depicted. For example, lamellar morphology is observed when the self-assembled diblock copolymer is symmetric, considering blocks of comparable volume. Increasing the degree of compositional asymmetry leads to the gyroid, cylindrical, and finally, spherical microphases.

1.3.2 Self-assembly in thin films

Thin films are polymer layers with a thickness varying from tenths to hundreds of nanometers. In addition of the parameters influencing the block copolymer self-assembly in bulk, the thin film morphology is also dictated by the surface energy of the interfaces of the thin film with the substrate onto which the film has been deposited and the medium on their upper side (generally air). Moreover, the geometrical constraints induced by the confinement (thickness) of the block copolymer chains in the thin film strongly command the self-assembly process [7, 72, 73]. Two examples were chosen to highlight the influence of these two factors on the self-assembly of thin films.

The first example demonstrates the effect of the interfaces on the morphology and more particularly on the orientation of the microphases. In general, the microdomains of the thin films are oriented parallel to the substrate because of a preferential interaction of one of the block copolymer sequences with the substrate (Figure 1.9a). A solution to avoid this situation is to remove the polymer-substrate and polymer-air preferential interactions. A common way to remove those preferential interactions is to confine the film between two non-preferential surfaces (Figure 1.9b) or to perform an annealing of the thin film, a perpendicular orientation of the microdomains is then obtained [74].

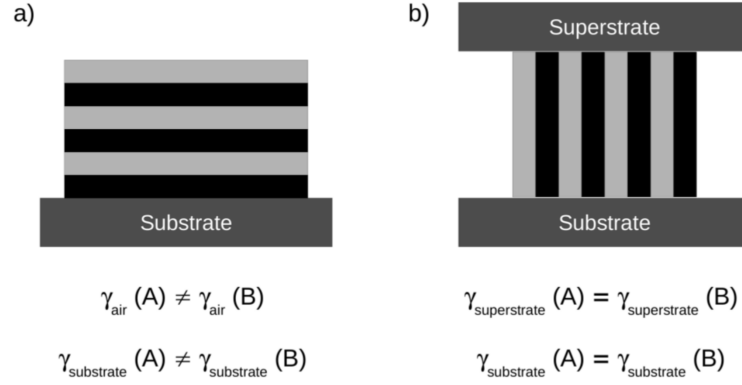


Figure 1.9: Influence of the interface on the orientation of lamellar microdomains for a AB diblock copolymer: (a) with, (b) without preferential interactions, γ = interfacial tension [75].

Figure 1.10 presents the effect of the confinement (or film thickness) into the film morphology. Thin films of PS-*b*-PB-*b*-PS of different thicknesses were spin-coated onto a silicon substrate. After the annealing of the films in chloroform, atomic force microscopy (AFM) pictures, in tapping mode, were recorded (a and b) [76]. A complete dependence of the film morphology to the film thickness is observed here. Indeed, the comparison of the AFM images with the film thickness profile (Figure 1.10c) clearly demonstrates the influence of the film thickness. The successive morphologies observed with an increase of the film thickness were: hexagonal pattern of PS cylinders oriented perpendicularly to the surface ($C\perp$), PS cylinders parallel to the substrate ($C\parallel$), perforated lamellae (PL), $C\perp$ again and finally, two layers of $C\parallel$.

The concept of annealing was introduced in the two previous examples. The annealing of a thin film is a process in which the mobility of the polymer chains in the thin film is restored. The polymer chains can thus rearrange to adopt a more thermodynamically favorable morphology. To understand the purpose of the annealing process, a closer look to the formation process of thin films is needed. Block copolymer thin films are usually prepared by the spin-coating technique. Drops of a solution of the polymer are deposited onto a spinning solid substrate (often

silicon wafers). The polymer film is spread by centrifugal force, and the volatile solvent is rapidly driven off. Most of the thin films obtained by this technique are unstructured, as the block copolymer chains do not have the time to arrange themselves in a well-defined morphology. The annealing of thin films is performed by the application of either mechanical flow fields [77], electric fields [78,79], gradients of solvents [68,80] or temperature gradient [81,82].

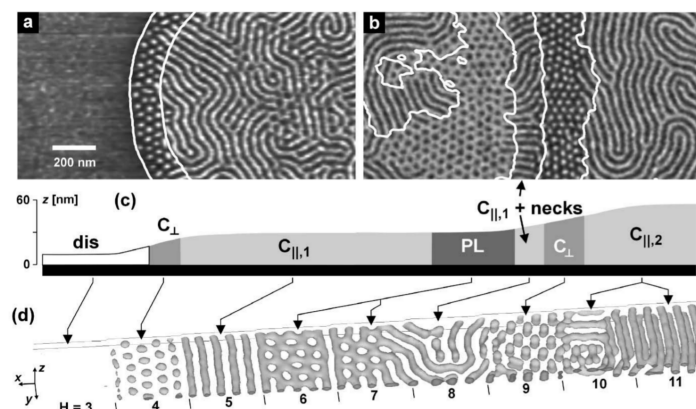


Figure 1.10: Influence of the film thickness on block copolymer morphology. Tapping-mode atomic force microscopy (AFM) phase pictures (a and b) of two PS-*b*-PB-*b*-PS thin films with different initial thicknesses after solvent annealing, thickness profile of the thin films (c) and simulation of the profile of the triblock copolymer film (d) [76].

1.3.3 Self-assembly in solution

The behavior of block copolymers in solution depends on the affinity of the blocks for the solvent. When the blocks of the copolymers are soluble in the selected solvent, the copolymer chains adopt a random-coil conformation (unimers). Such type of solvent is called a *non-selective solvent*. On the other hand, a *selective solvent* is defined as a thermodynamically good solvent for one of the block and precipitant for the other blocks. When dissolved in a selective solvent, the block copolymer chains self-assemble in micellar aggregates, where the insoluble blocks associate

to form the *core* of the micelles while the soluble sequences surround and stabilize the micellar core (micellar *corona*, Figure 1.11) [10, 83–85].

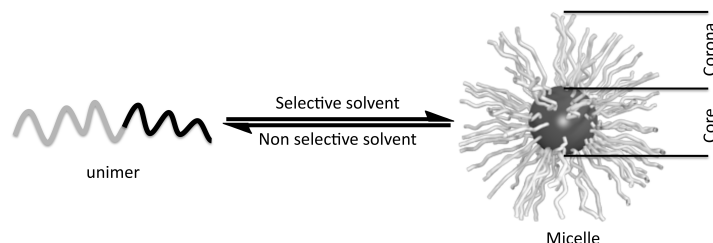


Figure 1.11: Schematic representation of an unimer and a micelle in solution.

The micellization of block copolymers is generally a dynamic process in which micelles and free unimers coexist in solution. Moreover, the polymer chains may reversibly transit from one state to the other as a function of the core forming polymer characteristics. This feature is particularly important for drug delivery applications since this dynamic equilibrium is involved in the undesired diffusion of the drug from the carrier (micellar core) to the solution. A suppression of this unimer/micelle equilibrium is observed when the micellar core is made of a polymer block possessing a high glass transition temperature (T_g) such as polystyrene. The structure is then "kinetically frozen" and may not represent the thermodynamic equilibrium. An other option to suppress the unimers/micelles equilibrium consists in the chemical cross-linking of the micellar core or corona [12, 86–88].

Block copolymer micelles can be prepared by two different methods, either by the direct dissolution of the polymer in the selective solvent or by the use of a non selective cosolvent. In the cosolvent method, the block copolymer is first dissolved in a non-selective solvent, and then the selective solvent is added dropwise in the solution to induce the precipitation of one of the blocks. The non-selective solvent is finally removed from the solution by dialysis or evaporation. On the other hand, the direct micellization process consists in the dissolution of the block copolymer powder in a selective solvent by stirring or via the sonication of the solution

The micellization of block copolymers is dependent on several physical and chemical parameters. The first factor is the concentration of polymer in the solution as it plays a major role in the micellization of block copolymers. Similarly to small molecular weight surfactants in solution, a *critical micellization concentration* (CMC) is observed during the self-assembly of block copolymers in solution (Figure 1.12). Below this concentration, the polymer chains are present as unimers and no micelles are observed. Above the CMC, the concentration of the unimers remains constant and the formation of micelles is observed. At a fixed temperature, the CMC is thus defined as the minimal concentration of copolymer in solution where the first micelle is formed. The definition of the CMC based on this criterion is idealistic. It is indeed particularly hard (or impossible) to precisely determine the exact concentration at which the first micelle is formed with experimental techniques. Therefore, it seems more reasonable to define the CMC as the concentration at which a sufficient number of micelles is formed to be detected by a given method [89]. As presented in figure 1.12 the increase of the polymer concentration in the solution can induce the formation of a so-called micellar gel where the micelles order themselves into lattices. A *critical gel concentration* (CGC) is thus defined and characterizes the behavior of the block copolymers in solution.

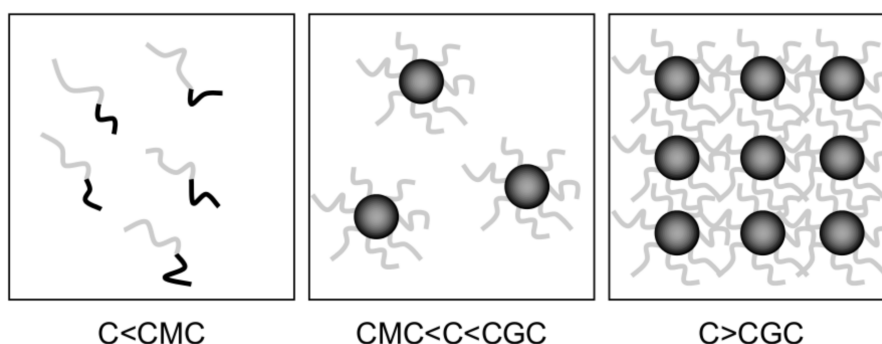


Figure 1.12: Illustration of the critical micelle concentration (CMC) and critical gel concentration (CGC) in a block copolymer solution [3].

The second factor is the temperature of the solution during the micellization. As previously mentioned, this process is a dynamic equilibrium between the free unimers and the micelles and this thermodynamic equilibrium is thus influenced by the temperature. Similarly to the CMC, the *critical micellization temperature* (CMT) may be defined for thermoresponsive polymers as the temperature, at a fixed polymer concentration, at which a sufficient number of micelles is formed to be detected by a given method.

1.3.3.1 Micellization in aqueous media

Considering the concept of the micellization, where the self-assembly is guided by the polymer/solvent affinity, block copolymer micelles can be generated either in aqueous or in organic media. Generally, the micellization of block copolymers takes place in water, as one of the major targeted applications of these systems is to carry and precisely deliver hydrophobic drugs [11–18]. When self-assembled in water, amphiphilic block copolymers are separated in three categories: the non-ionic amphiphilic, the amphiphilic with one ionic block and the "double-hydrophilic" block copolymers.

The first category is typically formed of block copolymers composed of one non ionic hydrophilic block, *e.g.* poly(ethylene oxide)(PEO), linked to a hydrophobic block, typically poly(propylene oxide)(PPO), poly(methyl methacrylate)(PMMA), poly(*tert*-butyl acrylate)(PtBA), poly(ϵ -caprolactone)(PCL) or polystyrene (PS). The micellization of block copolymers made of a combination of these blocks has been largely studied. The commercial availability and the interesting dependence of their micellization to the polymer concentration and to temperature explain the success story of the PPO-PEO di- and triblock copolymers [2, 90]. PS-PEO di- and triblock copolymers were largely studied as the high T_g of the polystyrene block allows the formation of frozen micelles and a wide range of morphologies (Figure 1.13) [10, 91]. However, the micelles formed from these copolymers are prone to secondary aggregation as reported by several groups. [92, 93] PCL-PEO copolymers have attracted much interest as they are biocompatible which makes them ideal candidates for drug delivery applications [94–97].

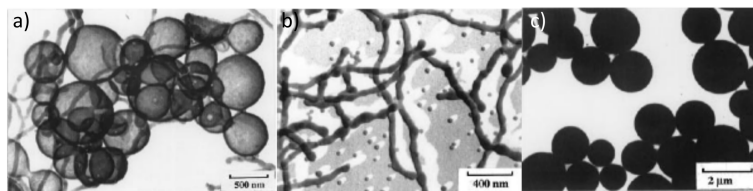


Figure 1.13: TEM images of vesicles (a), rodlike micelles (b) and large compound micelles formed from PS-*b*-PEO diblock copolymer. [91]

The second category is composed of one hydrophobic block similar to the ones described in the first category and of one polyelectrolytic block, typically poly[(meth)acrylic acid](P(M)AA), poly(styrene sulfonate) (PSS), poly(2-vinyl pyridine)(P2VP), poly(4-vinyl pyridine)(P4VP) or other aminated polymers such as poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA). The behavior in solution of micelles with an ionic corona is more complex than the micelles with a nonionic one, as the properties of these micelles are strongly influenced by the pH, the salt concentration and electrostatic interactions [10, 83, 85, 98]. Most of the studies on the micellization of anionic polyelectrolyte copolymer were realized on PS-*b*-PAA diblock copolymers [99–103]. In these studies, the effects of the PAA/PS ratio, the addition of salt and the micellization procedures on the micellar structures were investigated.

The third category of block copolymer is composed of two water soluble blocks. When dissolved in water, these block copolymers do not directly generate micellar structures, however one of the sequence of these copolymers is responsive to a physical or a chemical stimulus. When submitted to the adequate stimulus, the "double-hydrophilic" copolymer can be transformed into an amphiphilic copolymer and inversely. This transition thus allows either stimuli-induced micellization when the copolymer is shifted from the double-hydrophilic structure to the amphiphilic one, or micelle disruption when the amphiphilic block copolymer is transformed into a double-hydrophilic one. More information about stimuli-responsive polymers and their use for stimuli driven self-assembly in solution will be given in chapter 2.

1.3.3.2 Size and morphology of micelles

Extensive efforts were provided, over the time, to rationalize the micellar dimensions, *i.e.*, R_c , R_m and N_{agg} , with the block copolymers characteristics, *i.e.*, N_A and N_B in the case of A-B diblock copolymers with the sequence A forming the micellar core and B forming the micellar corona [2, 10, 84].

In this section, the results obtained from the theoretical investigations are briefly summarized. In the scaling theories, the R_c , R_m and N_{agg} are directly correlated to N_A and N_B . Two limiting case have been distinguished, the starlike and the "crew-cut" micelles. Crew-cut micelles are spherical micelles prepared from highly asymmetric block copolymers containing a short hydrophilic block. The micelles are then characterized by a large core and a short corona ($R_c > R_{corona}$), in contrast to the star-like micelles where the size of the corona is bigger or equal to the core size ($R_c \leq R_{corona}$) (Figure 1.14).

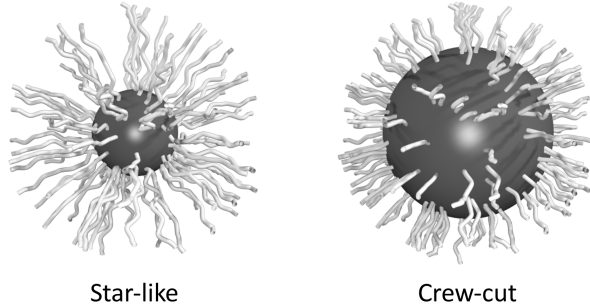


Figure 1.14: Schematic representation of star-like and crew-cut micelles.

The model for star like micelles was developed by Daoud and Cotton, equation 1.4 presents the relation between the micellar radius and the N_{agg} with the copolymer characteristics. One can see that R_m is dependent of the lengths of the sequences forming the core and the corona whereas the aggregation number is only function of the length of the core forming sequence [104].

$$R_m \sim N_A^{4/25} \times N_B^{3/5} \text{ and } N_{agg} \sim N_A^{4/5} \quad (1.4)$$

Zhulina and Birshtein developed a model for AB diblock copolymer. [105] Four regions were identified depending on the copolymer composition (Figure 1.15). The characteristics of region I correspond to crew-cut micelles and the region IV depicts the starlike micelles. The region II and III represent situation where the micelles adopt intermediate structures between the starlike and the crew-cut micelles. The results of their work for the region IV (starlike micelles) are in close agreement with the relations AB derived by Daoud-Cotton. For the crew-cut micelles, they showed that $R_c \sim N_A^{2/3}$ and the $N_{Agg} \sim N_A$.

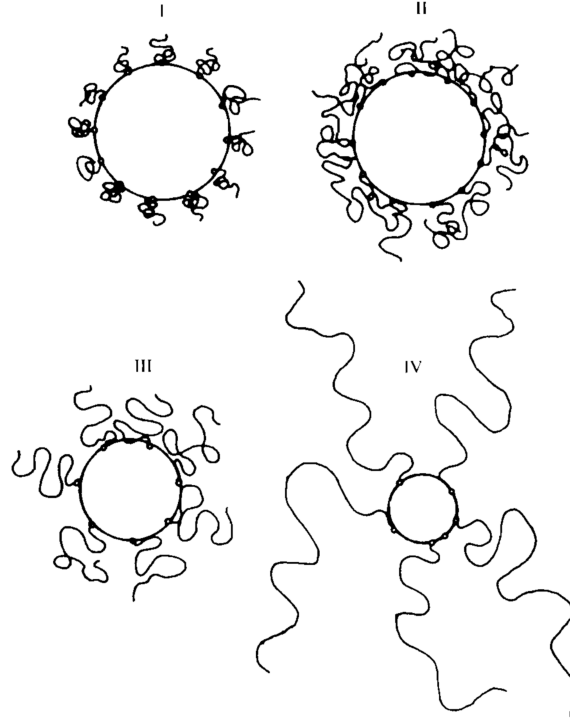


Figure 1.15: Schematic representation of micellar structure in region I-IV defined by Zhulina and Birshtein.

From an experimental point of view, the measure of the micelles size is not trivial and some analytical techniques does not permit to measure directly R_c and R_m . The micelles size is thus characterized by two other experimental parameters, the hydrodynamic radius (R_h) and the radius of gyration (R_g). " R_h is the radius of an equivalent sphere, experiencing during its Brownian motion in solution a friction f identical in magnitude to that of the scattering particle analyzed in dynamic light scattering (DLS) [106]". The gyration radius characterizes the mass distribution of the micelle around the mass centre of the particle.

Block copolymers generally self-assemble in spherical micelles. However, the dissolution of amphiphilic block copolymers in solution also generates other morphologies, like cylindrical micelles and vesicles (Figure 1.7). Extensive efforts have been devoted, over the years, to understand the rules that govern the self-assembly of block copolymers in solution and to control the morphology of the micelles.

Eisenberg's group is considered as a pioneer in the field of the morphology control. Their works on the PS-PAA crew-cut micelles has allowed a better understanding of the key parameters influencing micellar morphology. According to their works the morphology of a micelle depends on the stretching of the core-forming chains, the core/corona interfacial energy, and the repulsion among coronal chains [107].

Another approach to present these parameters is based on a geometrical point of view, where the morphology is influenced by the inherent molecular curvature of the structures and how this influences the packing of the copolymer chains [17]. A "packing parameter (p)" is then introduced (equation 1.5) where v is the volume of the hydrophobic chains, a_o is the area of the hydrophilic group and l_c is the length of the hydrophobic domain (Figure 1.16). According to the packing parameter of a given molecule, the morphology of the self-assembled structure can be predicted.

$$p = \frac{v}{a_o \times l_c} \quad (1.5)$$

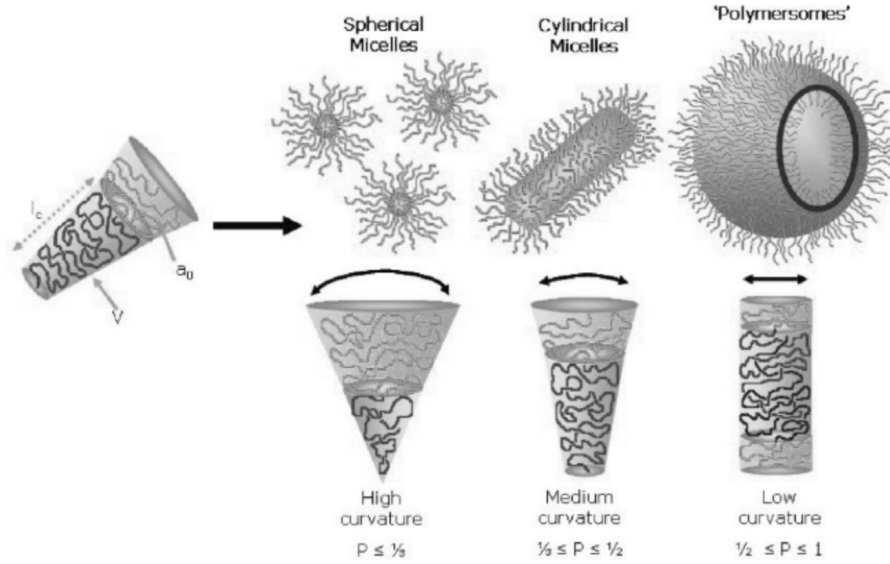


Figure 1.16: Influence of the packing parameter (p) on the morphology of the self-assembled nano-objects [17].

The two aforementioned approaches to determine and explain the morphology of self-assembled structures are not intuitive and do not permit an easy prediction of the morphology based on simple experimental informations, as the total degree of polymerization of the copolymer and the copolymer composition. Discher and Eisenberg, therefore, proposed simple and unifying rules for coil-coil copolymers based on the mass fraction of the hydrophilic block ($f_{hydrophilic}$): [108]

- spherical micelles are observed for $f_{hydrophilic} > 45\%$
- cylindrical micelles are observed for $f_{hydrophilic} < 50\%$
- vesicles are observed for $f_{hydrophilic} \sim 35\%$
- Inverted microstructures such as large compound micelles are observed for $f_{hydrophilic} < 25\%$

Moreover, the morphology of the micelles is also determined by "external" factors such the preparation method [109], the starting selective solvent [100] and added salts during the micellization [110]. An evolution of the micellar morphology is also monitored during the micellization process, when the ratio of non-selective to selective solvent is changed, as demonstrated by figure 1.17 [108].

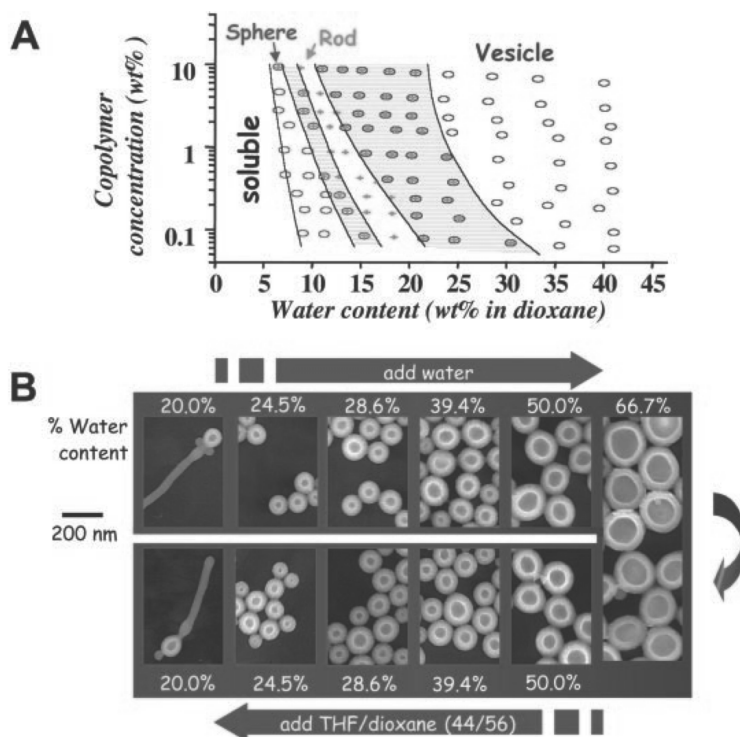


Figure 1.17: Phase diagram of $\text{PS}_{310}\text{-}b\text{-PAA}_{52}$ in dioxane/water mixtures (A, the grey regions between the sphere and rod phases and the rod and vesicle phases correspond to coexistence regions), reversibility of vesicle formation and growth process for $\text{PS}_{300}\text{-}b\text{-PAA}_{44}$ as a function of the selective/non selective solvent composition (B) [108].

1.3.3.3 Characterization of micelles

A large number of analytical techniques are used to characterize micellar structures. The different techniques can be classified either by their physical principles or by the informations they give about the micellar size and morphology. Table 1.1 presents a non-exhaustive list of analytical techniques used for the characterization of micelles and the information they give concerning the self-assembled structures.

As previously mentioned, micelles are characterized by several parameters. First of all, since the micelles are formed by the aggregation of polymer chains, the number of aggregated chains (N_{agg} = aggregation number) plays an important role on the micellar dimensions. N_{agg} is typically determined by static light scattering (SLS). The core is characterized by R_c whereas the R_m represent the radius of the micelles. These radii may be experimentally measured using microscopy techniques (AFM, TEM) or by Small-angle X-ray scattering (SAXS). In static light scattering, the second virial coefficient (A_2) gives information about the quality of the selective solvent for the coronal chains. The micellar gyration radius is typically determined by SLS and the hydrodynamic radius of the micelles by dynamic light scattering.

The CMCs of block copolymers were largely determined by fluorescence spectroscopy via the encapsulation of hydrophobic chromophores (pyrene, nile-red) into the micellar core [102,111,112]. Fluorescence spectroscopy was also used to monitor the micellar dynamics with the grafting of chromophores onto the polymer chains [10,113]. The UV-Vis spectroscopy is especially used for the characterization of stimuli-responsive polymers in solution, to monitor the response of the polymer to a stimulus. This technique is largely adapted for the determination of the *upper critical solubility temperature* (UCST) and the *lower critical solubility temperature* (LCST) of thermoresponsive polymer.

Category	Analytical technique	Physical state	Information about the micelles
Microscopy techniques	Atomic force microscopy (AFM)	Dry state	Morphology, R_m , R_c
	Transmission electron microscopy (TEM) [114]	Dry state	Morphology, R_m , R_c
	Cryo-TEM [114]	Frozen solution	Morphology, R_m , R_c
Scattering techniques	Dynamic light scattering (DLS) [106]	Solution	R_h , PDI
	Static light scattering (SLS) [106]	Solution	R_g , A_2 , M_w , N_{agg}
	Small-angle neutron scattering (SANS)	Solution	Morphology, R_g
	Small-angle X-ray scattering (SAXS)	Solution	Morphology, R_g , R_m , R_c
Spectroscopy techniques	UV-Vis	Solution	LCST, UCST
	Fluorescence	Solution	CMC, dynamics of the micelles
	Nuclear magnetic resonance (NMR)	Solution	Solvation of the core, R_h

^a R_h, R_g, A_2 stands respectively for hydrodynamic radius, radius of gyration and second virial coefficient.

Table 1.1: Analytical techniques used to characterized micelles. [83, 84, 115]

NMR may be used to determine the solvation of the micellar core since the signals corresponding to the core forming polymers are only observed when the core of the micelles is solvated. Moreover, a *Pulsed Field Gradient Spin-Echo* (PFG-SE) sequence or Diffusion-Ordered Spectroscopy (DOSY) allow the measurement of diffusion coefficient of molecules in NMR. The diffusion coefficient determined may thus be related by the stoke-Einstein law to the R_h of micellar systems.

1.4 Focus on the photochemistry

In this thesis, the formation of functional nanostructures in solution is driven by light irradiation. To understand the interaction of light with molecules, some elements of photochemistry are detailed below [116–118].

1.4.1 The wave-particle duality of light

To understand the interaction of light with organic and inorganic materials, the nature of the light irradiation must be understood. For a long time, scientists were not able to decide whether the light acted as a particles beam or a wave. Indeed, the light behaves like an electromagnetical wave characterized by a wavelength λ and a frequency ν (equation 1.6) and with an energy proportional to the frequency according to equation 1.7.

$$\lambda.\nu = c \quad (1.6)$$

$$E = h.\nu \quad (1.7)$$

where c is the light velocity and h is the Plank's constant. Meanwhile, light is also composed of a flow of particles called *photons*. The Einstein theory of relativity state that the energy of these photons is proportional to their masses:

$$E = m.c^2 \quad (1.8)$$

From the combination of equations that relate the energy of the wave character of light (equation 1.7) with the energy of the particle behavior of light 1.8, the equation of De Broglie (equation 1.9) is obtained which introduces the concept of *wave-particle duality*.

$$E = h.\nu = m.c^2 \implies \lambda = \frac{h}{p} \quad (1.9)$$

where $p = mc$. This relationship proves that light has a wave-particle duality as the wavelength is related to the mass of the photon. That equation goes further as it can be applied not only to light but also to every particles.

1.4.2 Photochemistry: light and chemistry

Photochemistry is based on a change in the electronic configuration of molecules by the absorption of one or several photons. Photochemical processes are observed when the energy of the light irradiation is similar to those of chemical bonds. Since light displays a broad electromagnetic spectrum, only a portion of the spectrum affects the electronic states of molecules. As presented in figure 1.18, photochemical process are possible with near-infrared (NIR), visible (Vis) or ultra-violet (UV) irradiations.

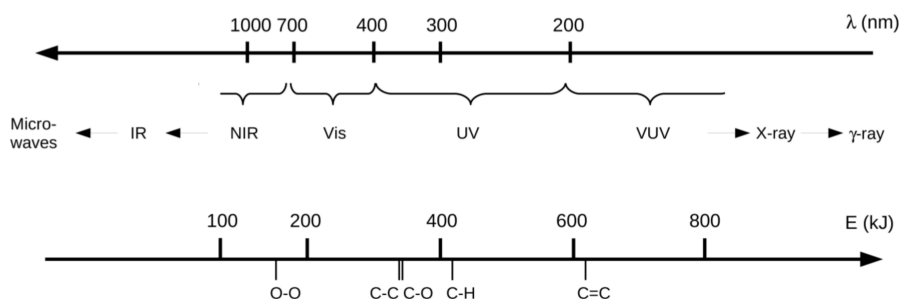


Figure 1.18: Electromagnetic continuum versus the energy of chemical bonding. [75]

The irradiation of molecules by light does not affect the atomic structure of the molecules and thus does not directly lead to chemicals reactions. The interaction of molecules with a light irradiation forces the promotion of one or several electrons of the molecules to higher energy orbitals. Figure 1.19a displays this process, originally in their ground state (E) the electrons reach an excited state (E^*) when the molecule

absorbs the energy of photons. The exchange of energy between the photons and molecules results from the interaction between the oscillating electric field of light and the transition moment of the molecule. The transition moment is a transient oscillating electric field induced in the molecule by the light wave. This exchange of energy is only possible when the frequency of the transition moment and the light frequency are the same. When the molecules are in excited states, they can relax by several processes including structural changes. The absorption of photons by molecules is thus the starting point of all photochemical processes. Figure 1.19b presents the typical electronic transitions observed for organic compounds. In the ground state, the electrons are present in the bonding orbitals of the molecule (σ , π) or as lone pairs (n). After excitation by light, one or several electrons can move from occupied molecular orbitals to unoccupied ones (σ^* , π^*).

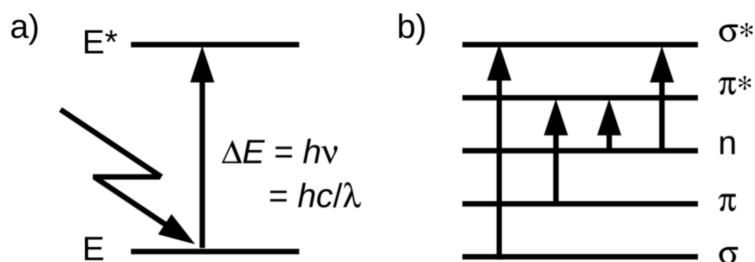


Figure 1.19: Absorption of light by a molecule (a) and typical electronic transitions in organic compounds (b) [75].

Jablonski diagrams display the variety of electronic states and relaxation processes possible for the excited molecules (Figure 1.20). In this diagram, the ground state (S_0) is the singlet state in which the energy of electronic state of the molecules is the lowest. The following states, S_1 and S_2 correspond to the singlet states of the $n\pi^*$ and the $\pi\pi^*$ transitions. In addition of the singlet states, the triplet states of the π^* and the $\pi\pi^*$ transitions are displayed by T_2 and T_1 . The aforementioned states represent the "stable" excited configurations but higher energetic vibrational states exist (dotted lines).

As these excited states are not the most stable electronic configurations of the molecules, the system tends to return in its ground state. Various radiative and non-radiative relaxation processes are possible. The excited system can dissipate the energy by the emission of photons (radiative relaxation). Fluorescence is observed when the relaxation occurs from a singlet state to the ground state ($S_1 \Rightarrow S_0$). Phosphorescence is monitored when the relaxation occurs from a triplet state to the ground state ($T_1 \Rightarrow S_0$).

In the non-radiative processes, the system dissipates the energy in the form of heat by vibration (internal conversion) or inter-system conversion. Finally the excess of energy can be dissipated by a change of the molecule structure leading to new products. Photochemistry focuses precisely on this type of relaxation.

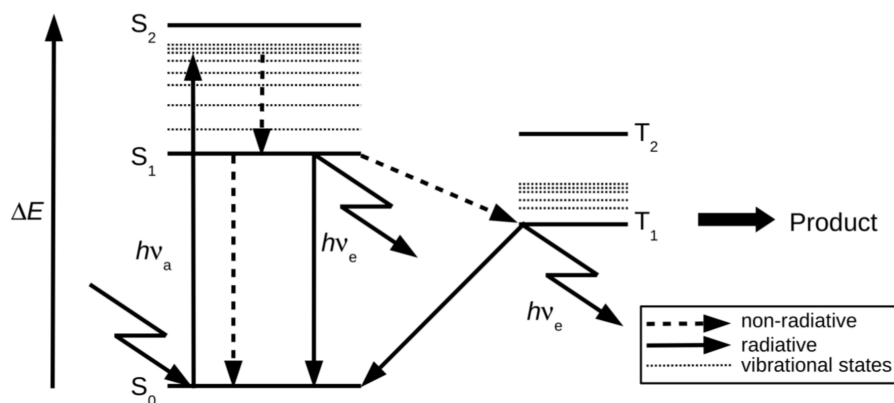


Figure 1.20: Jablonski state diagram. [75]

1.4.3 Photochemistry vs. thermochemistry

In traditional chemistry (thermochemistry), a chemical reaction is controlled by the energetic barriers which have to be overcome to transform the reagents into the products (full curve in Figure 1.21). This energetic barrier is called the *activation energy* (E_a) and dictates the rate of the reaction as demonstrated by the Arrhenius equation (eq. 1.10).

$$k = A. \exp \frac{-E_a}{RT} \quad (1.10)$$

In photochemistry, the excitation of the reactive molecules allows to easily overcome the activation energy as the excited states are often more energetic than their corresponding ground states. When looking at the energetical profiles of the photochemical reactions (dashed line in Figure 1.21), one can see that the energy of the excited state exceeds the activation energy of the reaction and thus the barrier is already overcome. Moreover, some reactions that are not possible in thermochemistry are allowed in photochemistry.

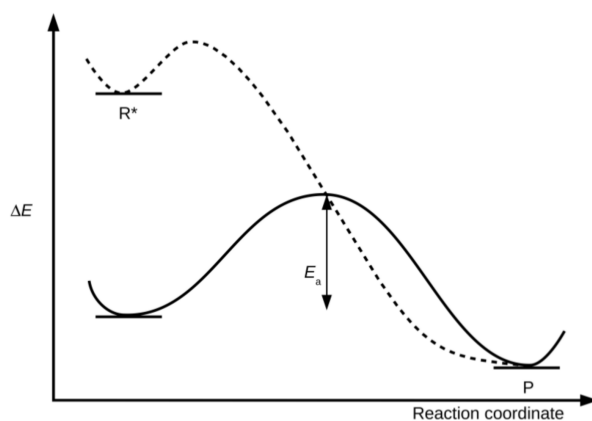


Figure 1.21: Energetic pathways from reagents (R) to products (p) in thermochemistry (solid line) and in photochemistry (dashed line). [75]

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FUNCTIONAL NANOSTRUCTURES IN SOLUTION

Abstract

The self-assembly of block copolymers in solution is the topic of extensive studies because of the high valued applications of the obtained nanostructures. Since, this thesis focuses on the use of stimuli to promote the formation of polymeric nanostructures in solution, This chapter provides a brief overview of the wide range of functional nanostructures made of block copolymers in solution. In the first part, the use of stimuli-responsive block copolymers to control the formation of micellar structures will be detailed. The second part deals with multi stimuli-responsive materials and how the design of block copolymers can influence the variety of nanostructures in solution. The last part of this chapter presents a special kind of nanostructures made of polymeric materials in solution: the nanocages or hollow nanocapsules.

2.1 Introduction

In the previous chapter, a brief overview of the self-assembly of block copolymers in solution was given (Section 1.3.3). Several parameters influencing the size and the morphology of the polymeric nanostructures were presented. However, the previously described systems exhibited a somewhat "static" behavior. Indeed, The self-assembly of these polymeric materials is only influenced by the polymer-solvent interactions and the micellar systems were only obtained with amphiphilic block copolymers. The appearance, in the mid 90's, of *smart* polymeric materials totally changed the story since the self-assembly of those kind of block copolymers can be modified based on environmental constraints [1–12]. A part of this chapter will thus focus on these stimuli-responsive block copolymers and on how the self-assembled nanostructures can be modified by variations in the surrounding environment. Moreover, section 2.3 will present more complex stimuli-responsive polymeric materials, where several different sensitive blocks are introduced in the copolymers. The self-assembly of such kind of polymeric systems gives rise to complex micellization processes known as "multiple and schizophrenic" micellization. These concepts and several examples of such type of copolymers will thus be presented.

In parallel to the research on stimuli-responsive block copolymers, the scientific community devotes an extensive effort on the study of vesicles in solution. Indeed, such nano-objects display similar structures than those found in cells, where the internal cavity can be seen as the cytoplasm and the core-shell structure of the vesicle represents the plasmic membrane. The study of these structures thus allows scientists to mimic nature and design systems where a compartmentalization of chemical processes is possible, like in a cell [13–15]. Since vesicles are obtained by the self-assembly of copolymers of a certain composition and that the coexistence of several morphologies is possible, chemists investigated different approaches to produce hollow nanocapsules. Section 2.4 presents a brief overview of the strategies to produce nanocages either thanks to direct polymerization process or via the self-assembly of polymeric materials.

2.2 Stimuli-responsive micelles

Despite the successful application of polymeric micelles as drug carrier, *in vitro* and *in vivo* studies pointed the limitations of these systems [1]. The premature release in the blood circulation of drugs from the micellar core or the inability of micelles to deliver the drugs into the adequate cells were the two commonly observed issues. The ability to trigger the micellization and especially the disruption of micelles has thus been particularly sought after over the last decades. Indeed, the control of the micellization of block copolymers allows the loading and precise release of active molecules, which is particularly interesting for biomedical applications as attested by the number of articles published in the last years [1–6].

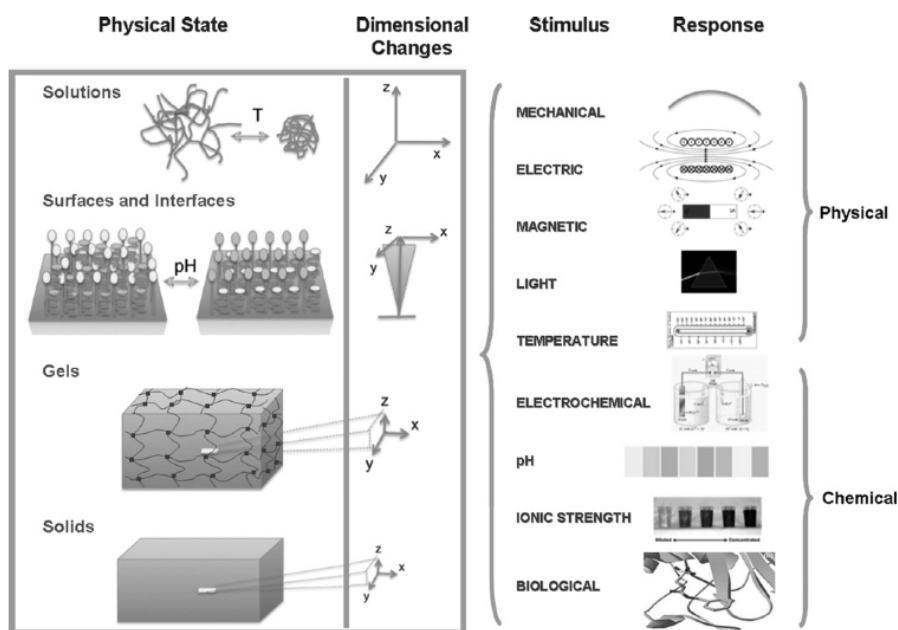


Figure 2.1: Schematic representation of dimensional changes in polymeric solutions, at surfaces and interfaces, in polymeric gels and polymer solids resulting from physical or chemical stimuli [9].

To overcome those limits, the appearance of stimuli-responsive polymers in the mid 90's allowed the formation of "smart" polymeric materials, which are able to modify their behaviors as a function of the surrounding medium. Typically, such polymers are able to show a sharp modification of their chemical and/or physical properties in response to slight changes of their environment such as those induced by variations of pH, temperature, ionic strength, light, *etc.* [7–12] As depicted in figure 2.1, the physical changes of stimuli-responsive materials may occur in solution, at surfaces and interfaces and in the solid state.

In addition of the aforementioned drug release application, stimuli-responsive micelles may be used as purification devices, where selected molecules are encapsulated in the micellar core during the stimuli induced micellization [12, 16]. Stimuli-responsive micelles are also largely used as sensors in solution, the grafting of chromophores on the stimuli-responsive backbone allows the monitoring of variation in the environment of the micelles by fluorescence and Förster resonance energy transfer (FRET) [12, 17, 18]. One of the other major application of micelles is the stabilization of inorganic nanoparticles. The use of stimuli-responsive polymers for nanoparticles allowed the regulation of the stability of colloidal suspensions [12, 18]. The stabilization of nanoparticles in solution is also interesting for medical applications since it is possible to stabilize metal nanoparticles in the micelles and to use them as imaging agents. Another successful application of stimuli-responsive micelles is their use in catalysis processes [12, 18]. The stabilization of metal nanoparticles in stimuli-responsive micelles allows to modulate the access of reactants to the catalyst and thus to modulate the reaction. Moreover this type of stabilized catalyst is particularly appreciated for tandem reactions.

This section focuses on the physical and chemical changes in solution of stimuli-responsive polymers and more particularly on the stimuli induced micellization and disruption of block copolymer micelles. Among the wide range of stimuli available, the responses of block copolymers to pH (Section 2.2.1), temperature (Section 2.2.2) and light (Section 2.2.3) are presented.

2.2.1 pH stimulus

The idea of using pH sensitive polymers as drug carrier rapidly spread in the scientific community. Indeed, an increase of the medium acidity is monitored during the transition from the blood circulation to the intracellular medium. Moreover, if an oral administration of the drug is considered, the use of a drug carrier stable at low pH (*i.e.* in the stomach) and unstable near physiological pH (*i.e.* at the intestines) is desired [1, 2]. Micelles responsive to pH were also successfully used in surface coating [19]. More particularly Grigoriev and coworkers designed an anti-corrosive coating where pH sensitive nanocarriers are loaded with corrosion inhibitor [20]. During the corrosion of surfaces, a local change in pH is observed. This pH modification induces the release of the inhibitors exactly at the corrosion-damaged sites. Elimination of the corrosive attack returns the nanoparticles to the sealed state, enabling effective storage and consumption of the inhibitor [20].

pH sensitive polymers are divided into two groups: polybases and polyacids. In water, the first class is composed of polymers bearing basic functions (*e.g.* amines), which are uncharged and thus hydrophobic at high pH and protonated (hydrophilic) when submitted to an acidification of the medium. On the other hand, polyacids are charged in basic media and protonated at low pH. Typically the transition from the soluble conformation to the insoluble one is function of the pKa of the protonatable group. The micellization of polybases thus occurs at pH above the pKa, where the chains are largely uncharged. Upon acidification of the medium, the ionization of the polymer chains causes an increase of the chain hydrophilicity and the electrostatic repulsions between generated charges lead to the destabilization of the micelles. The disruption of polyacid micelles is driven by the same physical and chemical principles, the only difference is that the micelle disruption occurs by a basification of the medium.

Table 2.1 presents a non-exhaustive list of pH sensitive polymers, their chemical structures are depicted in figure 2.2. The value of pH indicated in the second column represents the typical pH where the micelles

to unimers transition is observed. The five first polymers are polybases and the two last entries of the table present typical polyacid systems. In table 2.1, three examples of pH-sensitive bio-polymers are presented, poly(L-histidine), poly(glutamic acid) and chitosan. They are ideal candidates for drug delivery applications as they are biocompatible, non-toxic and biodegradable. [2] In the synthetic polypeptides class, other amino acids bearing protonatable groups might undergo a pH transition around their pKa such as aspartic acid (pKa=3.9), lysine (pKa=10.5), or arginine (pKa=12.5). Poly(L-histidine) and poly(glutamic acid) were selected as their pKa values, respectively 6 and 4.1, are around or below the physiological pH, which make them appropriate candidates for drug delivery applications. Chitosan is part of another class of biopolymers, the ionizable polysaccharides. Chitosan is the N-deacetylated derivative of chitin and is a copolymer consisting of 2-acetamido-2-deoxy-*b*-D-glucopyranose and 2-amido-2-deoxy-*b*-D-glucopyranose (Figure 2.2). Chitosan also presents biological activities such as good mucoadhesivity, hemostatic, and antibacterial properties [21].

Monomer	pH ^a	Copolymer	Ref
DMAEMA	< 12	PEO- <i>b</i> -PDMAEMA	[22]
		PDMAEAM- <i>b</i> -PNIPAm	[23]
DEA	< 12	Py-PDEA- <i>b</i> -PAP	[24]
Chitosan	< 9	chitosan- <i>g</i> -P(NIPAm- <i>co</i> -AA)	[25]
L-histidine	< 7.4	PHis- <i>b</i> -PEO	[26, 27]
Vinylpyridine	< 5	P2VP- <i>b</i> -PEO	[28–31]
		P2VP- <i>b</i> -PNIPAm	[32]
(Meth)acrylic acid	> 5	P(M)AA- <i>b</i> -PEO	[33, 34]
Glutamic acid	> 4	PGA- <i>b</i> -PEO	[35]
		PGA- <i>b</i> -PDMAEMA	[36, 37]

^a pH at which micelles are destabilized.

Table 2.1: Selected examples of pH responsive polymers.

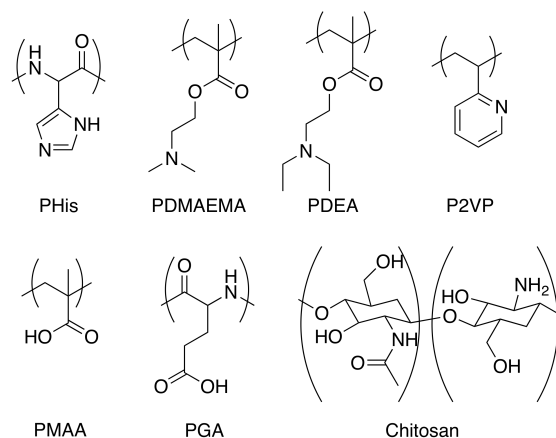


Figure 2.2: Stucure of selected pH responsive polymers.

In addition to the "simple" pH induced micellization, the creation of micelles with a pH sensitive core can be carried out with *Interpoly-electrolyte complexes* (IPECs). When block copolymers bearing positive and negative charges (called polyampholytic copolymers) are solubilized in water, the electrostatic interactions between the oppositely charged blocks induce the precipitation of the aggregated chains in the medium. This behavior is unfavorable for the formation of micelles, however the use of highly asymmetric polyampholytic copolymers with an excess of negative or positive charges gives rise to micellar structures with a core composed of IPEC and a corona formed with the uncomplexed segments. Since the ionization degree of the blocks is a function of the surrounding pH, the formation of the IPECs is only observed in a restricted pH region, where the blocks are partially or totally ionized. This type of pH sensitive IPECs micelles is observed for P2VP-*b*-P(M)AA [38] and PDMAEMA-*b*-P(M)AA copolymers [39, 40].

IPEC micelles may also be formed with mixture of polybases and polyacids as demonstrated by figure 2.3. In this example, chitosan (polybase) and PAA (polyacid) were mixed in water at a fixed pH and the charge ratio of the solution was varied. Figure 2.3a plots the evolution of the normalized scattered intensity (I_2/I_1) versus the charge ratio

(n^+/n^-) of the polymer solution. Figure 2.3b presents the hypothetical structure of the system with the variation of the charge ratio. This experiment clearly demonstrates the effect of the charge ratio, and thus of the solution pH, on the stability of the nanostructures in solution [21].

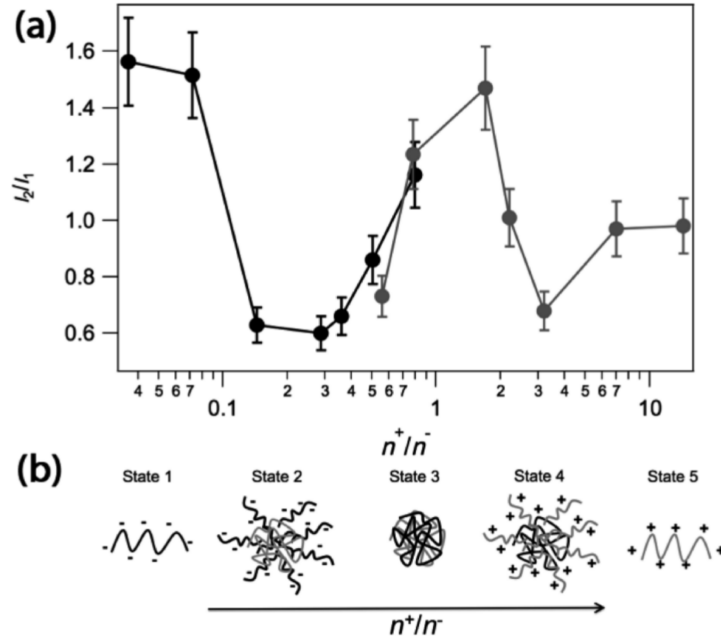


Figure 2.3: Evolution of the intensity ratio (I_2/I_1) with the charge ratio n^+/n^- during the titration of solutions of PAA by chitosan (black curve) and of chitosan by PAA (gray curve) (a). Sketches depicting the hypothetical nanoparticle structures while increasing the charge ratio n^+/n^- (b) [21].

2.2.2 Temperature stimulus

Temperature is the most widely used stimulus for environmental responsive polymer systems because in principle all polymers exhibit a response to temperature in solution. As mentioned in the introduction, the solubility of polymers in a solvent varies as a function of the polymer/solvent affinity. In the Flory-Huggins Mean-Field Theory, the parameter χ_{P-S} (Flory-Huggins parameter) represents the polymer/solvent

affinity and a thermal evolution of this parameter is practically observed according to equation 2.1:

$$\chi_{P-S} = Z \frac{\epsilon_{P-S} - (\epsilon_{P-P} + \epsilon_{S-S})}{2kT} \quad (2.1)$$

Where ϵ_{S-S} , ϵ_{P-P} and ϵ_{P-S} are the interaction energies for a solvent-solvent (S-S) contact, a polymer-polymer (P-P) contact and a polymer-solvent (P-S) contact, respectively. Z is the lattice parameter and k is the Boltzmann constant [41]. This equation states that when a negative χ_{P-S} is observed the polymer-solvent contacts are preferred and that, in general, the χ_{P-S} decreases when temperature is increased, *i.e.* the solubility of the polymer is better when the temperature is increased.

The phase diagram of a polymer solution highlights the unique property of temperature responsive polymers, the critical solution temperature. Figure 2.4a presents a typical phase diagram of a polymer solvent mixture, where the solubility of the polymer increases with temperature. The coexistence curve of this diagram presents region of composition and temperature across which a transition occurs from miscibility of the components to conditions where single-phase mixtures are metastable or unstable. This coexistence curve may be experimentally approximated to the cloud point temperature curve (T_{cp}), which report the temperature where a phase separation is macroscopically observed as a function of the polymer-solvent composition. Generally, the coexistence curve exhibits a parabolic shape where the maximum of the curve is called the *upper critical solution temperature* (UCST). The UCST is the temperature where the solute is miscible with a solvent in all proportions [42].

Although the solubility of a polymer in a solvent is generally increased when the temperature of the solution is raised, the opposite behavior is observed when the polymer and the solvent interact by hydrogen bonding. This may seem counter-intuitive but can be easily understood by the fact that the hydrogen bonds between the solvent and the polymer break at high temperature. The released water molecules increase the entropy of the overall system. A demixing of the solution is observed [41]. The phase diagram drawn in figure 2.4b presents the *lower critical so-*

lution temperature behavior (LCST). In this case the phase separation between the polymer and the solvent is observed when the temperature of the solution is raised. As previously mentioned, the thermo-responsive behavior of polymers depends on the polymer/solvent system, however this characteristic is particularly interesting in water for applications where a collapse of the polymer is needed at high temperature. The examples of thermo-responsive systems displayed below will only focus on polymers with an UCST or a LCST behavior in water.

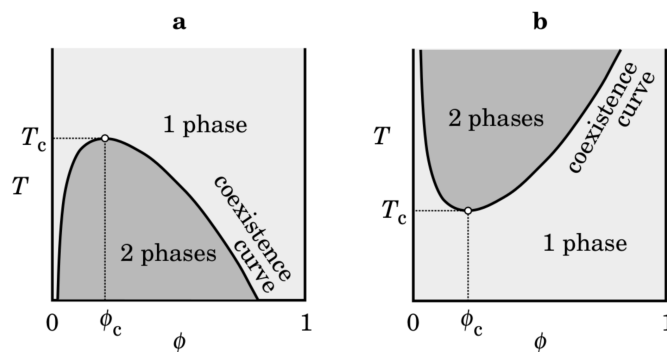


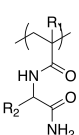
Figure 2.4: Phase diagrams of polymer solution on temperature-composition plane: (a) UCST-type phase diagram and (b) LCST-type phase diagram [41].

2.2.2.1 UCST polymers

Although the UCST behavior is the typical behavior of block copolymers in solution, the number of publication related to this topic is limited and especially for the study of UCST polymers in water [42]. This can be explained by the fact that most of the common polymers exhibit their UCST in organic solvents or at temperatures below 0°C or above 100°C in water. The lack of information on this topic was solved by Seuring *et al.* whom have recently published a review on polymers with upper critical solution temperature in aqueous solutions [42].

The most studied polymers with an UCST behavior in water are the poly(N-acryloylglycinamide) (PNAGA) derivatives and poly(betaine) (Fig-

ure 2.5). It was first revealed that PNAGA can form thermo-reversible gels in concentrated aqueous solutions [43]. The gels were formed as the consequence of hydrogen bonding between the monomer units of the polymer as demonstrated by the addition of hydrogen bond interrupting agents like thiocyanates or urea. This hydrogen bonding crosslinking of the PNAGA was further evidenced by analyzing the shifts of the CO and NH-bands in the Raman spectrum [44]. Although the PNAGA derivatives are the most studied UCST polymers, their application for the formation of micelles is limited [42].

Polymer [a]	M_n [b] kg mol ⁻¹	Cloud points [c]
 1 poly(N-acryloylglycinamide) R₁ = H, R₂ = H 2 poly(N-acryloylasparagineamide) R₁ = H, R₂ = CH₂CONH₂ 3 poly(N-acryloylglutamineamide) R₁ = H, R₂ = CH₂CH₂CONH₂ 4 poly(N-methacryloylasparagineamide) R₁ = CH₃, R₂ = CH₂CONH₂	16 17 22	22°C in pure water 25°C in 20x10⁻³ M Tris-HCl, pH8 2°C in in 40x10⁻³ M Tris-HCl, pH8 3°C in in 80x10⁻³ M Tris-HCl, pH8 13°C in pure water 33°C (LCST) in pure water 13°C in PBS 30°C (LCST) in PBS

[a] ammonium peroxodisulfate was used as initiator; [b] measured by GPC in 0.1 M NaNO₃ as eluent, calibration standard not given;
[c] c(polymer) = 0.2% (w/v), it was not specified whether it is the cloud point upon heating or cooling.

Figure 2.5: PNAGA derivatives and their UCST. Adapted from [42].

Polybases displays a UCST behavior in the presence of oppositely charged particles or multivalent ions. This was first demonstrated by Jia *et al.* on a PEO-*b*-P2VP block copolymer [45]. At low pH the pyridine functions of the P2VP block were protonated, the addition of peroxodisulfate ions below a critical temperature led to the aggregation of the P2VP block and the formation of stable micelles. The increase of the solution temperature, as well as the reduction of the peroxodisulfate, led to the disruption of the micelles. A similar behavior was presented by Plamper *et al.* for PEO-*star*-PDMAEMA in the presence of hexacyanocobaltate(III) (Figure 2.6) [46]. In presence of this cobalt salt, PDMAEMA exhibits UCST behavior in solution, moreover the UCST of the system can be suppressed by the irradiation of the solution, which induces an ion exchange and the formation of [Co(CN)₆•5H₂O]²⁻ [46,47]. Based on these reports, polyelectrolytes seem ideal candidates for the

formation of thermo-sensitive micelles with a UCST behavior although a delicate balance of ionic strength, pH, and counter-ion valency is required to observe it [42].

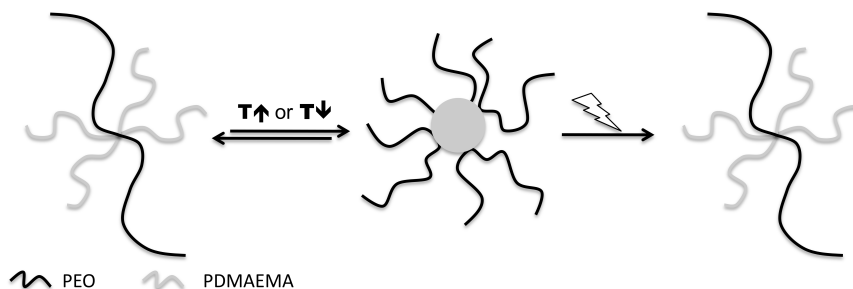


Figure 2.6: Behavior of PEO-*star*-PDMAEMA miktoarm star in pH 8 buffer and in the presence of $[\text{Co}(\text{CN})_6]^{3-}$. Adapted from [46].

2.2.2.2 LCST polymers

In contrast to polymeric systems showing an UCST, polymers displaying a LCST were highly studied as attested by the number of reviews dealing with this topic [1,2,6,7,9,17,48–51]. As previously mentioned the LCST in water is observed with polymeric systems bearing side groups (*e.g.* amide or ether groups) able to form hydrogen bonds with the surrounding water molecules.

The temperature driven transition from soluble to insoluble is a rather complex phenomenon involving both hydrophilic and hydrophobic interactions, this phenomenon is clearly explained by Aseyev *et al.* [49]. According to those authors, the thermo-responsive polymer is soluble in water at low temperature because of the hydrophilic side groups organize the surrounding water molecules by hydrogen bonding, leading to the formation of ordered hydration layers. This was attested by IR studies on PNIPAm where $\text{C}=\text{O}\cdots\text{HN}$, $\text{C}=\text{O}\cdots\text{H}_2\text{O}$, $\text{N}-\text{H}\cdots\text{OH}_2$ hydrogen bonds and non-hydrogen-bonded $\text{C}=\text{O}$ and $\text{N}-\text{H}$ groups signals were observed [52,53]. The hydrophilic/hydrophobic balance of the polymer is thus shifted to hydrophilic. Upon heating, the hydrogen bonds

between the polymer and the water molecules are destabilized and the water molecules are released in solution, allowing associative contacts between the newly exposed hydrophobic monomer units. The overall hydrophilic/hydrophobic balance of the polymer is shifted to hydrophobic and a phase separation is observed (Figure 2.7). From the Gibbs free energy point of view (ΔG_M), the demixing observed at a high temperature for LCST polymers is explained by the contribution of the enthalpic (ΔH_M) and entropic (ΔS_M) factors (equation 2.2).

$$\Delta G_M = \Delta H_M - T\Delta S_M \quad (2.2)$$

The enthalpy of the system before and after the demixing is positive, because of the destruction of P-S bonds and the creation of P-P and P-S bonds ($\Delta H_M > 0$). However, the entropy of the system largely increases since the water molecules are released in the solution, *i.e.* $\Delta S_M > 0$. Equation 2.2 thus becomes:

$$\Delta G_M = \Delta H_M - T\Delta S_M = \oplus - \oplus \times \ominus > 0 \quad (2.3)$$

Since the free energy of mixing is positive, a phase separation occurs. Equation 2.3 also demonstrates that the phase separation of LCST polymers is an entropically driven process.

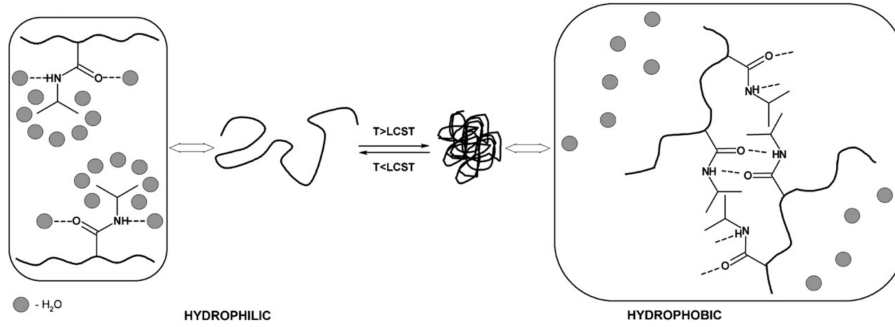


Figure 2.7: Representation of the solvation of a thermo-responsive polymer at low (left) and high temperature [50].

A wide range of polymers exhibits a LCST behavior in water: poly[(meth)acrylamide]s, poly(N-vinyl amide)s, poly(2-oxazoline)s, poly(ether)s, poly(vinylether)s and poly(phosphoester)s. A non exhaustive list of these polymers with their cloud point temperature is given by Aseyev *et al.* [49]. Among the large variety of LCST polymers, poly(N-isopropyl acrylamide) PNIPAm is the most famous and the most studied. The success story of this thermo-responsive polymer is explained by the sharp transition from the soluble to the collapsed state observed around 32°C and by the possibility to synthesize (co)polymers of NIPAAm by controlled radical polymerization techniques. With time, another class of thermo-responsive polymer was discovered: the poly[oligo(ethylene glycol) (meth)acrylates] (POEGA or POEGMA). These polymers are really interesting for biomedical applications because they are biocompatible and the monomers are commercially available. Moreover, the tuning of the materials LCST is easily achieved by the copolymerization of monomers with different oligo(ethylene glycol) length (Figure 2.8) [48, 54].

Polymer	Properties in Aqueous Environment	Commercial Availability
PMMA	Hydrophobic	Yes
PMEMA	Slightly hygroscopic	Yes
PMEO ₂ MA	LCST 26°C	Yes
PMEO ₃ MA	LCST 52°C	No
POEGMA ₃₀₀	LCST 64°C	Yes
POEGMA ₄₇₅	LCST 90°C	Yes

Figure 2.8: Properties of polymers prepared with oligo(ethylene glycol) methacrylates of various length. Adapted from [54].

In thermo-sensitive micelles, the responsive sequence can either constitute the core or the corona of the micelles. At a first glance, the idea of micelles with a thermo-responsive corona for encapsulation and delivery applications is counter-intuitive since the increase of temperature induces a collapse of the micelles. However, studies on a PNIPAm-*b*-PBMA revealed that micelles with a low T_g core (PBMA, $T_g < 20$ °C) and a thermo-sensitive corona are disrupted when the temperature of the solution is increased [55]. On the other hand, the same experiment realized on micelles formed from a high T_g core (PS, $T_g = 100$ °C) does not dis-

play the disruption of the micelles upon heating. Moreover, the release of active molecules was tested with the two systems, the PNIPAm-*b*-PBMA micelles released the drugs when heated above the LCST, while PNIPAm-*b*-PS micelles did not.

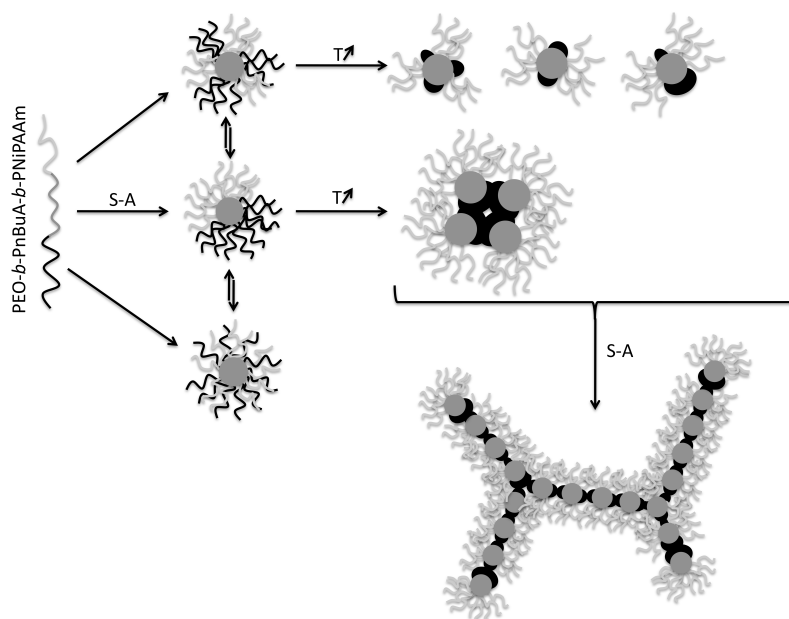


Figure 2.9: Use of thermal stimulus to induce super-structure formation from a PEO-*b*-PnBA-*b*-PNIPAm triblock copolymer. Adapted from [56].

Micelles with a hydrophobic core and a thermo-responsive corona are also used to induce a change of morphology and the formation of super-structures in solution. Müller *et al.* realized such super-structures with a PEO-*b*-PnBA-*b*-PNIPAm triblock thermo-responsive copolymer [56]. Figure 2.9 presents a schematic representation of the triblock copolymer, the self-assembly (S-A) in micelles with a PnBA core and a PEO/PNIPAm corona and the use of a thermal stimulus to form the superstructure in solution. Triblock copolymers, with the hydrophobic block localized between the two hydrophilic blocks, can self-assemble in solution into a mixture of three types of micelles: mixed (bottom), mul-

ticompartment (top) and Janus micelles (middle). When the thermal stimulus is applied the PNIPAm chains collapse and the superstructures are formed where the multicompartment and the Janus micelles arrange themselves according to the composition of their corona (Figure 2.9b).

Micelles with a thermo-responsive corona were successfully used for responsive catalysis [57, 58]. Wang and coworkers loaded PNIPAm-*b*-P4VP micelles with gold nanoparticles. They used these gold loaded micelles to realize the reduction of *p*-nitrophenol by NaBH₄ and monitor the rate of the reduction at temperature below and above the LCST of PNIPAm (Figure 2.10) [57].

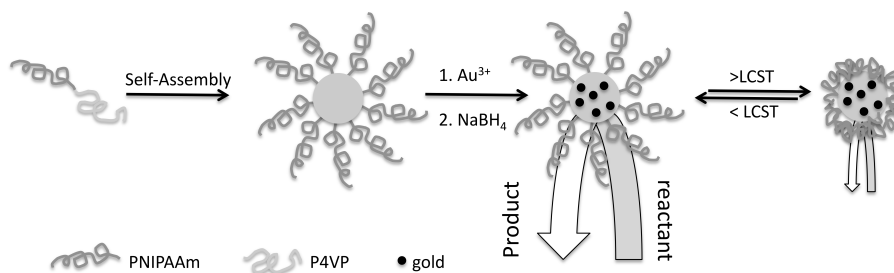


Figure 2.10: Use of thermo-responsive micelles as responsive catalyst. Adapted from [57].

Micelles with a thermo-responsive core were formed with several block copolymers including PNIPAm-*b*-PEO [59–62], PNIPAm-*b*-PAA [63], PNIPAm-*b*-POEGMA [64, 65], PEO-*b*-PMEO₂MA-*co*-PMAA [66], PNIPAAm-*b*-(PEA-*g*-PDEA) [67], PNEAm-*b*-PNIPAm-*b*-PMEO₂A [68]. In all these cases, the core of the micelles was formed after an increase of the solution temperature. In some cases, the system was designed with other stimuli-responsive blocks to induce multiple micellization processes. This type of systems will be described more precisely in section 2.3.

2.2.3 Light stimulus

The use of light to control the properties of copolymers has recently attracted much attention due to some unique features such as the possibility to control reactions from outside of the system, to localize this stimulus in time and space, the process does not need additional reagents and generates a limited amount of by-products. Moreover, irradiation parameters (wavelength, intensity) can be easily tuned depending on the photosensitive moieties used, insuring selectivity [69–71].

The design of light sensitive block copolymers is strongly influenced by the targeted applications. Indeed, several photosensitive moieties (nitrobenzyl, phenacyl and pyrenyl esters) might undergo an irreversible transformation during the irradiation whereas others can react reversibly (syropyrane and azobenzene moieties). Moreover, the location of the photo-responsive moiety in the copolymer strongly influences the type of response to irradiation (Figure 2.11) [72, 73]. The polymer has thus to be rationally designed to fit the targeted application.

As presented in figure 2.11, the major applications for light irradiation of polymeric materials in solution are the micelle disruption (a, b and c) and the micelle stabilization, by cross-linking of the core or the corona (d). Figure 2.12 presents the classification of several photo-responsive moieties in function of their response to light irradiation.

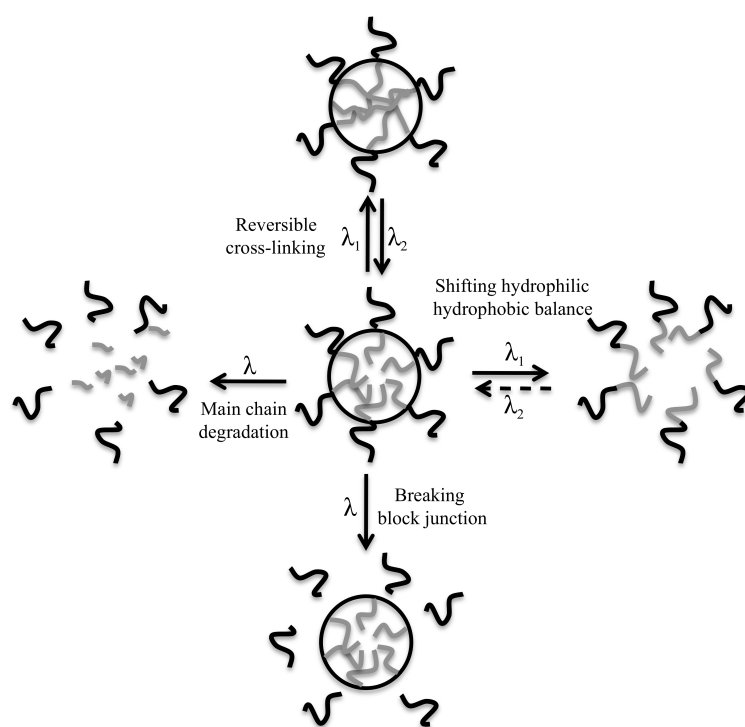


Figure 2.11: Schematic illustration of various types of light-responsive block copolymer micelles [73].

2.2.3.1 Micelle disruption

Light induced disruption may be realized on micelles prepared from block copolymers bearing photosensitive side groups or with linear photosensitive copolymers, where the photo-responsive moieties are introduced in the main chain. Moreover, the disruption can be reversible or irreversible depending on the removal of photochromic moieties with irradiation [73]. The examples of micelle disruption presented below are divided into two parts depending on the location of the photo-responsive moieties.

Disruption by a variation in the hydrophilic-hydrophobic balance

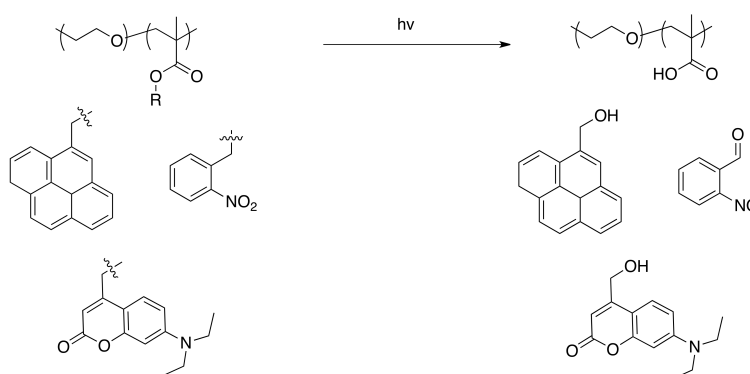


Figure 2.13: Irreversible change in the hydrophilic-hydrophobic balance of block copolymers by illumination.

The irreversible disruption of micelles is realized on copolymers bearing photocleavable moieties, *e.g.* coumarin, nitrobenzyl, phenacyl or pyrenyl. Most of the time these photocleavable moieties are present as side groups on one of the copolymer sequence (Figure 2.13). The disruption of the micelles is the consequence of the shift in the hydrophilic-hydrophobic balance of the block copolymer, where the hydrophobic sequence bearing the photocleavable side groups is transformed into an hydrophilic one, *e.g.* P(M)AA, upon irradiation. This approach was pioneered by Zhao *et al.* on block copolymers composed of a PEO block

and a block bearing either pyrenylmethyl [74], *o*-nitrobenzyl [75, 76] or coumarin methacrylate [77]. It has to be noted that pyrenylmethyl esters and phenacyls esters cleave themselves under irradiation via an intermolecular mechanism and thus require the presence of water or a protonic solvent, whereas the *o*-nitrobenzyl ester undergoes a photolysis by an intramolecular Norrish II mechanism.

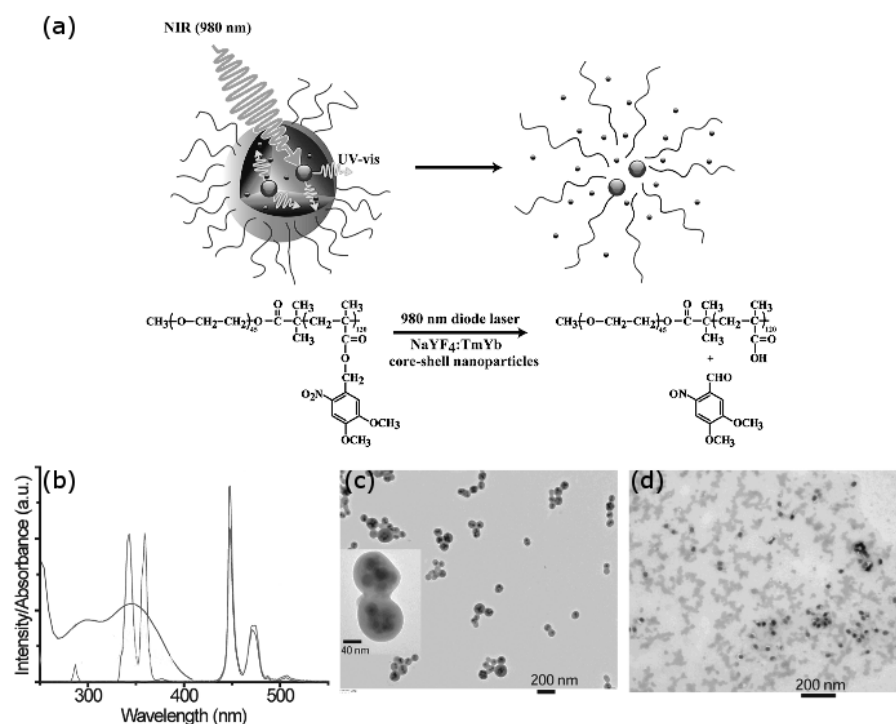


Figure 2.14: Schematic illustration of LID using NIR light excitation of UCNP to trigger dissociation of BCP micelles (a), absorption and emission of UCNP and absorption of the photocleavable block copolymer (b), TEM image of the micelles before (c) and after irradiation (d). Adapted from [78]

An interesting characteristics of *o*-nitrobenzyl and coumarin esters is that they can be cleaved not only by UV irradiation but also by near-infrared light (NIR) through a two photons absorption process. The NIR irradiation is appreciable for the control release of drugs since it is

less detrimental to healthy cells and it can have a deeper penetration through tissues compared to UV [75,77]. However, the cleavage of these moieties by NIR light is generally slow and inefficient. To solve this issue and combine the advantages of the two types of irradiation, Zhao and coworkers used upconverting nanoparticles (UCNPs) [78]. Such type of particles are excited by a two photons absorption process (NIR) and relax themselves by the emission of one higher energetic photon (UV). Micelles of poly(ethylene oxide)-*block*-poly(dimethoxynitrobenzyl methacrylate)(PEO-*b*-PDMNBA) were loaded with NaYF₄:TmYb UCNPs. When irradiated with a NIR light ($800 < \lambda < 1000$ nm) these nanoparticles absorb photons and become excited. These excited particles relax themselves by a radiative process where UV light is emitted ($\lambda_{\text{max}} \approx 350$ nm). The dimethoxynitrobenzyl esters can thus be cleaved, as the maximum of absorbance of these functions is around 350 nm (Figure 2.14).

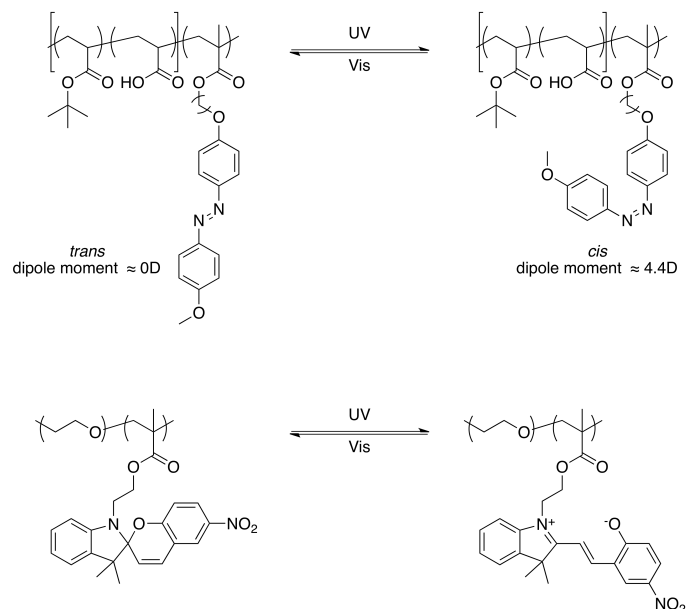


Figure 2.15: Reversible changes in the hydrophilic-hydrophobic balance of block copolymers induced by illumination.

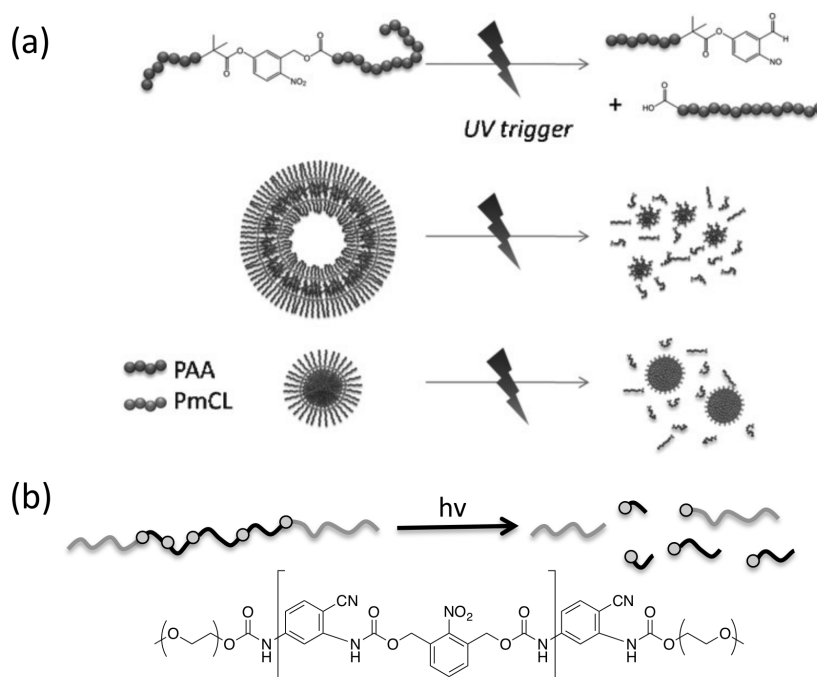
Similarly to the irreversible micelle disruption, the reversible light-induced process was realized with copolymers bearing photo-sensitive side groups. Azobenzene and spiropyran are the typical chromophores employed. The light induced disruption of micelles based on azobenzene relies on the *trans-cis* isomerization, where the *cis* isomer is more polar and thus more soluble in polar solvents than the *trans* isomer [79, 80]. The irradiation of spiropyran leads to the isomerisation of the spiropyran group to the charged merocyanine form which is soluble in polar solvents (Figure 2.15) [81–83].

Disruption by breaking of junction

Light induced disruption of micelles was also realized on linear photocleavable block copolymers by the breaking of the block copolymer junction. Similarly to the change in the hydrophilic-hydrophobic balance, the breaking of the junction may be reversible or irreversible depending of the photosensitive moiety.

The reversible breaking of the junction between the hydrophilic and the hydrophobic block was realized with inclusion complexes between azobenzene and cyclodextrine groups (Figure 2.12). The junction between the two blocks is formed when the azobenzene group at the end of one block is in the elongated *trans* conformation that complexes with the terminal cyclodextrine functionality of the other block. Upon UV irradiation, the azobenzene adopts the *cis* conformation, which induces the breaking of the junction. The complex, and the micelles, may be reformed by the irradiation by visible light [84, 85].

The irreversible breaking of a block copolymer junction was realized by the introduction of truxilic acid derivatives [86] and one or several *o*-nitrobenzyl esters [87–89] in the main chain of the block copolymers (Figure 2.12). Yang *et al.* used a truxilic acid derivative to induce the breaking of poly(ethylene oxide)-*b*-poly(*n*-butyl acrylate) and poly(ethylene oxide)-*b*-poly(cyclohexyl acrylate). Cabane *et al.* synthesized a PCL-*h*v-PAA copolymer with an *o*-nitrobenzyl junction between the PCL and the PAA blocks. This copolymer was self-assembled in



and photocleavable *o*-nitrobenzyl groups in a PEO-*b*-poly(disulfide-*alt*-nitrobenzene)-*b*-PEO triblock copolymer and to disrupt micelles made from this copolymer by UV and redox stimuli [90].

2.2.3.2 Micelle cross-linking

The stabilization of micelles is a topic of high interest in polymer science [91–94]. In addition to the traditional chemical cross-linking methods, the cross-linking of the core or shell of micelles is possible by a photo-induced approach. Indeed, Liu and coworkers were the firsts to propose the use of cinnamic esters (Figure 2.12) combined to a UV irradiation to stabilize the corona of poly(isoprene)-*b*-poly(cinnamoyl ethyl methacrylate)(PI-*b*-PCEMA) micelles [95]. Under UV irradiation, such photosensitive moieties undergo an irreversible [2+2] cyclization. With time, polymers bearing cinnamic esters were used for the cross-linking of micellar core and corona. Moreover, the combination of this photosensitive moiety with other light sensitive and stimuli-responsive copolymers was used for the stabilization of functional nanoparticles in solution [22, 89].

The major drawback of cinnamic esters is the irreversibility of the cross-linking. *In vitro* studies demonstrated that irreversible cross-linking may lead to a difficult release of the payloads [96]. This issue was solved using photosensitive moieties able to undergo a cyclization at one wavelength and subsequent breaking of the cycle at a different wavelength. Coumarin derivatives fit this description as two coumarin moieties combine themselves by a [2 + 2] cycloaddition at $\lambda > 310$ nm and the cyclobutane cycle breaks with lower wavelength irradiation at $\lambda < 260$ nm [97]. This strategy was largely explored by Zhao and coworkers to stabilize micelles by core and shell cross-linking [98–100].

The reversible photo-cross-linking using coumarin was also combined with other stimuli responsive copolymers, in particular with thermo-responsive copolymers [101–103]. He and coworkers synthesized a PEO-*b*-P(MEO₂MA-*alt*-CMA) copolymer by ATRP and the micellization of this copolymer was thermally driven. Above the LCST of PMEO₂MA,

the micelles are composed of a P(MEO₂MA-*alt*-CMA) core and a PEO corona. The irradiation of this copolymer at $\lambda > 310$ nm leads to the dimerization of the coumarin moieties and thus to the stabilization of the micellar core. The decrease of the temperature below the LCST allowed the formation of nanogel particles with both water-soluble core and shell as well as phototunable size by adjusting the cross-linking density through the decrosslinking using UV light at $\lambda < 260$ nm (Figure 2.17) [101].

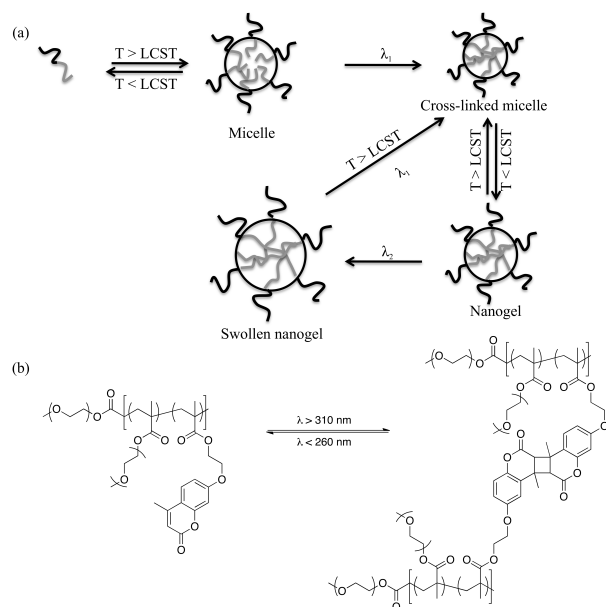


Figure 2.17: (a) Schematic illustration of the preparation and photo-controlled volume change of nanogel, (b) designed diblock copolymer bearing coumarin side groups for the reversible photo-cross-linking reaction [101].

It should be noted that the reversible cyclization of coumarin derivatives under irradiation was not only used for micellar stabilization but also for the photo-triggered release of drugs immobilized on a polymer backbone [104]. In this case, the authors synthesized a statistical copolymer of MMA and 7-(*tert*-butyldimethylsiloxy)-7'-(methacryloxy) dicoumarin. Chlorambucil was immobilized on the dicoumarin units by

a carbodiimide coupling after the deprotection of the (methacryloxy) dicoumarin. The release of the chlorambucil was promoted by the irradiation of the copolymer. For the interested reader, there are several reviews that appeared in the last few years either totally or in part dedicated to the use of photosensitive block copolymers [1–3, 6, 69–73, 97, 105–108].

2.3 Multistimuli-responsive micelles

One interesting feature of stimuli-responsive block copolymers is that some of them can display response to multiple changes in the environment. This characteristic gives rise to multistimuli-responsive micelles in solution. Among all the multistimuli-responsive block copolymers, two families can be distinguished depending on the location of the stimuli-responsive blocks in the copolymers. In the first family, the copolymer is composed of only one stimuli-responsive block able to respond to multiple stimuli. This multistimuli-responsive block can either be composed of only one monomer, able to change its behavior under several stimuli, or a statistical copolymer composed of two different stimuli-responsive monomers. The second family is characterized by at least two blocks able to respond independently to two different stimuli.

2.3.1 Block copolymers with one multistimuli-responsive block

In solution, block copolymers composed of only one multi-responsive block can undergo multiple micellization processes (Figure 2.18). In general, the multiple micellization processes give rise to micelles of the same nature, *i.e.* the core and the corona of the micelles formed are the same no matter what the nature of the stimulus is.

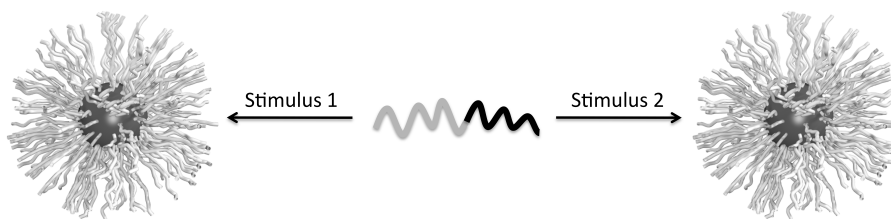


Figure 2.18: Schematic representation of a multiple micellization

Surprisingly, the multiple micellization of block copolymers is not largely reported in the literature. A majority of the examples displayed below have been discovered by different research groups, which studied the micellization of multistimuli-responsive block copolymers with only one stimulus. Generally, block copolymers composed of a polyacid or a polybase block are able to form micelles with similar core-corona structure by a pH stimulus or the addition of multivalent cations. Micelles with a P(M)AA core and a PEO corona were formed by the addition of multivalent cations [109–111] or the acidification of the solution [33, 34]. P2VP-*b*-PEO and P4VP-*b*-PEO are able to form micelles at low pH by the addition of Pd^{2+} cations [112, 113] and by the basification of the medium [28–31]. The driving force of the micellization of polyacid or polybase containing block copolymers by multivalent cations is the coordination of carboxylate or vinylpyridine (VP) units with metal ions. Another example of multiple micellization occurs with copolymers composed of a POEGMA sequence. Indeed POEGMA exhibits response to both phosphate and carbonate anions [114, 115] and to an increase of the temperature [48, 54]. In the case of the salt response of POEGMA, the driving force is the salting out of the oligo(ethylene glycol) side chain [110].

One special example of multiple micellization occurs for copolymers that self-assembles when submitted to Stimulus 1. In a next step, the micelles are disrupted upon application of Stimulus 2 and a cycle can subsequently be restarted by submitting the copolymer to Stimulus 1 again. Such type of behavior is called "*multiple micellization and dissociation process*" (Figure 2.19). Most of the time, this process is realized with block copolymers in which the responsive sequence is composed of a statistical copolymer of thermo-responsive monomers and other stimuli-responsive monomers (pH, light). The principle of the multiple micellization and dissociation process with a thermally induced micellization relies on the ability to tune the LCST of the system by the copolymerization of the thermo-responsive monomer with another monomer. Indeed, the statistical introduction of hydrophilic or hydrophobic monomers in a thermo-responsive polymer respectively increases or decreases the LCST of the accordingly obtained statistical copolymer [2, 7, 116].

The micellization of the block copolymer will thus be conducted by an increase of the temperature whereas the second stimulus will induce a shift in the hydrophilic-hydrophobic balance of the statistical copolymer block forming the core and will thus induce the disruption of the micelles.

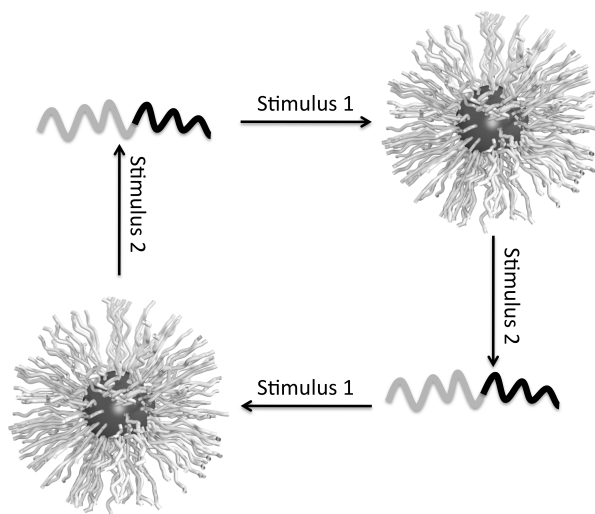


Figure 2.19: Schematic representation of the multiple micellization and dissociation process

A typical example of multiple micellization and dissociation process was developed by Zhao and coworkers, where a PEO-*b*-P(MEO₂MA-*co*-MAA) copolymer underwent multiple micellization and disruption cycles by combining temperature and pH triggers in water. Figure 2.20a presents the variations observed in the hydrodynamic diameter of the PEO-*b*-P(MEO₂MA-*co*-MAA) micelles formed by a continuous increase of the temperature and the addition of KOH [66]. The increase in temperature induces the collapse of the P(MEO₂MA-*co*-MAA) block and thus the micellization of the block copolymer. The addition of KOH, on the other hand, induces the deprotonation of the carboxylic acids of PMAA and thus a disruption of the micelles. A further increase in temperature leads to a second micellization process.

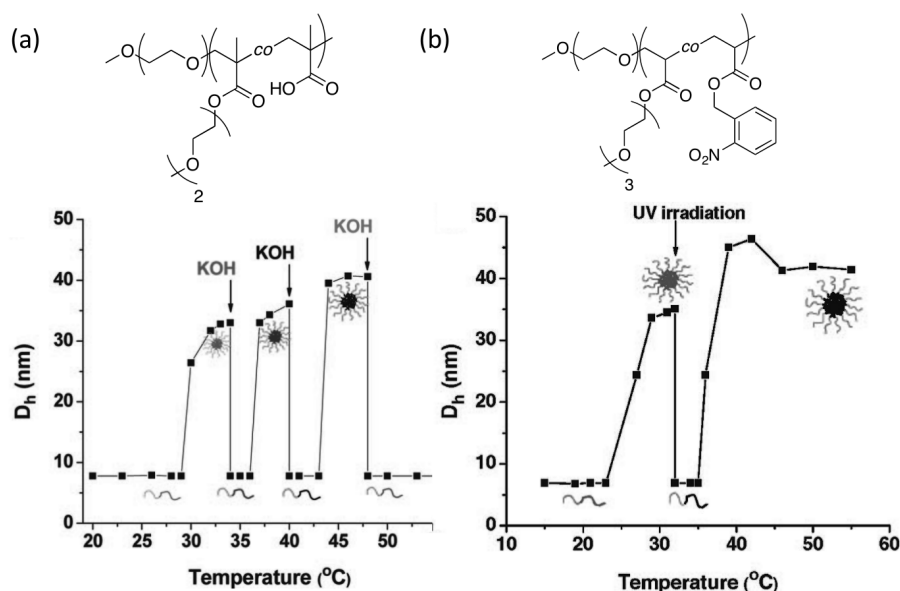


Figure 2.20: Multiple micellization and dissociation process of: (a) PEO-*b*-P(MEO₂MA-*co*-MAA) and PEO-*b*-P(MEO₃A-*co*-NBA). Adapted from [66, 117].

A similar approach was realized by the same group using temperature and light as the stimuli [117]. Figure 2.20b presents the variations of the hydrodynamic diameter of the PEO-*b*-P(MEO₃A-*co*-NBA) micelles formed by a continuous increase of the temperature and the irradiation of the solution. In this case, the disruption of the micelles is the consequence of the photocleavage of the PNBA units leading to the formation of a P(MEO₃A-*co*-AA) sequence.

2.3.2 Block copolymers with two responsive blocks

The second family is composed of copolymers in which each of the constituent blocks can be transformed via an adequate stimulus from a soluble to an insoluble state and, inversely, independently from the other blocks. Such types of block copolymers are called "Schizophrenic copolymers" [118]. In solution, these copolymers can exist in three different states: unimers, micelles, and inverse micelles in which the core-

corona structure is inverted compared to the regular micelles (Figure 2.21). The first example of schizophrenic copolymer was reported by Armes and coworkers, they studied the response in water of a poly[(N-morpholino) ethyl methacrylate]-*b*-poly[(diethylamino)ethyl methacrylate] (PMEMA-*b*-PDEA) diblock copolymer to the addition of Na₂SO₄ and pH [118].

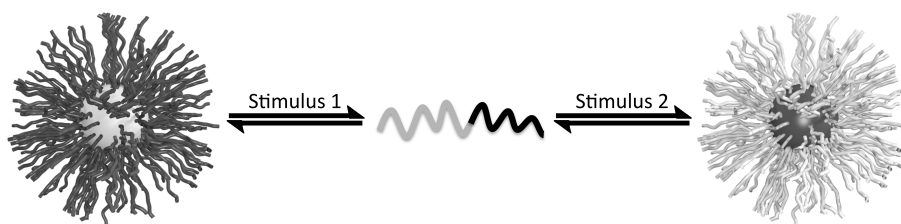


Figure 2.21: Schematic representation of the behavior in solution of a schizophrenic copolymer

Since the introduction of this concept in 1998, numerous examples of schizophrenic copolymers were published. Among all the stimuli available, schizophrenic copolymers with combination of pH and temperature stimuli were the most studied. In these cases, the thermo-responsive sequence is, most of the time, composed of PNIPAm, POEGMA or PMEMA and the pH sensitive sequence is containing PDMAEMA, PDEA, P2VP or PAA [32, 63, 67, 114, 119–125].

Several groups, including Armes and coworkers, have studied the micellization of schizophrenic copolymers with the combination of a pH and a salt stimulus on PMAA-*b*-POEGMA [114], poly[4-vinylbenzoic acid-*block*-2-(N-morpholino) ethyl methacrylate]] (PVBA-*b*-PMEMA) [126] and PDEA-*b*-PDMAEMA-*b*-PMEMA [127]. The combination of salt and temperature was used to demonstrate the schizophrenic behavior of poly[sodium 2- (acrylamido) -2- methylpropanesulfonate]-*b*-poly(N-isopropyl- acrylamide) (PAMPS-*b*-PNIPAm) in water [128]. In this case, the collapse of the PAMPS block was induced by the addition of Fe³⁺ ions, which create cross-linking points between the sulfonate functions of the PAMPS sequence. The same group studied the behavior in solution

of a salt-salt schizophrenic copolymer. On a PEO-*b*-PMAA, they demonstrated that micelles with a PMAA core are formed by the addition of Ca^{2+} and that the dialysis in EDTA solution disrupts the micelles. The inverse micelles were formed after the addition of phosphate in the solution [110].

To the best of our knowledge, only one example of schizophrenic copolymer using a light stimulus was published. Jin *et al.* synthesized a PSPMA-*b*-PMEO₂MA copolymer and demonstrated its schizophrenic behavior in water. Figure 2.22 presents the chemical structure of the polymer under UV and Visible irradiation and a schematic representation of the schizophrenic behavior of this polymer [82].

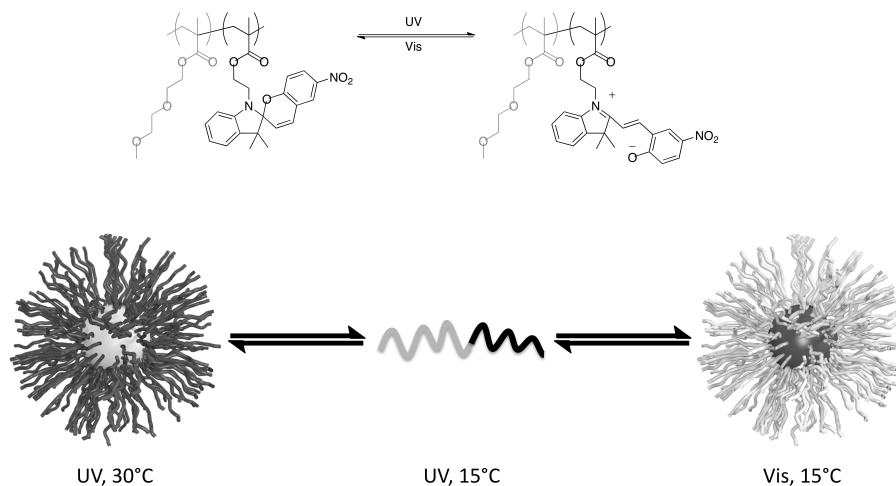


Figure 2.22: Schematic representation of the schizophrenic behavior in solution of PSPMA-*b*-PMEO₂MA. Adapted from [82].

Although schizophrenic copolymers often respond to two different stimuli, polymeric systems exhibiting a schizophrenic behavior in solution with only one stimulus were also produced. This was realized with zwitterionic block copolymers, composed of a polyacid block and a polybase block (Figure 2.23). Gohy and coworkers studied the response of PDMAEMA-*b*-PMAA copolymer to a variation of pH in water [40]. The authors demonstrated that a wide range of micellar struc-

tures may be obtained by the variation of pH including micelles with PMAA core and PDMAEMA corona in acidic medium and the hollow aggregates further associated into multicompartimentalized superaggregates in basic medium. Rodriguez-Hernandez and coworkers, synthesized a poly(glutamic acid)-*block*-polylysine (PGA-*b*-PLys) copolymer and studied its response to pH (Figure 2.23a) [37]. Du et al. synthesized a poly[(diethylamino)ethyl methacrylate]-*block*-poly(N-acryloyl-L-phenylalanine)(PDEA-*b*-PAP) copolymer and demonstrated the formation of vesicles with a cationic PDEA shell at acidic pH and with a anionic PAP shell in basic medium (Figure 2.23b) [24]. Bo and coworkers also reported the schizophrenic behavior of a PAA-*b*-P4VP diblock copolymer [129]. Temperature-temperature schizophrenic system were also developed. The synthesis of a block copolymer containing both LCST (PNIPAm) and UCST (3-[N-(3-methacrylamidopropyl)-N,N-dimethyl] ammoniopropane sulfonate, SPP) blocks was realized by Arotçaréna et al [130].

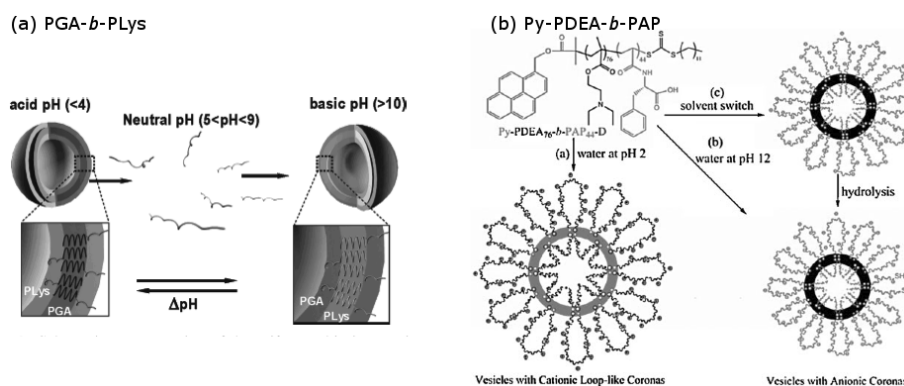


Figure 2.23: Behavior in water of a pH-responsive schizophrenic copolymer: (a) PGA-*b*-PLys and (b) Py-PDEA-*b*-PAP. Adapted from [24, 37].

Another interesting case was developed by Zhang and coworkers, they developed a schizophrenic system with at least three distinct "personalities" [131]. The studied copolymer is a poly(N-isopropyl- acrylamide)₂ [poly(N-vinylpyrrolidone)-*b*-poly(acrylic acid)]₂ miktoarm ((PNIPAm)₂ (PNVP-*b*-PAA)₂). When dissolved in water, this copolymer showed re-

sponse to variation of temperature and pH (Figure 2.24). The authors demonstrated that micelles with a PNIPAm core were formed at 45°C and the inverse micelles, with a PAA core, were formed at pH = 6. In addition to this schizophrenic behavior, they proved that their system presented a third "personality" at low pH and high temperature. PAA/PNIPAm complex core micelles were induced at pH = 6 and 45°C. However, under these conditions the PNVP block was not long enough to stabilize the micellar structure and the system precipitated.

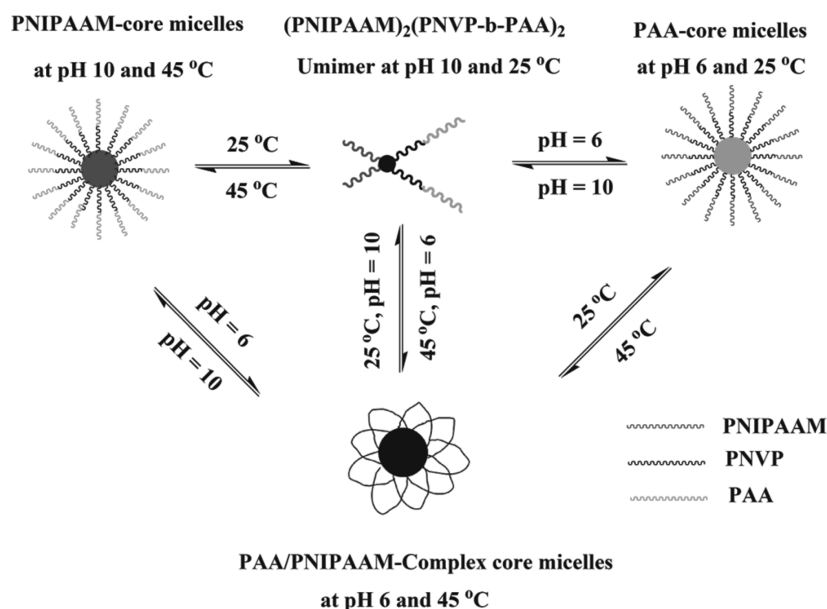


Figure 2.24: Schematic representation of the schizophrenic behavior of $(\text{PNIPAm})_2(\text{PNVP-}b\text{-PAA})_2$ [131].

Roy and coworkers developed a triple responsive schizophrenic copolymer [132]. They synthesized a poly(3-acrylamidophenylboronic acid)-*b*-poly(N-isopropylacrylamide)(PAPBA-*b*-PNIPAm) by RAFT. This copolymer when dissolved in water exhibited a schizophrenic behavior (Figure 2.25). The increase of the temperature forced the precipitation of the PNIPAm block and induced the formation of micelles with a PNIPAm core and a PAPBA corona. The inverse micelles, with the core com-

posed of PAPBA were formed when the pH of the solution was around 8-9. Moreover, copolymers bearing boronic acids are well known to undergo a reduction in pKa upon esterification with diols (*e.g.* glucose). The authors proved that the addition of glucose in the solution at pH = 8.7 led to the disruption of the micelles.

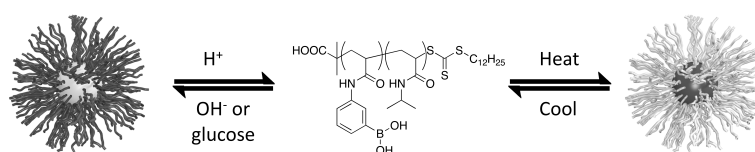


Figure 2.25: Schematic representation of the behavior in solution of PAPBA-*b*-PNIPAm. Adapted from [132]

2.4 Nanocages

In the last decade, the research on hollow nanostructures has attracted extensive attention [13, 133–136]. Hollow nanoparticles in solution can adopt different structures, such as cylindrical (called nanotubes) or spherical (often called hollow nanocapsules or nanocages). Chemists became interested in such type of structures since they may be seen as nature mimics. Indeed, nanocages may display some similarity to viruses, cells or even enzymes. Similarly to viruses, nanocages have a large empty core domain, which allows the encapsulation of large guest molecules or a large quantity of small guest molecules [137]. If the nanocages are functionalized with several catalysts, they can display correspondence to cells where compartmentalization of chemical processes and communication between compartments are observed [14]. The parallelism between nanocages and enzymes relies on the environmental constraints of the empty core domains to the guest molecules. Indeed, enzymes are particularly effective and selective because of the proximity between their recognition site and the catalytic site [138].

This section of the thesis mainly focuses on polymeric nanocages but several other type of hollow nanocapsules were produced over the years. Nanoreactors were produced by the self-assembly of small organic non-covalent systems (surfactants) or even by the modification of biomacromolecules (proteins and viruses) [14,138]. In this section, several preparation methods of polymeric hollow nanocapsules are detailed. The first part focuses on the formation of nanocages by direct polymerization. The second part of this section reviews some techniques involving the self-assembly of polymer to produce the hollowed nanocapsules.

2.4.1 Formation of nanocages by direct polymerization

One of the first approaches used to produce hollow nanocapsules was to polymerize monomers in a scaffold of a similar geometry. This concept was realized with liposomes or surfactant emulsions, as they are able to a certain degree, to solubilize hydrophobic monomers into the hydrophobic part of the bilayer. The subsequent polymerization leads to the formation of polymer chains entrapped into the bilayer and thus the polymeric structure adopts the geometry of the scaffold (Figure 2.26). Several groups used this concept for the formation of nanocages by the radical polymerization of styrenic and (meth)acrylate monomers [139–141].

This approach was also used for the formation of porous nanocages (Figure 2.26). Dergunov and coworkers synthesized polymer nanocapsules by free radical photopolymerization of 4-*tert*-butylstyrene and of *p*-divinylbenzene (DVB) cross-linker with or without porogen incorporated in the lipid bilayer [142]. Two types of porogen were used in their study. The first kind is glucose pentaacetate, which is inert toward the polymerization and thus forms the hydrophobic pores after the removal of the lipids. The second kind was characterized by a polymerizable moiety and a bulky porogen linked together by a degradable function. They used a molecule prepared by the coupling of glucose tetraacetate with 4-vinylbenzoic acid as the porogen. After the formation of the nanocapsules, the ester link is hydrolyzed in alkaline conditions and thus hydrophilic pores are obtained.

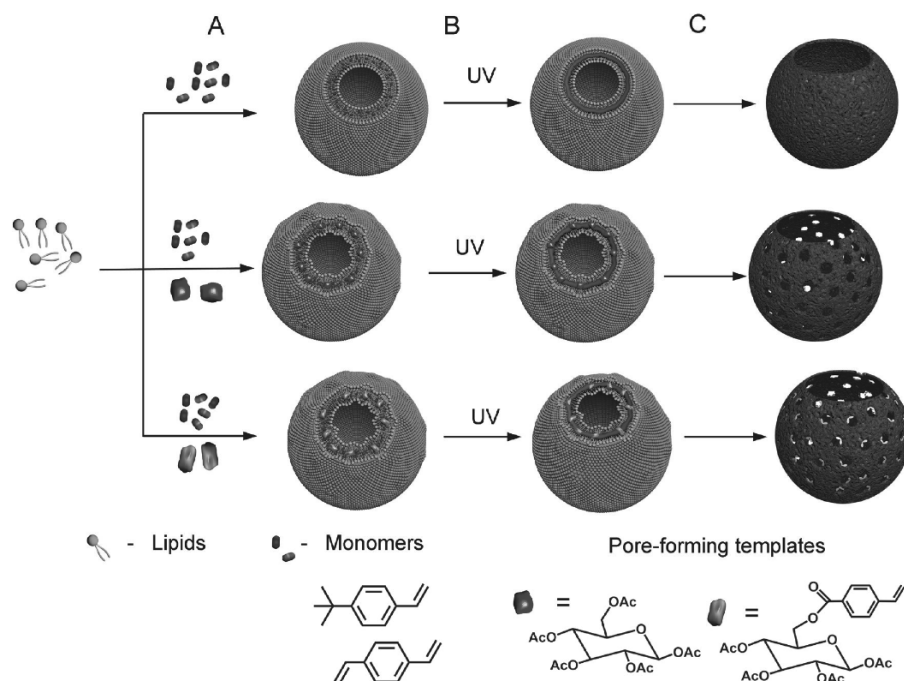


Figure 2.26: Schematic representation for the formation of nanocages by direct polymerization in liposomes [142].

Another procedure to produce hollow nanocapsules based on the direct polymerization is to use multi-step emulsion polymerization combined with dynamic swelling of the structure. Figure 2.27a presents the methodology of the multi-step emulsion polymerization and dynamic swelling method. PS/PDVB hollow particles were prepared by this methodology [134, 143]. In a first step, PS is prepared by emulsion polymerization. The core-corona structure was then obtained by the polymerization of a mixture of DVB and styrene. The dynamic swelling of the core was carried out by the dissolution of the structure in a good solvent, such as toluene, at temperatures higher than the T_g of PS. Under these conditions, the toluene swells the core of the structure leading to the expansion of the PS/PDVB corona. Upon the decrease of the temperature and the removal of the solvent, the PS chains forming the core are absorbed in the corona and the hollow nanocapsules are formed. Fig-

ure 2.27b presents a TEM image of ultrathin cross-sections of PS/PDVB nanocages formed by this technique. The presence of a empty core into the PS/PDVB nanocages is clearly demonstrated here (structure y).

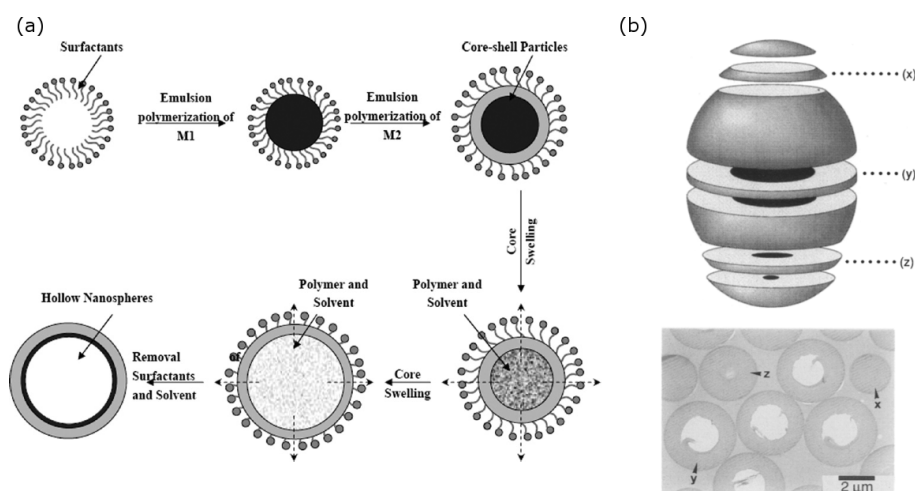


Figure 2.27: (a) Schematic representation of the preparation of nanocages via multi-steps emulsion polymerization and dynamic swelling method, (b) TEM image of ultrathin cross-sections of PS/PDVB nanocages. Adapted from [136,143].

One last technique of formation of nanocages based on the direct polymerization relies on the surface initiation of the polymerization. In this case, a nanoparticle is functionalized by a polymerization initiator: typically a SiO_2 nanoparticle bearing ATRP initiators (Figure 2.28). The polymerization is then conducted to yield a nanoparticle with surface grafted polymer. The polymer shell is then cross-linked and the SiO_2 nanoparticle etched with HF to produce the hollow nanocapsule [144–146]. In figure 2.28, the SiO_2 nanoparticle is decorated by a PS-*b*-PMMA block copolymer. The presence of this sacrificial PMMA outer shell prevents interparticle cross-linking during the UV irradiation and thus the aggregation of the PS nanospheres [144].

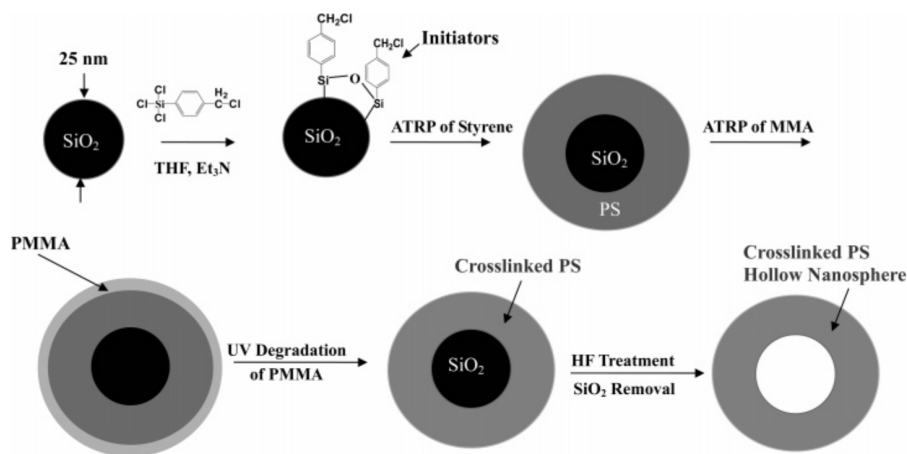


Figure 2.28: Schematic representation of the preparation of nanocages via surface initiated polymerization [144].

2.4.2 Formation of nanocages by self-assembly

2.4.2.1 Self-assembly via layer by layer

The layer by layer (lbl) technique is one of the most attractive technique for the preparation of hollow nanocapsules because the process is environmentally friendly, inexpensive, versatile and it is easy to tune the dimension of the nanocages [147–149]. The formation of nanocages by this technique relies on the consecutive adsorption of oppositely charged polyelectrolytes on the surface of a particle (Figure 2.29) to yield core-shell nanoparticle hybrids. The core of the particle may be afterwards destructed to form the nanocage.

Nanocages made of the successive deposition of poly(allyamine hydrochloride)(PAH) and poly(styrene sulfonate)(PSS) were extensively studied [150–152]. but other polyelectrolyte couples were studied, including PVP/PMAA [153, 154] and PDMA-*b*-PDEA/PDEA-*b*-PMAA [155].

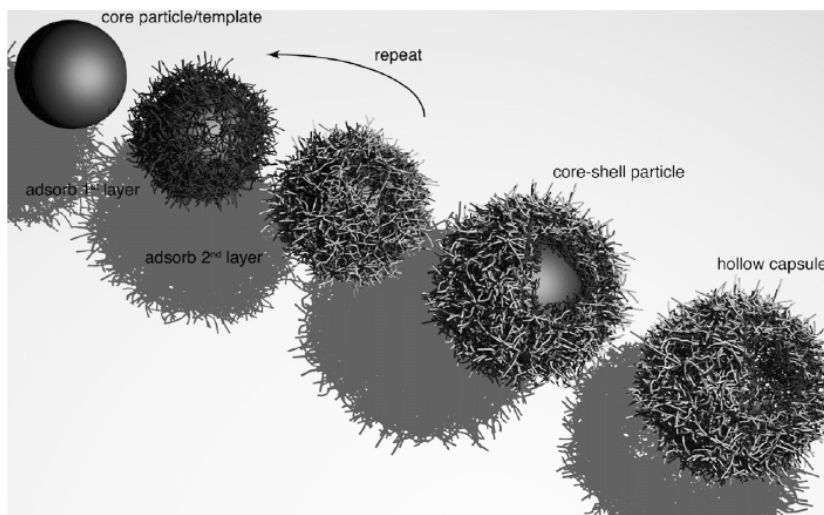


Figure 2.29: Schematic representation of the preparation of nanocages via layer by layer deposition [148].

The conditions of the core removal are strongly influenced by the chemical nature of the substrate. Spheres of weakly polymerized melamine-formaldehyde (PMF) were destroyed in aqueous media at pH below 1.6 [151, 152]. PVP/PMAA coated silver chloride microcrystals were treated with a mixture of KSCN/HNO₃ to remove the AgCl core [153]. SiO₂ particles were destroyed by HF treatment [153]. Gold nanoparticles were also used as a deposition substrate and the removal of the gold core was carried out in presence of KCN [150]. Adison et al. used CaCO₃ for the production of hollow PDMA-*b*-PDEA/PDEA-*b*-PMAA nanocapsules, the destruction of the CaCO₃ core was carried out with and EDTA solution in presence of HCl (pH = 1) [155].

2.4.2.2 Self-assembly of core/shell precursors

Most of the techniques of preparation of nanocages by the self-assembly of copolymers involve the selective degradation or the extraction of the core-forming chains to leave a membrane-like shell. The general procedure of formation of nanocages by this technique involves the synthesis and the self-assembly in solution of a block copolymer. The third step is

the cross-linking of the micelle shell. Finally the chains forming the core can be either degraded (Figure 2.30) or extracted if a cleavable junction is present between the blocks forming the core and the shell of the micelles (Figure 2.31).

The formation of nanocages by the self-assembly of block copolymers and a degradation of the core was largely studied by the group of Wooley [156–159]. They first developed a system based on a PI-*b*-PAA block copolymer. The dissolution of this copolymer in water leads to spherical micelles with a PI core and a PAA corona. The micelles were then stabilized by a chemical cross-linking of the PAA shell with a diamine compound. The degradation of the core was realized by the ozonolysis of polyisoprene the double bond followed by the dialysis of the accordingly obtained PAA nanocages against water to remove small-molecule byproducts. They also demonstrated the ability of the system to entrap active molecules and that the nanocages can be regioselectively modified [156, 157]. Indeed, they demonstrated that the grafting of phosphatidylethanolamine lipids by a Shift-base approach only led to the functionalization of the internal wall. Cheng and coworkers combined ROMP and NMP polymerization to synthesize a polynorbornene-*graft*-PI-*b*-PAA. The PI-*b*-PAA methodology was then applied to this copolymer to produce unimolecular micelles and nanocages [158].

Recently, the self-assembly and core degradation methodology was applied to a poly(N-(acryloyloxy)succinimide-*co*-N-acryloylmorpholine)-*b*-poly(L-lactic acid) P(NAS-*co*-NAM)-*b*-PLA. When dissolved in water, this copolymer self-assembles in spherical micelles. The shell of the micelles was then cross-linked by the reaction of a diamine with the N-(acryloyloxy)succinimide function. The core of the shell cross-linked micelles was degraded by enzymatic degradation in presence of proteinase K (Figure 2.30) [159].

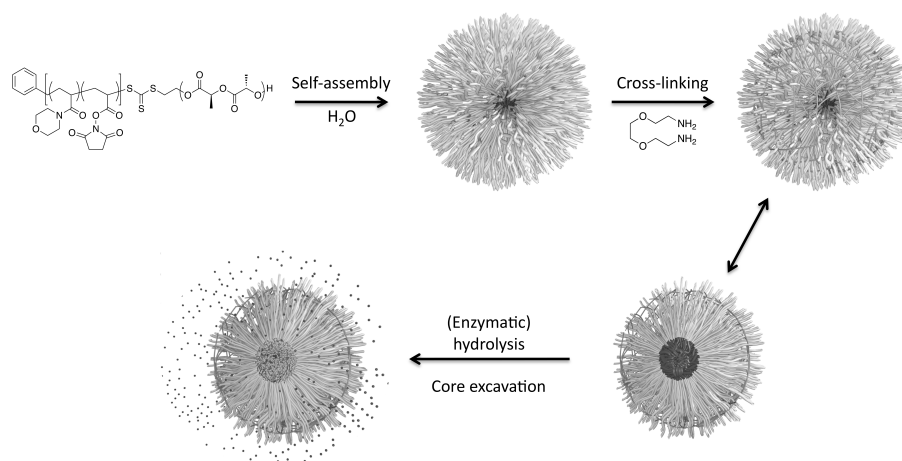


Figure 2.30: Schematic representation of the preparation of nanocages via self-assembly of a block copolymer and degradation of the micellar core. Adapted from [159].

The other approach to produce empty core structures in solution relies on a copolymer characterized by a cleavable junction between its two sequences [160–163]. The first example of nanocage formation by this approach was developed by Shanmugananda *et al.* [160]. They synthesized a PS-*b*-PAA where the two sequences are linked together by a thermally labile C-ON bonds (nitroxide function). The cleavage of the function was then triggered by heating at 125°C for 24 h in a sealed Schlenk tube (in toluene-acetic acid, 80+20 v/v) in presence of ascorbic acid. The extraction of the PS core was realized by suspending the nanocages in THF.

O'Reilly and coworkers adopted a strategy relying on supramolecular copolymers to produce PAA nanocages [161–163]. Figure 2.31 presents the strategy of formation of the nanocages. An asymmetric bis-terpyridine Ru(II) PAA-*b*-PS copolymer was synthesized by NMP. This copolymer was self-assembled in water (i) and the shell of the micelle was cross-linked with a diamine (ii). The cleavage of the bis-terpyridine Ru(II) complex was realized in presence of N-hydroxyethylthylenediamine triacetic acid sodium salt (HEEDTA), which is a competitive binder for the Ru(II)

ions. The dialysis of the shell cross-linked micelles in a THF/water mixture allowed the extraction of the PS core [161].

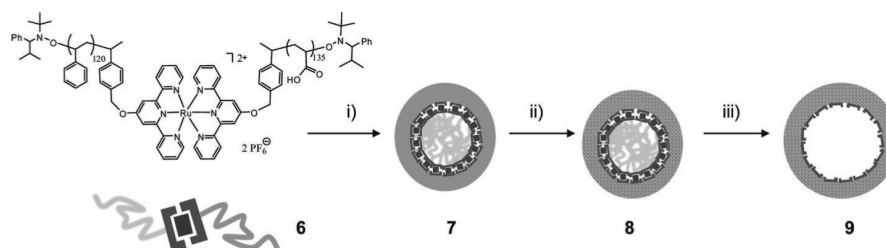


Figure 2.31: Schematic representation of the preparation of nanocages via self-assembly of a block copolymer and extraction of the micelle core [161].

A similar methodology was adopted by this group for the production of nanocages from a PS-*b*-PAA copolymer where the two blocks are linked together by a "pincer"-pyridine- palladium(II) complex. The cleavage of the Pd(II) complex was carried out in presence of a phosphate buffer solution at pH = 5.0 [162, 163].

2.4.2.3 Self-assembly of core/shell copolymer

The production of nanocages by the direct self-assembly of block copolymers is also possible. The simplest example of hollow nanocapsules obtained by the self-assembly of block copolymers is a vesicle. Indeed, amphiphilic block copolymers can self-assemble in vesicle by a fine tuning of the experimental parameters, such as the relative block length, solvent composition, and solution concentration (cfr. Section 1.3.3.2, Chapter 1).

The formation of nanocages was also realized by a structural reorganization of the micelles during a drying process (Figure 2.32). This approach was realized by Petzetakis and coworkers, they synthesized a PLA-*b*-PAA copolymer by the combination of ROP and RAFT. This copolymer was self-assembled in water, leading to the formation of mi-

celles (spherical and cylindrical). They observed the reorganization of the system and the formation of hollow structures when the micelles were slowly dried onto a substrate. They proposed that this reorganization and the spontaneous cavitation of the nanoparticles is the consequence of hydrogen-bonding between the polylactide core and poly(acrylic acid) corona, leading to the contraction of the core on the corona. They also observed that these hollow nanoparticle slowly rearrange, to form micellar structures, during re-suspension in water [164].

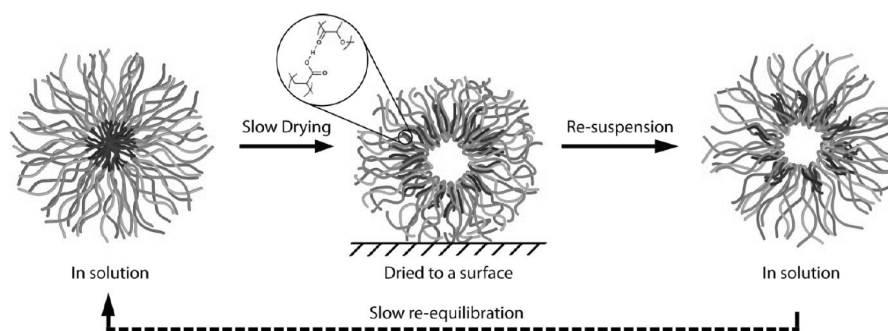


Figure 2.32: Schematic representation of the preparation of nanocages via a structural reorganization during drying [164].

Another approach to prepare hollow nanocapsules via the self-assembly of block copolymers was developed by Sugihara and coworkers [165]. They synthesized a PEO-*b*-PDMAEMA-*b*-PMPC (poly(2-(methacryloyloxy)ethyl phosphorylcholine)) triblock copolymer. This copolymer was self-assembled in a mixture of isopropanol and water (9:1), the micelles formed in these conditions have a PMCP core, a PDMAEMA shell and a PEO corona. The shell of the micelles was chemically cross-linked by BIEE to yield shell cross-linked micelles. The dialysis of the micelles solution against water leads to the solubilization of the PMCP block. The migration of this block into the corona allowed the formation of the desired nanocages (Figure 2.33a).

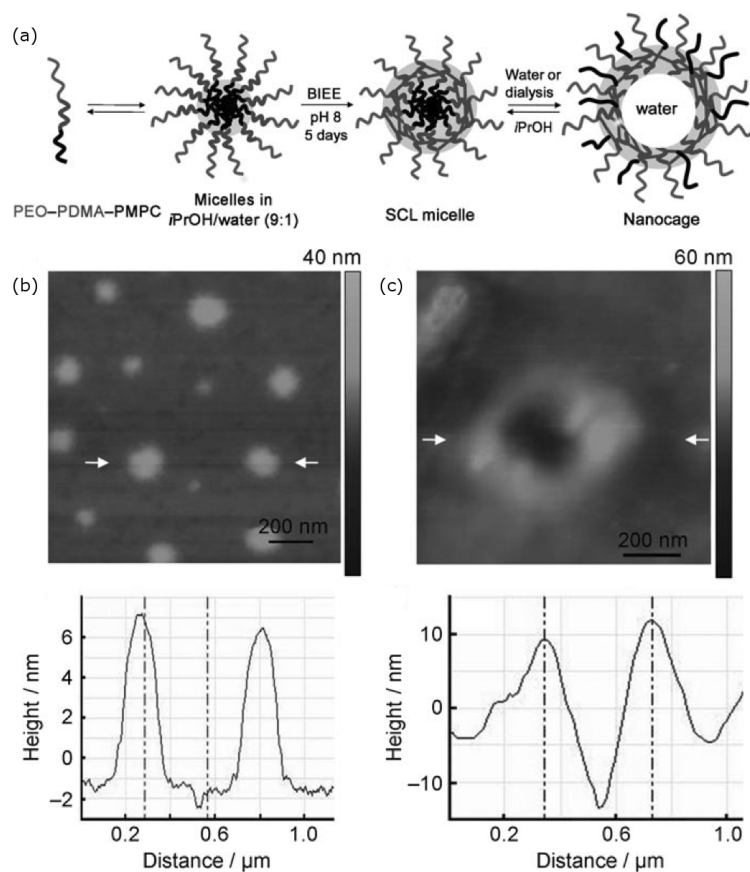


Figure 2.33: Formation of nanocages by a non destructive one pot process: Schematic representation of the formation strategy (a), AFM image and cross section profile of the SCL micelle (b) and nanocage (c). Adapted from [165]

2.5 Conclusions

This chapter has highlighted the interest of functional nanostructures in solution. The influence of the copolymer design on the self-assembled nanostructures and on the properties of these nanostructures was discussed.

In the first section of this chapter, the concept of stimuli-responsive copolymer was presented. These copolymers have the ability to modify their solubility in function of the physical and chemical properties of their surrounding medium, giving the possibility to trigger the micellization-disruption processes. An overview of pH responsive copolymers was given and the ability of a mixture of polyacids and polybases to interact and form interpolyelectrolyte complexes, which are also influenced by the pH of the environment was presented. In a second part, thermo-responsive polymers were presented as well as the LCST and UCST behaviors. Finally, polymers exhibiting response to light were discussed. We have seen that the micelle disruption may either be reversible or irreversible and that the location of the photosensitive moieties influence the disruption process. Photo cross-linkable moieties were also presented.

The second section of this chapter focused on copolymers sensitive to more than one stimulus. It was pointed out that the design of the copolymer strongly influences the behavior of those copolymers in solution. Indeed, multiple micellization processes are observed when only one sequence of the copolymer is sensitive to multiple stimuli. On the other hand, copolymers where the two blocks respond to a stimulus independently from the other blocks, exhibit a schizophrenic behavior in solution. Schizophrenic copolymer systems with a response to salt-salt, salt-pH, pH-pH, pH-temperature, temperature-temperature, temperature-light and temperature-salt stimuli were presented.

Finally, a brief review of the techniques to produce nanocages was presented. Hollow nanocapsules can be produced by polymerization techniques, such as polymerizations in emulsion or polymerizations at the surface of nanoparticles. Techniques involving the self-assembly of block copolymers were also presented. We have seen that block copolymers may be used as precursors for nanocages, where the blocks forming the core are removed from the structures. Polymeric systems where the self-assembly of copolymers directly produces nanocages were also presented.

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CHAPTER
THREE

OBJECTIVES

Abstract

The general aim of this thesis is to use photocleavable block copolymers and light to promote the formation of functional nanostructures in solution. In this chapter, the general strategies for micellization and disruption of micelles induced by light irradiation as well as the formation of nanocages using light are presented.

3.1 The project

This thesis focuses on the *use of a light stimulus to control the formation of functional nanostructures in solution*. The formation and the disruption of micelles in solution will be studied on copolymers bearing photocleavable side groups. The irradiation of these copolymers in solution will induce the cleavage of the side groups and thus a variation in the block copolymer polarity. The formation of micelles will be monitored when the hydrophobic/hydrophilic balance of the block copolymer will be changed from "double-hydrophilic" to amphiphilic. Inversely, when the irradiation induce the formation of a double-hydrophilic copolymer from an amphiphilic one, the disruption of micelles will be observed.

Hollow nanocapsules will be formed with a second family of block copolymers, which are characterized by the introduction of a photocleavable junction between the two blocks. These copolymers, when dissolved in a selective solvent, will self-assemble into micelles. After a chemical cross-linking of the corona, the cleavage of the junction will be promoted by light irradiation. The extraction of the polymer forming the core will allow the generation of nanocages with a functional internal wall.

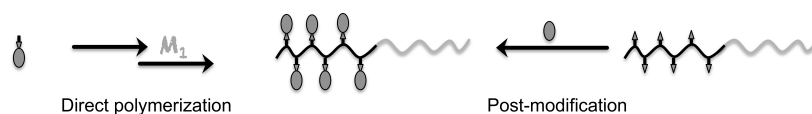
The creation of the nanostructures will proceed in three distinctive steps: the synthesis of the photocleavable block copolymers, the study of their properties in solution and their response to light irradiation.

3.2 Synthesis of photocleavable block copolymers

The synthesis of two families of photosensitive block copolymers is proposed in this thesis. The first family is characterized by the presence of a sequence bearing photocleavable side groups. The photosensitive moieties are photolabile protecting groups of carboxylic acids. Therefore, the photocleavable sequence of the investigated copolymers is composed of *o*-nitrobenzyl acrylate (PNBA), dimethoxynitrobenzyl acrylate (PDMNBA) or *p*-methoxyphenacyl methacrylate (PMPMA). Depending on the aimed application, either light induced micellization or light induced disruption, the other block is respectively composed of polystyrene

(PS) or poly[oligo(ethylene glycol) methacrylate] (POEGMA). The synthesis of these block copolymers is presented in chapter 4. The copolymers bearing *o*-nitrobenzyl derivatives (PNBA-*b*-PS and PDMNBA-*b*-PS) are synthesized by post functionalization of a PAA-*b*-PS precursor [1]. We selected this synthetic approach because our group previously demonstrated that the controlled radical polymerization of monomers bearing a nitroaromatic group is a big challenge [2]. On the other hand, copolymers bearing *p*-methoxyphenacyl side groups (PMPMA-*b*-POEGMA and PMPMA-*b*-PS) are synthesized by direct polymerization since the absence of nitroaromatic groups in their chemical structure, which strongly perturb radical polymerization, is an undeniable advantage for the synthesis of well-defined polymers (Figure 3.1).

Block copolymer bearing photocleavable side groups



Linear photocleavable block copolymer

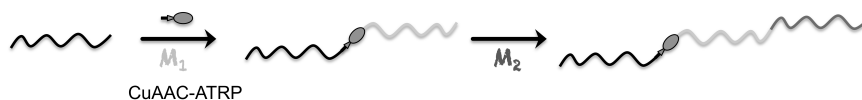


Figure 3.1: Synthetic pathway for the production of photocleavable block copolymers.

The second family of photocleavable block copolymers is characterized by the introduction of a photocleavable junction between the two sequences of the copolymer (Figure 3.1). The introduction of the photocleavable junction between the blocks is realized by a "one-pot" CuAAC-ATRP reaction. This approach allows simultaneously the polymerization of a polymer sequence at one end of the photocleavable junction and the attachment of the other sequence by a Huisgens 1,3 dipolar cycloaddition on the other end of the junction [3, 4]. The advantage of this synthetic strategy relies in the fact that it allows simultaneously the synthesis of block copolymers and the introduction of a photocleavable

junction between the two blocks in a "one-pot" process. Moreover, our group demonstrated that a wide range of block copolymers may be synthesized by this technique [3]. The formation of diblock and triblock copolymers is considered in this thesis (chapter 5). In the case of the diblock copolymers, the hydrophobic block is composed of polystyrene or poly(*tert*-butyl acrylate) and the hydrophilic block is composed of either poly(acrylic acid) or poly(dimethylaminoethyl methacrylate) (PS-*hv*-PAA or PtBA-*hv*-PDMAEMA). The triblock copolymers are built via the growth of a POEGMA sequence on the PtBA-*hv*-PDMAEMA copolymer.

3.3 Light induced micellization

The micellization promoted by light irradiation (LIM) is performed on the block copolymers bearing photocleavable moieties in organic media. The investigated copolymers are PNBA-*b*-PS, PDMNBA-*b*-PS and PMPMA-*b*-PS. These copolymers when dissolved in a non-selective solvent (chloroform) are in their random coil conformation (unimers). Upon light illumination, the cleavage of the side chromophores occurs, unmasking the acrylic acid (AA) functions. As a result, the photosensitive block is turned into a fully hydrophilic PAA block, which is insoluble in the used solvent. Self-assembly thus occurs, forming micelles made of a PAA core and a PS corona (Figure 3.2). The advantages a photocleavable approach to induce the micellization of block copolymer compared to other methods are that the reaction is triggered from outside the system, the micellization is realized without the addition of chemical reagents and finally that the irradiation is a "on-off" process. Moreover, the direct micellization of photocleavable block copolymers by light was never reported.

Chapter 6 presents the results obtained for the LIM. The ability of the photosensitive sequence to cleave upon irradiation is studied by UV-Vis spectroscopy and ^1H NMR spectroscopy. The objects formed after irradiation are investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Moreover, the ability of the system to trap hydrophilic molecules into the micellar core is examined by fluorescence spectroscopy.

3.4 Light induced micelle disruption

The light induced disruption of micelles (LID) is presented in chapter 7. The investigated diblock copolymer is composed of a photosensitive PMPMA sequence and a thermosensitive POEGMA sequence. This block copolymer self-assembles in water to form micelles with a PMPMA core and a POEGMA corona. The irradiation of this copolymer allows the cleavage of the *p*-methoxyphenacyl side groups and the formation of a fully hydrophilic PMAA-*b*-POEGMA copolymer alongside with the micelles disruption (Figure 3.3a). Furthermore, the PMAA-*b*-POEGMA obtained after the irradiation displays a rich stimuli-responsive behavior in solution, including schizophrenic behavior [5–8]. Indeed, the PMAA sequence is well-known to respond to a pH variation and the addition of multivalent cations. On the other hand, the POEGMA sequence exhibits a salting-out of the oligo(ethylene glycol) side groups upon the addition of salts in addition of the LCST behavior (Figure 3.3b). The stimuli-responsive behavior of this diblock copolymer is rather unique since a large number and diversity of stimuli (light, salt, temperature and pH) may be applied to influence the self-assembly of the system. Moreover, the the contribution of an easy synthesis and a rich stimuli-responsive behavior make the PMPMA-*b*-POEGMA copolymer rather interesting.

In chapter 7, the optimization of the micellization process of the PMPMA-*b*-POEGMA copolymer is presented. Information on the photo-induced disruption is gathered by DLS, TEM and UV-Vis spectroscopy. In addition, the response of the accordingly obtained PMAA-*b*-POEGMA copolymer to pH, temperature, calcium (Ca^{2+}), and phosphate (PO_4^{3-}) ions is demonstrated. Finally, the ability of this system to act as a stimuli-responsive nanocarrier for hydrophobic drugs is demonstrated.

3.5 Formation of nanocages driven by light irradiation

The formation of nanocages is realized on linear photocleavable block copolymers. The different investigated copolymers are designed with a hydrophobic (PS or PtBA) and a hydrophilic part (PAA, PDMAEMA or PDMAEMA-*b*-POEGMA) linked by a photocleavable junction. Upon dissolution in water, these copolymers self-assemble in micelles with the photocleavable junction localized precisely at the core-corona interface (PS-*hv*-PAA and PtBA-*hv*-PDMAEMA) or at the core-shell interface (PtBA-*hv*-PDMAEMA-*b*-POEGMA) (Figure 3.4). The corona (for diblock) or the shell (for triblock) is then cross-linked using a diamine or a dihalide according to the chemical nature of the considered block [9,10]. These shell-crosslinked micelles can be submitted to light irradiation, leading to the cleavage of the *o*-nitrobenzyl ester junction and the detachment of the core from the shell. The extraction of the core allows the formation of the desired nanocages.

To the best of our knowledge, a photocleavable approach was never investigated to produce hollow nanocapsules. The advantage of this approach compared to the methodology presented in chapter 2 is the fact that the cleavage of the copolymer junction is a soft technique that does not require any reagents. Moreover, the photocleavable approach allows the formation of nanocages with an empty core patterned by carboxylic acid function, which can be afterwards easily functionalized.

The formation of PAA, PDMAEMA and PDMAEMA-*b*-POEGMA by the photocleavable approach is presented in chapter 8. The response of the different investigated copolymers to light irradiation is first studied in non-selective solvent to determine the optimal photocleavage conditions. The formation of the PAA nanocages from PS-*hv*-PAA is then investigated. The third section of the chapter focuses on the formation of PDMAEMA and PDMAEMA-*b*-POEGMA nanocages. Finally, the possibility to functionalize the internal cavity of PDMAEMA and PDMAEMA-*b*-POEGMA nanocages is presented.

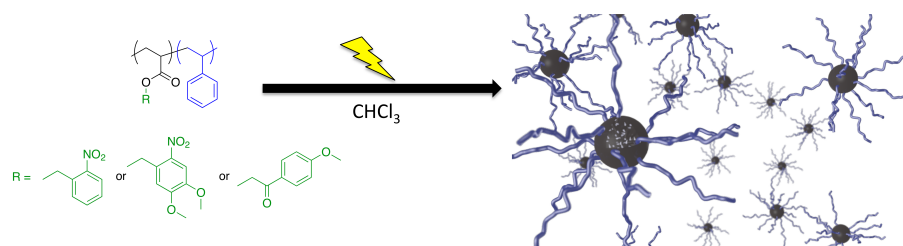


Figure 3.2: Schematic representation of light-induced micellization of block copolymers bearing photocleavable side groups.

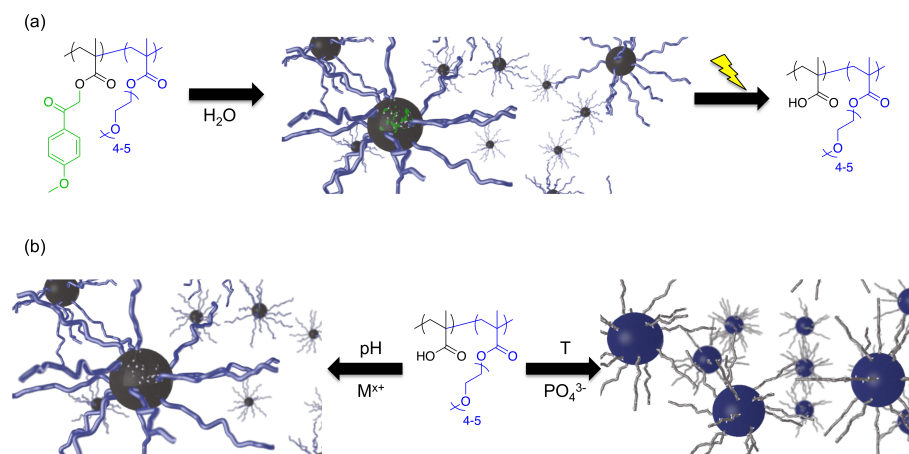


Figure 3.3: Schematic representation of light-induced disruption of micelles (a) and of the multistimuli responsive behavior of the accordingly obtained block copolymer (b).

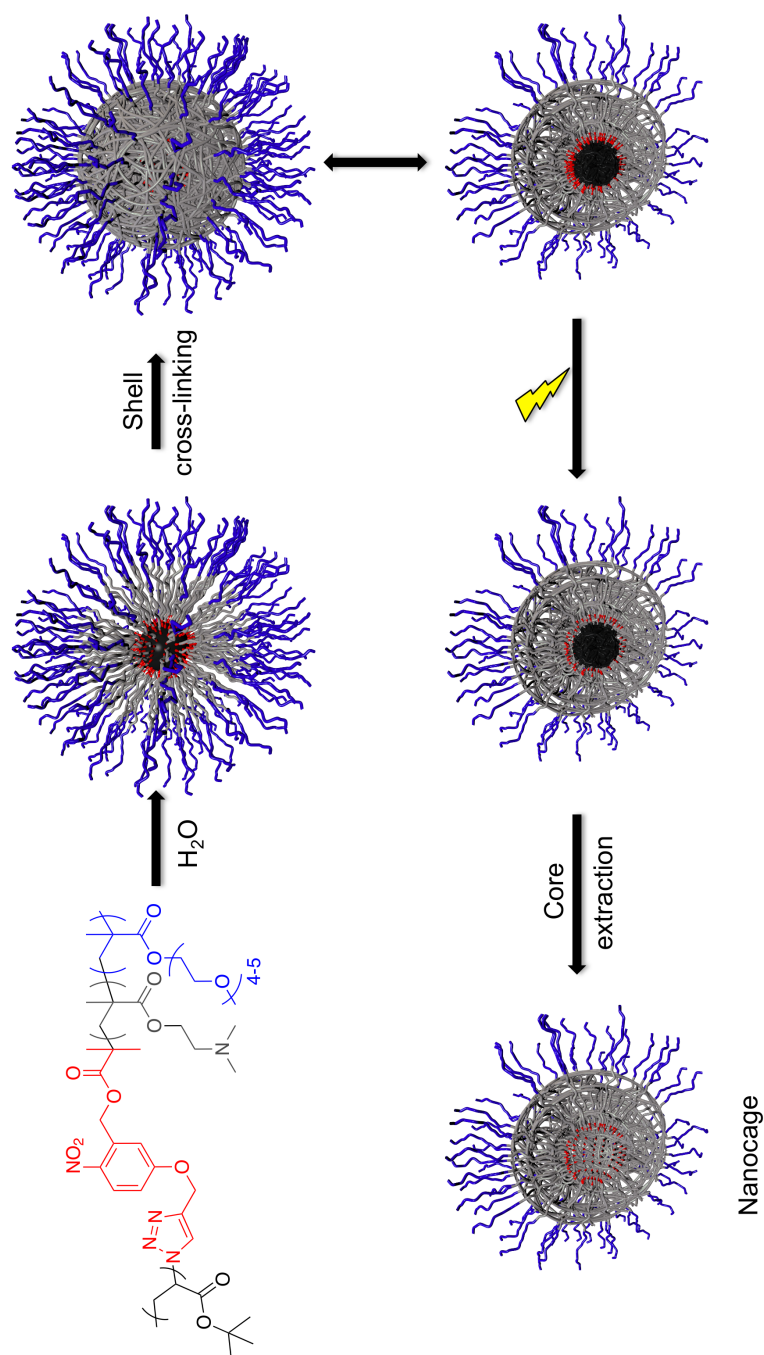


Figure 3.4: Schematic representation of the formation of nanocages driven by light irradiation.

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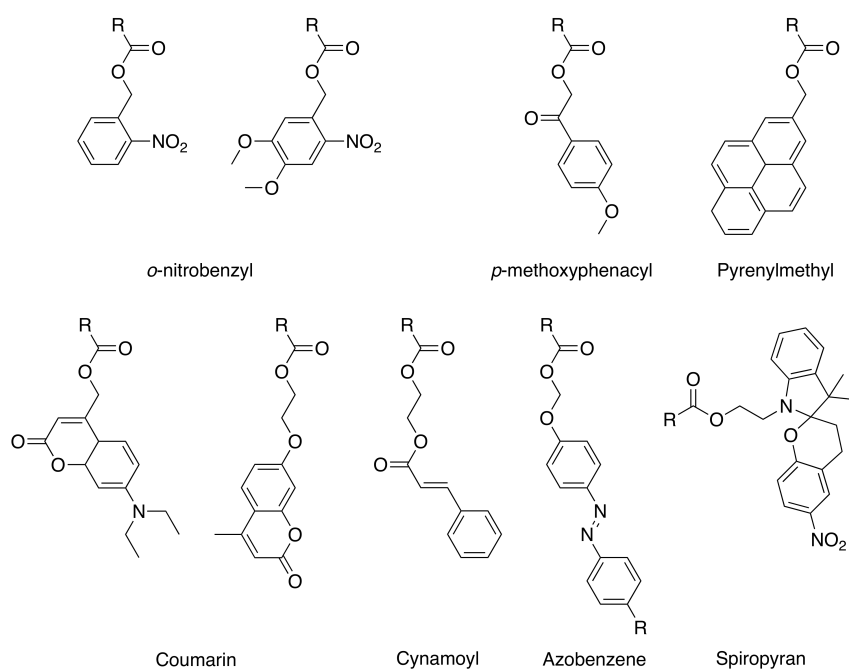
SYNTHESIS OF DIBLOCK COPOLYMERS
BEARING PHOTOCLEAVABLE SIDE GROUPS

Abstract

In this chapter, the synthesis of diblock copolymers made of a linear sequence and a sequence bearing photocleavable side groups is described. The first section deals with the synthesis of block copolymers bearing o-nitrobenzyl derivatives obtained via the grafting of o-nitrobenzyl or dimethoxynitrobenzyl moieties onto PAA-b-PS precursors. The second section focuses on the synthesis of block copolymers bearing p-methoxyphenacyl esters by direct polymerization (ATRP). In a first part, several catalytic systems have been screened to synthesize poly(p-methoxyphenacyl methacrylate)(PMPMA) in a controlled way. In the second part, several monomers were copolymerized from a PMPMA macroinitiator to evidence the possibility of preparing copolymers with monomers belonging to the three main categories (styrenic, acrylate and methacrylate).

4.1 Introduction

In this chapter the synthesis of diblock copolymers bearing photocleavable side groups is presented. The copolymers are composed of three different types of photocleavable moieties: *ortho*-nitrobenzyl esters, dimethoxynitrobenzyl esters and *para*-methoxyphenacyl esters (Figure 4.1).



employed to synthesize the targeted block copolymers. On the other hand, many polymerization techniques have a limited functional-group tolerance. The presence of these functional groups may completely prevent controlled polymerization processes. Protected monomers may be used for the formation of functional polymers, but the deprotection step may not necessarily be quantitative and may also affect the structural integrity of the polymer backbone.

Direct polymerization approach

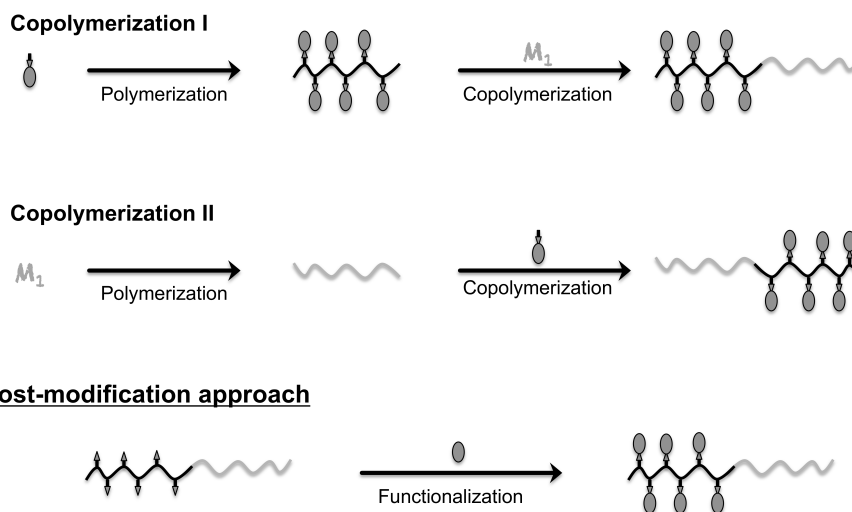


Figure 4.2: Synthetic pathways toward block copolymers bearing functional side groups

Among the different photoresponsive moieties presented in figure 4.1, the direct polymerization approach was applied for the synthesis of polymers bearing azobenzene [1–4], coumarin [5–11], cynamonyl [12], *o*-nitrobenzyl [13–24], *p*-methoxyphenacyl [25–27], pyrenyl [28, 29] and spiropyran side groups [2, 30–32].

The post-modification approach allows the production of copolymers that would not be produced by the direct polymerization approach. This approach is totally adapted to the creation of library of block copolymers. A wide range of chemical reactions can be used for the functionalization (nucleophilic substitution [33,34], reaction with activated esters [35–37], Michael addition [38], Click reactions [38–41], *etc.*). The drawbacks of the post-modification approach are the increased number of synthetic steps and the fact that the functionalization step may not necessarily be quantitative. Polymers bearing azobenzene [42–45], cynamonyl [46,47], *o*-nitrobenzyl [33,48–51], *p*-methoxyphenacyl [34,52], and spiropyran [53] side groups were produced by a post-modification approach.

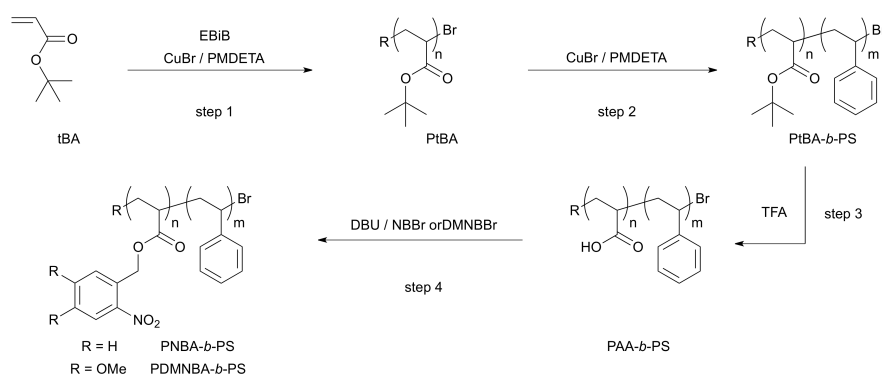
In this chapter, both approaches are used depending on the type of photocleavable moieties. The block copolymers bearing *o*-nitrobenzyl side groups are produced by post-modification and the block copolymers bearing *p*-methoxyphenacyl side groups are synthesized by direct polymerization.

4.2 Synthesis of copolymers bearing *o*-nitrobenzyl side groups

In this section the synthesis of block copolymers bearing *o*-nitrobenzyl derivatives as side groups is detailed. Those block copolymers are produced by a post-modification approach based on the grafting of *o*-nitrobenzyl bromide or dimethoxynitrobenzyl bromide on the carboxylic acid function of a poly(acrylic acid)-*block*-polystyrene copolymer (PAA-*b*-PS). The synthetic pathway to the photocleavable block copolymers is presented in scheme 4.1.

The PAA-*b*-PS precursor was prepared in three steps. A PtBA was first synthesized by ATRP (step 1). This PtBA homopolymer was then used as a macroinitiator for the polymerization of styrene by ATRP. Thirdly, the PAA-*b*-PS precursor was obtained by unmasking the carboxylic functions by an acidic hydrolysis of the *tert*-butyl protecting groups of the PtBA sequence (step 3). Finally, the photocleavable block

copolymers bearing *o*-nitrobenzyl side groups were obtained by a nucleophilic substitution of *o*-nitrobenzyl bromide (NBBBr) or 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBBBr) by the carboxylic acid function of the diblock precursor (step 4). DMNBBBr was selected because the quantum yield of dimethoxynitrobenzyl esters is higher than the nitrobenzyl esters. Moreover, the λ_{max} of dimethoxynitrobenzyl esters is located at 350 nm, it is thus possible to cleave the side groups with lower energetic lamps.

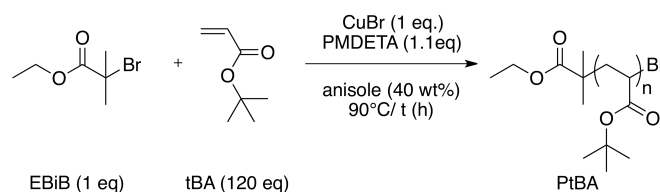


Scheme 4.1: Synthetic pathway towards PNBA-*b*-PS and PDMNBA-*b*-PS copolymers: polymerization of tBA (step 1), copolymerization of styrene (step 2), acidic hydrolysis of the *tert*-butyl side groups of the PtBA sequence (step 3), grafting of the *o*-nitrobenzyl moieties on the block copolymer (step 4).

As previously mentioned, this type of approach is not the most direct way towards the targeted photocleavable block copolymers. Indeed, the most straightforward approach would be to directly polymerize the desired photocleavable monomer (the nitrobenzyl acrylate or the dimethoxynitrobenzyl acrylate) and then copolymerize styrene. However, Dr. Schumers demonstrated that their polymerization in a controlled way remains challenging due to inhibition and retardation effects originating from the nitro-aromatic moieties [18]. Moreover, the feasibility of the post modification approach has already been demonstrated during the master thesis [54].

4.2.1 PtBA synthesis

Scheme 4.2 presents the experimental conditions for the polymerization of tBA by ATRP. Polymerizations were conducted in anisole at 90 °C using CuBr/PMDETA as the catalytic system and EBiB as the initiator.



Scheme 4.2: Poly(*tert*-butyl acrylate) synthesis.

Table 4.1 presents the results for the tBA polymerization. Well-defined PtBA were obtained with molar masses varying from 4,000 to 14,000 g/mol (M_n NMR) and PDI lower than 1.22. Except for PtBA₁₁₂ (Entry 3) the monomer conversions were limited to 50% to avoid the loss of reactive chain-ends. Indeed, high monomer conversion during polymerization induces loss of the active end groups leading to dead polymer chains [55].

Entry ^a	PtBA ^b	t (h)	conv ^b (%)	M_n (NMR) (g/mol)	M_n ^c (g/mol)	PDI ^c
1	PtBA ₃₁	0.5	26	4,000	7,100	1.22
2	PtBA ₄₃	1	33	5,700	7,100	1.20
3	PtBA ₁₁₂	2.5	76	14,400	16,600	1.10

^a Reaction conditions: EBiB/CuBr/PMDETA/tBA (molar ratio) = 1/1/1.1/120, anisole 40wt%, T = 90°C.

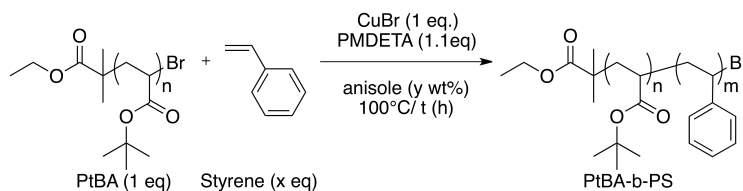
^b DPs and conversions were determined by ¹H NMR.

^c M_n s and PDIs were determined by GPC using a PS calibration.

Table 4.1: Experimental conditions and results for the polymerization of tBA by ATRP.

4.2.2 PtBA-*b*-PS synthesis

The previously synthesized PtBA were used as macroinitiator for the copolymerization of styrene. Scheme 4.3 presents the general conditions of the copolymerizations. They were carried out in anisole at 100 °C. CuBr/PMDETA was used as the catalytic system.



Scheme 4.3: Poly(*tert*-butyl acrylate)-*b*-polystyrene synthesis.

Table 4.2 presents the conditions and the results for the copolymerization of styrene starting from a PtBA macroinitiator. Three different PtBA-*b*-PS copolymers with molar masses between 10,600 and 27,500 g/mol (M_n (NMR)) and PDIs lower than 1.21 were obtained with these conditions.

A look at the initiation efficiency (e_i) column clearly demonstrates the effect of the monomer conversion during the PtBA polymerization on the active chain-end lost. Indeed, a decrease of 19% for e_i is observed between the PtBA₄₃-*b*-PS₄₉ (Entry 2, $e_i = > 99\%$, tBA conversion = 33%) and the PtBA₁₁₂-*b*-PS₁₂₅ (Entry 3, $e_i = > 80\%$, tBA conversion = 76%). Entry 1 shows a rather surprising result for the initiation efficiency ($e_i = 66\%$). This low value is most probably due to an ageing (loss of chain-end with time) of the macroinitiator as the copolymerization was realized more than 6 months after the PtBA synthesis for this sample.

Entry ^a	PtBA- <i>b</i> -PS ^b	styrene (eq.)	anisole (wt%)	t (h)	conv ^b (%)	M_n (NMR) (g/mol)	M_n^c (g/mol)	PDI ^c	e_i^d (%)
1	PtBA ₃₁ - <i>b</i> -PS ₁₆₈	300	60	1.25	37	21,500	21,000	1.14	66
2	PtBA ₄₃ - <i>b</i> -PS ₄₉	200	65	4	32	10,600	11,600	1.15	>99
3	PtBA ₁₁₂ - <i>b</i> -PS ₁₂₅	300	60	2.5	33	27,500	21,600	1.21	80

^a Reaction conditions: PtBA/CuBr/PMDETA (molar ratio) = 1/1/1.1, T = 100°C.

^b DPs and conversions were determined by ¹H NMR.

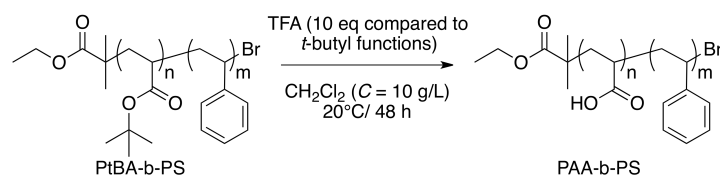
^c M_n s and PDIs were determined by GPC using a PS calibration.

^d e_i = initiation efficiency = DP(PS)/DP_{th}(PS)

Table 4.2: Results for the polymerization of styrene with a PtBA macroinitiator by ATRP.

4.2.3 Hydrolysis of PtBA-*b*-PS

The third step of the diblock copolymer synthesis is the hydrolysis of the *tert*-butyl functions of the PtBA sequence in acidic condition. Scheme 4.4 presents the experimental conditions for the hydrolysis. Tri-fluoroacetic acid (TFA) was used as a proton donor. The reaction was conducted in CH₂Cl₂ for 48h at 20°C.



Scheme 4.4: Hydrolysis of poly(*tert*-butyl acrylate)-*b*-polystyrene.

The hydrolysis reaction was performed on the previously synthesized copolymers and an average hydrolysis degree of 95% was obtained under these conditions.

Entry ^a	PtBA- <i>b</i> -PS	→	PAA- <i>b</i> -PS	e_H^b (%)
1	PtBA ₃₁ - <i>b</i> -PS ₁₆₈	→	PAA ₃₁ - <i>b</i> -PS ₁₆₈	95
2	PtBA ₄₃ - <i>b</i> -PS ₄₉	→	PAA ₄₃ - <i>b</i> -PS ₄₉	96
3	PtBA ₁₁₂ - <i>b</i> -PS ₁₂₅	→	PAA ₁₁₂ - <i>b</i> -PS ₁₂₅	95

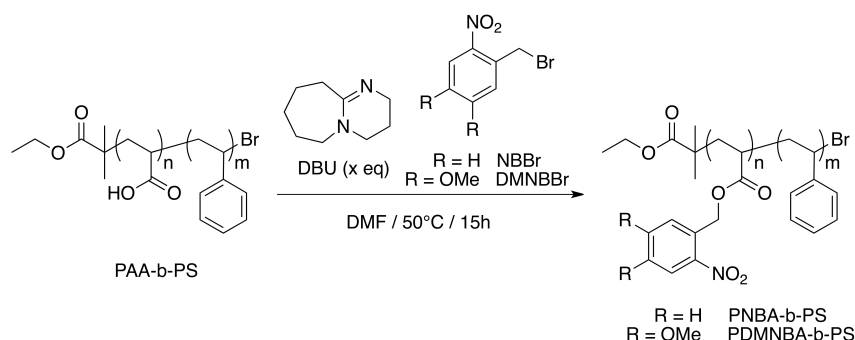
^a Reaction conditions: $C_{\text{PtBA-}b\text{-PS}} = 10 \text{ g/L}$ in CH₂Cl₂, TFA = 10 eq. compared to tBA, T = 20°C, 48h.

^b e_H = hydrolysis yield determined by ¹H NMR.

Table 4.3: Results for the hydrolysis of poly(*tert*-butyl acrylate)-*b*-polystyrene.

4.2.4 Grafting of the *o*-nitrobenzyl derivatives on PAA-*b*-PS

The last synthetic step towards block copolymers bearing *o*-nitrobenzyl side groups is the grafting of the nitrobenzyl derivatives onto the carboxylic acid functions of the PAA sequence. Scheme 4.5 presents the general conditions of the grafting reaction.



Scheme 4.5: Grafting of the *o*-nitrobenzyl derivatives on PAA-*b*-PS.

The grafting was carried out via the nucleophilic substitution ($\text{S}_{\text{N}}2$) of the carboxylic acid functions of the PAA sequence onto *o*-nitrobenzyl bromide derivatives. The first step of this nucleophilic substitution requires a base to deprotonate the carboxylic acid thus forming the reactive carboxylate. As the carboxylate is a poor nucleophile, the base has to be non nucleophilic and bulky. Indeed, a nucleophilic base would substitute the bromide of the *o*-nitrobenzyl bromide derivative. Moreover, the bulky character of the base is needed to increase the nucleophilic character of the carboxylate function, as it will allow a total dissociation of the ionic pair. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was selected as it complies with these requirements. In addition, DMF was chosen as the solvent because it is a good solvent for PAA-*b*-PS and its high polarity increases the ionic pair dissociation. In 1990, Shimokawa and coworkers were the first to use that approach for the synthesis of homo-poly(nitrobenzyl methacrylate) (PNBMA) starting from a poly(methacrylic acid) (PMAA) precursor [33].

Entry ^a	copolymers	C ^b (mol/L)	DMNBBr (eq.)	M _n ^c (g/mol)	PDI ^c	e _g ^d (%)
1 ^e	PNBA ₃₁ - <i>b</i> -PS ₁₆₈	0.05	2.2	21,700	1.14	97
2	PDMNBA ₃₁ - <i>b</i> -PS ₁₆₈	0.05	2.2	24,100	1.17	97
3	PDMNBA ₄₃ - <i>b</i> -PS ₄₉	0.09	4.4	14,000	1.17	92
4	PDMNBA ₁₁₂ - <i>b</i> -PS ₁₂₅	0.09	1.6	24,900	1.25	91

^a Performed in DMF with x eq. of DBU (x = DMNBBr eq.) at 50 °C for 15 h.^b C = Concentration of carboxylic acid in the reaction medium.^c M_ns and PDIs were determined by GPC using a PS calibration.^d e_g = grafting yield determined by ¹H NMR.^e Performed with *o*-nitrobenzyl bromide.**Table 4.4:** Results for the grafting of the *o*-nitrobenzyl derivatives onto PAA-*b*-PS.

Table 4.4 presents the different conditions and results obtained for the grafting of the *o*-nitrobenzyl moieties onto the PAA-*b*-PS copolymer. The functionalizations were conducted in DMF at 50°C for 15 h with *o*-nitrobenzyl bromide (NBBBr) or 4,5-dimethoxy-2-nitrobenzyl bromide (DMNBBBr). All the different experiments led to high grafting yields (> 90%).

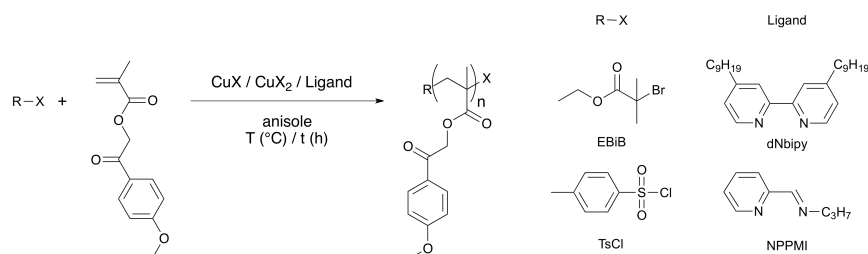
4.3 Synthesis of copolymers bearing *p*-methoxyphenacyl side groups

The previous section demonstrated that the synthesis of block copolymers bearing *o*-nitrobenzyl side groups is possible via a post modification approach. However, this approach is not the more straightforward way to produce block copolymers bearing photocleavable side groups. Since the control polymerization of *o*-nitrobenzyl acrylate is difficult because the monomer bears an nitro function, which usually quenches the radical polymerization process. We thus decided to focus on the synthesis of a photocleavable monomer that does not bear such type of function: *p*-methoxyphenacyl methacrylate. In the literature, the synthesis of polymers bearing phenacyl derivatives is limited to the post functionalization approach [34, 52] and the direct polymerization by FRP [25–27]. This section is focused on the direct synthesis of well-defined block copolymers bearing *p*-methoxyphenacyl moieties by ATRP.

In section 4.3.1, the homopolymerization of *p*-methoxyphenacyl methacrylate (MPMA) by ATRP is investigated. Various catalytic systems have been tested in order to obtain PMPMA polymers in a controlled way. Section 4.3.2 deals with the synthesis of diblock copolymers composed of one block of PMPMA and a second block of polystyrene, poly(*tert*-butyl acrylate), poly(methyl methacrylate) or poly[oligo(ethylene glycol) methacrylate], evidencing the possibility to copolymerize monomers from the three main families.

4.3.1 Polymerization of *p*-methoxyphenacyl methacrylate

As the controlled behavior of the polymerization of MPMA by ATRP was never demonstrated, several catalytic systems were studied. Scheme 4.6 presents the different parameters that were evaluated to obtain controlled polymerization of MPMA by ATRP: the copper halide, the initiator and the ligand.



Scheme 4.6: Polymerization of *p*-methoxyphenacyl methacrylate.

At first, the polymerization was carried out with copper (I) bromide and the screened parameters were the ligand (dNbipy, NPPMI), the temperature (40 to 70°C), the addition of copper (II) bromide and the monomer concentration (40, 50 or 90 wt% in anisole). Table 4.5 presents the different conditions and results for the polymerization of MPMA with CuBr.

Except for two sets of conditions (Entries 1 and 8), the accordingly obtained polymers exhibit good to reasonable PDI. The first set of experiments was realized with 4,4'-dinonyl-2,2'-dipyridyl (dNbipy) which is a moderately activating ligand in ATRP [56]. The high PDI of entry 1 can be explained by the low temperature used for the polymerization (40°C). Indeed, it is well-known that the activation rate constant of the initiator in ATRP is a function of the temperature, *i.e.* the initiation is slow [57]. The decrease of the temperature in this case leads to an increase of the initiation duration, which is undesired in CRP as the initiation should be fast and efficient. In the second experiment, the temperature was increased to 60°C and the solvent/monomer ratio was

increased to 90 wt%. With these conditions, a well-defined PMPMA was produced. Unfortunately the high dilution of the reaction medium was not adapted to the production of PMPMA in large scale.

The second set of experiments (entries 3 to 6) was realized with N-(n-propyl)-2-pyridylmethanimine (NPPMI) and CuBr_2 . This catalytic system, $\text{CuBr}/\text{CuBr}_2/\text{NPPMI}$, was chosen to decrease the propagation rate of the polymerization. Indeed, NPPMI is one of the less active ligand for ATRP [56] and the presence of Cu(II) species during ATRP induces a decrease of the apparent rate of propagation ($k_{\text{p,app}}$) [58]. Under these conditions, relatively well-defined homopolymers were produced with M_n ranging from 19,600 to 32,900 g/mol. However, GPC chromatograms (Figures 4.3b-d) revealed an asymmetry toward the high elution times (low molar masses), suggesting the occurrence of side reactions and therefore a not fully controlled behavior. The best results were obtained at 70°C with NPPMI as ligand, and 0.1 equivalents of CuBr_2 (Table 4.5, Entries 3 and 4). Under these conditions, polymers with PDIs lower than 1.15 and exhibiting a symmetrical elution peak in the chromatogram (Figure 4.3a) were obtained. However, problems of reproducibility were met and an important difference between the theoretical and experimental molar masses was observed, indicating a non-controlled behavior.

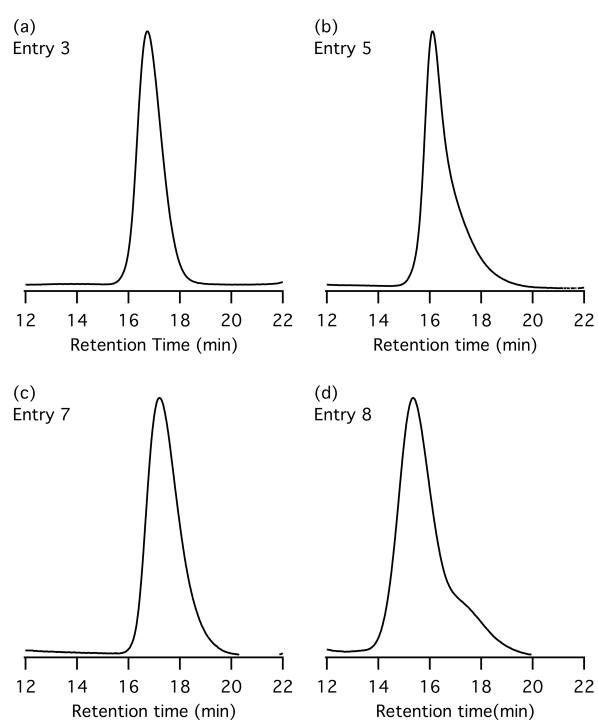
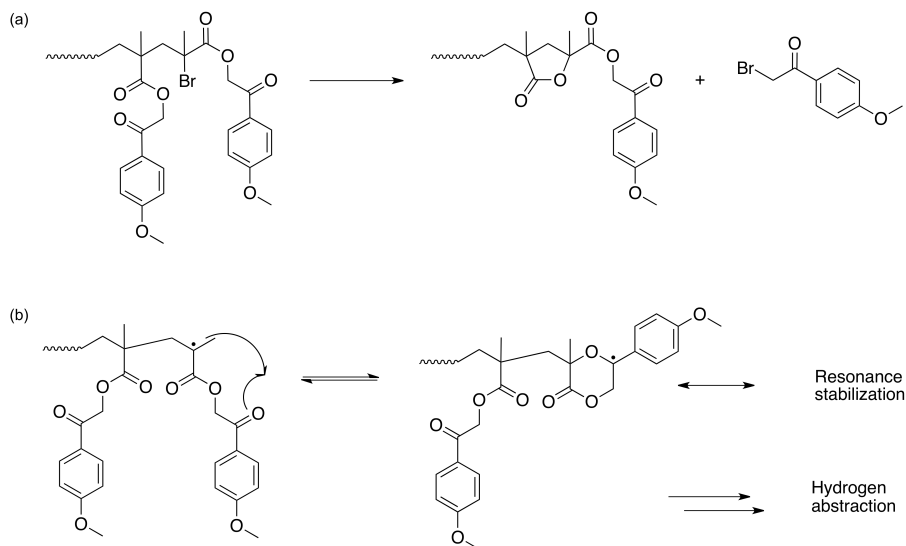


Figure 4.3: GPC chromatograms of PMPMA produced by ATRP catalyzed by CuBr (Table 4.5).

Entry ^a	EBiB/CuBr/CuBr ₂ /Ligand/MPMA (Molar ratio)	Ligand	T (°C)	t (h)	conv ^b (%)	M _n (th) (g/mol)	M _n ^c (g/mol)	PDI ^c
1 ^d	1/1/0/2/100	dNbipy	40	1.5	84	19,700	34,200	1.92
2 ^e	1/1/0.1/2.2/100	dNbipy	60	2	38	9,000	11,500	1.21
3	1/1/0.1/3.3/100	NPPMI	70	9	38	9,000	26,400	1.15
4	1/1/0.1/3.3/100	NPPMI	70	5	42	9,900	19,600	1.14
5	1/1/0.1/3.3/150	NPPMI	70	8	70	24,400	32,900	1.36
6	1/1/0.1/3.3/150	NPPMI	70	6	61	20,000	25,600	1.33
7	1/0.5/0/1.5/150	NPPMI	60	5.5	35	12,400	16,100	1.25
8	1/0.5/0/1.5/150	NPPMI	60	5.5	46	16,000	54,000	1.97

^a Reactions were performed in anisole (50wt%).^b Conversions were determined by ¹H NMR.^c M_ns and PDIs were determined by GPC using a PS calibration.^d Reactions were performed in anisole (40wt%).^e Reactions were performed in anisole (90wt%).**Table 4.5:** Results for the polymerization of MPMA by ATRP with CuBr.

Coskun's group has investigated the nature of the end-groups of poly(phenacyl methacrylate) produced by FRP and ATRP [26, 27]. According to their findings, the formation of a lactone cycle at the chain-end can occur during the early stage of the polymerization. This lactone is formed either by the removal of phenacyl bromide (Scheme 4.7a) or by the attack of the chain-end radical onto the carbonyl of a phenacyl side group (Scheme 4.7b). As in ATRP the radical concentration in the reaction medium is low, the probability of the radical lactone cyclization is not considered as the major deactivation process. Deactivation of the chains through *p*-methoxyacetophenone bromide abstraction and cyclization into a lactone is thus hypothesized in the case of ATRP (Scheme 4.7a). This side reaction, which occurs rather early in the polymerization process, leads to the formation of dead chains of low molar mass, which explains the tailing at high elution times observed in the chromatograms of figure 4.3.



Scheme 4.7: Side reactions occurring during the polymerization of *p*-methoxyphenacyl methacrylate: (a) removal of *p*-methoxyphenacyl bromide, (b) radical cyclization.

New sets of experiments were carried out to avoid side reactions (Table 4.5, Entries 7-8). In these experiments, the amount of CuBr was decreased compared to the initiator in order to decrease the concentration of radicals in the reaction medium. With these conditions, similar results were observed (asymmetry in the GPC trace, reproducibility issue and difference between the theoretical and experimental molar masses). These experiments demonstrate that a decrease of the radical concentration in the reaction medium does not reduce the side reactions, and thus emphasize the hypothesis of the *p*-methoxyacetophenone bromide abstraction. In an effort to prevent this abstraction of halide during polymerization, the polymer-halide bond strength was increased by replacing the bromide by a chloride. The catalytic system was thus changed to CuCl/dNbipy and TsCl was used as the initiator (Scheme 4.6). For the first polymerization test, we selected dNbipy as the ligand to reduce the activity of the catalyst since methacrylate monomers are highly reactive in controlled radical polymerization.

Table 4.6 presents the conditions and results for the polymerization of MPMA with CuCl/dNbipy. The different reactions were conducted in anisole (50wt%) at 60°C with molar ratio of TsCl/CuCl/dNbipy = 1/1/2. With these conditions, polymers with molar masses ranging from 10,600 to 26,000 g/mol and low PDIs < 1.14 were obtained. In addition to GPC and ¹H NMR, the polymers were analyzed by a GPC coupled to a multiangle laser light scattering (MALLS) to determine their absolute molar masses. The degree of polymerization of the different PMPMA was determined by GPC-MALLS as no characteristic peaks of the chain-ends are observed in the ¹H NMR spectra. The low PDIs of the obtained PMPMA and the symmetric peak observed in the GPC chromatograms give us clues that the polymerization of MPMA with CuCl/dNbipy as the catalytic system is controlled.

Entry ^a	MPMA (eq.)	t (h)	conv (%)	M _n (th) (g/mol)	M _n ^b (g/mol)	M _n (MALLS) (g/mol)	PDI ^b	DP ^c
1	150	8	61	21,600	19,900	26,000	1.13	110
2	140	4.75	67	22,200	20,900	23,400	1.10	99
3	100	4.5	50	11,800	12,500	14,500	1.10	61
4	100	2.75	31	7,400	9,100	10,600	1.10	44

^a Reaction conditions: TsCl/CuCl/dNbipy (molar ratio) = 1/1/2, anisole (50 wt%), T = 60°C.

^b M_ns and PDIs were determined by GPC using a PS calibration.

^c Determined by GPC-MALLS.

Table 4.6: Results for the polymerization of MPMA by ATRP with CuCl.

Finally, to confirm the controlled behavior of the polymerization, a kinetic study was realized with the conditions presented in Entry 1 of table 4.6. Figure 4.4a presents the semi-logarithmic plot of conversion vs. time. A linear variation of the conversion with time is observed, indicating a constant concentration of active species in the medium. Moreover, the molar masses can be predicted since they increase linearly with conversion (Figure 4.4b). All these observations confirm the controlled behavior of the polymerization of MPMA with the CuCl/dNbipy catalytic system in the studied conversion range.

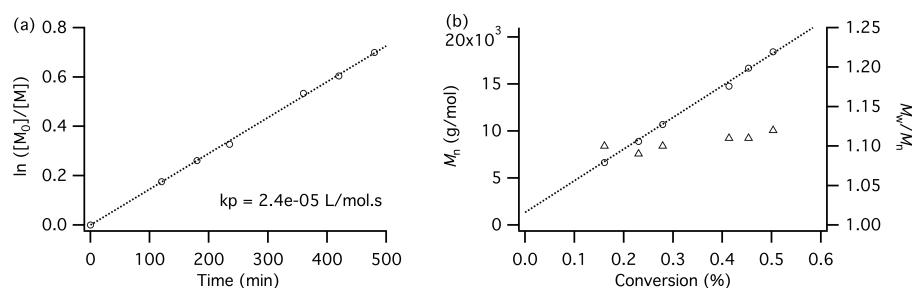


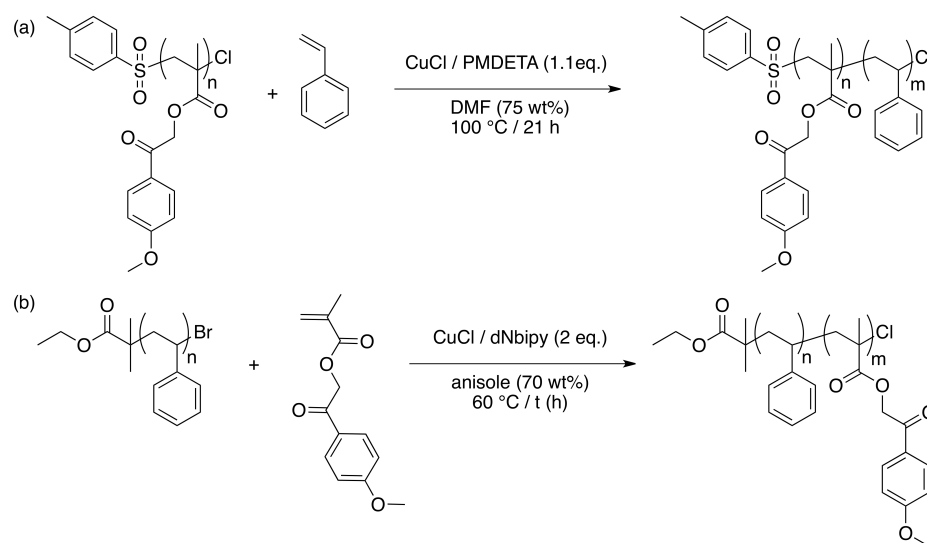
Figure 4.4: (a) Kinetic plot for the ATRP of MPMA using CuCl/dNbipy as catalytic system, (b) dependence of molar mass (M_n , O) and polydispersity index (M_w/M_n , Δ) on monomer conversion. Reaction conditions: TsCl/CuCl/dNbipy/MPMA = 1/1/2/150 T = 60°C in anisole (50 wt%).

4.3.2 Synthesis of diblocks copolymers containing poly(*p*-methoxyphenacyl methacrylate)

This section deals with the synthesis of diblock copolymers composed of a PMPMA block and a second block of polystyrene, poly(*tert*-butyl acrylate), poly(methyl methacrylate) or poly[oligo(ethylene glycol) methacrylate]. These monomers were selected to demonstrate that a wide range of diblock copolymers containing poly(*p*-methoxyphenacyl methacrylate) can be produced by ATRP, and that the PMPMA previously synthesized are effective macroinitiators and can thus reinitiate a polymerization.

4.3.2.1 copolymerization with styrene

The copolymerization was first studied with styrene. Scheme 4.8 presents the different approaches used for the synthesis of *p*-methoxyphenacyl methacrylate and styrene copolymers.



Scheme 4.8: Synthesis of PMPMA-*b*-PS: (a) copolymerization of styrene starting from a PMPMA macroinitiator, (b) copolymerization of MPMA starting from a PS macroinitiator.

Table 4.7 presents the conditions and results for the investigated copolymerizations. In a first time, the copolymerization of styrene starting from a PMPMA macroinitiator was carried out with CuCl/PMDETA as the catalyst in DMF (75wt%) at 100°C (Entry 1). Under these conditions, the polymerization is not controlled and a badly defined polymer is produced (PDI = 6.66). Figure 4.5a presents the GPC chromatograms of the starting PMPMA and of the obtained PMPMA-*b*-PS. In this figure, we can observe the presence of several high molar mass populations indicating the occurrence of side reactions during the polymerization, most probably styrene self-initiation at high temperature.

Entry	copolymer	t (h)	conv (%)	M_n (th) (g/mol)	M_n^a (g/mol)	PDI ^a
1 ^b	PMPMA ₁₁₀ - <i>b</i> - PS ₂₇₂	21	74	56,800	98,000	6.66
2 ^c	PS ₈₇ - <i>b</i> -PMPMA ₁₅₄	4.5	74	35,200	37,500	1.29

^a M_n s and PDIs were determined by GPC using a PS calibration.

^b Conditions: PMPMA-Cl/CuCl/PMDETA/styrene (molar ratio) = 1/1/1.1/400, DMF 75 wt%, T = 100°C.

^c Conditions: PS-Br/CuCl/dNbipy/MPMA = 1/1/2/150, anisole 50 wt%, T = 60°C.

Table 4.7: Results for the synthesis of PMPMA-*b*-PS.

Another approach was thus followed for the preparation of copolymers composed of PMPMA and PS (Scheme 4.8b). Starting from a PS-Br macroinitiator, MPMA was polymerized with the CuCl/dNbipy catalytic system in anisole (60 wt%) at 60 °C (Entry 2). The conditions used for the copolymerization of MPMA were the same as for the homopolymerization except for the initiator. The presence of bromide at the chain-end of the macroinitiator has two effects on the polymerization. First, an increase of the polymerization rate is observed. Second, a slight tailing for the GPC chromatogram of the PS-*b*-PMPMA is observed (see Figure 4.5b). This tailing indicates that a loss of phenacyl bromide occurs during the polymerization as a mixture of both bromine and chlorine atoms caps the polymer chain during the polymerization [59]. Even if side reactions occur during the polymerization, relatively well-defined PS-*b*-PMPMA block copolymers with PDI < 1.3 are nevertheless obtained.

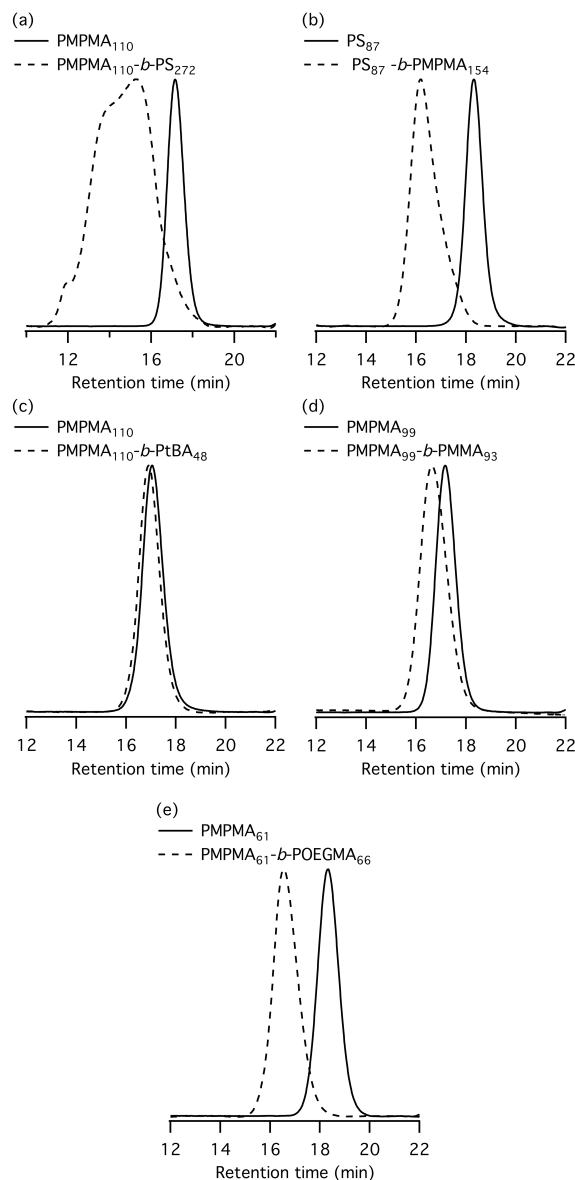
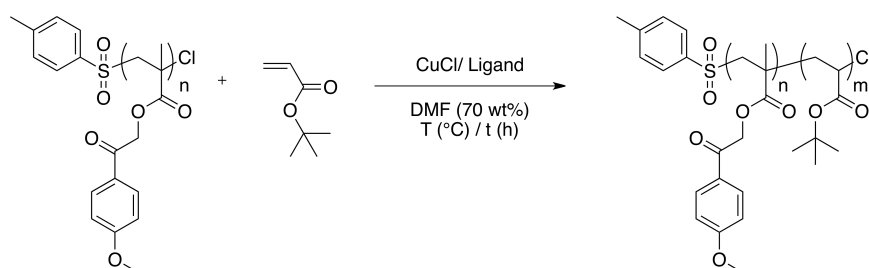


Figure 4.5: Typical GPC chromatograms of macroinitiators (solid curves) and of diblock copolymers (dashed curves): (a) copolymerization of styrene starting from PMPMA (Entry 1, Table 4.7), (b) copolymerization of MPMA starting from PS (Entry 2, Table 4.7), (c) copolymerization of tBA starting from PMPMA (Entry 4, Table 4.8), (d) copolymerization of MMA starting from PMPMA (Entry 1, Table 4.9), (e) copolymerization of OEGMA starting from PMPMA (Entry 3, Table 4.9) .

4.3.2.2 Copolymerization with *tert*-butyl acrylate

The second type of investigated monomer belongs to the acrylate family. Indeed, the copolymerization of *tert*-butyl acrylate from a PMPMA macroinitiator was investigated (Scheme 4.9). The effects of the catalytic system and of the temperature have been investigated to obtain well-defined copolymers.



Scheme 4.9: Copolymerization of tBA starting from a PMPMA macroinitiator.

Table 4.8 presents the conditions and the results for the copolymerization of tBA. The polymerization was first carried out with CuCl/PMDETA in DMF (70 wt%). The effect of temperature was investigated for this condition by performing the reaction at 70, 60 and 50 °C. The PDIs of the obtained copolymer is decreasing when the reaction temperature is decreased from 70 °C to 60 °C (Entries 1 and 2). The decrease of the temperature to 50 °C does not have a major effect on the copolymer PDI (Entries 2 and 3). Finally, the effect of the catalytic system on the copolymerization of tBA has been investigated by using CuCl/dNbipy at low temperature (50 °C). These conditions yield a PMPMA-*b*-PtBA with a narrow PDI (Entry 4, PDI = 1.15). The average DP of the second block was calculated using the DP of the PMPMA, previously determined, and the PMPMA/second block ratio determined by ¹H NMR.

Entry ^a	PMPMA- <i>b</i> -PtBA ^b	tBA (eq.)	T (°C)	t (h)	conv (%)	<i>M</i> _n (th) (g/mol)	<i>M</i> _n ^c (g/mol)	<i>M</i> _n (MALLS) (g/mol)	PDI ^c
1	PMPMA ₁₁₀ - <i>b</i> -PtBA ₂₀₅	200	70	8	93	49,700	43,200	49,700	1.50
2	PMPMA ₁₁₀ - <i>b</i> -PtBA ₂₀₅	300	60	4	65	50,800	44,900	47,300	1.32
3	PMPMA ₁₁₀ - <i>b</i> -PtBA ₂₈₂	345	50	5	60	52,300	49,000	55,900	1.31
4 ^d	PMPMA ₁₁₀ - <i>b</i> -PtBA ₄₈	200	50	3.5	15	32,100	24,700	24,400	1.15

^a Reaction conditions: PMPMA-Cl/CuCl/PMDETA (molar ratio) = 1/1/1.1, DMF 70 wt%.

^b Determined by ¹H NMR.

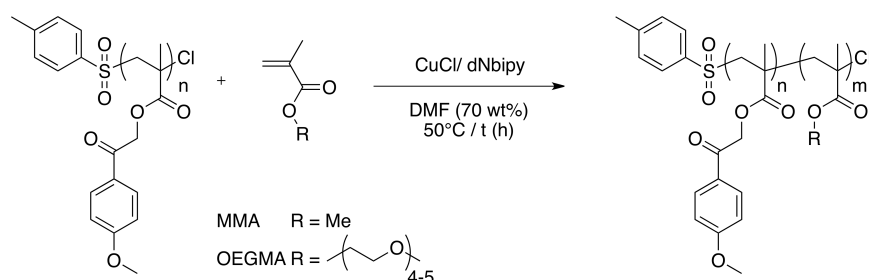
^c *M*_ns and PDIs were determined by GPC using a PS calibration.

^d PMPMA-Cl/CuCl/dNbipy = 1/1.15/2.3.

Table 4.8: Results for the copolymerization of tBA starting from a PMPMA macroinitiator.

4.3.2.3 Copolymerization with methacrylate monomers: MMA and OEGMA

Finally, the copolymerization with methacrylate monomers was explored with methyl methacrylate (MMA) and oligo(ethylene glycol) methacrylate (Scheme 4.10).



Scheme 4.10: Copolymerizations of MMA and OEGMA starting from a PMPMA macroinitiator.

Table 4.9 presents the conditions and the results for the copolymerization of methacrylate monomers starting from a PMPMA macroinitiator. The copolymerization was first prospected with methyl methacrylate and CuCl/dNbipy in DMF (70wt%) at 50°C (Entry 1). With these conditions, a well-defined PMPMA₉₉-*b*-PMAA₉₃ with a $M_n = 31,100$ g/mol and a PDI = 1.20 was synthesized. As the copolymerization was controlled, these conditions were applied to the copolymerization of OEGMA (Entries 2-4). The resulting PMPMA-*b*-POEGMA diblock copolymers were obtained with molar masses ranging from 30,200 to 35,500 g/mol and with PDIs lower than 1.30. The presence of a slight excess of CuCl in the reaction medium seems to lead to the formation of copolymers with narrower molar mass distribution (Entries 2-3). The average DP of the second blocks were calculated using the DP of the PMPMA (previously determined) and the PMPMA/second block ratio determined by ¹H NMR.

Entry ^a	PMPMA- <i>b</i> -polymer ^b	monomer (eq.)	t (h)	conv (%)	<i>M</i> _n (th) (g/mol)	<i>M</i> _n ^c (g/mol)	<i>M</i> _n (MALLS) (g/mol)	PDI ^c
1 ^d	PMPMA ₉₉ - <i>b</i> -PMAA ₉₃	345	1.5	29	33,400	29,100	31,100	1.20
2 ^e	PMPMA ₉₉ - <i>b</i> -POEGMA ₄₈	100	1.3	38	34,800	31,300	35,500	1.21
3	PMPMA ₆₁ - <i>b</i> -POEGMA ₆₆	100	4.5	59	29,800	27,100	30,200	1.27
4	PMPMA ₄₄ - <i>b</i> -POEGMA ₆₆	100	4.5	60	28,600	23,500	N.D.	1.26

^a Reaction conditions: PMPMA-Cl/CuCl/dNbipy (molar ratio) = 1/1/2, DMF 70 wt%, T = 50°C.

^b Determined by ¹H NMR.

^c *M*_ns and PDIs were determined by GPC using a PS calibration.

^d Molar ratio of PMPMA-Cl/CuCl/dNbipy = 1/1.15/2.3.

^e Molar ratio of PMPMA-Cl/CuCl/dNbipy = 1/1.2/2.8.

Table 4.9: Results for the copolymerization of MMA and OEGMA starting from a PMPMA macroinitiator.

4.4 Conclusions

The focus of this chapter was the synthesis of diblock copolymers bearing photocleavable side groups. The photo-responsive behavior of this type of copolymers in solution will allow the change of polarity of one of the block copolymer sequence and will thus promote their micellization (Chapter 6) or on the contrary the micelles disruption (Chapter 7) by light.

In the first section of this chapter, the synthesis of copolymers bearing *o*-nitrobenzyl side groups was detailed. The synthetic strategy relies on the post-modification of PAA-*b*-PS precursor copolymers. The feasibility of this approach has been demonstrated by the quantitative grafting of either 2-nitrobenzyl bromide or 4,5-dimethoxy-2-nitrobenzyl bromide on the PAA sequence of the diblock precursors.

The second section focused on the synthesis by direct polymerization of copolymers bearing *p*-methoxyphenacyl side groups. In the first part the homopolymerization of MPMA was optimized by screening different experimental parameters. The best results were obtained by using TsCl/CuCl/dNbipy as the catalytic system, which gave rise to a well-controlled behavior, and led to homopolymers with low PDI. In the second part, copolymerization with different monomers was investigated. The copolymerization of (meth)acrylate monomers starting from a PMPMA macroinitiator was studied. Several polymerization parameters were screened and optimized to allow the synthesis of well-defined block copolymers. Diblock copolymers composed of polystyrene and poly(*p*-methoxyphenacyl methacrylate) were obtained with a halide exchange approach, starting from a PS-Br macroinitiator with CuCl/dNbipy as the catalytic system.

This part of the thesis was devoted to the synthesis of well-defined photocleavable copolymers. The synthesis of block copolymers bearing *o*-nitrobenzyl side groups via the post modification of a PAA-*b*-PS precursor was here improved in comparison to the master thesis results to obtain a quantitative grafting of different *o*-nitrobenzyl derivatives.

Moreover, the control polymerization by ATRP of *p*-methoxyphenacyl methacrylate was demonstrated for the first time. The living character of the PMPMA has allowed the synthesis of new photocleavable block copolymers.

Experimental part

Materials and instrumentation. Styrene (Sty, Aldrich, 99 %), *tert*-butyl acrylate (tBA, Acros, 99 %), methyl methacrylate (MMA, Aldrich, 99 %) and poly(ethylene glycol) methyl ether methacrylate (OEGMA, $M_n = 300$ g/mol, Aldrich, 99%) were passed through activated basic alumina (Acros) columns prior use. N,N-dimethylformamide (DMF, Aldrich, 99.8 %) was distilled on CaH₂ prior use. Triethylamine (Et₃N, Fischer Scientific, >98%) was dried over KOH. 4,5-dimethoxy-2-nitrobenzyl bromide (DMNNBr, Acros, 97 %) and 2-nitrobenzyl bromide (NBBR, Acros, 97%) was dried in vacuo at 30°C for 48 h prior to use. N-(n-propyl)-2-pyridylmethanimine (NPPMI) was synthesized as described in ref [60]. CuBr (Aldrich, 99.999 %), CuBr₂ (Aldrich, 99.999 %), CuCl (Aldrich, 99.999 %), ethyl 2-bromoisobutyrate (EBiB, Acros, 98 %), tosyl chloride (TsCl, Fulka, >99%), anisole (Acros, 99 %), N,N,N',N'',N'''-pentamethyldiethylenetriamine (PMDETA, Aldrich, 98%), 4,4'-dinonyl-2,2'-dipyridyl (dNbipy, Aldrich, 97 %), ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA, Fluka, 97 %), 2-bromo-4'-methoxyacetophenone (Aldrich, 97%), methacrylic acid (Acros, 99.5%), trifluoroacetic acid (TFA, Acros, 99 %), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, Acros, 98 %) and all the other chemicals were used as received.

Proton and carbon nuclear magnetic resonance (NMR) spectra were acquired on a 300 MHz or 500 MHz Bruker Avance II. Infrared (IR) spectra were recorded on thin films deposited onto sodium chloride plates with a FT-IR spectrometer (Shimadzu FTIR-8400S). Molar masses (M_n) and polydispersity indices (PDI) of the polymers were measured on an Agilent gel permeation chromatography (GPC) system equipped with an Agilent 1100/1200 pump (35°C; eluent: DMF; flow rate of 1 mL/min), an Agilent differential refractometer, a BTC BI-MwA Molecular Weight Analyzer and two PSS GRAM columns (Beads 10 μ ; porosity of column 1: 1000 Å; porosity of column 2: 100 Å). The calibration was performed using polystyrene standards.

Section 4.2

General procedure for the polymerization of poly(*tert*-butyl acrylate):

Under argon, a round-bottom flask, with a stopcock, containing CuBr (82 mg; 0.57 mmol; 1 equiv.) was filled with a solution containing *tert*-butyl acrylate (tBA; 9.97 mL; 68.68 mmol; 120 equiv.), EBiB (112 mg; 0.57 mmol, 1 equiv.), PMDETA (109 mg; 0.62 mmol; 1.1 equiv.), and anisole (5.90 mL; 40 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 90°C for 30 min (tBA conversion = 26 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the

reaction mixture to air. The reaction mixture was diluted with CH_2Cl_2 and washed with an aqueous solution of EDTA (0.04 M). The organic phase was dried over MgSO_4 , filtered and the solvent was removed under reduced pressure. The residue was precipitated in $\text{MeOH:H}_2\text{O}$ (50:50), filtered and dried in vacuo at 35°C for 24 h, affording a white solid (1.67 g, 70%).

M_n (GPC) = 7,100 g/mol; PDI (GPC) = 1.22. ^1H NMR (300 MHz, CDCl_3): δ (ppm) 4.1 (m, 3H, $\text{CH}_2\text{-O}$, CH-Br), 2.3- 2.0 (b, 30 H, CH backbone), 1.9-1.3 (s, 341H, CH_2 backbone, CH_3 t-Butyl). M_n (NMR) = 4,000 g/mol.

General procedure for the synthesis of poly(*tert*-butyl acrylate)-*b*-polystyrene:

Under argon, a round bottom flask, with a stopcock, containing CuBr (12 mg; 0.08 mmol; 1 equiv.) was filled with a solution containing $\text{PtBA}_{31}\text{-Br}$ (332 mg; 0.08 mmol, 1 equiv.), styrene (2.61 g; 25.10 mmol; 300 equiv.), PMDETA (16 mg; 0.09 mmol; 1.1 equiv.) and anisole (784 mg; 30 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 100°C for 4 h (styrene conversion = 37 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was diluted with CH_2Cl_2 and washed with an aqueous solution of EDTA (0.04 M). The organic phase was dried over MgSO_4 , filtered and the solvent was removed under reduced pressure. The residue was precipitated in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white powder (1.04 g, 81 %).

M_n (GPC) = 21,000 g/mol; PDI (GPC) = 1.14. ^1H NMR (500 MHz, CDCl_3): δ (ppm) 7.30-6.30 (b, 840H, H_{Ar} ; PS), 4.1 (m, 2H, $\text{CH}_2\text{-O}$; Chain end), 2.3- 1.1 (b, 1076H, CH backbone, CH_2 backbone, CH_3 t-Butyl). M_n (NMR) = 21,500 g/mol.

General procedure for the synthesis of poly(acrylic acid)-*b*-polystyrene:

In a round bottom flask, $\text{PtBA}_{31}\text{-b-PS}_{168}$ (974 mg; 1.40 mmol in tBA units; 1 equiv.) was dissolved in dichloromethane (97.4 mL, 0.01 M). TFA (1.46 mL, 19.72 mmol, 14 equiv.) was added and the solution was stirred at 20°C for 24 h. The solvent was removed under reduced pressure and the residue was kept in a minimum amount of DMF and dialyzed against methanol. The polymer was precipitated in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white powder (813 mg, 91%).

^1H NMR (300 MHz, DMF-d_7): δ (ppm) 7.3-6.1 (b, 840H, H_{Ar} ; PS), 2.2 (b, 38H, CH backbone PAA), 2.1- 1.1 (b, 654H, CH_2 backbone PAA + PS). M_n (NMR) = 19,700 g/mol.

General procedure for the synthesis of poly(4,5-dimethoxy-2-nitrobenzyl acrylate)-*b*-polystyrene:

Under argon, in a Schlenk tube, PAA₃₁-*b*-PS₁₆₈ (252 mg; 0.40 mmol in AA units; 1 equiv.) was dissolved in DMF (7.91 mL, 0.05 M). DMNBBBr (242 mg, 0.88 mmol, 2.2 equiv.) and DBU (112 μ L, 0.88 mmol, 2.2 equiv.) were added and the solution was stirred at 50°C for 15 h. AcOH (28 μ L, 0.47 mmol, 1.2 equiv.) was added to the medium. The mixture was then diluted with CH₂Cl₂ and washed with distilled water. The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The polymer was precipitated in methanol, filtered and dried in vacuo at 35°C for 24 h, affording a light yellow powder (247 mg, 76 %).

M_n (GPC) = 26,900 g/mol; PDI (GPC) = 1.14. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.6-7.4 (b, 32H, H_{Ar}, PDMNBA), 7.2-6.1 (b, 840H, H_{Ar}; PS), 5.3-4.9 (b, 61H, CH₂-O, PDMNBA), 4.1-3.8 (b, 212H, CH₃-O, PDMNBA), 2.7-2.4 (b, 30H, CH backbone PDMNBA), 2.3-1.1 (b, 678H, backbone PDMNBA + PS). M_n (NMR) = 20,500 g/mol. Grafting efficiency = 97%. FTIR (film, cm⁻¹): 3082-2850 (C_{Ar}-H, PS), 1737 (C=O, PNBA), 1525 (NO₂, PDMNBA), 1492 (C_{Ar}=C_{Ar}, PS), 1452 (C_{Ar}=C_{Ar}, PS), 1348 (NO₂, PDMNBA), 1228-1153 (C-O, PDMNBA).

Section 4.3

Synthesis of the *para*-methoxyphenacyl methacrylate:

Methacrylic acid (3.50 mL; 0.041 mol; 1.1 equiv.) and DMF (103 mL) were placed in a 250 mL flask. Triethylamine (6.30 mL, 0.045 mol; 1.2 equiv) and 2-bromo-4-methoxyacetophenone (8.59 g, 0.038 mol; 1 equiv) were then added. The reaction mixture was stirred for 1 h at 20°C, and then water (100 mL) and ethyl acetate (200 mL) were added. The aqueous phase was extracted three times with ethyl acetate, and the organic phases were collected and washed ten times with water. The organic phase was dried with MgSO₄, filtered, and the solution was concentrated under reduced pressure. The brownish liquid was filtered on silica (eluent: CH₂Cl₂) and the solvent was removed under reduced pressure. The slightly yellow solid obtained was purified by recrystallization in hexane (8.65 g, 89%).

¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.89 (d, 2H, 8.9 Hz, H_{Ar}), 6.94- (d, 2H, 8.9 Hz, H_{Ar}), 6.25 (t, 1H, 1.5 Hz, CH₂=), 5.65 (s, 1H, CH₂), 5.35 (s, 2H, O-CH₂-CO), 3.86 (s, 3H, O-CH₃), 2.00 (s, 3H, CH₃-C=). ¹³C NMR (300MHz, CHCl₃): δ (ppm) 190.75 (C=O,phenacyl), 166.91 (C=O ester), 164.11 (C_{Ar}-OMe), 135.71 (=C-MeCOO), 130.18 (C-H phenyl), 127.40 (C_{Ar}-CO), 126.73 (CH₂=), 114.13 (C-H phenyl), 66.05 (O-CH₂-CO), 55.60 (O-CH₃), 18.41 (CH₃-C=). MS (APCI): m/z = 235 ([M+H]⁺), 148 ([C₉H₉O₂]⁺), 127 ([C₆H₇O]⁺), 69 ([C₄H₅O]⁺).

General procedure for the polymerization of poly(*p*-methoxyphenacyl methacrylate) with CuBr:

Under argon, a round-bottom flask, with a stopcock, containing CuBr (8.1 mg; 0.056 mmol; 1 equiv.) and CuBr₂ (1.4 mg; 0.006 mmol; 0.11 equiv.) was filled with a solution containing *p*-methoxyphenacyl methacrylate (MPMA; 1.32 g; 5.64 mmol; 100 equiv.), EBiB (11 mg; 0.056 mmol; 1 equiv.), NPPMI (27.9 mg; 0.19 mmol; 3.3 equiv.), and anisole (1.33 mL; 50 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 70°C for 5 h (MPMA conversion = 42 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated in Et₂O twice, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.43 g, 76%).

M_n (GPC) = 19,600 g/mol; PDI (GPC) = 1.14. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.90-7.70 (b, 2H, H_{Ar}), 6.90-6.70 (b, 2H, H_{Ar}), 5.50-5.10 (b, 2H, O-CH₂-CO), 3.90-3.70 (b, 3H, O-CH₃), 2.45- 1.70 (b, 2H, CH₂ backbone), 1.50-1.00 (b, 3H, CH₃ backbone).

General procedure for the polymerization of poly(*p*-methoxyphenacyl methacrylate) with CuCl:

Under argon, a round-bottom flask, with a stopcock, containing CuCl (8.3 mg; 0.084 mmol; 1 equiv.) was filled with a solution containing *p*-methoxyphenacyl methacrylate (MPMA; 2.75 g; 11.73 mmol; 140 equiv.), tosyl chloride (TsCl; 16 mg; 0.08 mmol; 1 equiv.), dNbipy (68.5 mg; 0.17 mmol; 2 equiv.), and anisole (2.76 mL; 50 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 60°C for 5 h (MPMA conversion = 67 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated in Et₂O twice, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (1.586 g, 82%).

M_n (GPC) = 20,900 g/mol; PDI (GPC) = 1.10. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.90-7.70 (b, 2H, H_{Ar}), 6.90-6.70 (b, 2H, H_{Ar}), 5.50-5.10 (b, 2H, O-CH₂-CO), 3.90-3.70 (b, 3H, O-CH₃), 2.45- 1.70 (b, 2H, CH₂ backbone), 1.50-1.00 (b, 3H, CH₃ backbone).

General procedure for the synthesis of poly(*p*-methoxyphenacyl methacrylate)-*b*-poly(*tert*-butyl acrylate):

Under argon, a round-bottom flask, with a stopcock, containing CuCl (2.3 mg; 0.023 mmol; 1 equiv.) was filled with a solution containing *tert*-butyl acrylate (tBA; 0.68 mL; 4.6 mmol; 200 equiv.), PMPMA-Cl ($M_n = 19,900$, DPI = 1.13; 0.46 g; 0.023 mmol; 1 equiv.), dNbipy (19 mg; 0.046 mmol; 2 equiv.), and DMF (1.90 mL; 75 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 50°C for 3 h 30 (tBA conversion = 15 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated in MeOH:H₂O 75:25, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.30 g, 55%).

M_n (GPC) = 24,700 g/mol; PDI (GPC) = 1.15. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.90-7.70 (b, 2H, H_{Ar} (PMPMA)), 6.90-6.70 (b, 2H, H_{Ar}), 5.50-5.10 (b, 2H, O-CH₂-CO (PMPMA)), 3.90-3.70 (b, 3H, O-CH₃ (PMPMA)), 2.45-0.90 (b, 8H, CH backbone (PMPMA + PtBA) + CH₃ (PtBA)).

General procedure for the synthesis of poly(*p*-methoxyphenacyl methacrylate)-*b*-poly(methyl methacrylate):

Under argon, a round-bottom flask, with a stopcock, containing CuCl (2.8 mg; 0.028 mmol; 1.15 equiv.) and dNbipy (23.6 mg; 0.058 mmol; 2.3 equiv.) was filled with a solution containing methyl methacrylate (MMA; 0.93 mL; 8.7 mmol; 345 equiv.), PMPMA-Cl ($M_n = 20,900$, DPI = 1.10; 0.53 g; 0.025 mmol; 1 equiv.) and DMF (2.4 mL; 70 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 50°C for 1 h 30 (MMA conversion = 29 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white powder (0.48 g, 61%).

M_n (GPC) = 29,100 g/mol; PDI (GPC) = 1.20. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.90-7.70 (b, 2H, H_{Ar} (PMPMA)), 6.90-6.70 (b, 2H, H_{Ar} (PMPMA)), 5.50-5.10 (b, 2H, O-CH₂-CO (PMPMA)), 3.90-3.70 (b, 3H, O-CH₃ (PMPMA)), 3.70-3.50 (b, 3H, O-CH₃ (PMMA)), 2.45- 0.60 (b, 10H, CH₂+CH₃ backbone (PMPMA + PMMA)).

General procedure for the synthesis of poly(*p*-methoxyphenacyl methacrylate)-*b*-poly(oligoethylene glycole methacrylate):

Under argon, a round-bottom flask, with a stopcock, containing CuCl (2.6 mg; 0.026 mmol; 1.2 equiv.) and dNbipy (25.3 mg; 0.062 mmol; 2.8 equiv.) was filled with a solution containing poly(ethylene glycol) methyl ether methacrylate (OEGMA; 0.63 mL; 2.2 mmol; 100 equiv.), PMPMA-Cl ($M_n = 20,900$, $PDI = 1.10$; 0.44 g; 0.022 mmol; 1 equiv.) and DMF (2.1 mL; 70 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 50°C for 1 h 15 (MMA conversion = 38 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al_2O_3 (eluent: CH_2Cl_2). The solvent was removed under reduced pressure. The residue was precipitated in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white powder (0.72 g, 81%).

M_n (GPC) = 31,300 g/mol; PDI (GPC) = 1.21. 1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.90-7.70 (b, 2H, H_{Ar} (PMPMA)), 6.90-6.70 (b, 2H, H_{Ar} (PMPMA)), 5.50-5.10 (b, 2H, O- CH_2 -CO (PMPMA)), 4.20-4.00 (b, 1H, CO-O- CH_2 (POEGMA)), 3.90-3.50 (b, 6H, O- CH_2 - CH_2 (POEGMA)+O- CH_3 (PMPMA)), 2.45-0.60 (b, 4H, CH_2 + CH_3 backbone (PMPMA + POEGMA)).

General procedure for the synthesis of poly(*p*-methoxyphenacyl methacrylate)-*b*-polystyrene:

Under argon, a round-bottom flask, with a stopcock, containing CuCl (2.1 mg; 0.021 mmol; 1 equiv.) was filled with a solution containing PMPMA ($M_n = 22,300$ g/mol; $PDI = 1.13$; 0.47 g; 0.021 mmol; 1 equiv.), styrene (0.97 mL; 8.5 mmol; 400 equiv.), PMDETA (4.8 μ L; 0.023 mmol; 1.2 equiv.), and DMF (4.87 mL; 75 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 100°C for 21 h (styrene conversion = 60 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al_2O_3 (eluent: CH_2Cl_2). The solvent was removed under reduced pressure. The residue was precipitated in MeOH twice, filtered and dried in vacuo at 35°C for 24 h (0.55 g, 87%).

M_n (GPC) = 98,000 g/mol; PDI (GPC) = 6.66. 1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.90-7.70 (b, 2H, H_{Ar} (PMPMA)), 7.20-6.30 (b, 14H, H_{Ar} (PS + PMPMA)), 5.60-5.00 (b, 2H, O- CH_2 -CO (PMPMA)), 3.95-3.40 (b, 3H, O- CH_3 (PMPMA)) 2.40-1.10 (b, 12H, H backbone, PS + PMPMA).

General procedure for the polymerization of polystyrene:

Under argon, a round-bottom flask, with a stopcock, containing CuBr (14.9 mg; 0.104 mmol; 1 equiv.) was filled with a solution containing styrene (4.76 mL; 41.5 mmol; 400 equiv.), EBiB (20.3 mg; 0.104 mmol; 1 equiv.), PMDETA (19.8 mg; 0.11 mmol; 1.1 equiv.), and anisole (1.09 mL; 20 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 100°C for 6 h (styrene conversion = 23 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was diluted with CH₂Cl₂ and washed with an aqueous solution of EDTA (0.04 M). The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The residue was precipitated in MeOH twice, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.83 g, 83%).

M_n (GPC) = 9,100 g/mol; PDI (GPC) = 1.07. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.20-6.10 (b, 5H, H_{Ar}), 2.40- 1.10 (b, 3H, H backbone).

General procedure for the synthesis of polystyrene-*b*-poly(*p*-methoxyphenacyl methacrylate):

Under argon, a round-bottom flask, with a stopcock, containing CuCl (2.4 mg; 0.024 mmol; 1 equiv.) was filled with a solution containing *p*-methoxyphenacyl methacrylate (MPMA; 0.85 g; 3.6 mmol; 150 equiv.), PS-Br (M_n = 9,100 g/mol; PDI = 1.07; 0.22 g; 0.024 mmol; 1 equiv.), dNbipy (19.8 mg; 0.048 mmol; 2 equiv.), and anisole (1.99 mL; 70 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 60°C for 4 h 30 (MPMA conversion = 74 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated in Et₂O twice, filtered and dried in vacuo at 35°C for 24 h. Residual homo-PS was removed by extraction with selective a solvent (Et₂O), affording a white solid (0.55 g, 87%).

M_n (GPC) = 37,500 g/mol; PDI (GPC) = 1.29. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.90-7.70 (b, 288H, H_{Ar} (PMPMA)), 7.20-6.30 (b, 723H, H_{Ar} (PS + PMPMA)), 5.60-5.00 (b, 296H, O-CH₂-CO (PMPMA)), 3.95-3.40 (b, 441H, O-CH₃ (PMPMA)) 2.40-1.10 (b, 981H, H backbone, PS + PMPMA).

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SYNTHESIS OF LINEAR PHOTOCLEAVABLE
BLOCK COPOLYMERS

Abstract

The synthesis of linear photocleavable block copolymers is presented here. The copolymers are characterized by the introduction of a photocleavable junction at the interface of a hydrophobic block [polystyrene or poly(tert-butyl acrylate)] and a hydrophilic block [poly(acrylic acid or poly(2-(dimethylamino)ethyl methacrylate)]. In addition to the diblock copolymers, the synthesis of a PtBA-hv-PDMAEMA-b-POEGMA triblock copolymer is also reported. The introduction of the photocleavable junction is realized by a CuAAC-ATRP simultaneous reaction. The targeted application of this type of linear photocleavable block copolymers is the formation of hollowed nanocapsules in solution.

5.1 Introduction

In this chapter, the synthesis of the second family of photocleavable polymers is detailed. This family is characterized by the presence of a photocleavable junction, an *o*-nitrobenzyl ester, between the hydrophobic and the hydrophilic sequences of the block copolymers. This type of block copolymers is meant for the formation of nanocages. Indeed, after the self-assembly in water and a cross-linking of the micellar corona, a light irradiation will allow the cleavage of the junction and the extraction of the core to yield nanocages.

From a synthetic point of view, the introduction of a junction between two sequences of a block copolymer can be realized by different approaches. The coupling between two end functionalized polymers [1–5] and the use of a functionalized macroinitiator [6–10] are largely used for the introduction of a junction between the copolymer blocks. The approach selected in this thesis, relies on a simultaneous "one pot" *copper assisted azide-alkyne cycloaddition/atom transfer radical polymerization* process (CuAAC/ATRP, Figure 5.1) [11–13].

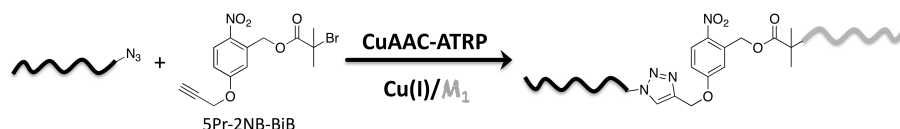


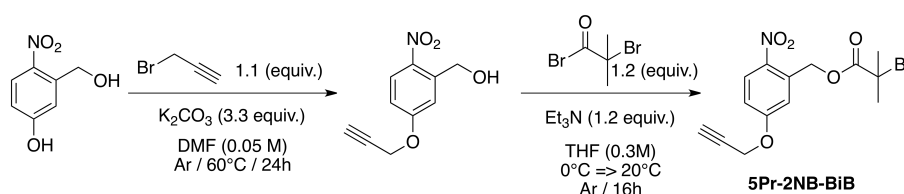
Figure 5.1: Synthetic strategy for the production of linear photocleavable block copolymers.

This "one pot" CuAAC/ATRP process was first demonstrated by Haddleton's group [14,15]. The simultaneity of the "click" and the polymerization reactions is enabled by the nature of the reaction catalyst. Indeed, both reactions are catalysed by copper(I) halide complexed to a ligand (often CuBr/PMDETA). The advantages of this process in contrast with the two step strategy is that complex copolymers and polymers architectures may be produced in only one synthetic step [16,17].

The targeted linear photocleavable block copolymers are synthesized using 5-propargylether-2-nitrobenzyl bromoisobutyrate (5Pr-2NB-BiB in Figure 5.1). This photocleavable initiator has a propargyl function, which can react with azide terminated polymers to form a triazole function. The other end of the photocleavable initiator is composed of a α -haloester (bromoisobutyrate function), which is a typical ATRP initiator.

The following sections present the synthesis of the desired linear photocleavable block copolymers. The first section presents the synthesis of the photocleavable initiator. In the second section, the synthesis of the first amphiphilic block copolymer, composed of a polystyrene and a poly(acrylic acid) (PS-*hv*-PAA), is presented. The third section focuses on the synthesis of polystyrene-*hv*-poly[(dimethylamino)ethyl methacrylate]-*block*-poly[oligo (ethylene glycol) methacrylate] (PS-*hv*-PDMAEMA-*b*-POEGMA). In the last section of this chapter, the synthesis of poly(*tert*-butyl acrylate)-*hv*-poly[(dimethylamino)ethyl methacrylate]-*block*-poly[oligo (ethylene glycol) methacrylate] triblock copolymer is presented (PtBA-*hv*-PDMAEMA-*b*-POEGMA).

5.2 Synthesis of the photocleavable initiator



Scheme 5.1: Photocleavable initiator synthesis.

The synthesis of the photocleavable initiator was conducted in two steps (Scheme 5.1) [12,18]. In the first step, the 5-hydroxy-2-nitrobenzyl alcohol reacted with propargyl bromide in a K_2CO_3 /DMF mixture to selectively etherify the phenol function. The second step consisted in the

esterification of the previously synthesized 5-propargylether-2-nitrobenzyl alcohol by bromo-isobutyrate bromide in dry THF to obtain the desired 5-propargylether-2-nitrobenzyl bromoisobutyrate (5Pr-2NB-BiB).

5.3 Synthesis of PS-*hv*-PAA

The polystyrene-*hv*-poly(acrylic acid) diblock copolymer is obtained via a four steps strategy (Figure 5.2). In the first step, the synthesis of polystyrene by ATRP is realized. The chain-end of this PS is modified by an azide function in a second step. The third step of the synthesis is the CuAAC/ATRP reaction with the photocleavable initiator (5Pr-2NB-BiB) and *tert*-butyl acrylate to produce a PS-*hv*-PtBA copolymer. Finally the *tert*-butyl side groups of the PtBA block are hydrolyzed under acidic conditions to obtain the desired PS-*hv*-PAA copolymer. The results presented here were realized during the Master thesis of Roland Duchêne [19].

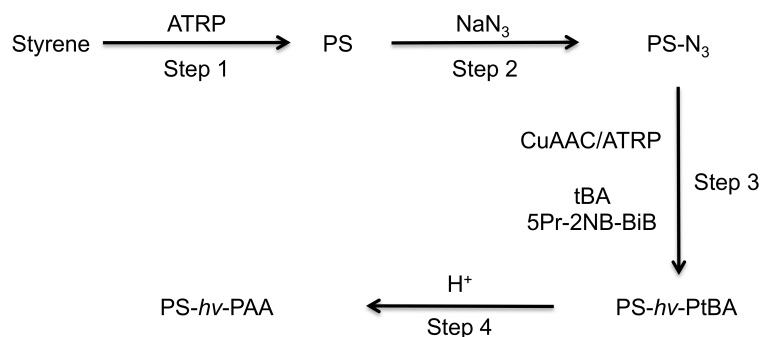


Figure 5.2: Synthetic strategy for the synthesis of PS-*hv*-PAA.

5.3.1 PS synthesis

The polystyrene was produced by ATRP with CuBr/PMDETA as the catalyst and ethyl 2-bromoisobutyrate (EBiB) as the initiator. Considering the role of the polystyrene block in the nanocage formation, the molar mass of the targeted polymer was maintained low ($M_n \approx 5,000$

g/mol) to facilitate its further extraction. Moreover, the polymerization conditions were carefully chosen to ensure a quantitative functionalization of the chain-end during the second step of the synthesis. The temperature of the polymerization was set at 90°C to avoid styrene self-initiation during the polymerization [20]. The monomer conversion was maintained low (no more than 20%), to limit the chain-end loss during the polymerization [21, 22].

Entry ^a	PS ^b	t (h)	conv ^b (%)	M_n ^c (g/mol)	PDI ^c
1	PS ₄₆	4	12	4,950	1.09

^a Reaction conditions: EBiB/CuBr/PMDETA/Sty (molar ratio) = 1/0.5/0.6/250, anisole 20wt%, T = 90°C.

^b DP and conversion were determined by ¹H NMR.

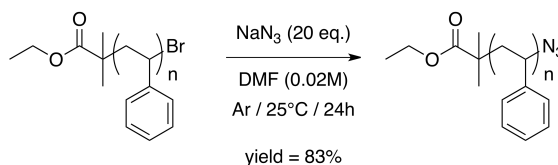
^c M_n and PDIs were determined by GPC using a PS calibration.

Table 5.1: Synthesis of PS by ATRP.

According to these specifications, styrene polymerization was conducted in anisole (20 wt%) at 90°C with the molar ratios of EBiB/CuBr/PMDETA/Sty = 1/0.5/0.6/250. Table 5.1 presents the conditions and the results for the polymerization of styrene by ATRP. Under these conditions, a well-defined polystyrene with a low polydispersity index (PDI ≤ 1.10) and a molar mass around 5,000 g/mol was produced.

5.3.2 PS-N₃ synthesis

The chain-end of the polystyrene was further functionalized with sodium azide (NaN₃ = 20 equivalents of the brominated chain-end) in dry N,N'-dimethylformamide (DMF) at 25°C for 24h (Scheme 5.2).



Scheme 5.2: Synthesis of the PS-N₃.

^1H NMR analysis demonstrated the quantitative functionalization of the polystyrene chain-end (Figure 5.3). Indeed, a shift of the geminal proton (H_a) to the right is observed in the ^1H NMR spectrum of the PS- N_3 indicating a quantitative functionalization (right). However, a closer look at the α (H_b) and ω (H_a) chain-end integrals show the presence in the sample of around 10% of dead polystyrene chains.

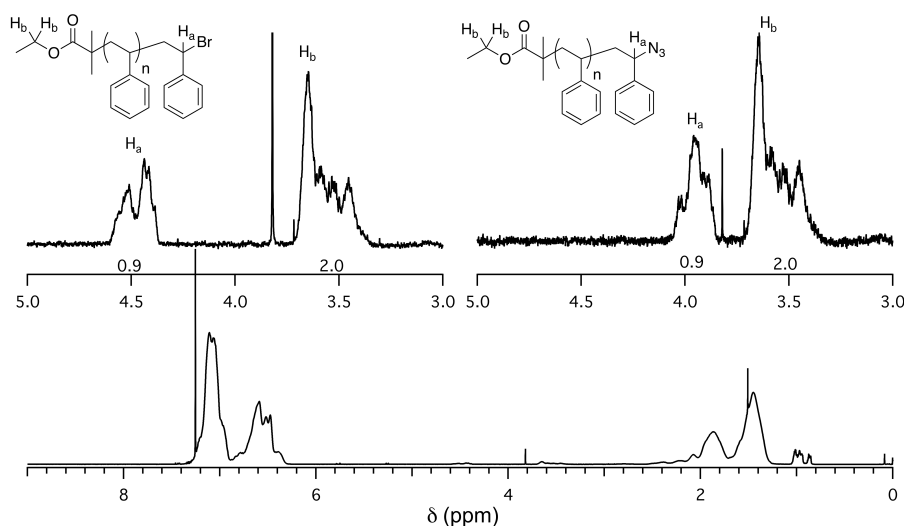
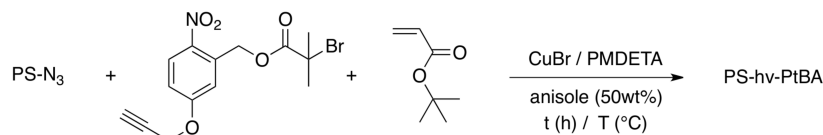


Figure 5.3: Chain-end analysis realized by ^1H NMR: PS₄₃-Br (left) and PS₄₆-N₃ (right).

The ^1H NMR analysis of the PS-Br clearly demonstrated that this dead polymer was already present after the styrene polymerization (left). Indeed, the polymerization of various monomers with CuBr/PMDETA displays the same chain-end loss, resulting from hydrogen radical abstraction of PMDETA ligand as previously reported [23, 24]. The presence of this dead polystyrene chains does not represent an issue for the next synthetic step because it will not reinitiate the polymerization and will thus not produce a photocleavable block copolymer. Moreover, it can easily be removed from the PS- $h\nu$ -PAA copolymer.

5.3.3 Synthesis of PS-*hv*-PtBA by CuAAC/ ATRP

The second step of the synthesis of the PS-*hv*-PAA photocleavable block copolymer is the "one pot" CuAAC/ATRP reaction. In this step, the azide functionalized polystyrene is brought together with the photocleavable initiator (5Pr-2NB-BiB) and *tert*-butyl acrylate. In the presence of a copper complex (CuBr/PMDETA), the photocleavable initiator will couple with the azide terminated polystyrene by a CuAAC reaction on one side and initiate the tBA polymerization on the other side (Scheme 5.3).



Scheme 5.3: Synthesis of the PS-*hv*-PtBA.

Table 5.2 presents the conditions and the results of the CuAAC/ATRP with tBA. The reactions were performed in anisole (50 wt%) at 80 and 90°C with an equimolar ratio of the PS-N₃ and 5Pr-2NB-BiB. An excess of the catalyst with respect to the initiator (5Pr-2NB-BiB/CuBr/PMDETA = 1/1.5/1.8 molar ratio) was used during the polymerization in order to avoid any competition between the CuAAC and the ATRP reactions [18]. With these conditions three different PS-*hv*-PtBA with good to reasonable PDI's and molar masses ranging from 9,200 to 22,600 g/mol were obtained.

The efficiency of the coupling reaction was determined by ¹H NMR (500 MHz) on the crude copolymer, by comparing the integrations of the CH₂-O-C=O chain-end of PS (H_a) with the signal of the aromatic protons (H_{Ar}) of the junction. Figure 5.4 shows the NMR spectrum of the crude PS₄₆-*hv*-PtBA₅₀ (Table 5.2, Entry 2). The click efficiency is equal to 89 % (H_{Ar} × 100) when HH_a is calibrated to 2 (2 protons).

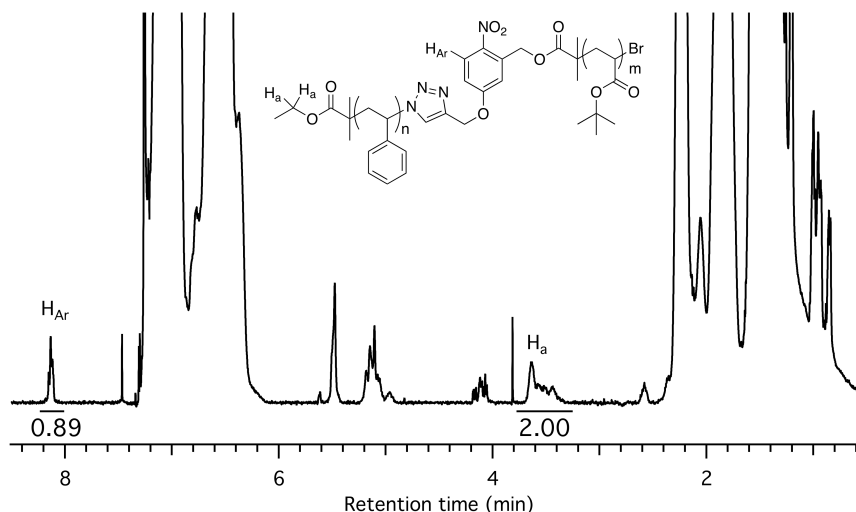


Figure 5.4: ^1H NMR spectrum of the crude $\text{PS}_{46}\text{-}h\nu\text{-PtBA}_{50}$ (Table 5.2, Entry 2).

The ^1H NMR of the three samples demonstrated that the click efficiencies are limited to 89 % (Table 5.2). This is the consequence of the presence of the 10% of dead chain-end in the PS_{46} polymer (as demonstrated in Figure 5.3). Moreover, the presence of this dead polystyrene chains is also visible in the GPC chromatogram of the $\text{PS}_{46}\text{-}h\nu\text{-PtBA}_{50}$. Indeed, figure 5.5a shows the presence of a second population at higher retention time which corresponds perfectly to the starting polystyrene.

Entry ^a	PS- <i>hv</i> -PtBA ^b	T (°C)	t (h)	conv ^b (%)	M _n (NMR) (g/mol)	M _n ^c (g/mol)	PDI ^c	e _c ^b (%)
1	PS ₄₆ - <i>hv</i> -PtBA ₃₀	80	4.5	12	9,200	8,900	1.12	88
2	PS ₄₆ - <i>hv</i> -PtBA ₅₀	80	15	20	11,700	12,700	1.15	89
3	PS ₄₆ - <i>hv</i> -PtBA ₁₃₅	90	15	54	22,600	21,500	1.30	89

^a Reaction conditions: PS-N₃/5Pr-2NB-BiB/CuBr/PMDETA/tBA (molar ratio) = 1/1/1.5/1.8/300, anisole 50wt%.

^b DP, conversion and coupling efficiency (e_c) were determined by ¹H NMR.

^c M_n and PDIs were determined by GPC using a PS calibration.

Table 5.2: Synthesis of PS-*hv*-PtBA by the CuAAc/ATRP "one pot" reaction.

5.3.4 Hydrolysis of PS-*hv*-PtBA

The last step of the synthesis of the PS-*hv*-PAA copolymer is the hydrolysis under acidic conditions of the *tert*-butyl function of the PtBA sequence. This hydrolysis was performed with the same conditions than the hydrolysis of the PtBA-*b*-PS copolymer (Section 4.2.3 of Chapter 4). The copolymer was dissolved in dichloromethane in the presence of trifluoroacetic acid. The samples were stirred for 24h at 25 °C. A near quantitative hydrolysis ($\text{DH} = 95\%$) of the *tert*-butyl groups was observed for the three samples under these conditions. The traces of homo-polystyrene and homo-PAA were removed by washing the crude polymer with selective solvents (DCM/MeOH washing cycles).

The GPC chromatogram of the hydrolyzed copolymer demonstrates that the hydrolysis under these conditions does not lead to the hydrolysis of the ester function of the photocleavable junction. Indeed, only one population is observed in the GPC curve after the hydrolysis and the selective solvent extractions (Figure 5.5b).

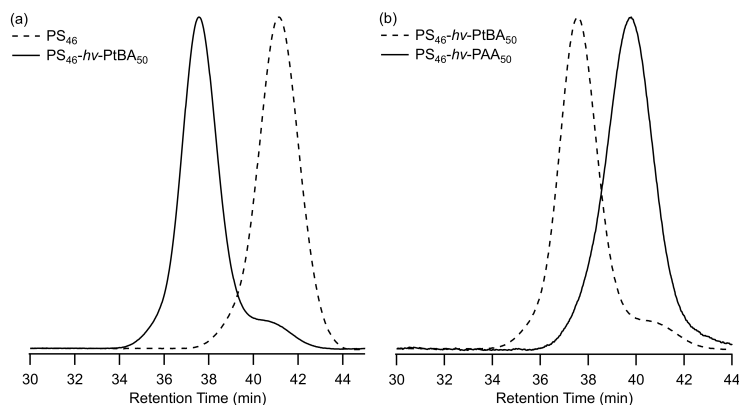


Figure 5.5: GPC chromatograms for the PS-*hv*-PAA synthesis: (a) copolymerization of tBA by CuAAC/ARTP, (b) hydrolysis of the PS-*hv*-PtBA.

5.4 Synthesis of PS-*hv*-PDMAEMA-*b*-POEGMA

The second type of photocleavable copolymer is a triblock copolymer composed of a polystyrene block linked to a poly[2(dimethylamino)ethyl methacrylate] block via the photocleavable junction and a third poly[oligo (ethylene glycol) methacrylate] block (PS-*hv*-PDMAEMA-*b*-POEGMA). This copolymer is designed to produce PDMAEMA nanocages with a POEGMA corona after the irradiation of the crosslinked micelles and the extraction of the PS cores.

The synthesis of this copolymer is conducted in four steps as represented in figure 5.6. The first step is the synthesis of polystyrene by ATRP. In a second step the chain-end of the polystyrene block is functionalized by an azide function. The third step of the synthesis is the CuAAC/ATRP simultaneous reaction to produce a PS-*hv*-PDMAEMA diblock. Finally, this diblock copolymer is used as macroinitiator for the polymerization of OEGMA by ATRP.

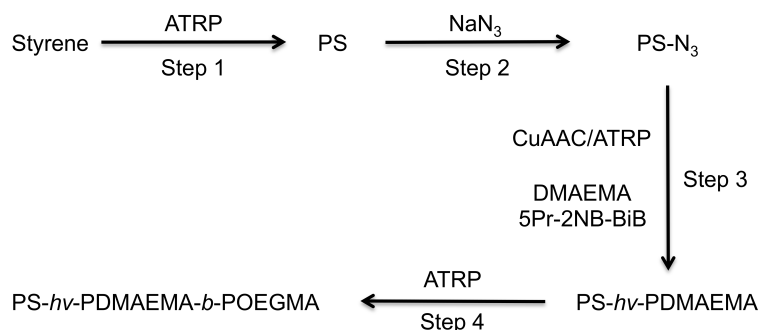


Figure 5.6: Synthetic strategy for the production of PS-*hv*-PDMAEMA-*b*-POEGMA.

Since the two first steps of the synthesis are the same as the synthesis of the PS-*hv*-PAA copolymer, they will not be presented here. The key step of the synthesis is the CuAAC/ATRP reaction to produce a PS-*hv*-PDMAEMA copolymer. To synthesize this copolymer with a low PDI and a quantitative coupling of the PS-N₃ to the photocleavable initiator, several polymerization parameters were studied:

- the copper halide: CuBr and CuCl,
- the ligand: dNbipy and PMDETA,
- the polymerization temperature,
- the solvent,
- the initiator/monomer addition sequence,
- the CuAAC/ATRP procedure for the copolymer synthesis.

As demonstrated by figure 5.8, the variation of these parameters did not permit the production of well-defined copolymers. Indeed, the GPC chromatograms of the different copolymers show a tailing at low molar masses in the best cases and the presence of a second population in the worst cases. The PDI's of the copolymers range from 1.2 to 1.6 and the coupling efficiencies varied from 50 to 90%. Moreover in some experiments, the copper (I) species was complexed by the monomer instead of the ligand, as the polymerization medium displayed the typical color of copper complexed to mono or bidentate aminated ligands.

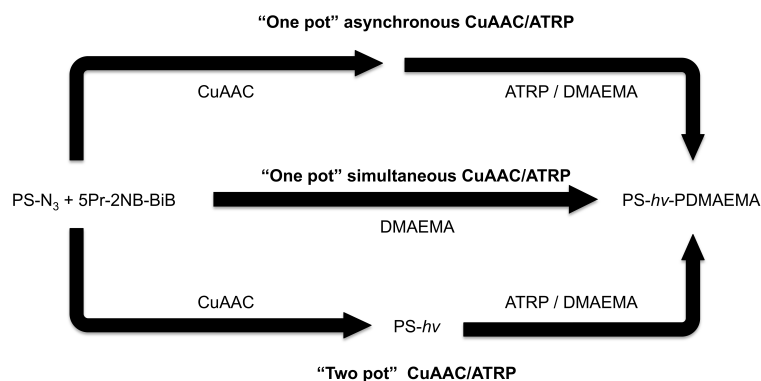


Figure 5.7: Synthetic strategies for the production of PS-*hν*-PDMAEMA-*b*-POEGMA.

In addition to the study of the effect of the catalytic system over the polymerization, the CuAAC/ATRP procedures were varied. As mentioned in the introduction, the fact that the catalyst is the same for the CuAAC and the ATRP reactions allows us to finely tune the sequence of reactions. It is, actually, possible to perform these two reactions with a "one pot" simultaneous approach, a "one pot" asynchronous way or a "two pots" process. Figure 5.7 presents the typical sequence of reactions for our system. The three procedures were tested and the "one pot" simultaneous approach reveals itself to be the more promising. Indeed, the GPC curves of the asynchronous and the two step processes show the presence of two populations in the crude copolymer obtained after synthesis (Figure 5.8d and e). Moreover, the results of the two step process are disappointing as only a small part of the macroinitiator has initiated the polymerization.

The simplest explanation for the presence of two populations in the crude copolymer is the presence of dead chain-ends in the polystyrenes synthesized in the first step of the synthesis. Indeed, the polystyrene samples used for the synthesis of the PS-*hv*-PAA and the PS-*hv*-PDMAEMA were synthesized with the same catalytic system (CuBr/PMDETA). To avoid this problem, we decided to change the first block of the triblock copolymers and thus to target a PtBA-*hv*-PDMAEMA-*b*-POEGMA copolymer. This choice was made because PtBA is also a hydrophobic polymer and that acrylate monomers have a less tendency to loose their chain-end compared to styrenic monomers.

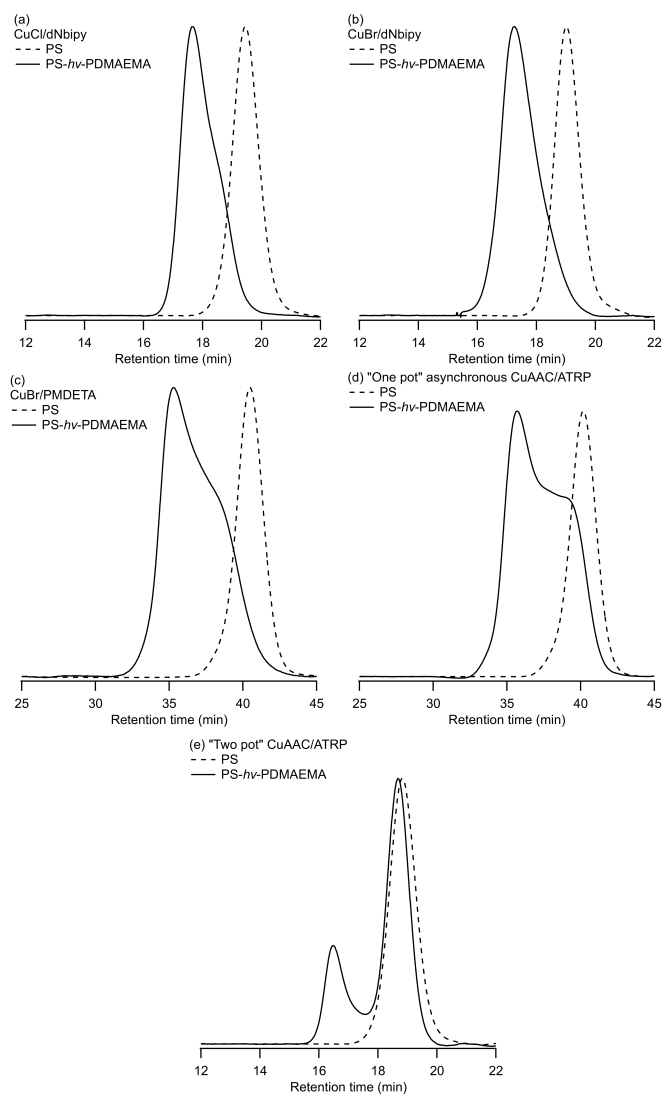


Figure 5.8: GPC chromatograms of PS- $h\nu$ -PDMAEMA copolymers produced by CuAAC/ATRP: (a) CuCl/dNbipy, (b) CuBr/dNbipy, (c) CuBr/PMDETA, (d) "one pot" asynchronous CuAAC/ATRP, (d) "two pots" CuAAC/ATRP.

5.5 Synthesis of PtBA-*hv*-PDMAEMA-*b*-POEGMA

The synthetic strategy for the production of the PtBA-*hv*-PDMAEMA-*b*-POEGMA is similar to the one previously described for the synthesis of PS-*hv*-PDMAEMA-*b*-POEGMA copolymers (Figure 5.6). The only difference lies in the first step of the synthesis where a poly(*tert*-butyl acrylate) is prepared instead of a polystyrene.

5.5.1 PtBA synthesis

The PtBA block was synthesized by ATRP with CuBr/PMDETA as the catalyst and EBiB as the initiator. Similarly to the styrene polymerization for the PS-*hv*-PAA synthesis, the tBA polymerization conditions were finely tuned to limit the loss of active chain-ends. The polymerizations were then conducted at 80°C and the conversion was limited to 50%.

Entry ^a	PtBA ^b	tBA (eq.)	t (h)	conv ^b (%)	M_n (NMR) (g/mol)	M_n^c (g/mol)	PDI ^c
1	PtBA ₆₈	200	4	31	8,900	9,400	1.13
2	PtBA ₁₁₈	190	8	40	15,300	14,200	1.12
3 ^d	PtBA ₆₆	250	2	24	8,700	8,400	1.22
4 ^d	PtBA ₁₄₅	250	4	51	18,800	17,200	1.15

^a Reaction conditions: EBiB/CuBr/PMDETA (molar ratio) = 1/1/1.1, anisole = 40 wt% T = 80°C.

^b DP and conversion were determined by ¹H NMR.

^c M_n and PDIs were determined by GPC using a PS calibration.

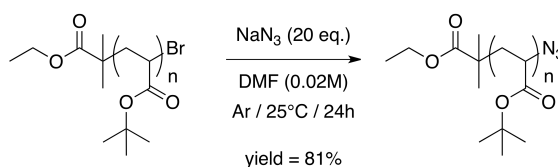
^d Performed with 20 wt% of anisole.

Table 5.3: Synthesis of PtBA by ATRP.

Table 5.3 presents the conditions and the results for the polymerization of tBA by ATRP. Well-defined PtBA were obtained with molar masses varying from 8,700 to 18,800 g/mol (M_n (NMR)) and PDI lower than 1.22.

5.5.2 PtBA-N₃ synthesis

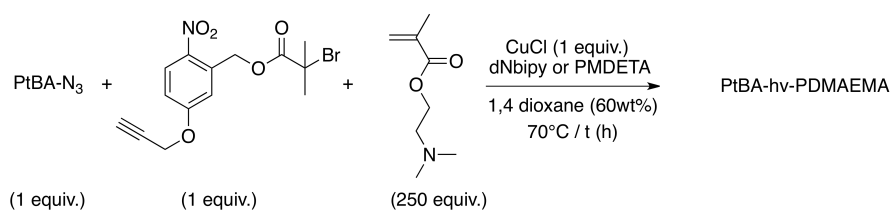
The functionalization of the PtBA chain-end by an azide function was carried out under the same conditions as the polystyrene functionalization. The polymers were dissolved in dry DMF and stirred at 25 °C for 24h in the presence of sodium azide (Scheme 5.4). A quantitative functionalization of the PtBA chain-ends was obtained with these conditions.



Scheme 5.4: Synthesis of the PtBA-N₃.

5.5.3 Synthesis of PtBA-*hv*-PDMAEMA by CuAAC/ATRP

Scheme 5.5 presents the general conditions for the synthesis of PtBA-*hv*-PDMAEMA by the CuAAC/ATRP.



Scheme 5.5: Synthesis of the PtBA-*hv*-PDMAEMA copolymers by CuAAC/ATRP.

Some parameters of the reactions were tuned in regard of the issues observed during the synthesis of the PS-*hv*-PDMAEMA. One of the first observations was that the polymerization of the DMAEMA was fast and

has an uncontrolled behavior when catalyzed by CuBr. To solve this issue, copper chloride was used as the catalyst and the molar ratio of the CuCl/initiator was reduced to slow down the polymerization. Another issue was the complexation of the copper by the amine functions of DMAEMA. This characteristic has two major effects, the impossibility to remove the copper from the polymer during the polymerization and a reduction of the polymerization overall speed. The second effect is not a real issue for the polymerization but the random character of this complexation does not allow the reproducibility of the polymerization. To solve this issue, the copper/ligand complex was preformed for the synthesis of the PtBA-*hν*-PDMAEMA. Copper(I) chloride was dissolved in a small amount (300 μ L) of a previously degazed solution of PMDETA, this solution was allowed to stir for 30 min before the introduction of the other reactants.

The polymerizations were carried out at 70°C in 1,4-dioxane (60 wt%) with the PtBA-N₃/5Pr-2NB-BiB/CuCl/DMAEMA molar ratio equal to 1/1/ 1/250. The screened parameters were the polymerization time and the ligand (PMDETA and dNbipy). It has to be noted that the ligand was used in excess to ensure a total complexation of the copper by the ligand and thus avoid the formation of a Cu/monomer complexes.

Table 5.4 presents the conditions and the results for the synthesis of PtBA-*hν*-PDMAEMA by CuAAC/ATRP. The three first experiments were conducted with CuCl/PMDETA as the catalyst. Well-defined block copolymers with molar masses (M_n (NMR)) ranging from 21,700 to 40,800 g/mol and with low PDI's were produced under these conditions. Moreover, a quantitative coupling was monitored ($e_c > 98\%$). Finally, the GPC chromatograms do not show the presence of homo PtBA in the crude PtBA-*hν*-PDMAEMA (dashed curve in Figure 5.9a, Entry 2).

The synthesis was also realized with CuCl/dNbipy as the catalyst (Entries 4 and 5). The copolymers synthesized with this catalytic system have molar masses ranging from 20,800 to 29,000 g/mol and low PDI's (PDI < 1.15). As in the synthesis with PMDETA, the coupling

is quantitative and a symmetric peak with no traces of homo PtBA is observed in the GPC curve (dashed curve in Figure 5.9b, Entry 4)

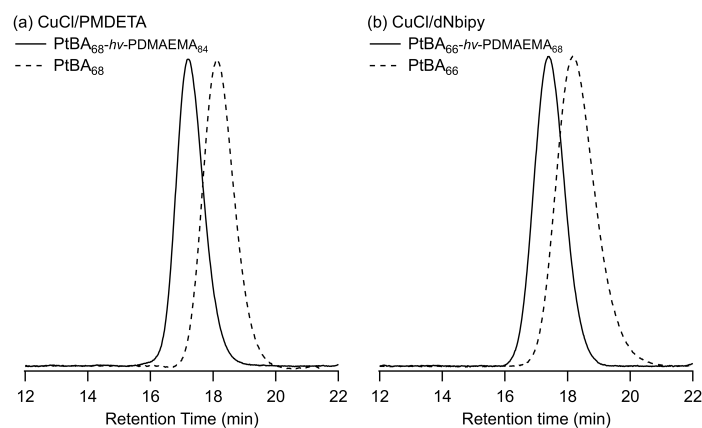


Figure 5.9: GPC chromatograms of PtBA-*h**v*-PDMAEMA synthesized by CuAAC/ATRP: (a) CuCl/PMDETA, (b) CuCl/dNbipy.

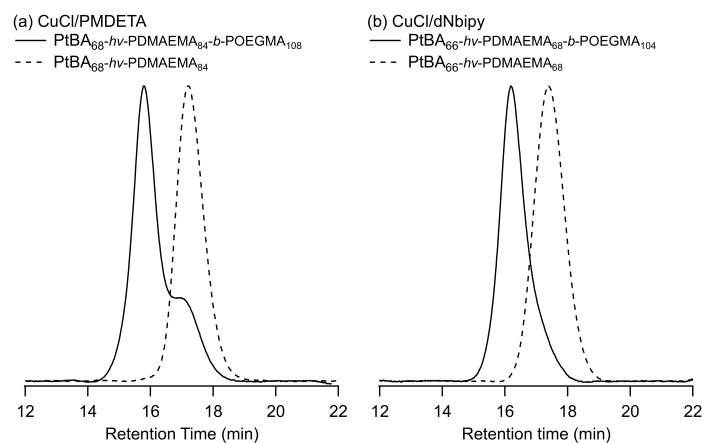


Figure 5.10: GPC chromatograms of PtBA-*h**v*-PDMAEMA-*b*-POEGMA synthesized by ATRP: (a) CuCl/PMDETA, (b) CuCl/dNbipy.

Entry ^a	PS ^b	t (min)	conv ^b (%)	M_n (NMR) (g/mol)	M_n^c (g/mol)	PDI ^c	e_c^b (%)
1	PtBA ₆₈ - <i>h</i> <i>v</i> -PDMAEMA ₇₂	33	23	21,700	16,600	1.13	99
2	PtBA ₆₈ - <i>h</i> <i>v</i> -PDMAEMA ₈₄	40	36	23,800	18,600	1.13	98
3	PtBA ₁₁₈ - <i>h</i> <i>v</i> -PDMAEMA ₁₄₁	90	54	40,100	26,500	1.16	ND
4 ^d	PtBA ₆₆ - <i>h</i> <i>v</i> -PDMAEMA ₆₈	150	22	20,800	16,400	1.15	99
5 ^d	PtBA ₁₄₅ - <i>h</i> <i>v</i> -PDMAEMA ₅₇	120	18	29,000	24,500	1.13	98

^a Reaction conditions: PtBA-N₃/5Pr-2NB-BiB/CuCl/PMDETA/DMAEMA (molar ratio) = 1/1/1/2/250, dioxane = 60 wt%, T = 70°C.

^b DP, conversion and coupling efficiency (e_c) were determined by ¹H NMR.

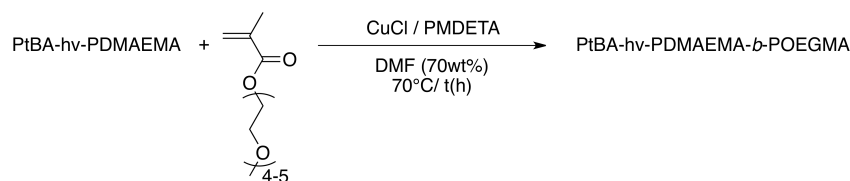
^c M_n and PDIs were determined by GPC using a PS calibration.

^d Performed with PtBA-N₃/5Pr-2NB-BiB/CuCl/dNbipy/DMAEMA (molar ratio) = 1/1/1/2.2/250

Table 5.4: Synthesis of PtBA-*h**v*-PDMAEMA copolymers by CuAAC/ATRP.

5.5.4 PtBA-*hv*-PDMAEMA-*b*-POEGMA synthesis

The last step for the PtBA-*hv*-PDMAEMA-*b*-POEGMA synthesis is the polymerization of the OEGMA starting from the diblock photocleavable copolymer. Scheme 5.6 presents the general conditions for the copolymerization of OEGMA. The polymerizations were first carried out at 70°C in DMF with CuCl/PMDETA as catalyst. The conditions and results of this copolymerization are presented in table 5.5 (Entry 1 to 3).



Scheme 5.6: Synthesis of the PtBA-*hv*-PDMAEMA-*b*-POEGMA copolymers by ATRP.

From entries 1 to 3, one can conclude that the copolymerizations carried out in DMF with CuCl/PMDETA do not demonstrate a controlled behavior. Indeed, badly defined PtBA-*hv*-PDMAEMA-*b*-POEGMA, with PDI's ranging from 1.3 to 1.5, were produced. A closer look at the GPC chromatograms of these triblock copolymers inform us that the large PDI's are not really the consequence of an uncontrolled behavior of the OEGMA polymerization. Figure 5.10a presents the GPC curves of the PtBA₆₈-*hv*-PDMAEMA₈₄-*b*-POEGMA₁₀₈ copolymers (full curve) and of the PtBA₆₈-*hv*-PDMAEMA₈₄ macroinitiator (dashed curve). The chromatogram of the triblock clearly demonstrates the presence of a mixture of diblock and triblock copolymers. The large amount of diblock copolymer, that has not reinitiated the polymerization, can explain the large PDI's observed for the polymerization in these conditions.

Entry ^a	PtBA- <i>hv</i> -PDMAEMA- <i>b</i> -POEGMA ^b	t (h)	conv ^b (%)	M_n (NMR) (g/mol)	M_n^c (g/mol)	PDI ^c
1	PtBA ₆₈ - <i>hv</i> -PDMAEMA ₇₂ - <i>b</i> -POEGMA ₈₃	2	43	46,300	42,300	1.32
2	PtBA ₆₈ - <i>hv</i> -PDMAEMA ₈₄ - <i>b</i> -POEGMA ₁₀₈	2.5	52	56,200	42,100	1.49
3	PtBA ₁₁₈ - <i>hv</i> -PDMAEMA ₁₄₁ - <i>b</i> -POEGMA ₇₇	1.5	42	63,200	33,800	1.46
4 ^d	PtBA ₆₆ - <i>hv</i> -PDMAEMA ₆₈ - <i>b</i> -POEGMA ₁₀₄	2.25	48	52,100	38,000	1.23
5 ^d	PtBA ₁₄₅ - <i>hv</i> -PDMAEMA ₅₇ - <i>b</i> -POEGMA ₁₀₄	2.5	55	60,200	53,500	1.32

^a Reaction conditions: PtBA-*hv*-PDMAEMA/CuCl/PMDETA/OEGMA (molar ratio) = 1/1.1/1/200, DMF = 70 wt% T = 70°C.

^b DP, conversion and were determined by ¹H NMR.

^c M_n and PDIs were determined by GPC using a PS calibration.

^d Reaction conditions: PtBA-*hv*-PDMAEMA/CuCl/dNbipy/OEGMA (molar ratio) = 1/1/2/200, EtOH = 70 wt% T = 70°C.

Table 5.5: Synthesis of PtBA-*hv*-PDMAEMA-*b*-POEGMA copolymers by ATRP.

To solve this issue the optimization of the polymerization conditions was carried out with model PDMAEMA-*b*-POEGMA copolymers. Figure 5.11 presents the GPC curves of the PDMAEMA-*b*-POEGMA copolymers synthesized to optimize the copolymerization of OEGMA. The presence of unreacted diblock copolymer is either the consequence of low initiation efficiency during the OEGMA copolymerization or simply a loss of the active chain-end during the synthesis of the PtBA-*hν*-PDMAEMA. To test these hypotheses, the synthesis of the model PDMAEMA-*b*-POEGMA was conducted with two different catalytic systems: CuCl/PMDETA and CuCl/dNbipy.

The first step for the synthesis of the model PDMAEMA-*b*-POEGMA copolymers is the synthesis of the PDMAEMA macroinitiator. These reactions were realized with the exact conditions of the PtBA-*hν*-PDMAEMA synthesis, at 70°C in 1,4-dioxane (60wt%), the nature of the copper/ligand complex being the only varied parameter. The GPC chromatogram of the PDMAEMA₁₁₂ synthesized with CuCl/PMDETA shows a tailing of the curve at low molar masses even if the polymer displays a narrow molar masses distribution (PDI = 1.13, Figure 5.11a). On the other hand, when the polymerization is carried out with CuCl/dNbipy the PDI increases slightly but the GPC curve shows a symmetric peak (PDI = 1.18, Figure 5.11b).

These polymers were then used as macroinitiators for the polymerization of OEGMA. The polymerizations were carried out at 70°C in EtOH with PDMAEMA/CuCl/OEGMA = 1/1/200 and the effects of the ligand and of the macroinitiator on the polymerization were studied. The first column of figure 5.11 presents the results of the copolymerization of OEGMA when the PDMAEMA was synthesized using CuCl/PMDETA. The top GPC curve presents the analysis of the PDMAEMA-*b*-POEGMA obtained after the ARTP with CuCl/PMDETA (Figure 5.11c). In this curve, we can notice the presence of a large amount of unreacted macroinitiator, similarly to the synthesis of the PtBA-*hν*-PDMAEMA-*b*-POEGMA triblock copolymer. Similar results are observable when the copolymerization is carried out with CuCl/dNbipy as the catalyst (bottom, Figure 5.11d).

The second column of figure 5.11 presents the results of the copolymerization of OEGMA when the PDMAEMA was synthesized using CuCl/dNbipy. Similarly to the previous experiments, the copolymerizations were carried out with PMDETA (top GPC curves, Figure 5.11e) and dNbipy (bottom, Figure 5.11f). When the PMDETA is used for the copolymerization, a tailing at low molar masses is observed in the GPC chromatogram and the PDI of the PDMAEMA-*b*-POEGMA is rather high (PDI = 1.31). On the other hand, a well-defined PDMAEMA-*b*-POEGMA is obtained with CuCl/dNbipy as the catalyst (PDI = 1.23).

In regards of these experiments, the presence of unreacted diblock copolymer, in the synthesis of PtBA-*hv*-PDMAEMA-*b*-POEGMA, is the consequence of a loss of active chain-ends during the synthesis of the PtBA-*hv*-PDMAEMA when CuCl/PMDETA is used as the catalyst. To avoid this loss of chain-end the synthesis of the PtBA-*hv*-PDMAEMA should be carried out with CuCl/dNbipy as in the Entries 4 and 5 of table 5.4. Moreover, the study on the model PDMAEMA-*b*-POEGMA copolymers showed that the copolymerization is more controlled when CuCl/dNbipy is used for the polymerization of OEGMA.

Finally, entries 4 and 5 of table 5.5 present the conditions and the results for the synthesis of the triblock copolymer according to these specifications. Well-defined PtBA-*hv*-PDMAEMA-*b*-POEGMA copolymers were produced with molar masses ranging from 52,100 to 60,200 g/mol. Figure 5.10b presents the GPC chromatogram of the PtBA₆₆-*hv*-PDMAEMA₆₈-*b*-POEGMA₁₀₄ copolymer (Entry 4). One can see that only one population is observed in the GPC curve of this triblock.

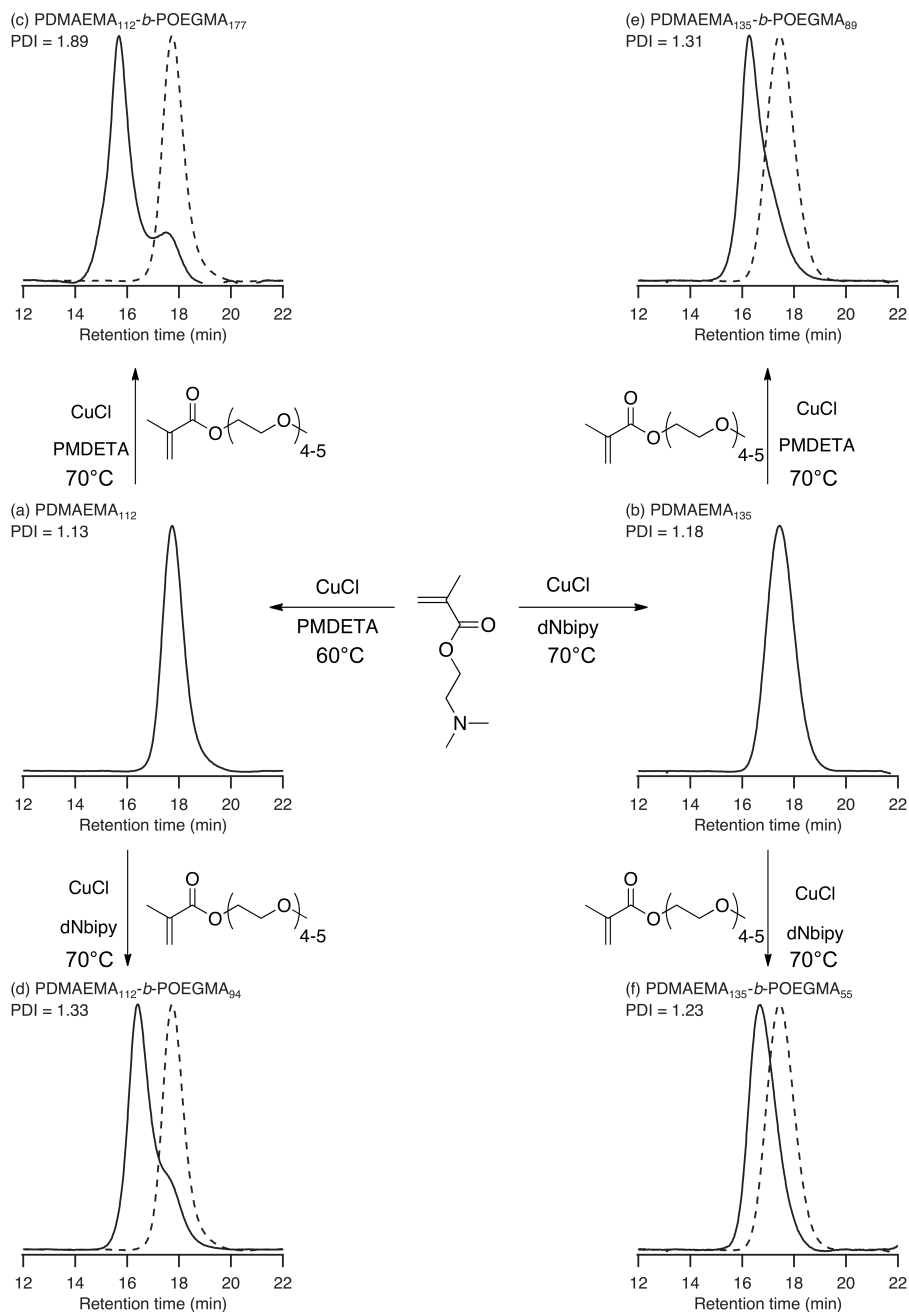


Figure 5.11: Optimization of the OEGMA polymerization on a model PDMAEMA-*b*-POEGMA copolymer.

5.6 Conclusions

This chapter presented the synthesis of linear photocleavable diblock and triblock copolymers. The targeted application of these type of copolymers is the formation of nanocages in solution (Chapter 8). The synthetic strategy for the introduction of a photocleavable junction between the two blocks of the copolymer relies on a "one pot" simultaneous CuAAC/ATRP reaction.

The photocleavable initiator is composed of an alkyne function on one side and a α -haloester on the other side. These functions were introduced on the photocleavable initiator by a two step synthesis starting from a commercial 5-hydroxy-2-nitrobenzyl alcohol.

The first copolymer was composed of a polystyrene block and a poly(acrylic acid) block. The synthesis of this copolymer was conducted in four steps. A polystyrene, synthesized by ATRP, was functionalized by an azide function. The copolymerization of tBA was then realized by the "one pot" CuAAC/ATRP process. The diblock copolymer was finally hydrolyzed in acidic conditions to obtain the desired PS-*hv*-PAA. This synthesis demonstrated the care that has to be taken during each individual synthetic step. Indeed, we were unable to synthesize a PS-*hv*-PtBA diblock copolymer without traces of homo-polystyrene. The presence of this homo-PS was the consequence of a chain-end loss in the first synthetic step. Fortunately, the homo-PS was easily removed from the PS-*hv*-PAA copolymer.

The second family consisted of triblock copolymer composed of polystyrene, poly[2-(dimethylamino)ethyl methacrylate] and poly[oligo(ethylene glycol) methacrylate]. In this case we were unable to synthesize the desired triblock copolymer, because of the homo-PS contamination in the crude diblock copolymer. To avoid this problem of chain end loss, the initially targeted triblock copolymer was modified by replacing the polystyrene block by a poly(*tert*-butyl acrylate) one.

The last section of this chapter focused on the synthesis of PtBA-*hv*-PDMAEMA-*b*-POEGMA triblock copolymers. The synthesis of the PtBA and PtBA-N₃ was presented. Well-defined PtBA-*hv*-PDMAEMA were synthesized by the CuAAC/ATRP process, without any traces of homo PtBA. The last part of this section presented the synthesis of the triblock copolymers by ATRP. In this last part, the optimization of the polymerization of the PDMAEMA and of the POEGMA blocks was realized to obtain pure triblock copolymers. Finally, two well-defined PtBA-*hv*-PDMAEMA-*b*-POEGMA triblock copolymers were successfully obtained.

Experimental part

Materials and instrumentation. Styrene (St, Aldrich, 99 %), *tert*-butyl acrylate (tBA, Acros, 99 %), 2-(dimethylamino)ethyl methacrylate (DMAEMA, Aldrich, 98 %) and poly(ethylene glycol) methyl ether methacrylate (OEGMA, $M_n = 300$ g/mol, Aldrich, 99%) were passed through activated basic alumina (Acros) columns prior use. N,N-dimethylformamide (DMF, Aldrich, 99.8 %), 1,4-dioxane (Acros, 99+%) and tetrahydrofuran (THF, Aldrich, 99.9%) were distilled on CaH₂ prior use. Triethylamine (Et₃N) was dried over KOH. Potassium carbonate (K₂CO₃, 99 %) was dried 24 h in a vacuum oven at 100°C. 5-hydroxy-2-nitrobenzyl alcohol (Aldrich, 97 %) was dried in vacuo prior to use. Propargyl bromide (Aldrich, 80 wt% in toluene), sodium azide (NaN₃, Acros, 99 %), CuBr (Aldrich, 99.999 %), CuCl (Aldrich, 99.999 %), ethyl 2-bromoisobutyrate (EBiB, Acros, 98 %), anisole (Acros, 99 %), N,N,N',N'',N'''-pentamethyldiethylenetriamine (PMDETA, Aldrich, 98%), 4,4'-dinonyl-2,2'-dipyridyl (dNbipy, Aldrich, 97 %), trifluoroacetic acid (TFA, Acros, 99 %) and all the other chemicals were used as received.

Proton and carbon nuclear magnetic resonance (NMR) spectra were acquired on a 300 MHz or 500 MHz Bruker Avance II. Infrared (IR) spectra were recorded on thin films deposited onto sodium chloride plates with a FT-IR spectrometer (Shimadzu FTIR-8400S). Molar masses (M_n) and polydispersity indices (PDI) of the polymers were measured on an Agilent gel permeation chromatography (GPC) system equipped with an Agilent 1100/1200 pump (35°C; eluent: DMF; flow rate of 1 mL/min), an Agilent differential refractometer and two PSS GRAM columns (Beads 10 μ ; porosity of column 1: 1000 Å; porosity of column 2: 100 Å) and on a Waters SEC system equipped with a Waters 510 pump and a 410 differential refractometer (35°C; eluent: DMF or DMF with NH₄PF₆ (2.5 mM)); flow rate of 0.5 mL/min). The calibration were performed using polystyrene standards.

Section 5.2

Synthesis of 5-propargylether-2-nitrobenzyl alcohol:

A mixture of 5-hydroxy-2-nitrobenzyl alcohol (4.48 g, 26.5 mmol, 1 equiv.) and K₂CO₃ (11.0 g, 79.4 mmol, 3 equiv.) was stirred in dry DMF (81.9 mL) for 1 hour at 60°C. Propargyl bromide (80 % in toluene, 2.83 mL, 31.7 mmol, 1.2 equiv.) was added dropwise. The mixture was stirred for 24 hours at 60°C. DMF was removed under reduced pressure. The residue was dissolved in AcOEt and washed with water. The organic phases were combined, dried over MgSO₄, filtered and the solvent was removed in vacuo. The yellow-brown solid was recrystallized in AcOEt/cyclohexane, affording slightly iridescent white crystals (3.67 g, 72 %).

¹H NMR (300 MHz, (DMF-d₇): δ (ppm) 8.53 (d, 1H, $J = 9.0$ Hz, H_{A_r}), 7.60

(d, 1H, $J = 2.9$ Hz H_{Ar}), 7.21 (d, 1H, $J = 9.0$ and 2.9 Hz, H_{Ar}), 5.76 (t, 1H, $J = 5.5$ Hz, $C\equiv C-H$), 5.10 (s, 2H, $-CH_2-OH$), 5.04 (d, 2H, $J = 5.4$ Hz, $C\equiv C-CH_2-$), 3.71 (s, 1H, $-OH$). ^{13}C NMR (75 MHz, DMF- d_7): δ (ppm) 143.1 ($C_{Ar}-NO_2$), 140.8 ($C_{Ar}-CH_2-$), 127.8 ($H-C_{Ar}$), 114.4 ($H-C_{Ar}$), 113.5 ($H-C_{Ar}$), 78.7 ($H-C\equiv C-$), 78.6 ($H-C\equiv C-$), 61.3 ($C\equiv C-CH_2$), 56.7 (H_2C-OH).

Synthesis of 5-propargylether-2-nitrobenzyl bromoisobutyrate:

A solution of bromoisobutyrate bromide (1.03 mL, 8.31 mmol, 1.2 equiv.) in dry THF (8.5 mL) was added dropwise to a stirred solution of 5-propargylether-2-nitrobenzyl alcohol (1.34 g, 6.92 mmol, 1 equiv.) and dry Et_3N (1.16 mL, 8.31 mmol, 1.2 equiv.) in dry THF (16.8 mL) at $0^\circ C$ and under argon. The mixture was slowly brought to $20^\circ C$ and stirred overnight. Distilled water (500 μL) was added dropwise to quench the reaction. THF was removed under reduced pressure and the residue was dissolved in DCM and washed with water. The organic phases were combined, dried over $MgSO_4$, filtered and the solvent was removed in vacuo. The yellow-brown residue was purified on flash chromatography (eluent: $CHCl_3$) affording a light yellow solid (2.15 g, 90%).

1H NMR (300 MHz, $CDCl_3$): δ (ppm) 8.19 (d, 1H, $3J = 9.1$ Hz, H_{Ar}), 7.26 (s, 1H, H_{Ar}), 7.02 (d, 1H, $3J = 9.1$ Hz, H_{Ar}), 5.64 (s, 2H, $-CH_2-OCO-$), 4.80 (d, 2H, $4J = 2.1$ Hz, $C\equiv C-CH_2-$), 2.60 (d, 1H, $-C\equiv C-CH$), 2.01 (s, 6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 170.8 ($C=O$), 161.8 ($C_{Ar}-O-$), 140.4 ($C_{Ar}-NO_2$), 135.3 ($C_{Ar}-CH_2-$), 127.9 ($H-C_{Ar}$), 114.5 ($H-C_{Ar}$), 113.4 ($H-C_{Ar}$), 77.1 ($H-C\equiv C-$), 77.0 ($H-C\equiv C-$), 64.4 ($-CH_2-OCO-$), 56.4 ($C\equiv C-CH_2$), 55.7 ($C-Br$), 30.8 (CH_3).

Section 5.3

General procedure for the synthesis of PS-Br:

Under argon, a round-bottom flask, with a stopcock, containing $CuBr$ (47.6 mg; 0.330 mmol; 0.5 equiv.) was filled with a solution containing styrene (17.3 g; 165 mmol; 400 equiv.), $EBiB$ (129.4 mg; 0.660 mmol; 1 equiv.), $PMDETA$ (69.2 mg; 0.390 mmol; 0.6 equiv.), and anisole (4.34 mL; 20 wt%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at $90^\circ C$ for 4 h (styrene conversion = 12 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was diluted with CH_2Cl_2 and washed with an aqueous solution of EDTA (0.04 M). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was precipitated in MeOH twice, filtered and dried in vacuo at $35^\circ C$ for 24 h, affording a white solid (2.90 g, 88%).

$PS_{46}-Br$: M_n (GPC) = 4,950 g/mol; PDI (GPC) = 1.09. 1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.20-6.10 (b, 230H, H_{Ar}), 4.60-4.20 (b, 1H, $CH-Br$), 3.70-3.20

(b, 2H, CH₂-OCO 2.40-1.67 (b, 45H, CH backbone) 1.65-1.10 (b, 92H, CH₂ backbone).

General procedure for the synthesis of PS-N₃:

To a solution of PS₄₆-Br (570 mg, 0.12 mmol, 1 equiv.) in dry DMF (5.76 mL, 0.02 M), sodium azide (150 mg, 2.30 mmol, 20 equiv.) was added. The solution was stirred under argon at 25°C for 24 h. Distilled water (50 mL) was added to the mixture inducing the precipitation of the polymer which was extracted with CH₂Cl₂. The organic phases were combined, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was precipitated in MeOH, filtered and dried one night in vacuo at 35°C, affording a white powder (540 mg, 95 %).

PS₄₆-N₃: ¹H NMR (300 MHz, CDCl₃): δ (ppm) 7.20-6.10 (b, 230H, H_{Ar}), 4.10-3.75 (b, 1H, CH-N₃), 3.70-3.20 (b, 2H, CH₂-OCO) 2.40-1.67 (b, 45H, CH backbone) 1.65-1.10 (b, 92H, CH₂ backbone).

General procedure for the synthesis of PS-*hv*-PtBA:

Under argon, a Schlenk tube containing CuBr (15.7 mg; 0.110 mmol; 1.5 equiv.) was filled with a solution containing tert-butyl acrylate (tBA; 2.80 g; 21.89 mmol; 300 equiv.), PMDETA (22.8 mg; 0.131 mmol; 1.8 equiv.), PS₄₆-N₃ (326 mg; 0.073 mmol, 1 equiv.) and anisole (2.80 mL; 50 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was stirred for 30 minutes and was then frozen. Under argon, a solution of the 5Pr-2NB-BiB in anisole (235 μL (C = 100 g/L), 0.073 mmol, 1 equiv.) was added. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 80°C for 6 h (tBA conversion = 54 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath. The reaction mixture was diluted with CH₂Cl₂ and washed with an aqueous solution of EDTA (0.04 M). The organic phase was dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was precipitated twice in MeOH:H₂O 80:20, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (1.020 g, 82 %).

PS₄₆-*hv*-PtBA₁₃₅: M_n (GPC) = 22,600 g/mol; PDI (GPC) = 1.30. e_c = 89%, ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (d, 1H, J = 9 Hz, Junction: H_{Ar} in *ortho* of the NO₂), 7.20-6.10 (b, 230H, PS: H_{Ar}, junction: H_{Ar}), 5.47 (m, 2H, junction: CH₂-OCO) 5.20-5.10 (b, 3H, junction: O-CH₂-triazole, PS: CH-triazole), 4.20-4.00 (m, 1H, PtBA: CH-Br), 3.70-3.20 (b, 2H, PS: CH₂-OCO) 2.35-2.15 (b, 134H, PtBA_{backbone}: CH), 2.10-1.10 (b, 408H, PtBA_{backbone}: CH₂, PS_{backbone}: CH₂ + CH) 1.44 (bs, 1215H, PtBA: CH₃).

General procedure for the synthesis of PS-*hv*-PAA:

In a round bottom flask, PS₄₆-*hv*-PAA₁₃₅ (544 mg; 3.27 mmol in tBA units; 1 equiv.) was dissolved in dichloromethane (54.4 mL, 0.06 M). TFA (2.42 mL, 32.67 mmol, 10 equiv.) was added and the solution was stirred at 25°C for 24 h. The solvent was removed under reduced pressure and the residue was kept in a minimum amount of DMF and dialyzed against methanol. The polymer was precipitated in hexane and filtered. Residual homo-PS was removed by extraction with CH₂Cl₂. The copolymer was dried in vacuo at 35°C for 24 h, affording a white powder (307 mg, 84%).

PS₄₆-*hv*-PAA₁₃₅: ¹H NMR (300 MHz, DMF-d₇): δ (ppm) 7.2-6.3 (b, 230H, PS: H_{Ar}), 2.40-3.0 (b, 135H, PAA_{backbone}: CH), 2.1- 1.1 (b, 270H, PAA_{backbone}:CH₂, PS_{backbone}: CH₂ + CH). M_n (NMR) = 14,900 g/mol.

Section 5.4

General "one pot" simultaneous procedure for the synthesis of PS-*hv*-PDMAEMA:

Under argon, a Schlenk tube containing CuCl (3.50 mg; 0.035 mmol; 1.5 equiv.) was filled with a solution containing 2-(dimethylamino)ethyl methacrylate (DMAEMA; 367 mg; 2.338 mmol; 300 equiv.), dNbipy (29.6 mg; 0.075 mmol; 3.1 equiv.), PS₃₉-N₃ (99.1 mg; 0.023 mmol, 1 equiv.) and anisole (0.55 mL; 60 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was stirred for 30 minutes and was then frozen. Under argon, a solution of the 5Pr-2NB-BiB in anisole (266 μ L (C = 30 g/L); 0.023 mmol; 1 equiv.) was added. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 70°C for 6 h (DMAEMA conversion = 14 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated twice in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.130 g, 48 %).

PS₃₉-*hv*-PDMAEMA₄₅: M_n (GPC) = 11,400 g/mol; PDI (GPC) = 1.19. e_c = 57%, ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (m, 1H, Junction: H_{Ar} in *ortho* of the NO₂), 7.20-6.20 (b, 197H, PS: H_{Ar}, junction: H_{Ar}), 5.42 (m, 2H, junction: CH₂-OCO) 5.12 (b, 3H, junction: O-CH₂-triazole, PS: CH-triazole), 4.05 (b, 90H, PDMAEMA: COO-CH₂), 2.57 (b, 90H, PDMAEMA: CH₂-N-(Me)₂) 2.28 (b, 270H, PDMAEMA: Et-N-(CH₃)₂), 2.20-1.20 (b, 207H, PDMAEMA_{backbone}: CH₂, PS_{backbone}: CH₂ + CH) 1.20-0.8 (b, 135H, PDMAEMA_{backbone}: CH₃).

General "one pot" asynchronous procedure for the synthesis of PS-*hv*-PDMAEMA:

Under argon, a Schlenk tube containing CuBr (7.10 mg; 0.049 mmol; 1.05 equiv.) was filled with a solution containing 5Pr-2NB-BiB (168 μ L (C = 100 g/L); 0.047 mmol; 1 equiv.), PMDETA (9.80 mg; 0.057 mmol; 1.2 equiv.), PS₆₁-N₃ (311 mg; 0.047 mmol, 1 equiv.) and anisole (2.23 mL; 75 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 50°C for 2 h and was then frozen. Under argon, 2-(dimethylamino)ethyl methacrylate (DMAEMA; 795 μ L; 4.717 mmol; 100 equiv.), previously degassed, was added. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 70°C for 2 h (DMAEMA conversion = 39 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated twice in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.504 g, 82 %).

PS₆₁-*hv*-PDMAEMA₄₁: M_n (GPC) = 13,300 g/mol; PDI (GPC) = 1.34. e_c = 71%, ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (m, 1H, Junction: H_{Ar} in *ortho* of the NO₂), 7.20-6.20 (b, 307H, PS: H_{Ar}, junction: H_{Ar}), 5.42 (m, 2H, junction: CH₂-OCO) 5.12 (b, 3H, junction: O-CH₂-triazole, PS: CH-triazole), 4.05 (b, 82H, PDMAEMA: COO-CH₂), 2.57 (b, 82H, PDMAEMA: CH₂-N-(Me)₂) 2.28 (b, 246H, PDMAEMA: Et-N-(CH₃)₂), 2.20-1.20 (b, 265H, PDMAEMA_{backbone}: CH₂, PS_{backbone}: CH₂ + CH) 1.20-0.8 (b, 123H, PDMAEMA_{backbone}: CH₃).

General "two pot" procedure for the synthesis of PS-*hv*-PDMAEMA:

General procedure for the synthesis of PS-*hv*

Under argon, a Schlenk tube containing CuBr (14.6 mg; 0.102 mmol; 0.5 equiv.) was filled with a solution containing PMDETA (21.2 mg; 0.122 mmol; 0.6 equiv.), PS₆₆-N₃ (1.432 g; 0.204 mmol, 1 equiv.), 5Pr-2NB-BiB (72.5 mg; 0.204 mmol, 1 equiv.) and DMF (2.91 mL, C = 0.07 M) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 40°C for 16 h. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated in MeOH, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (1.28 g, 88 %).

PS₆₆-*hv*: M_n (GPC) = 7,600 g/mol; PDI (GPC) = 1.09. e_c = 89%, ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (m, 1H, junction: H_{Ar} in *ortho* of the NO₂), 7.20-6.20 (b, 332H, PS: H_{Ar}, junction: H_{Ar}), 5.42 (m, 2H, junction: CH₂-OCO) 5.12 (b, 3H, junction: O-CH₂-triazole, PS: CH-triazole), 3.70-3.20 (b, 2H, CH₂-

OCO 2.40-1.67 (b, 45H, PS_{backbone}: CH) 1.65-1.10 (b, 92H, PS_{backbone}: CH₂).

General procedure for the synthesis of PS-*hv*-PDMAEMA

Under argon, a Schlenk tube containing CuCl (5.10 mg; 0.052 mmol; 1 equiv.) was filled with a solution containing 2-(dimethylamino)ethyl methacrylate (DMAEMA; 1.78 g; 10.303 mmol; 200 equiv.), PMDETA (9.80 mg; 0.057 mmol; 1.1 equiv.), PS_{66-hv} (397 mg; 0.052 mmol, 1 equiv.) and anisole (2.69 mL; 60 wt-%) previously degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 70°C for 2 h (DMAEMA conversion = 36 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated twice in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.712 g, 90 %).

PS_{66-hv}-PDMAEMA₇₈: M_n (GPC) = 9,000 g/mol; PDI (GPC) = 1.69. ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (m, 1H, Junction: H_{Ar} in *ortho* of the NO₂), 7.20-6.20 (b, 332H, PS: H_{Ar}, junction: H_{Ar}), 5.42 (m, 2H, junction: CH₂-OCO) 5.12 (b, 3H, junction: O-CH₂-triazole, PS: CH-triazole), 4.05 (b, 156H, PDMAEMA: COO-CH₂), 2.57 (b, 156H, PDMAEMA: CH₂-N-(Me)₂) 2.28 (b, 468H, PDMAEMA: Et-N-(CH₃)₂), 2.20-1.20 (b, 354H, PDMAEMA_{backbone}: CH₂, PS_{backbone}: CH₂ + CH) 1.20-0.8 (b, 234H, PDMAEMA_{backbone}: CH₃).

Section 5.5

General procedure for the synthesis of PtBA-Br:

Under argon, a round-bottom flask, with a stopcock, containing CuBr (54.5 mg; 0.380 mmol; 1 equiv.) was filled with a solution containing *tert*-butyl acrylate (tBA; 13.8 mL; 94.98 mmol; 250 equiv.), EBiB (74.1 mg; 0.380 mmol, 1 equiv.), PMDETA (72.4 mg; 0.418 mmol; 1.1 equiv.), and anisole (2.18 mL; 40 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 80°C for 2h (tBA conversion = 24 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath and exposing the reaction mixture to air. The reaction mixture was diluted with CH₂Cl₂ and washed with an aqueous solution of EDTA (0.04 M). The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The residue was precipitated in MeOH:H₂O (50:50), filtered and dried in vacuo at 35°C for 24 h, affording a white solid (2.2 g, 73%).

PtBA₆₆: M_n (GPC) = 8,400 g/mol; PDI (GPC) = 1.22. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 4.1 (m, 3H, CH-Br + CH₂-OC=O), 2.3- 2.0 (b, 65H, PtBA_{backbone}: CH), 1.9-1.3 (s, 726H, PtBA_{backbone}: CH₂, *t*-butyl: CH₃). M_n (NMR) = 8,700 g/mol.

General procedure for the synthesis of PtBA-N₃:

To a solution of PtBA₄₆-Br (2.19 g, 0.253 mmol, 1 equiv.) in dry DMF (12.7 mL, 0.02 M), sodium azide (330 mg, 5.07 mmol, 20 equiv.) was added. The solution was stirred under argon at 25°C for 24 h. Distilled water (50 mL) was added to the mixture inducing the precipitation of the polymer which was extracted with CH₂Cl₂. The organic phases were combined, dried over Na₂SO₄, filtered and the solvent was removed under reduced pressure. The residue was precipitated in water, filtered and dried one night in vacuo at 35°C, affording a white powder (1.77, 81 %).

PtBA₆₆: M_n (GPC) = 8,400 g/mol; PDI (GPC) = 1.22. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 4.1 (m, 2H, CH₂-OC=O), 2.3- 2.0 (b, 66H, PtBA_{backbone}: CH), 1.9-1.3 (s, 726H, PDMAEMA_{backbone}: CH₂, *t*-butyl: CH₃).

General procedure for the synthesis of PtBA-*hv*-PDMAEMA:

Under argon, a Schlenk tube containing CuCl (7.50 mg; 0.076 mmol; 1 equiv.) and dNbipy (68.1 mg; 0.167 mmol; 2.2 equiv.) was filled with previously degassed 1,4-dioxane (300 μ L). The mixture was degassed by one freeze-pump-thaw cycle and allowed to stir for 30 min. To the Schlenk tube, a solution containing 2-(dimethylamino)ethyl methacrylate (DMAEMA; 2.98 g; 18.93 mmol; 250 equiv.), 5Pr-2NB-BiB (270 μ L (C = 100 g/L); 0.076 mmol; 1 equiv.), PtBA₆₆-N₃ (653 mg; 0.076 mmol, 1 equiv.) and 1,4-dioxane (3.45 mL; 60 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 70°C for 2 h 30 (DMAEMA conversion = 22 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated twice in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.798 g, 60 %).

PtBA₆₆-*hv*-PDMAEMA₆₈: M_n (GPC) = 16,400 g/mol; PDI (GPC) = 1.15. e_c = >99%, ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (d, 1H, junction: H_{Ar} in *ortho* of the NO₂), 7.82 (s, 1H, junction: =CH triazole), 7.08 (s, 1H, junction: H_{Ar}), 7.03 (m, 1H, junction: H_{Ar}) 5.44 (m, 2H, junction: CH₂-OCO) 5.23 (b, 3H, junction: O-CH₂-triazole, PtBA: CH-triazole), 4.03 (b, 136H, PDMAEMA: COO-CH₂), 2.54 (b, 136H, PDMAEMA: CH₂-N-(Me)₂) 2.25 (b, 408H, PDMA-EMA: Et-N-(CH₃)₂), 2.10-1.45 (b, 334H, PDMAEMA_{backbone}: CH₂, PtBA_{backbone}: CH₂ + CH), 1.40 (s, 594H, PtBA-*t*-butyl: CH₃, 1.20-0.8 (b, 204H, PDMAEMA_{backbone}: CH₃). M_n (NMR) = 20,800 g/mol.

General procedure for the synthesis of PtBA-*hv*-PDMAEMA-*b*-POEGMA:

Under argon, a Schlenk tube containing CuCl (1.50 mg; 0.015 mmol; 1 equiv.) and dNbipy (12.3 mg; 0.030 mmol; 2 equiv.) was filled with previously degassed ethanol (300 μ L). The mixture was degassed by one freeze-pump-thaw cycle and allowed to stir for 30 min. To the Schlenk tube, a solution containing poly(ethylene glycol) methyl ether methacrylate (OEGMA; 904 mg; 3.014 mmol; 200 equiv.), PtBA₆₆-*hv*-PDMAEMA₆₆ (296 mg; 0.015 mmol, 1 equiv.) and ethanol (2.52 mL; 70 wt-%) previously degassed by three freeze-pump-thaw cycles. The mixture was degassed by three freeze-pump-thaw cycles, filled with argon and stirred in an oil bath at 70°C for 2 h 15 (OEGMA conversion = 48 %). The polymerization was quenched by quickly cooling the tube in a water-ice bath. The reaction mixture was filtered on neutral Al₂O₃ (eluent: CH₂Cl₂). The solvent was removed under reduced pressure. The residue was precipitated twice in hexane, filtered and dried in vacuo at 35°C for 24 h, affording a white solid (0.569 g, 78 %).

PtBA₆₆-*hv*-PDMAEMA₆₈-*b*-POEGMA₁₀₄: M_n (GPC) = 38,000 g/mol; PDI (GPC) = 1.23. ¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.13 (d, 1H, junction: H_{Ar} in *ortho* of the NO₂), 7.82 (s, 1H, junction: =CH triazole), 7.08 (s, 1H, junction: H_{Ar}), 7.03 (m, 1H, junction: H_{Ar}) 5.44 (m, 2H, junction: CH₂-OCO) 5.23 (b, 3H, junction: O-CH₂-triazole, PtBA: CH-triazole), 4.03 (b, 340H, POEGMA: COO-CH₂, PDMAEMA: COO-CH₂), 3.8-3.57 (b, 1456H, POEGMA: O-CH₂), 3.54 (b, 208H, POEGMA: COO-CH₂-CH₂), 3.37 (s, 312H, POEGMA: O-CH₃), 2.54 (b, 136H, PDMAEMA: CH₂-N-(Me)₂) 2.25 (b, 408H, PDMAEMA: Et-N-(CH₃)₂), 2.10-1.45 (b, 542H, POEGMA_{backbone}: CH₂ PDM-AEMA_{backbone}: CH₂, PtBA_{backbone}: CH₂ + CH), 1.40 (s, 594H, PtBA_{t-butyl}: CH₃, 1.20-0.8 (b, 516H, POEGMA_{backbone}: CH₃, PDMAEMA_{backbone}: CH₃). M_n (NMR) = 52,100 g/mol.

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LIGHT INDUCED MICELLIZATION

Abstract

This chapter focuses on the use of light to promote the micellization of block copolymers bearing photocleavable side groups. The considered copolymers, synthesized in chapter 4, are composed of a polystyrene block and a photo-sensitive poly(o-nitrobenzyl acrylate), poly(dimethoxy-nitrobenzyl acrylate) or poly(p-methoxyphenacyl methacrylate) block. In the first section, the light induced micellization principles are presented. The second section focuses on the micellization of copolymers composed of o-nitrobenzyl derivatives. In the third section, the photo-sensitive behavior of the p-methoxyphenacyl moiety is described and the micelles formed after light irradiation are characterized. Finally, the ability of those systems to trap active molecules into the micellar core is demonstrated.

6.1 Introduction

This chapter deals with the photocleavage of block copolymers bearing photolabile side groups and more particularly on the use of a light stimulus to promote the formation of micelles in solution. The light induced micellization strategy consists first in the dissolution of the photocleavable block copolymers in a non selective solvent of the blocks, *i.e.* the polymer chains will adopt their random coil conformation in solution (unimers). Upon light illumination, the cleavage of the side chromophores occurs, unmasking the acrylic acid (AA) functions. As a result, the hydrophobic photo-responsive block is turned into a fully hydrophilic PAA block, which is insoluble in the used solvent (chloroform). Self-assembly thus occurs, forming micelles made of a PAA core and a PS corona (Figure 6.1).

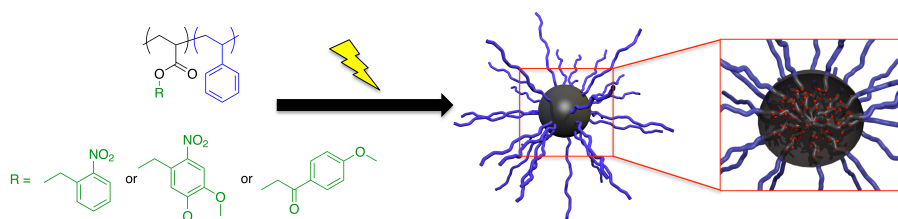


Figure 6.1: Schematic representation of the light induced micellization.

Although light-responsive block copolymers have been largely used for different applications such as micellar shell cross linking [1,2], micellar disruption [3,4] and drug release [5,6], only few examples of light-induced micellization have been reported [7–10]. To the best of our knowledge, only two examples of direct, *i.e.*, starting from unimers, light-induced micellization have been reported so far. The first one is based on the photolysis of a poly(*tert*-butoxystyrene)-*block*-polystyrene diblock copolymer (PBSt-*b*-PSt) promoted by a photoacid generator [8,9], and the second one deals with photo-induced micellization thanks to an electron transfer from poly(4-pyridinemethoxy-methylstyrene)-*block*-polystyrene to a diaryliodonium salt in organic media [10].

In the following sections, the light induced micellization will be presented for the block copolymers composed of a sequence bearing *o*-nitrobenzyl (Section 6.2) or *p*-methoxyphenacyl (Section 6.3) side groups and a sequence of polystyrene. Moreover, the ability of those systems to capture active molecules in the micellar core will be demonstrated (Section 6.4)

6.2 Copolymers bearing *o*-nitrobenzyls moieties

The *o*-nitrobenzyl derivatives are well-known photo-protecting groups in organic chemistry [11]. Upon irradiation *o*-nitrobenzyl esters cleave themselves into carboxylic acid group and nitrosobenzaldehyde counterparts according to a Norrish II mechanism (Figure 6.2).

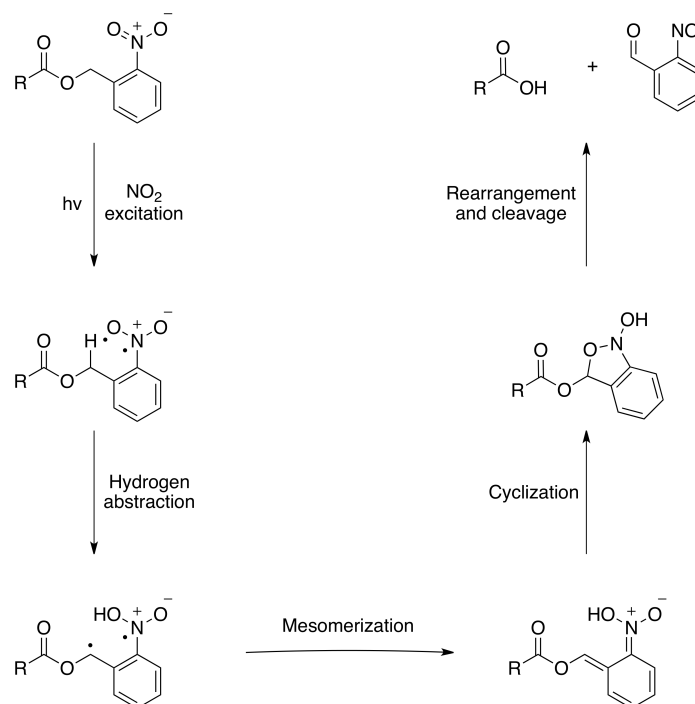


Figure 6.2: Mechanism of the photocleavage for *o*-nitrobenzyl esters. Adapted from Ref [12, 13].

The mechanism of photocleavage of *o*-nitrobenzyl esters starts by the excitation of the nitro function by an UV irradiation (250 - 400 nm). This excitation leads to $n\pi^*$ or $\pi\pi^*$ transitions and the formation of biradicals. After an intra-molecular hydrogen abstraction, the *aci*-nitro tautomer is formed. The isomerization of this tautomer allows then a cyclization reaction. Finally, the rearrangement of the cyclic intermediate enables the cleavage of the ester functions and a release of the carboxylic acid and the nitrosobenzaldehyde counterpart.

The light induced micellization (LIM) was realized on the copolymers bearing *o*-nitrobenzyl and dimethoxynitrobenzyl side groups (Table 4.4, Chapter 4). The irradiation wavelength used for the different copolymer samples was selected according to the λ_{max} of the photosensitive moieties (Figure 6.3). The photocleavage has then been carried out at $\lambda = 300$ and 365 nm, respectively for PNBA-*b*-PS and PDMNBA-*b*-PS.

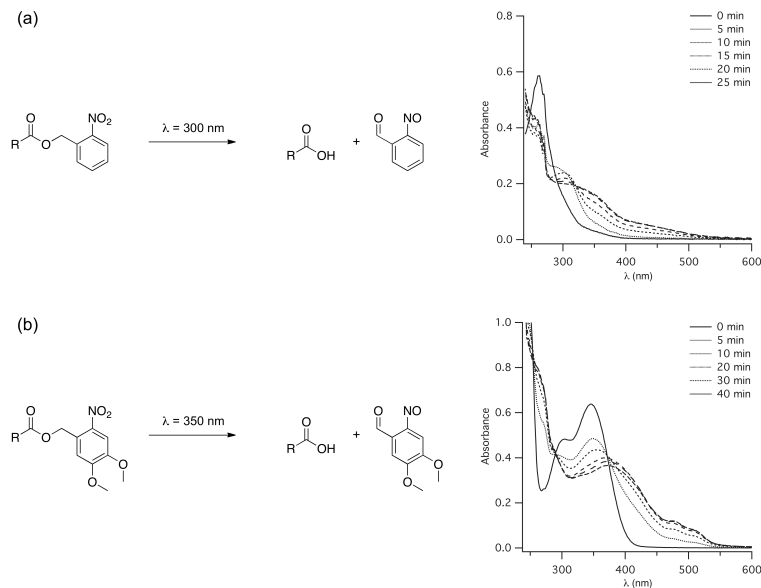


Figure 6.3: Evolution of the UV-vis spectra with irradiation time of a solution in CHCl_3 of : (a) PNBA₃₁-*b*-PS₁₆₈ ($C = 0.1$ g/L, $\lambda = 300$ nm) and (b) PDMNBA₃₁-*b*-PS₁₆₈ ($C = 0.02$ g/L, $\lambda = 365$ nm).

Figure 6.3a presents the evolution of the UV-Vis spectra with irradiation time of a solution of PNBA₃₁-*b*-PS₁₆₈ ($C = 0.1$ g/L in CHCl₃, $\lambda = 300$ nm). Before irradiation, a band at $\lambda_{\text{max}} = 260$ nm, corresponding to the absorption of PS and 2-nitrobenzyl esters, is observed. The irradiation of the sample leads to a decrease of this band intensity (cleavage of the *o*-nitrobenzyl esters) while a band appears at $\lambda = 450$ nm, corresponding to the appearance of 2-nitrosobenzaldehyde in the solution. The photocleavage of the PDMNBA-*b*-PS samples shows a similar trend (Figure 6.3b). The irradiation was realized on PDMNBA₃₁-*b*-PS₁₆₈ in CHCl₃ ($C = 0.02$ g/L) at $\lambda = 365$ nm. The UV-Vis spectra present a decrease of the band intensity at $\lambda_{\text{max}} = 365$ nm with irradiation time, corresponding to the cleavage the 4,5-dimethoxy-2-nitrobenzyl ester functions. Meanwhile, the formation of the 4,5-dimethoxy-2-nitrosobenzaldehyde in the medium is monitored by the appearance of new bands between 400 and 500 nm.

Beside UV-Vis spectroscopy, DLS and TEM evidenced the formation of micelles with a PAA core and a PS corona after irradiation. Figure 6.4 presents the CONTIN size distribution histograms before and after irradiation (left) and the typical TEM images of the micelles obtained after irradiation (right). The photocleavage was carried out in chloroform at a concentration of 0.5 g/L in polymer. In figure 6.4a, the evolution of the size distribution with irradiation time is monitored for the PDMNBA₄₃-*b*-PS₄₉ sample ($\lambda = 365$ nm, 5.10 mW/cm²). Before irradiation, a population at 3 nm is observed, corresponding to unimers in solution. With irradiation time, the disappearance of this population is observed combined by the appearance of a population at 70 nm (30 min and 3h of irradiation), corresponding to the micelles with a PAA core and a PS corona. The photocleavage was stopped when the signal from the unimers was no longer observed in the CONTIN size distribution histogram (16h). At the end of the cleavage process, the formed micelles are characterized by a hydrodynamic radius (R_h) of 85 nm.

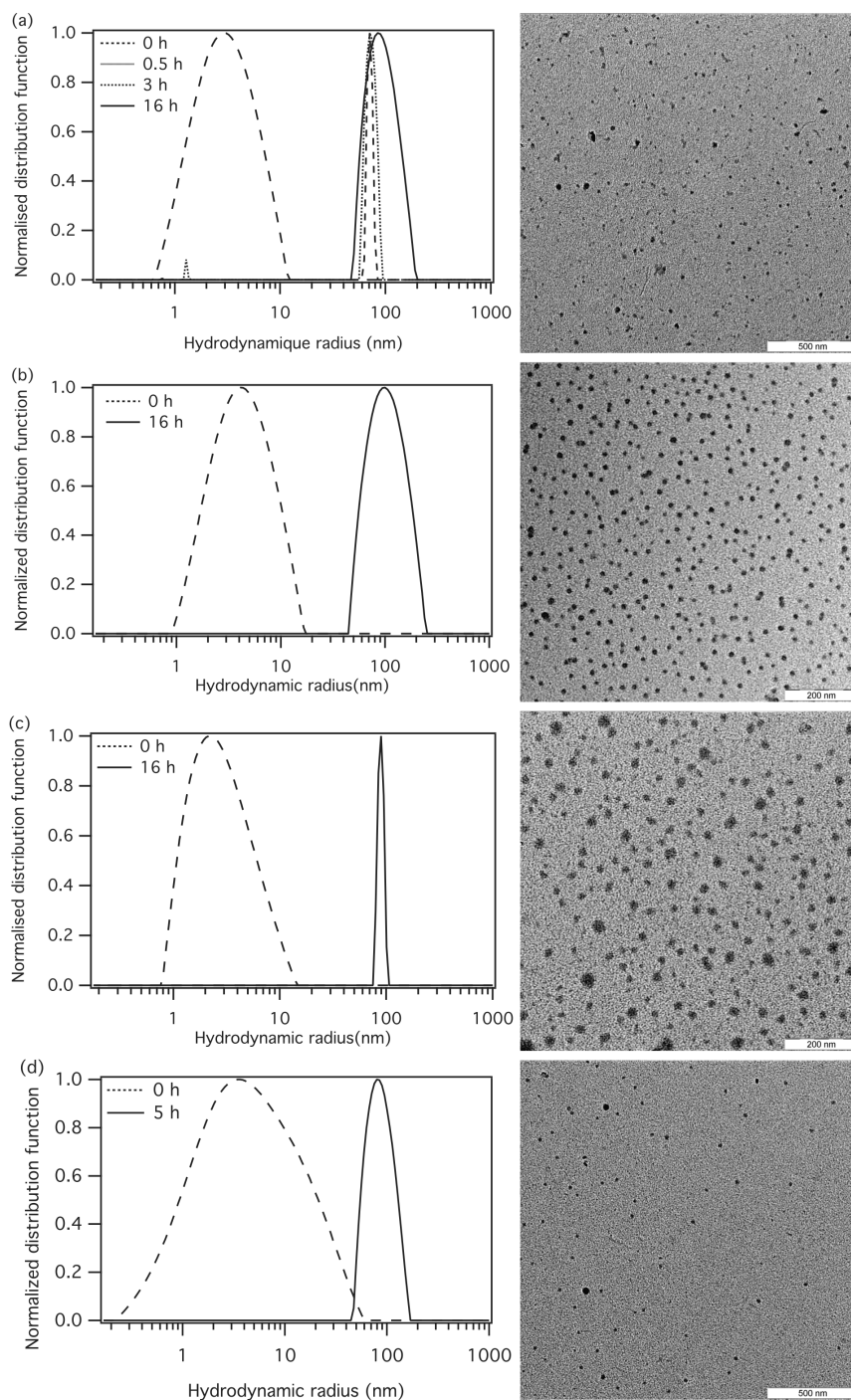


Figure 6.4: Evolution of the CONTIN size distribution histograms and typical TEM images of micelles obtained after the irradiation of solutions (0.5g/L in CHCl_3) of (a) PDMNBA₄₃-*b*-PS₄₉, (b) PDMNBA₁₁₂-*b*-PS₁₂₅, (c) PDMNBA₃₁-*b*-PS₁₆₈ and (d) PNBA₃₁-*b*-PS₁₆₈.

The photocleavage was realized on PDMNBA₁₁₂-*b*-PS₁₂₅ and PDMNBA₃₁-*b*-PS₁₆₈ solutions with similar conditions (Figure 6.4b and c). Formation of micelles was monitored by a transition in R_h from 4 to 100 nm for PDMNBA₁₁₂-*b*-PS₁₂₅ and from 2 to 90 nm for PDMNBA₃₁-*b*-PS₁₆₈.

The photocleavage of PNBA₃₁-*b*-PS₁₆₈ was carried out at $\lambda = 300$ nm (41.6 mW/cm²) in CHCl₃ (C = 0.5 g/L). We can notice in figure 6.4d that the population centered at 4 nm disappears after 5h of irradiation in favor of a larger size population centered at 80 nm.

In addition to DLS, the micelles formed after irradiation were imaged by TEM. Figure 6.4 presents the typical TEM images of the micelles obtained after photocleavage. The average radius of these micelles is equal to 10 ± 1.1 nm for PDMNBA₄₃-*b*-PS₄₉ (a), 11 ± 1.0 nm for PDMNBA₁₁₂-*b*-PS₁₂₅ (b), 14 ± 2.8 nm for PDMNBA₃₁-*b*-PS₁₆₈ (c) and 13 ± 1.0 nm for PNBA₃₁-*b*-PS₁₆₈ (d).

At a first look, the results of DLS and TEM measurements are rather disturbing. Indeed, the PAA-*b*-PS micelles formed by photocleavage of precursor copolymers have quite the same size in solution (see DLS results) and in the dried state (see TEM results) whatever the composition of the starting block copolymers. Moreover, the size of the micelles was found to be constant upon dilution as measured by DLS. These observations suggest that "frozen" non-equilibrium micelles are resulting from the photo-driven micellization process. In this case, the formation of "frozen" micelles can be understood by the fact that the micellar cores are formed by PAA blocks. Hydrogen-bonding between these PAA blocks results in quasi core-crosslinked micelles with no possibility of reorganization. A clue of this incapacity of reorganization is also found in the evolution of size distribution diagrams with irradiation time (Figure 6.4a), as the size of the micelles is fixed in the early stage of the LIM process and no significant change in size is observed with an increase of the irradiation time. Hence, the usual trends and scaling laws observed for block copolymer micelles could not be followed here.

Finally, a large discrepancy between hydrodynamic sizes (hydrodynamic radii around 100 nm) and micellar sizes in the dried state (TEM radii around 10-15 nm) is observed for the different samples. This could indicate that the micelles formed upon light irradiation are characterized by highly swollen cores. Indeed, the micellar cores could be swollen by solvent molecules and by the nitrosobenzaldehyde derivatives resulting from the photocleavage. These swollen micellar cores would collapse during the drying process needed for TEM specimen preparation.

6.3 Copolymers bearing *p*-methoxyphenacyls moieties

Traditionally used for the photo-release of caged phosphates, phenacyl esters are characterized by a simple chemical structure and an efficient release of the masked acid functions [11]. The irradiation of such moieties leads to the destruction of the ester bonds and the release of carboxylic acid groups and acetophenone counterparts. Compared to the *o*-nitrobenzyl ester, the cleavage of phenacyls ester proceeds by an extra-molecular mechanism involving a hydrogen donor (*e.g.* isopropanol in Figure 6.5).

The mechanism for the photocleavage of *p*-methoxyphenacyl esters starts by the excitation of the ketone function by UV irradiation (280 - 330 nm). This excitation leads to $n\pi^*$ transition and the formation of biradicals [14]. After an extra-molecular hydrogen abstraction from isopropanol, the ketyl radical is formed. This resulting ketyl radical decays through a second-order process, perhaps involving the attack of the radical donor into the aromatic. The rearrangement of this intermediate allows the cleavage of the ester bond and the production of the carboxylic acid group and a 1-phenylethenol counterpart. Finally, the acetophenone is obtained after keto-enol equilibrium.

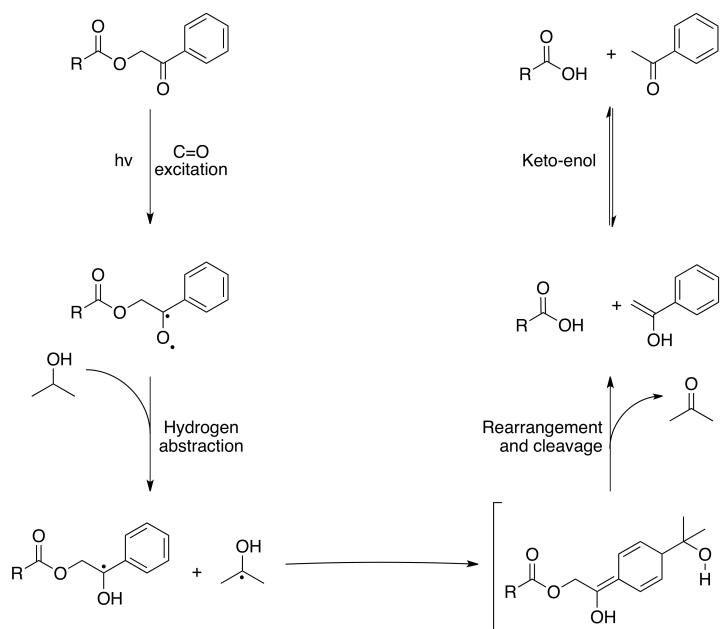


Figure 6.5: Mechanism of the photocleavage for phenacyls esters. Adapted from Ref [11,15].

The photocleavage ($\lambda = 300$ nm) of PMPMA has been carried out in CHCl_3 stabilized by ethanol and followed by UV-Vis spectroscopy. In this experiment, ethanol plays the role of the hydrogen donor [16]. Figure 6.6a illustrates the typical evolution of the UV-Vis absorption spectra of the PMPMA polymer upon irradiation. A decrease in the intensity of the absorption band with irradiation time is observed, corresponding to the cleavage of the *p*-methoxyphenacyl side groups. Similar results were obtained for the photocleavage of PS-*b*-PMPMA (Figure 6.6b).

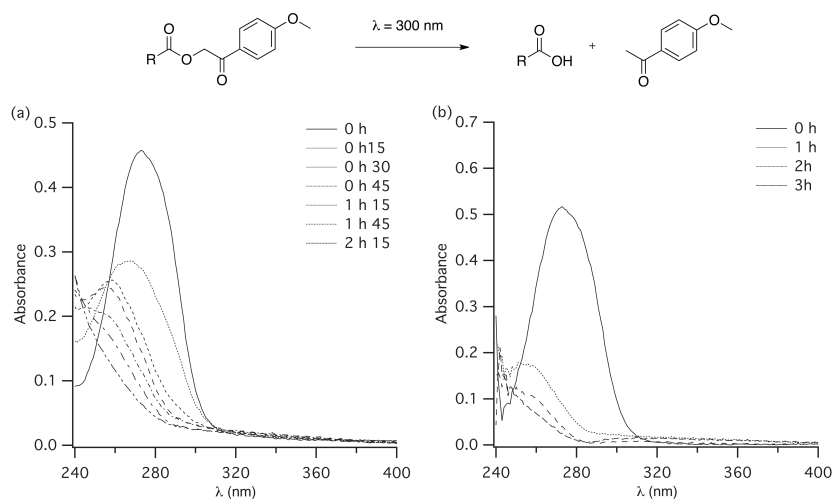


Figure 6.6: Evolution with irradiation time of the UV-Vis spectra of a solution irradiated at 300 nm in CHCl_3 of: (a) PMPMA₁₁₀ ($C = 5 \times 10^{-3}$ g/L), (b) PS₈₇-*b*-PMPMA₁₅₄ ($C = 1 \times 10^{-2}$ g/L)

In addition to UV-Vis spectroscopy, ^1H NMR experiments were carried out to characterize the photocleavage side products. This experiment was realized by the irradiation at $\lambda = 300$ nm of PMPMA for 40h ($C = 3$ g/L in CHCl_3). Since the resulting hydrophilic poly(methacrylic acid) is insoluble in chloroform, the polymer could easily be separated from the photoproducts of low molar masses by filtration. Figure 6.7 presents the overlay of the ^1H NMR spectra of PMPMA before irradiation (bottom), the polymer fraction after irradiation (middle) and the low molar masses photoproducts (top). The spectrum of the polymer fraction is typical of a PMAA in d_7 -DMF. Moreover, the spectrum of the low molecular masses photoproducts reveals that the major side-product is *p*-methoxyacetophenone.

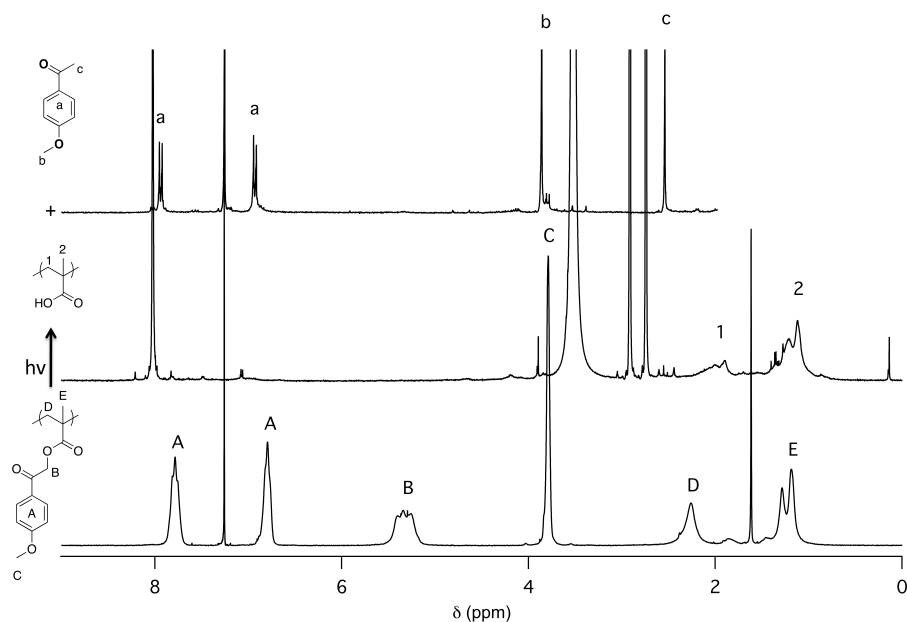


Figure 6.7: ^1H NMR spectra of the products generated after 40 h of irradiation of a PMPMA solution: (bottom) Starting PMPMA (CDCl_3), (middle) polymer fraction ($d_7\text{-DMF}$), (top) low molar mass photoproduct fraction (CDCl_3) ($C = 3 \text{ g/L}$ in CHCl_3).

Finally, the LIM process was applied on PS-*b*-PMPMA copolymers. Figure 6.8 presents the results for the irradiation at 300 nm of a solution of PS₈₇-*b*-PMPMA₁₅₄ in chloroform ($C = 0.5 \text{ g/L}$). The CONTIN size distribution diagram (Figure 6.8a) shows the presence of a population centered at 5 nm before irradiation (unimers). With irradiation time, a decrease of this population at the expense of larger size populations (80 nm after 45 min of irradiation) is observed. At higher irradiation time, several populations are observed at 100, 560 and 5900 nm in the CONTIN size distribution diagram. The presence of these populations, with a large hydrodynamic radius, could indicate the precipitation of the cleaved copolymer.

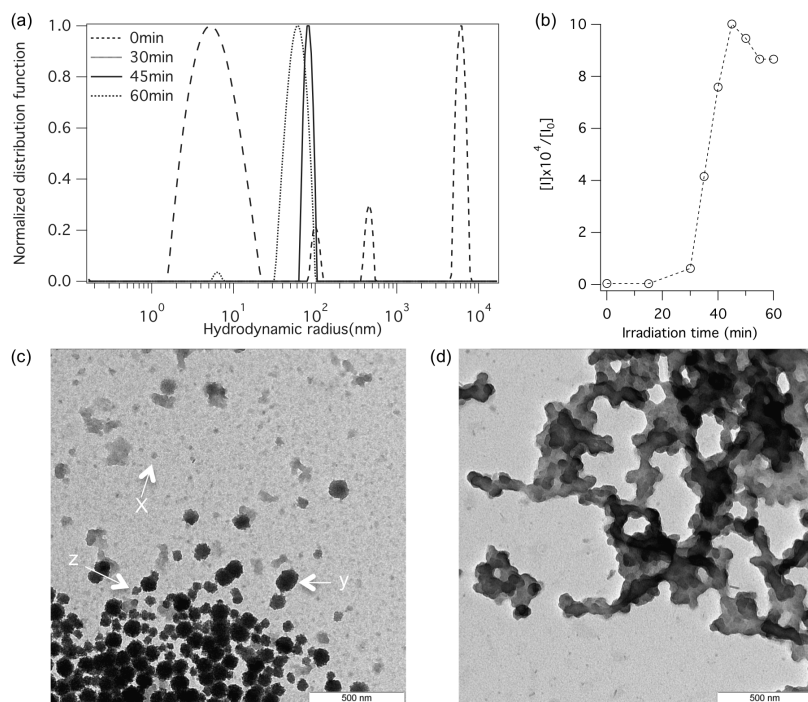


Figure 6.8: (a) Evolution of the CONTIN size distribution histogram, (b) evolution of the normalized scattered intensity and TEM image of the micelles obtained after 30 min (c) and 60 min (d) of irradiation of a solution of $\text{PS}_{87}\text{-}b\text{-PMPMA}_{154}$ in CHCl_3 .

This hypothesis is emphasized by the graph of the normalized scattered intensity (I/I_0) versus irradiation time (Figure 6.8b). Indeed, an increase of the scattered intensity typically indicates either an increase of the number of nanostructures in solution or the formation of larger nano-objects. In this case, the decrease in the scattered intensity observed after 40 min of irradiation can be explained by the decreased amount of nanostructures in solution due to precipitation, as an increase of the objects sizes is recorded.

Finally, transition electron microscopy confirmed the precipitation of the polymer with the formation of large aggregates of micelles (Figure 6.8c and d). Indeed, the TEM image of the sample irradiated for 30 min

reveals the presence of three types of nano-object (x, y and z in Figure 6.8c) are observed. The respective radius of the nano-objects equals to 17 ± 3 nm, 25 ± 5 nm and 49 ± 6 nm for the x, y and z structures. On the basis of these sizes, the structures x could correspond to isolated micelles whereas the structure y and z could be clusters of micelles. Moreover, the irradiation of the sample for 60 minutes clearly demonstrated the aggregation of the micelles since a large structure is observed (Figure 6.8d).

6.4 Applications: light induced encapsulation

Following the successful light induced micellization process, the ability of the system to capture active molecules in the micellar core was investigated. The concept of light induced encapsulation was studied by fluorescence spectroscopy by the irradiation of a solution containing the photocleavable copolymer and a dye.

The choice of the fluorescent dye is not trivial, as it has to fulfill several conditions. Indeed, the chromophore has to interact preferentially with the hydrophilic core created during the irradiation and the irradiation should not induce a modification of its optical properties. Coumarine 343 (C343) satisfied this two imperatives and has been selected to perform the experiments. The affinity of C343 for the core of PAA-*b*-PS micelles has been verified by the encapsulation via direct dissolution. This experiment was realized by the addition of PAA₄₃-*b*-PS₄₉ in a stirred solution of C343 in CHCl₃ ($C_{polymer}=0.1$ g/L, $C_{C343}=0.3$ mg/L). Figure 6.9a presents the emission fluorescence spectrum of C343 before (0h) and after the addition of the PAA-*b*-PS (1h and 20h). The decrease in the intensity reflects the encapsulation of C343 into the PAA micellar core. The dye stability was monitored by the irradiation of a solution of coumarin 343 ($C_{C343}=0.3$ mg/L) at 365 nm for 1h. Figure 6.9b displays the variation in the fluorescence intensity with irradiation time. After 60 min of irradiation, the intensity decreases by 10%, which is acceptable considering the huge variations of the intensity observed in the light induced encapsulation experiments (Figure 6.10).

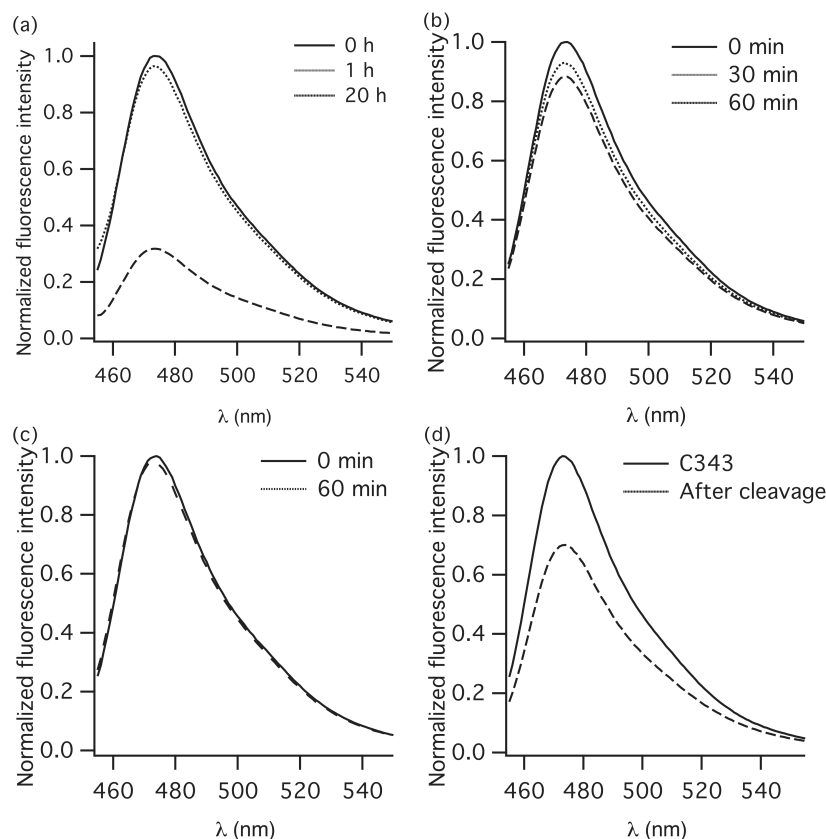


Figure 6.9: Fluorescence spectra of coumarin 343 (0.3 mg/L in CHCl_3) before and after: (a) its encapsulation into micelles of PAA-*b*-PS formed by direct dissolution ($C_{\text{polymer}}=0.1$ g/L); (b) irradiation at 365 nm; (c) stirring in a solution containing PAA-*b*-PS micelles ($C_{\text{polymer}}=0.1$ g/L); (d) stirring in a solution containing 4,5-dimethoxy-2-nitrosobenzaldehyde (irradiated solution of PDMNBA₄₃-*b*-PS₄₉).

In addition several experiments were carried out to ensure that no side phenomenons, like the diffusion of the dye into the micellar core or the well know fluorescence quenching effect of the nitrosobenzyl counterpart [17, 18], interfere with the encapsulation measurements. The diffusion test was performed by adding C343 to a solution of the PAA₄₃-*b*-PS₄₉ micelles in CHCl_3 , the solution was stirred for 60 min and the solution fluorescence was monitored every 10 min. Figure 6.9c demon-

strates that the dye could not diffuse into the micellar core, as no significant decrease of the intensity was observed. Finally, the fluorescence quenching effect of the nitrosobenzyl compounds was evaluated by the addition of coumarin into a solution of PDMNBA₄₃-*b*-PS₄₉ previously irradiated for 60 min. In figure 6.9d, a decrease of about 30% of the signal has been observed. This decrease indicates the existence of some fluorescence quenching due to the nitrosobenzyl compound and should be kept in mind when performing encapsulation experiments.

After verification of the above-mentioned requirements, the encapsulation of coumarin 343 via light-induced micellization has been demonstrated. The dye was added to a solution of the photocleavable block copolymer in chloroform (0.1 g/L). The mixture was then irradiated at 365 nm in order to induce the micellization and trap the fluorescent dye at the same time.

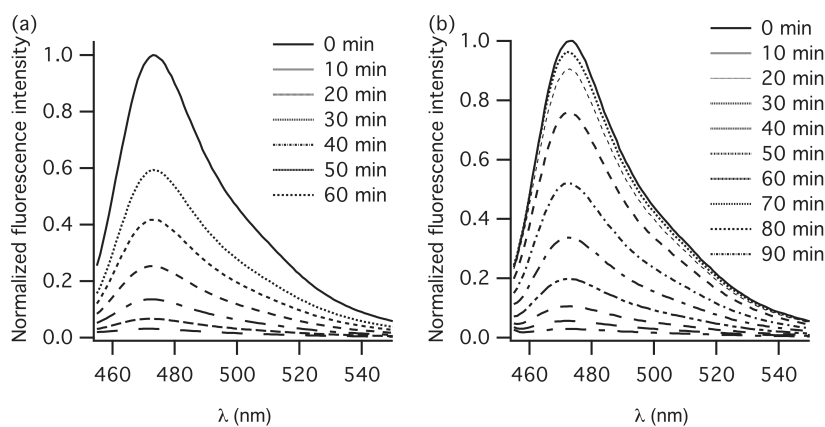


Figure 6.10: Evolution of the fluorescence spectra of coumarin 343 (0.3 mg/L) during its encapsulation via the light-induced micellization of (a) PDMNBA₄₃-*b*-PS₄₉, (b) PNBA₃₁-*b*-PS₁₆₈ ($C_{polymer}=0.1$ g/L in CHCl₃, $\lambda = 365$ nm).

The encapsulation was followed by fluorescence spectroscopy and figure 6.10 shows the progressive decrease of the fluorescence signal of C343 ($\lambda_{\text{ex}} = 444 \text{ nm}$) with the irradiation time of a PDMNBA₄₃-*b*-PS₄₉ (a) and a PNBA₃₁-*b*-PS₁₆₈ solution (b). After respectively 60 and 90 minutes of irradiation, the total disappearance of the signal evidences the successful incorporation of the dye into the micelles. In addition, these results also proved the interest of the light-induced approach compared to the encapsulation by direct micellization of PAA-*b*-PS. Indeed, in the latter case, it took 20 hours to observe a decrease of 70% in the fluorescence signal, while only 1 hour was needed to totally suppress the fluorescence with the light-induced approach. This clearly demonstrates a faster and a more efficient trapping process with the light-induced micellization.

The validity of the light induced encapsulation approach was not demonstrated with the copolymers bearing *p*-methoxyphenacyl side groups because the cleavage of these copolymers requires an irradiation at 300 nm. At this wavelength, the requirement of the chromophore stability is not fulfilled for most of the common dyes (coumarin, rhodamine and acrydine derivatives), *i.e.* a huge modification of their optical properties is monitored.

6.5 Conclusions

In this chapter, the principles of the light induced micellization process were presented. The LIM process was first studied on copolymers bearing *o*-nitrobenzyl derivatives (PNBA-*b*-PS and PDMNBA-*b*-PS). UV-Vis spectroscopy demonstrated the successful photocleavage of these block copolymers. The combination of DLS and TEM measurements allowed the characterization of the micelles formed during the irradiation.

In section 6.3, light induced micellization was demonstrated with copolymers bearing *p*-methoxyphenacyl moieties. The photocleavable characteristics of such photosensitive moieties was studied with UV-Vis and ^1H NMR spectroscopy on a homo-PMPMA. DLS and TEM measurements demonstrated the formation of micelles during the irradiation of a PS-*b*-PMPMA copolymer. However, the copolymer composition was an issue for the formation of stable micelles in solution.

Finally, the ability of the system to act as a trapping agent was demonstrated by the encapsulation of coumarin 343 into the micellar cores produced during the irradiation of the block copolymers in solution. The screening of the side phenomenons for the encapsulation (chromophore stability during irradiation, nitrobenzyl quenching effect, *etc...*) allowed to fully understand the encapsulation experiments. Moreover, the comparison with the encapsulation by a direct dissolution approach proved that this light-induced encapsulation is a fast and reliable process to trap molecules and makes it fully adapted for applications such as *in situ* product removal or product separation [19,20].

Experimental part

Materials and instrumentation. coumarin 343 (C343, Acros Organics, laser grade) and all the other chemicals were used as received.

Proton nuclear magnetic resonance (^1H NMR) spectra were acquired on a 300 MHz Bruker Avance II. Irradiations were performed with 3 Rayonet photochemical reactor lamps (maximum emission wavelength at $\lambda = 300$ and 365 nm). The intensity received by the sample was respectively equal to 41.6 and 5.1 mW/cm² and was measured with a radiometer RM12 from Dr Gröbel UV-elektronik GmbH equipped with a UVA (315-400 nm) and UVB (280-315 nm) sensors. UV-visible spectra were recorded on a Varian Cary 50 Conc. spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer. Dynamic light scattering (DLS) measurements were performed at an angle of 90° on a Malvern CGS-3 apparatus equipped with a He-Ne laser with a wavelength of emission at $\lambda = 632.8$ nm. The temperature was controlled at 25°C. TEM was performed on a LEO 922 microscope, operating at 120 kV accelerating voltage in bright field mode. Samples for TEM experiments were prepared by drop casting micelles solution on TEM grids (SPI, carbon coated copper, mesh: 200). The grids were then dried for 15 h. The samples were then exposed to RuO₄ vapors during 15 min for staining.

Section 6.2 and 6.3

Typical procedure for the photocleavage in solution (UV-Vis):

A solution of PNBA₃₁-*b*-PS₁₆₈ in CHCl₃ (C = 0.1 g/L) was irradiated under stirring at 300 nm. The photocleavage was followed by UV-visible spectroscopy until no change was observed in the shape of the spectra (t(min) = 0, 5, 10, 15, 20, 25).

Typical procedure for the photocleavage in solution (DLS):

A solution of PDMNBA₄₃-*b*-PS₄₉ in CHCl₃ (C = 0.5 g/L) was irradiated under stirring at 365 nm. The photocleavage was followed by DLS until the CONTIN size analysis showed the complete disappearance of the peak corresponding to the unimers (3-5 nm) and 10 μL of the solution was dropped casted on TEM grids.

Typical procedure for the photocleavage in solution (^1H NMR):

A solution of PMPMA₁₁₀ in CHCl₃ (C = 3 g/L) was irradiated under stirring at 300 nm for 40 h. The solution was then filtered on 0.2 μm PTFE filter. The filtered solution was concentrated under reduced pressure and the low molar mass fraction was dispersed on CDCl₃. The polymer fraction was

redissolved by passing d7-DMF through the filter. The two fractions were then analysed by ^1H NMR.

Section 6.4

Typical procedure for the encapsulation of the dye into PAA-*b*-PS micellar core:

To a quartz cell was filled with a solution of coumarin 343 ($C = 0.3 \text{ mg/L}$) in CHCl_3 stabilized with EtOH, PAA₄₃-*b*-PS₄₉ was added ($C = 0.1 \text{ g/L}$). The solution was stirred and the encapsulation was followed by fluorescence spectroscopy ($t \text{ (h)} = 0, 1, 20$).

Typical procedure the dye stability measurements:

A quartz cell was filled with a solution of coumarin 343 ($C = 0.3 \text{ mg/L}$) in CHCl_3 stabilized with EtOH. The solution was irradiated at 365 nm. The photocleavage was followed by fluorescence spectroscopy ($t \text{ (min)} = 0, 10, 20, 30, 40, 50, 60$).

Typical procedure for the diffusion of the dye into PAA-*b*-PS micellar core:

To a quartz cell was filled with a solution of PAA₄₃-*b*-PS₄₉ micelles in CHCl_3 stabilized with EtOH ($C = 0.1 \text{ g/L}$), coumarin 343 was added ($C = 0.3 \text{ mg/L}$). The solution was stirred and the diffusion of the dye into the micellar core was followed by fluorescence spectroscopy ($t \text{ (min)} = 0, 10, 20, 30, 40, 50, 60$).

Typical procedure for the investigation of the fluorescence quenching effect:

To a quartz cell was filled with previously irradiated solution of PDMNBA₄₃-*b*-PS₄₉ in CHCl_3 stabilized with EtOH ($C = 0.1 \text{ g/L}$), coumarin 343 was added ($C = 0.3 \text{ mg/L}$). The solution was stirred for 60 min and fluorescence spectrum was recorded.

Typical procedure for light induced encapsulation of the dye into the micellar core:

A quartz cell was filled with a solution of PDMNBA₄₃-*b*-PS₄₉ ($C = 0.1 \text{ g/L}$) and with coumarin 343 ($C = 0.3 \text{ mg/L}$) in CHCl_3 stabilized with EtOH. The solution was irradiated at 365 nm. The photocleavage was followed by fluorescence spectroscopy ($t \text{ (min)} = 0, 10, 20, 30, 40, 50, 60$).

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LIGHT INDUCED MICELLE DISRUPTION

Abstract

In this chapter, the use of light to disrupt micelles composed of block copolymers bearing photocleavable side groups is studied. The considered copolymers, synthesized in chapter 4, are composed of a photosensitive and hydrophobic poly(p-methoxyphenacyl methacrylate) block and a thermosensitive and hydrophilic poly[oligo(ethylene glycol) methacrylate] block. In the first part of this chapter, the micellization of these copolymers is studied by DLS and TEM. The part focuses on the disruption of the micelles by light. Finally, the multi-responsive behavior of the accordingly obtained PMAA-b-POEGMA is demonstrated.

7.1 Introduction

Although the stimuli induced micellization and disruption are two antagonist processes, they rely on the same principles. Indeed, these processes are based on the stimuli driven transition of a block copolymer from its amphiphilic form to a "double-hydrophilic" one and inversely. The disruption of micelles is observed when the amphiphilic block copolymer is transformed into a double-hydrophilic one. This transition is particularly interesting in the biomedical and pharmaceutical domains for controlled drug delivery applications.

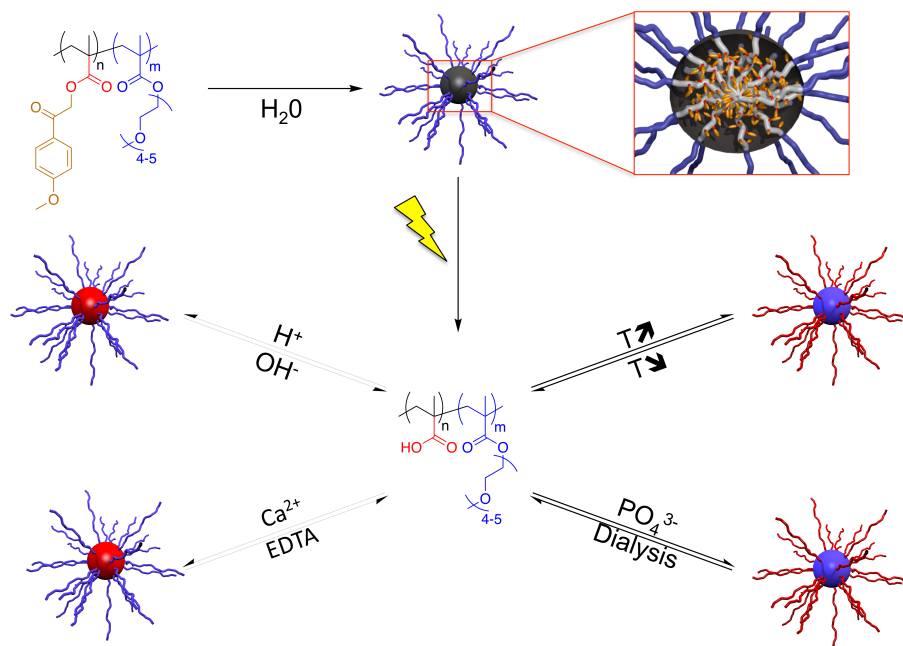


Figure 7.1: Schematic representation of the light induced disruption of PMPMA-*b*-POEGMA micelles and of the multi-stimuli responsive behavior of the PMAA-*b*-POEGMA copolymer in water.

In this chapter, micelle disruption is promoted by light. The investigated diblock copolymers are composed of a poly(*para*-methoxyphenacyl methacrylate) sequence (PMPMA) and of a poly[oligo (ethylene glycol) methacrylate] sequence (POEGMA). Upon dissolution in water, these

block copolymers self-assemble in micelles with a PMPMA core and a POEGMA corona. The irradiation of these micelles induces the cleavage of the *p*-methoxyphenacyl side groups leading to the production of a double-hydrophilic PMAA-*b*-POEGMA block copolymer and thus the micelle disruption (Figure 7.1). Indeed, *p*-methoxyphenacyl esters are well-known photolabile protecting groups of carboxylic acids and caged phosphates [1–5].

In addition to the light induced disruption of PMPMA-*b*-POEGMA micelles, the rich stimuli-responsive and schizophrenic behavior of the accordingly obtained PMAA-*b*-POEGMA is studied [6, 7]. Indeed, the PMAA block is a pH [8–10] and a multivalent cations responsive polymer [11–14] and the POEGMA block shows a salt responsive behavior [6] in addition to its well-known thermosensitive behavior [15–18]. The formation of micelles with a POEGMA core and a PMAA corona is then studied by a thermal stimulus and by the addition of PO_4^{3-} anions to the solution. On the other hand, the formation of the inverse micelles with a PMAA core and a POEGMA corona is promoted by the lowering of pH and by the addition of Ca^{2+} cations to the solution.

This chapter is divided into three sections. The first one focuses on the micellization of the PMPMA-*b*-POEGMA copolymers. In the second part of the chapter, the response of these block copolymers to light irradiation is studied by DLS, UV-Vis spectroscopy and TEM. Moreover the ability of the system to release active molecules upon irradiation is demonstrated with ibuprofen. The last section deals with the schizophrenic behavior of the PMAA-*b*-POEGMA obtained after irradiation.

7.2 Micellization of PMPMA-*b*-POEGMA

As demonstrated by Eisenberg and coworkers, the size and the morphology of micelles in solution are influenced by the preparation methods [19, 20]. The optimization for the micellization process of PMPMA-*b*-POEGMA is presented below. Two preparation methods were investigated in MilliQ water (pH = 6): direct dissolution (DD) and the cosol-

vent method (CS). In the cosolvent method, the block copolymer was first dissolved in a non-selective solvent (DMF), then the selective solvent (H_2O) was added dropwise in the solution to induce the precipitation of the PMPMA blocks. The non-selective solvent was finally removed from the solution by dialysis. On the other hand, the direct micellization process consisted in the dissolution of the block copolymer powder in a selective solvent by stirring or sonication of the solution.

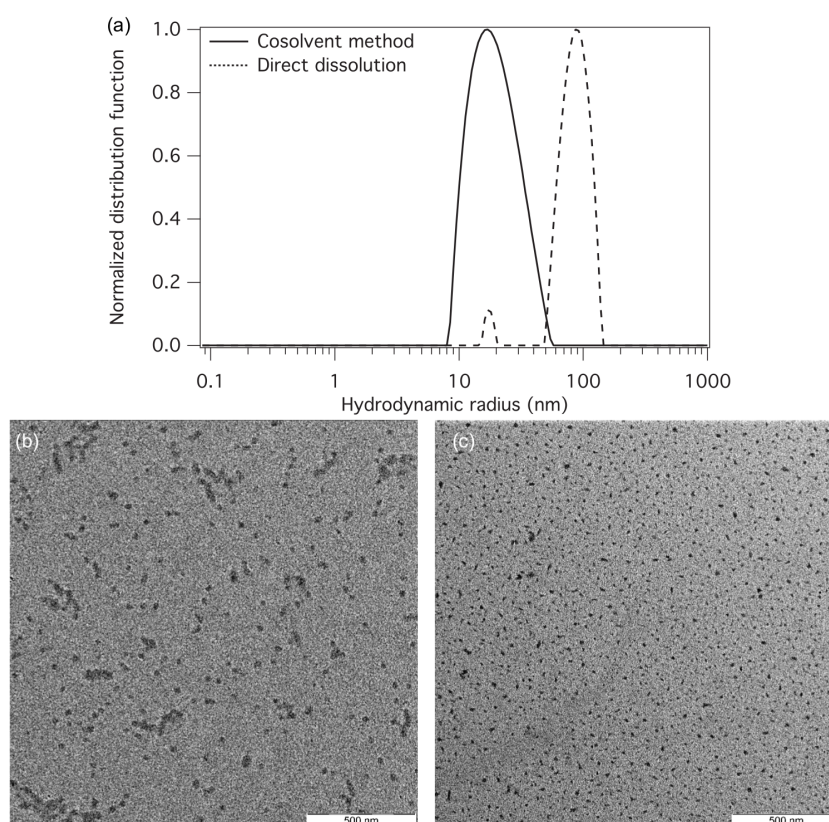


Figure 7.2: Effect of the preparation method on the micellization of PMPMA₄₄-*b*-POEGMA₆₆ in water ($C = 0.5$ g/L, pH = 6): (a) CONTIN size distribution diagram of micelles prepared by DD and CS method, TEM images of micelles prepared by DD (b) and CS (c).

Figure 7.2 presents the DLS (a) and TEM (b and c) results for the micellizations by different preparation methods. The CONTIN size

distribution diagrams shows the hydrodynamic sizes of PMPMA₄₄-*b*-POEGMA₆₆ (Entry 4, Table 4.9) micelles prepared either by the cosolvent method (full curve) or the direct dissolution process (dashed curve). The CONTIN size diagram of micelles prepared by the cosolvent method shows one population centered at 17 nm. On the other hand, the CONTIN analysis of micelles prepared by direct dissolution shows two populations, the one centered at 17 nm corresponds to isolated micelles and the one at $R_h = 87$ nm corresponds to aggregates of micelles. In addition to DLS, TEM images of the micelles prepared by both methods were recorded. The image of micelles prepared by direct dissolution are characterized by the presence of clusters of micelles (Figure 7.2b, $R_{TEM} = 12 \pm 1.2$ nm). Figure 7.2c clearly demonstrates the absence of clusters when micelles are prepared by the cosolvent method ($R_{TEM} = 10 \pm 0.9$ nm). Similar results were observed for the micellization of the PMPMA₆₁-*b*-POEGMA₆₆ and PMPMA₉₉-*b*-POEGMA₄₈ copolymers (Entries 2 and 3, Table 4.9).

Regarding the conclusions of these sets of experiments, the cosolvent method was then selected for the micellization of the different PMPMA-*b*-POEGMA copolymers. The effect of the pH during the micellization and the dialysis of the micelles was then investigated. Indeed, a neutral or slightly basic pH is needed during the light induced disruption to ensure that the carboxylic acids of the accordingly obtained PMAA sequence are in their negatively charged carboxylate form. Figure 7.3 presents the CONTIN size distribution diagram of PMPMA₆₁-*b*-POEGMA₆₆ (a) and PMPMA₄₄-*b*-POEGMA₆₆ (b) micelles prepared by the cosolvent method at pH = 6, 8 and in a pH = 8 buffer solution (Borax = sodium tetraborate/hydrochloric acid). In these experiments, a significant change in the size distribution is observed when the buffer solution is used. Indeed, when the dialysis is realized in the buffer solution, a second population at higher apparent hydrodynamic radius ($R_{h,app} \approx 70$ nm) is observed. This population is most probably the consequence of an aggregation of micelles due to a salting out effect on the POEGMA corona. Moreover, this effect seems to be more pronounced when the buffer solution is used for the micellization process of PMPMA₄₄-*b*-POEGMA₆₆ (Figure 7.3b).

Based on these results, the best micellization procedure is the cosolvent method realized at $\text{pH} = 8$. Indeed, DLS and TEM analysis showed that the micelles formed in these conditions are isolated and have a relatively narrow size distribution ($\text{PDI} = 0.099$ and 0.101 respectively for $\text{PMPMA}_{61}\text{-}b\text{-POEGMA}_{66}$ and $\text{PMPMA}_{44}\text{-}b\text{-POEGMA}_{66}$). The light induced disruption of these micelles is detailed in the next section.

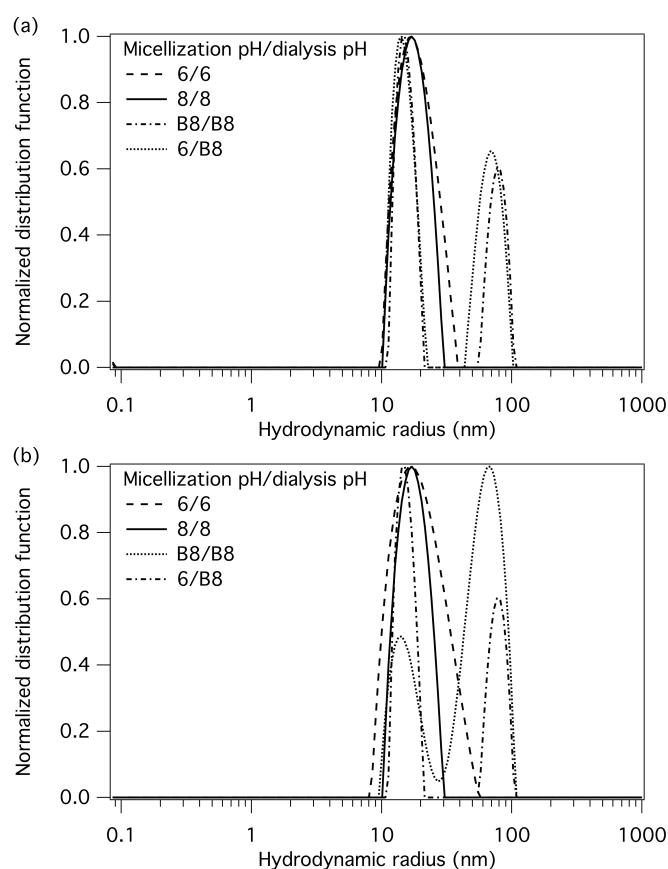


Figure 7.3: Effect of the pH on the micellization by the cosolvent method of : (a) $\text{PMPMA}_{61}\text{-}b\text{-POEGMA}_{66}$ and (b) $\text{PMPMA}_{44}\text{-}b\text{-POEGMA}_{66}$ ($C = 0.5 \text{ g/L}$).

7.3 Disruption of the PMPMA-*b*-POEGMA micelles by light

The irradiation of the PMPMA-*b*-POEGMA copolymers leads to the cleavage of the phenacyl side groups according to the mechanism presented in figure 6.5 (Chapter 6). Figure 7.4 presents the evolution of the UV-Vis spectra with irradiation time of an aqueous solution of PMPMA-*b*-POEGMA. The typical behavior of the irradiated PMPMA is observed here (*cfr.* Figure 6.6, Chapter 6), *i.e.* the decrease of the band centered at $\lambda_{max} = 270$ nm corresponding to the cleavage of the phenacyl ester bonds.

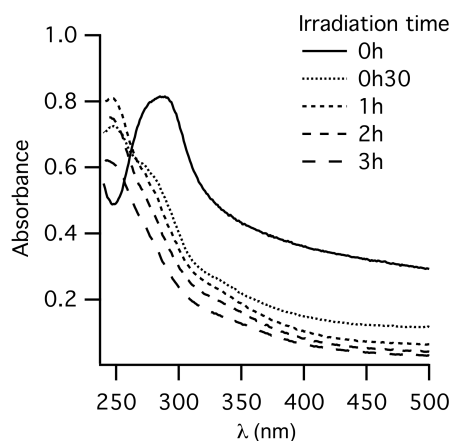


Figure 7.4: Evolution with irradiation time of the UV-Vis spectra of a solution of PMPMA₉₉-*b*-POEGMA₄₈ in water ($C = 0.03$ g/L) irradiated at 300 nm.

In addition to the cleavage of the phenacyl ester functions, the UV-Vis experiments also highlighted the micelle disruption. Indeed, a variation in the absorbance is monitored at 500 nm. Since the copolymer does not absorb light in this region, the relatively high turbidity observed here is the consequence of the presence in solution of large objects (micelles) that scatter the light. The absorbance decreasing with irradiation time indicates the disappearance in the medium of these nano-objects and evidences thus the disruption of the micelles.

7.3.1 Irradiation at pH = 8

The irradiation of the micelles formed by the cosolvent method at pH = 8 was realized with the PMPMA₆₁-*b*-POEGMA₆₆ and PMPMA₄₄-*b*-POEGMA₆₆ samples. Figure 7.5 presents the results of the light induced disruption of PMPMA₄₄-*b*-POEGMA₆₆ micelles. The cleavage was followed by DLS. The graph of the normalized scattered intensity ($[I]/[I_0]$) versus the irradiation time (Figure 7.5b) allows the monitoring of the photo-disruption process. Indeed, the $[I]/[I_0]$ ratio is influenced by the micellar size and the micellar concentration and the density of the micellar core. In the case of a micelle disruption, a decrease in the intensity indicates a reduction of the micelle size since the concentration in the sample is fixed and that the object size decreases. The irradiation was maintained until the $[I]/[I_0]$ ratio reached a plateau. In this case, no variation was observed after 30 h of irradiation time.

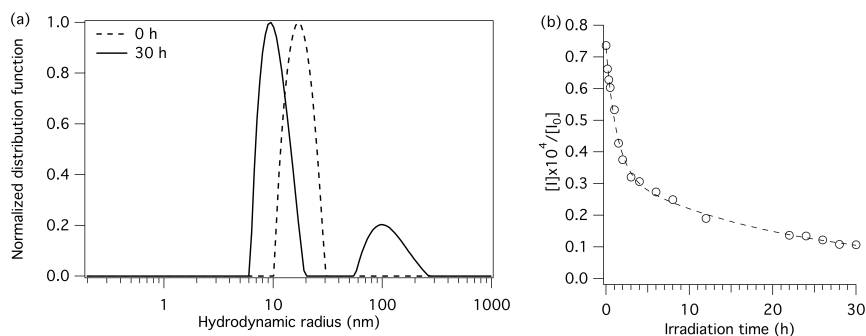


Figure 7.5: Light induced disruption of PMPMA₆₁-*b*-POEGMA₆₆ ($C = 0.5$ g/L) micelles at pH = 8: (a) CONTIN size distribution diagram before (dashed) and after 30h of irradiation (full), (b) graph of the normalized scattered intensity versus irradiation time.

The CONTIN size distribution diagram (Figure 7.5a) displays a slight decrease in the hydrodynamic radius of the micelles in solution from 17 nm to 9.5 nm. This decrease in the R_h was expected, but the size of the objects in solution after irradiation is unexpectedly high for unimers in solution. The presence of objects of this size in solution after irradiation can be explained by the formation of micelles with a PMAA core

and a POEGMA corona during the cleavage. An acidification of the solution was actually monitored during the cleavage, with a variation from pH = 8 to 3. The PMAA formed after irradiation is a well-known pH responsive polymer which is insoluble in water below pH = 4 [21]. Similar results were obtained for the photo-disruption of PMPMA₆₁-*b*-POEGMA₆₆ micelles. As a consequence of this acidification, the light induced disruption of PMPMA-*b*-POEGMA micelles was realized in a pH = 8 buffer solution.

7.3.2 Irradiation in a buffer solution

As demonstrated in the micellization section, the use of a buffer solution leads to the formation of clusters of micelles. On the other hand, this buffer solution will ensure the formation of carboxylates during the irradiation and therefore the disruption of the micelles into PMAA-*b*-POEGMA unimers.

Figure 7.6 presents the analysis by DLS and TEM of PMPMA₄₄-*b*-POEGMA₆₆ (a), PMPMA₆₁-*b*-POEGMA₆₆ (b) and PMPMA₉₉-*b*-POEGMA₄₈ (c) micelles in a buffer solution (borax 1.25 mM). As previously demonstrated the clusters of micelles are observable in the CONTIN size distribution diagrams (left) and in the TEM images (right). The apparent hydrodynamic radii ($R_{h,app}$) of the isolated micelles are respectively 17, 14 and 69 nm. While the radius of the dried micelles determined by transmission electron microscopy is respectively 16 ± 1.8 , 12 ± 1.6 and 22 ± 3.9 nm.

With the exception of PMPMA₉₉-*b*-POEGMA₄₈, the micellar sizes determined by DLS and TEM are consistent. The large apparent hydrodynamic radius observed in DLS for PMPMA₉₉-*b*-POEGMA₄₈ is most probably the consequence of the presence of non resolved contributions from two type of objects with similar sizes, *i.e.* isolated micelles and relatively small micellar clusters.

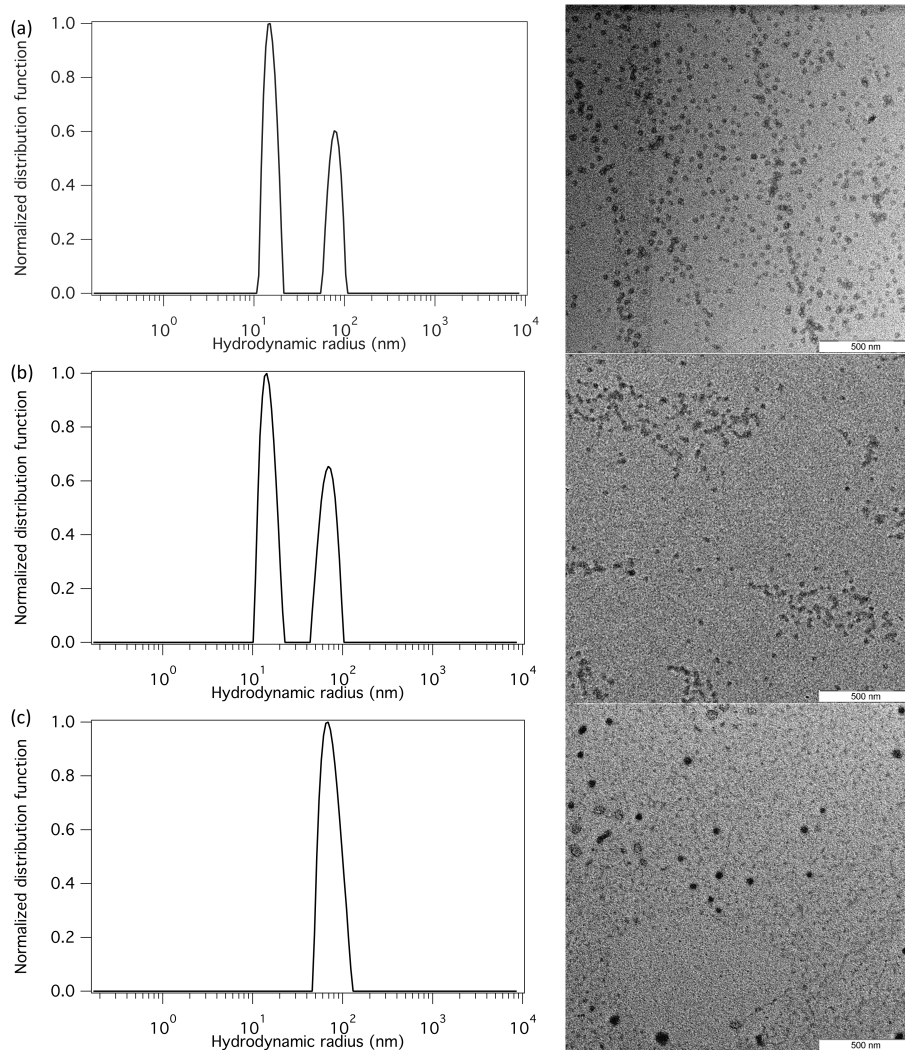


Figure 7.6: CONTIN size distribution diagrams and corresponding TEM images of micelles ($C = 0.5$ g/L) formed by the cosolvent method in a buffer solution (borax 1.25mM): (a) PMPMA₄₄-*b*-POEGMA₆₆, (b) PMPMA₆₁-*b*-POEGMA₆₆ and (c) PMPMA₉₉-*b*-POEGMA₄₈ ($C = 0.1$ g/L).

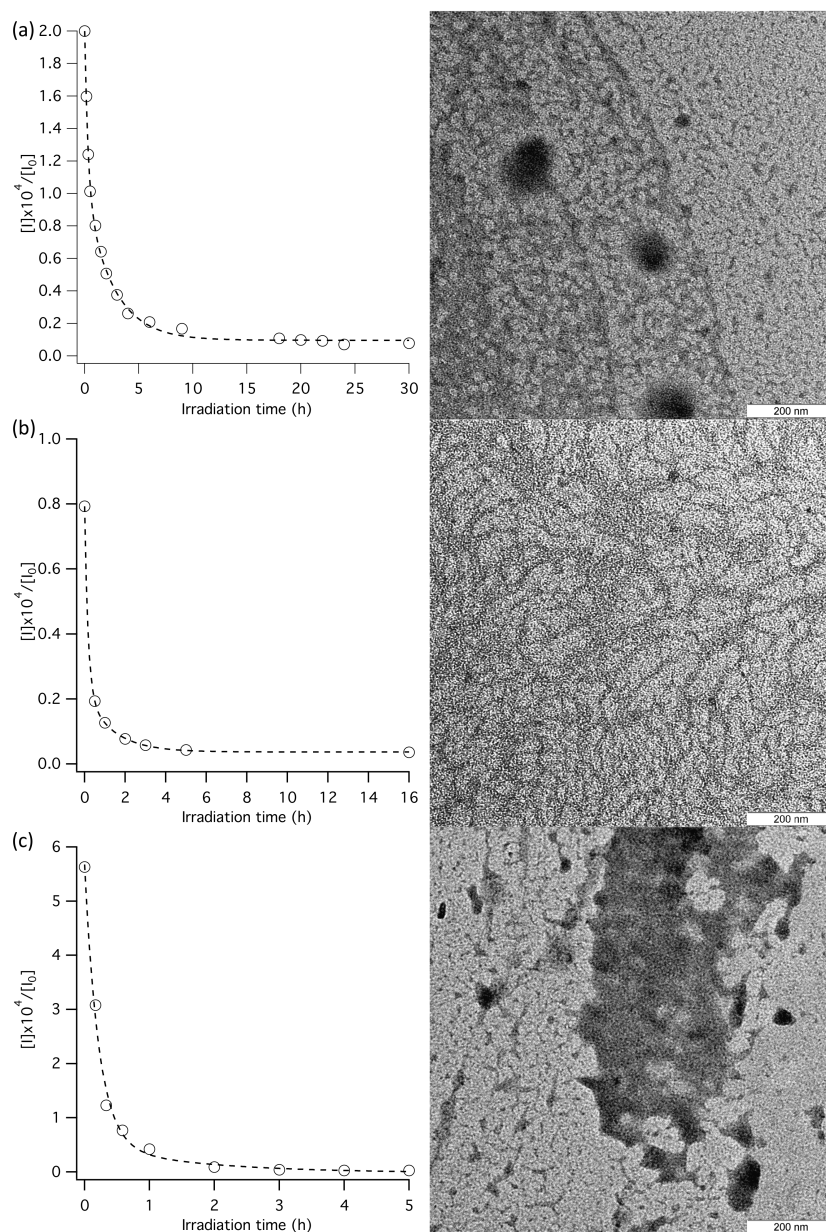


Figure 7.7: Evolution of the normalized scattered intensity with irradiation time and TEM images after irradiation of: (a) PMPMA₄₄-*b*-POEGMA₆₆, (b) PMPMA₆₁-*b*-POEGMA₆₆ and (c) PMPMA₉₉-*b*-POEGMA₄₈ ($C = 0.1$ g/L).

Finally, the irradiation of these micelles was realized at $\lambda = 300$ nm. The photocleavage was monitored by DLS and the normalized scattered intensity was monitored to follow the disruption of the micelles. Figure 7.7 presents the evolution of the normalized scattered intensity with irradiation time for the disruption of PMPMA₄₄-*b*-POEGMA₆₆ (a), PMPMA₆₁-*b*-POEGMA₆₆ (b) and PMPMA₉₉-*b*-POEGMA₄₈ (c) micelles. The decrease in $[I]/[I_0]$ observed here is similar to the LID at pH = 8 and highlights the disruption of the micelles. However, in comparison with the Light induced disruption at pH = 8, the scattered intensity substantially falls down and stabilizes at lower values. This observation suggests the absence of nano-objects in solution after irradiation. In addition, TEM images confirmed the disappearance of the micelles in solution after irradiation since a nonstructured polymer film was observed but no micelles.

7.3.3 Applications: light induced release

Following the successful light induced disruption of the PMPMA-*b*-POEGMA micelles, the ability of the system to release hydrophobic molecules upon irradiation was investigated. This concept was studied by the irradiation of a solution of micelles loaded with ibuprofen and the release of ibuprofen in solution was followed by turbidimetry, as ibuprofen is insoluble in water.

Figure 7.8 presents the results of the light induced release of ibuprofen in water. The loaded micelles were prepared by the direct dissolution of PMPMA₉₉-*b*-POEGMA₄₈ ($C = 1\text{g/L}$) in pH = 8 buffer water in presence of ibuprofen ($C = 1\text{g/L}$). The loaded micelles were then dialyzed against the buffer solution to remove all traces of the unloaded ibuprofen. A sample of the unloaded micelles was prepared by the same procedure. The photo-induced release was then monitored by UV-Vis spectroscopy in transmittance mode and the turbidity of the solution plotted versus the irradiation time (Figure 7.8a). One can notice that the turbidities of the loaded and unloaded micelle solutions are high before irradiation, respectively 69 and 34 %. The irradiation of these solutions leads to a decrease of the turbidity of approximately 30%. This decrease in the

solution turbidity is the consequence of the micelle disruption. After 3 h of irradiation, one can note an increase of the solution turbidity for the loaded micelles and a decrease of the turbidity for the unloaded micelles. Those opposite behaviors clearly demonstrate the ability of the system to release active molecules. Indeed, the decrease observed for the unloaded micelles is due to the formation of unimers whereas the monitored increase of turbidity is the consequence of the release of the ibuprofen molecules in the medium.

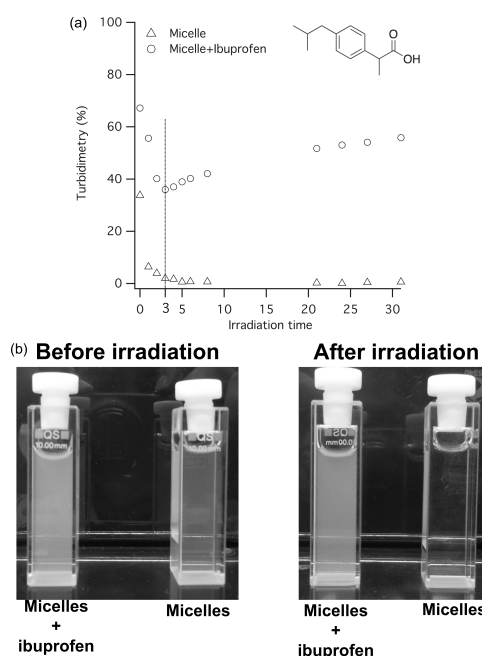


Figure 7.8: Light induced release of ibuprofen in water: (a) turbidity of the solution versus irradiation time ($\lambda = 600$ nm), (b) picture showing the solution of loaded and unloaded micelles before and after irradiation ($C_{\text{PMPMA-}b\text{-POEGMA}} = 1\text{g/L}$, $C_{\text{ibuprofen}} = 1\text{g/L}$).

Moreover, this light induced release can be observed visually. Figure 7.8b presents the picture of the loaded (left cell) and unloaded micelles (right cell) before and after irradiation. For the loaded micelles, the solution before and after cleavage are relatively similar whereas a transition from a turbid to a cristal clear solution is observed in the case of unloaded micelles.

7.4 Schizophrenic behavior of PMAA-*b*-POEGMA

Since the irradiation led to a double hydrophilic PMAA-*b*-POEGMA copolymer, existing as unimers in water buffered at pH = 8, several other stimuli were applied to this solution in order to induce further micellization. As presented in figure 7.1, micelles with a POEGMA core and a PMAA corona are obtained by an increase of the medium temperature or an addition of phosphate salts in the solution. The inverse micelles with a PMAA core and a POEGMA corona are formed by an acidification of the medium or an addition of multivalent cations in the solution.

As presented in the introduction, a "Schizophrenic block copolymer" can exist in three different states in solution: unimers, micelles and inverse micelles and can reversibly transit between these three states. Considering these aspects the reversible character of the PMAA-*b*-POEGMA micellization will also be demonstrated for the different stimuli.

7.4.1 Micelles with a POEGMA core

In the case of the thermally induced micellization of the PMAA-*b*-POEGMA copolymer, the driving force is the destabilization of the H-bonds between the oligo(ethylene glycol) side groups and the surrounding water molecules, resulting in the observation of a lower critical solution temperature (LCST) for the POEGMA block. This thermo-responsive behavior is well documented [15–18] and valuable in solution or for the production of stimuli-responsive surfaces [22, 23].

Figure 7.9 presents the results for the thermally induced micellization of the PMAA₉₉-*b*-POEGMA₄₈ copolymer in water buffered at pH = 8 ($C = 0.1$ g/L). The cloud point of the diblock copolymer was determined by DLS, using the evolution of the scattered intensity with temperature (Figure 7.9a). The cloud point was found to be located at 57 °C which is in agreement with the reported LCST of the POEGMA homopolymer [16]. In addition to the thermally induced micellization, the scattered intensity was monitored during the cooling of the solution.

Although a clear hysteresis is observed, the values of $[I]/[I_0]$, before, and after the transition ($T = 25 \Rightarrow 70 \Rightarrow 25^\circ\text{C}$) are almost the same, which demonstrates the reversibility of the thermally induced micellization.

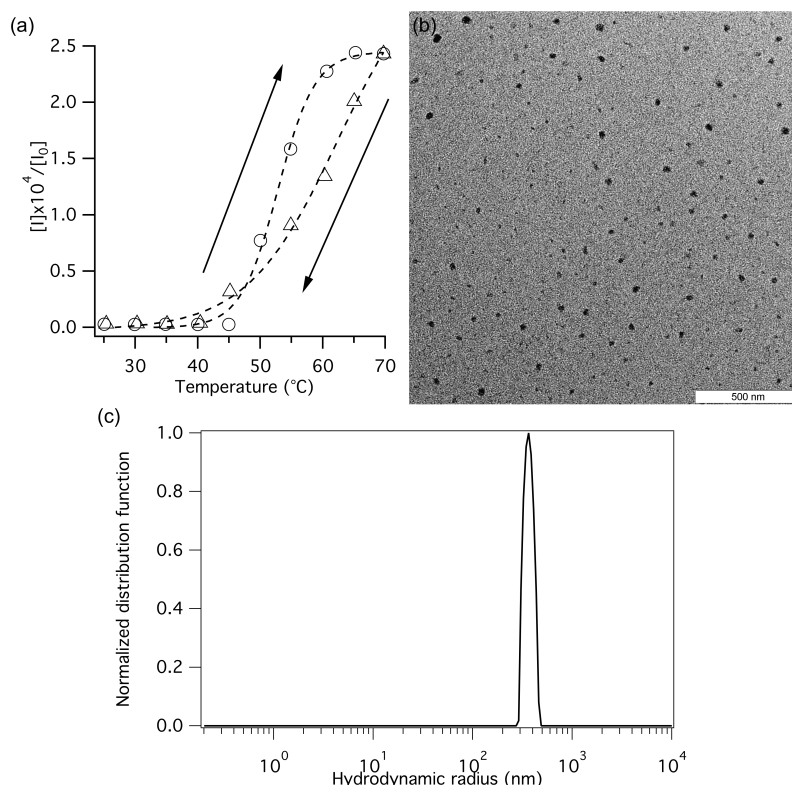


Figure 7.9: Response of PMAA₉₉-*b*-POEGMA₄₈ ($C = 0.1$ g/L in pH = 8 buffered water) to a thermal stimulus: (a) evolution of the normalized scattered intensity, (b) TEM image and (c) CONTIN size distribution diagram of micelles obtained at 65 $^\circ\text{C}$.

Figures 7.9b and c present the TEM image and the CONTIN size distribution diagram of the PMAA-*b*-POEGMA micelles obtained at 65 $^\circ\text{C}$. These micelles are characterized by an apparent hydrodynamic radius of 370 nm ($\text{PDI} = 0.100$) and a R_{TEM} of 16 ± 2.6 nm. The value obtained for R_{TEM} is indeed in agreement with the formation of spherical

micelles from diblock copolymers with characteristic features similar to the ones of the sample investigated here [24, 25]. However, the $R_{h,app}$ measured by DLS is much too large compared to the block copolymer composition. The discrepancy between the $R_{h,app}$ and the R_{TEM} , and especially the large value of the hydrodynamic radius, is the consequence of the presence, at $pH = 8$, of the negatively charged carboxylates in the micellar corona, which induces the so-called polyelectrolyte effect. Indeed, electrostatic interactions in polyelectrolyte micelles create an electrostatic field, which fluctuates with the Brownian motion of the micelles and reversely influences their motion dynamic by slowing it down. This results in the so-called "slow-mode" in the distribution of diffusion coefficients. As a consequence, $R_{h,app}$ is much larger for polyelectrolyte micelles [26, 27].

Several factors can influence this polyelectrolyte effect including the decrease of the polymer concentration in solution or the addition of mono-valent cation. Those experiments were not conducted here as these two factors may change the LCST of the POEGMA [16, 28].

The second approach to promote the collapse of the POEGMA block and the micellization is to add a phosphate salt in the medium. In this case, the driving force is the salting-out of the oligo(ethylene glycol) side groups [6, 13, 29]. Phosphate salts were chosen as they have a pronounced salting out effect, in agreement with the Hofmeister series [30].

Figure 7.10 presents the results of the micellization for the PMAA₉₉-*b*-POEGMA₄₈ in buffered water ($C = 0.1$ g/L). The micellization of this block copolymer was observed after the addition of 4.4×10^{-4} mol of K_3PO_4 to the solution. The reversibility of the micellization was controlled by measuring the solution scattered intensity after dialysis against a buffered solution to remove the phosphate salts. In the graph of the scattered intensity, the values of $[I]/[I_0]$, before micellization (o), and after dialysis (+) are almost the same, which demonstrate the reversibility of the phosphate induced micellization. The results obtained by TEM and DLS show that polyelectrolyte micelles were obtained with a $R_{h,app}$ of 182 nm ($PDI = 0.038$) and a R_{TEM} of 6 ± 0.9 nm (Figure 7.10b and c).

The discrepancy between TEM and DLS results can be again understood by a pronounced polyelectrolyte effect due to the charged PMAA corona.

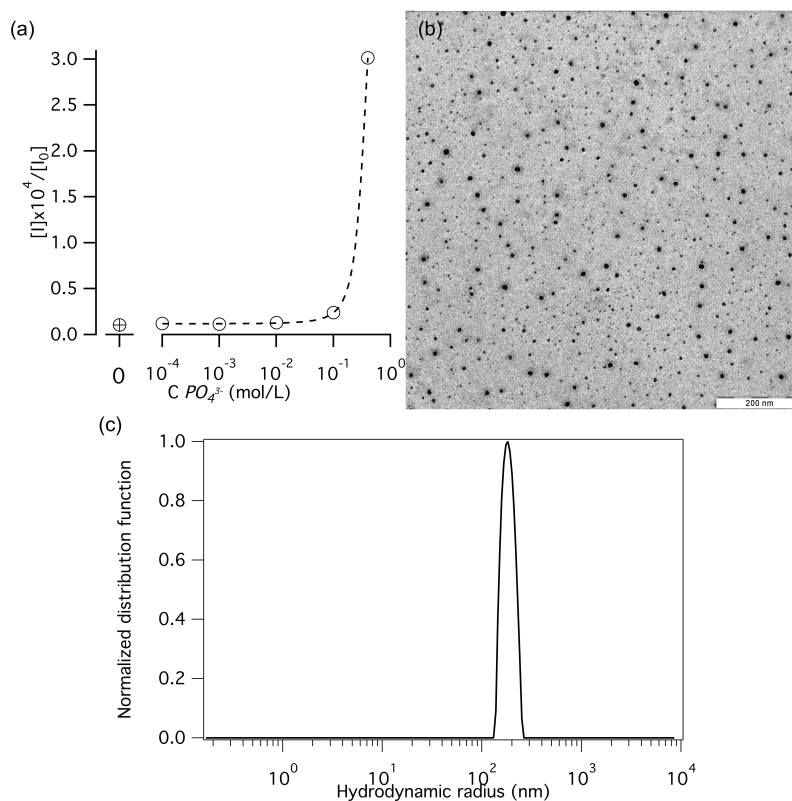


Figure 7.10: Response of PMAA₉₉-*b*-POEGMA₄₈ ($C = 0.1$ g/L in buffered water) to an addition of PO_4^{3-} : (a) evolution of the normalized scattered intensity, (b) TEM image and (c) CONTIN size distribution diagram of micelles obtained after an addition 4.4×10^{-4} mol of K_3PO_4 to the solution.

7.4.2 Micelles with a PMAA core

The pH-induced micellization is triggered by the protonation at low pH values of the PMAA block that lowers considerably its solubility in aqueous medium and induces its aggregation into micellar cores. Before the pH induced micellization, a dialysis of the block copolymer solution was realized to remove all traces of the original buffer salt.

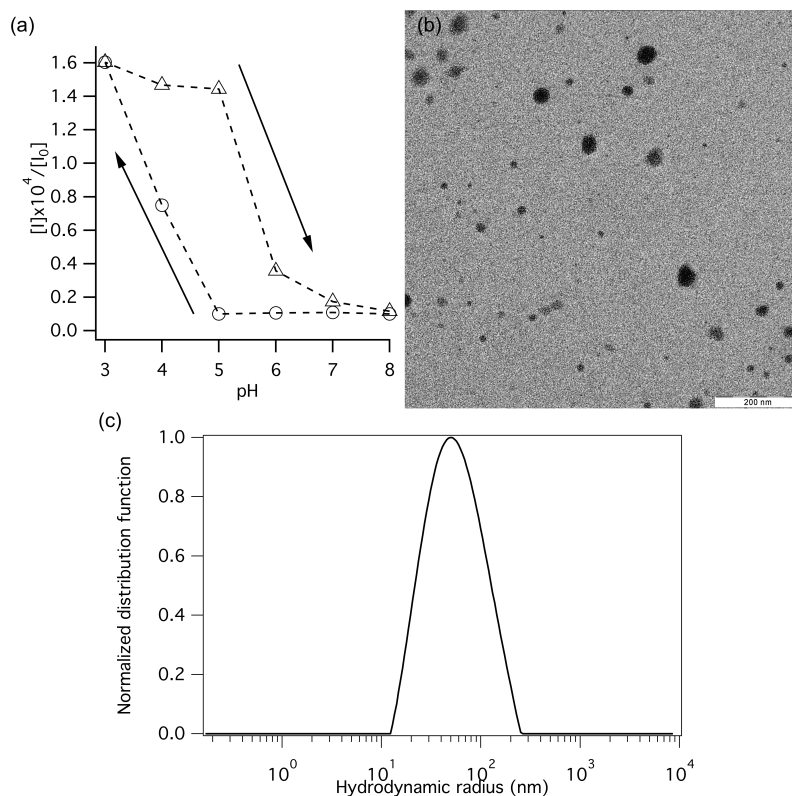


Figure 7.11: Response of PMAA₉₉-*b*-POEGMA₄₈ ($C = 0.1$ g/L in water) to a variation of pH: (a) evolution of the normalized scattered intensity, (b) TEM image and (c) CONTIN size distribution diagram of micelles obtained at pH = 3.

Figure 7.11 presents the results of the micellization of PMAA₉₉-*b*-POEGMA₄₈ induced by a pH stimulus. Similarly to the previous stimuli

induced micellization, the normalized scattered intensity was monitored to follow the micellization and to ensure the reversibility of this process (Figure 7.11a). The micellization was realized by the addition of HCl in the solution and the disruption of the micelles was triggered by an addition of NaOH. The sharp increase of $[I]/[I_0]$ observed a pH value under 4 indicates the formation of micellar structures. This critical pH is in agreement with the pKa value of PMAA, indicating that the protonated PMAA blocks are able to aggregate into micellar cores. After the basification of the medium $[I]/[I_0]$ attained a similar value than the polymer solution before micellization.

At pH = 3, micelles with a $R_{h,app}$ of 50 nm (PDI = 0.209) and a R_{TEM} of 12 ± 4.9 nm are observed. Although TEM and DLS experiments do not lead to the same values, the previously observed polyelectrolyte effect is no longer observed at pH = 3 confirming the protonation of the PMAA blocks. The discrepancy between $R_{h,app}$ and R_{TEM} is no longer due to a polyelectrolyte effect, but to some aggregated micelles, as observed in the TEM picture (Figure 7.11b).

The other approach to induce the micellization of the PMAA-*b*-POEGMA copolymers into micelles with the PMAA core is to add divalent cations (Ca^{2+}) into the copolymer solution. In this case the calcium cations are complexed by the carboxylate functions of the PMAA sequence [11, 13]. This experiment was realized by the addition of $CaCl_2$ to a solution of PMAA₉₉-*b*-POEGMA₄₈ in buffered water ($C = 0.1$ g/L). The micellization of the block copolymer occurred after addition of 3.3×10^{-4} mol of $CaCl_2$ to the solution. The accordingly obtained micelles were characterized by a $R_{h,app}$ of 151 nm (PDI = 0.326) and a R_{TEM} of 6 ± 1.2 nm (Figure 7.12b and c). The discrepancy between DLS and TEM results could be again explained by the formation of a small amount of aggregated micelles. The reversibility of the micellization was also monitored by DLS after dialysis of the micelles in an EDTA solution (0.1M). As it can be observed in figure 7.12a, the value of $[I]/[I_0]$ are similar before (o) and after (+) the micellization.

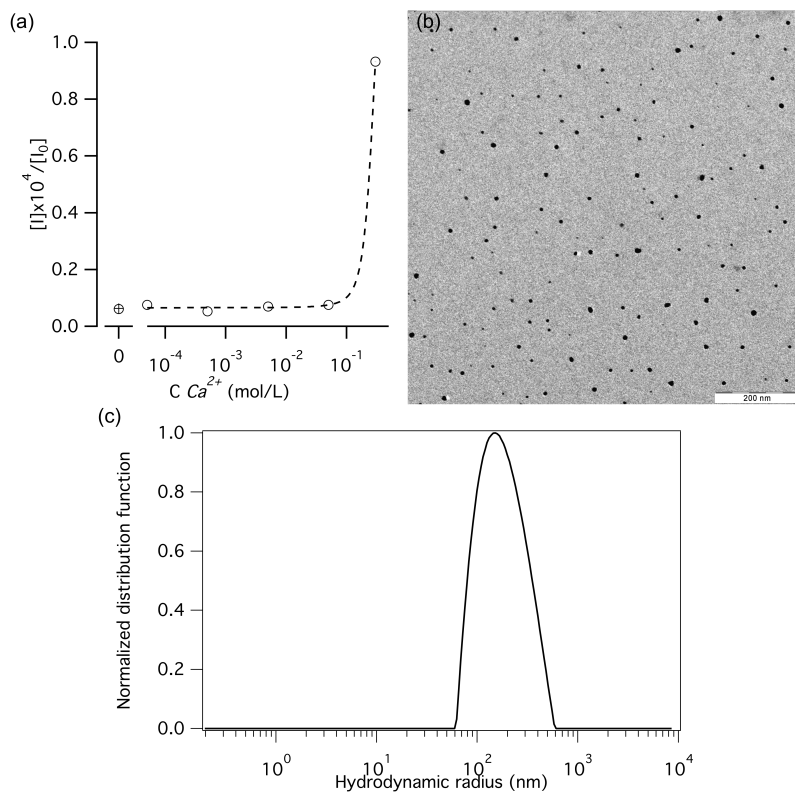


Figure 7.12: Response of PMAA₉₉-*b*-POEGMA₄₈ ($C = 0.1$ g/L in buffered water) to an addition of Ca^{2+} : (a) Evolution of the normalized scattered intensity, (b) TEM image and (c) CONTIN size distribution diagram of micelles obtained after an addition 3.3×10^{-4} mol of CaCl_2 to the solution.

7.5 Conclusions

The different parameters influencing the micellization of the PMPMA-*b*-POEGMA copolymer were described here. The effect of the micellization technique on the aggregation of the copolymer in solution was demonstrated with the cosolvent and the direct micellization methods. Dynamic light scattering and TEM experiments also showed the effect of a buffered medium on the micellar aggregation compared to the micellization in water.

Considering the light induced disruption of the PMPMA-*b*-POEGMA micelles, the first experiments clearly demonstrated the requirement for a buffer solution during the photocleavage to totally disrupt the micelles and produce PMAA-*b*-POEGMA unimers.

Section 7.3.3 proved the interest of such a system to carry hydrophobic molecules in solution and release them on demand. Indeed, the direct dissolution was used for the loading of ibuprofen into the PMPMA core of the micelles. The light induced release of the ibuprofen molecules in water was monitored by turbidimetry.

In section 7.4, the multi-responsive behavior of the accordingly obtained PMAA-*b*-POEGMA was studied by the application of various stimuli including temperature, pH, calcium cations, and phosphate anions. The schizophrenic behavior of this copolymer was demonstrated since all the stimuli induced micellization processes were reversible.

In summary, the system developed in this chapter is maybe one of the best example of the large varieties of nanostructures that can be obtained from stimuli responsive block copolymers in solution. Indeed, the number and diversity of the applied stimuli make our system unique compared to previously described stimuli-responsive micelles.

Experimental part

Materials and instrumentation. All the chemicals were used as received. The buffer solution was prepared by the following procedure: To a 2.5 mM solution of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (Reidel de Haën) in distilled water, 205 ml of a 0.1 M HCl solution (Fischer Scientific, 36wt%) were added and the solution was then made up to 1L with distilled water.

Proton nuclear magnetic resonance (^1H NMR) spectra were acquired on a 300 MHz Bruker Avance II. Irradiations were performed with 3 Rayonet photochemical reactor lamps (maximum emission wavelength at $\lambda = 300$ nm). The intensity received by the sample was equal to 41.6 mW/cm^2 and was measured with a radiometer RM12 from Dr Gröbel UV-electronik GmbH equipped with a UVB (280-315 nm) sensors. UV-visible spectra were recorded on a Varian Cary 50 Conc. spectrophotometer. Dynamic light scattering (DLS) measurements were performed at an angle of 90° on a Malvern CGS-3 apparatus equipped with a He-Ne laser with a wavelength of emission at $\lambda = 632.8$ nm. TEM was performed on a LEO 922 microscope, operating at 120 kV accelerating voltage in bright field mode. Samples for TEM experiments were prepared by drop casting micelles solution on TEM grids (SPI, carbon coated copper, mesh: 200). The grids were then dried for 15 h. The samples were then exposed to RuO_4 vapors during 15 min for staining.

Section 7.2

Typical procedure for the micellization of PMPMA-*b*-POEGMA by direct dissolution:

A 4mL vial equipped with a magnetic barrel was filled with 200 μL of a solution of PMPMA₉₉-*b*-POEGMA₄₈ in chloroform ($C = 10 \text{ g/L}$). The copolymer solution was then concentrated under vacuum to remove all traces of CHCl_3 . The vials was filled with 4 mL of MilliQ water ($C_{\text{final}} = 0.5 \text{ g/L}$) and the mixture was stirred for 24h. The micelles were characterized by DLS and TEM.

Typical procedure for the micellization of PMPMA-*b*-POEGMA by the cosolvent method:

PMPMA₉₉-*b*-POEGMA₄₈ was dissolved in DMF at a concentration of 0.5 g/L. A volume of water equal to 3 times the DMF volume was then added dropwise to induce aggregation of the insoluble PMPMA blocks, followed by the addition of a volume of water equal to the DMF volume in one shot to "freeze" the micelles ($C_{\text{final}} = 0.1 \text{ g/L}$). Subsequently, the DMF/water solution was dialyzed against a buffer solution ($\text{pH} = 8$) for 24 hours replacing the water at least three times (Spectra-Por dialysis bags, cutoff 3500 Da). The

final concentration of the copolymer in the buffer solution was set to 0.1 g/L. The micelles were characterized by DLS and TEM.

Section 7.3

Typical procedure for the photocleavage in solution (UV-Vis):

A solution of PMPMA₉₉-*b*-POEGMA₄₈ in buffered water ($C = 3 \times 10^{-2}$ g/L) was irradiated under stirring at 300 nm. The photocleavage was followed by UV-visible spectroscopy until the disappearance of the peak centered at $\lambda_{\max} = 270$ nm ($t(h) = 0, 0.5, 1, 2, 3$).

Typical procedure for the photocleavage in solution (DLS):

A solution of PMPMA₉₉-*b*-POEGMA₄₈ in a buffer solution ($C = 0.1$ g/L) was irradiated under stirring at 300 nm (38.5 mW/cm²). The photocleavage was followed by DLS until the scattered intensity reached a plateau at low values. 10 μ L of the solution was drop casted on TEM grids.

Section 7.3.3

Typical ibuprofen loading procedure in PMPMA-*b*-POEGMA micelles:

A 4 mL vial equipped with a magnetic stirrer was filled with 400 μ L of a solution of PMPMA₉₉-*b*-POEGMA₄₈ in chloroform ($C = 10$ g/L) and 400 μ L of a solution of ibuprofen in chloroform ($C = 10$ g/L). The solution was then concentrated under vacuum to remove all traces of CHCl₃. The vial was filled with 4 mL of MilliQ water ($C_{\text{PMPMA-}b\text{-POEGMA}}$ and $C_{\text{ibuprofen}} = 1$ g/L) and the mixture was stirred for 24h. Finally, the solution was dialyzed against a buffer solution (pH = 8) for 24 hours replacing the water at least three times (Spectra-Por dialysis bags, cutoff 3500 Da) to remove all traces of the unloaded ibuprofen.

Typical procedure for the light induced release of ibuprofen in solution:

A solution of loaded micelles in buffered water ($C_{\text{PMPMA-}b\text{-POEGMA}} = 1$ g/L) was irradiated under stirring at 300 nm. The ibuprofen release was monitored by UV-visible spectroscopy (transmittance mode, $\lambda = 600\text{nm}$) until a decrease of the transmittance was observed ($t(h) = 0 \Rightarrow 30$).

Section 7.4

Typical procedure for the thermally induced micellization:

A solution of the previously irradiated copolymer ($C = 0.1$ g/L in a buffer solution) was heated by 5°C step. The micellization was followed by DLS and the micelles were imaged by TEM.

Typical procedure for the pH induced micellization:

To a solution of the previously irradiated and dialyzed copolymer ($C = 0.1$ g/L), an aqueous solution of HCl was added until pH reached the value of 3. The micellization as a function of the pH was followed by DLS and the micelles were imaged by TEM. The reversibility of the pH-induced micellization was tested by the addition of an aqueous solution of NaOH.

Typical procedure for the salt induced micellization:

To a solution of the previously irradiated copolymer ($C = 0.1$ g/L in a buffer solution), either an aqueous solution of CaCl_2 or K_3PO_4 was added dropwise. The micellization was followed by DLS at different salt concentrations and the micelles were imaged by TEM.

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FORMATION OF NANOCAGES BASED ON
PHOTOCLEAVABLE BLOCK COPOLYMERS

Abstract

The formation of nanocages by light is presented here. The strategy is based on the self-assembly of linear photocleavable block copolymers combined with light irradiation. The light stimulus induces the cleavage of a junction between the core and the corona of shell cross-linked micelles. After the irradiation, the core-forming polymer may be extracted to obtain the desired nanocage. Three types of nanocages are presented here. Nanocages with a shell made of PAA or PDMAEMA are respectively built from PS-hv-PAA or PtBA-hv-PDMAEMA diblock copolymers. The third type of nanocage, composed of a PDMAEMA shell and of a POEGMA corona, is obtained by the self-assembly of PtBA-hv-PDMAEMA-b-POEGMA triblock copolymers.

8.1 Introduction

As presented in chapter 2, several strategies can be used to build polymeric nanocages. Our approach relies on the self-assembly of block copolymer precursors and on the extraction of the core forming polymer. The strategy we elaborated is similar to the one developed by O'Reilly and coworkers with the exception that the junction between the core and the corona is not a metal-ligand complex but a photocleavable *o*-nitrobenzyl ester [1–3].

Figure 8.1 presents our strategy to produce hollow nanocapsules. In the first step the linear photocleavable block copolymer, represented here by a triblock copolymer, is self-assembled in water to produce spherical micelles. The photocleavable junction is localized precisely at the core-corona interface. The micellar shell is then chemically cross-linked. The shell-crosslinked micelles can afterwards be submitted to light irradiation, leading to the cleavage of the *o*-nitrobenzyl ester junction and the detachment of the core from the shell. The last step is the extraction of the core-forming polymer to obtain the desired nanocages.

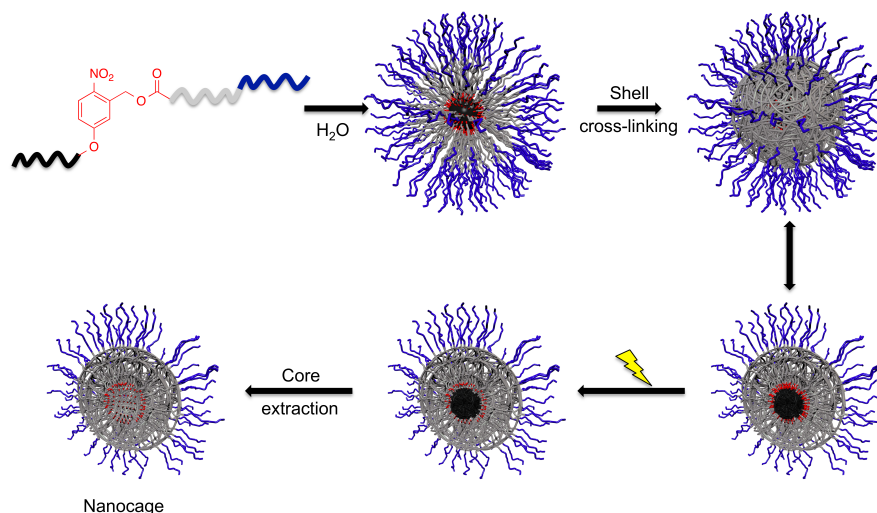


Figure 8.1: Schematic representation for the formation of nanocages driven by light.

The synthesis of the copolymers used as precursors of the nanocages was presented in chapter 5. In the first part of this chapter, the photocleavage of the block copolymers is studied in non-selective solvents to determine the optimal irradiation conditions. Section 8.3 presents the formation of PAA nanocages from PS-*hv*-PAA copolymers. In section 8.4, the formation of PDMAEMA and PDMAEMA-*b*-POEGMA nanocages from PtBA-*hv*-PDMAEMA diblock and PtBA-*hv*-PDMAEMA-*b*-POEGMA triblock copolymers is presented. In the last section of the chapter, the ability of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages to respond to pH and thermal stimuli is detailed. Moreover, the functionalization of the internal cavity of the nanocage is demonstrated.

8.2 Photocleavage in non-selective solvents

The optimization of the photocleavage conditions was carried out with the PtBA₁₁₈-*hv*-PDMAEMA₁₄₁ diblock copolymer in EtOH (*C* = 2.5 g/L). Upon irradiation, this copolymer dissociates into two homopolymers, one PDMAEMA bearing the deprotected carboxylic acid function and one PtBA bearing the nitrosobenzaldehyde counter part (Figure 8.2).

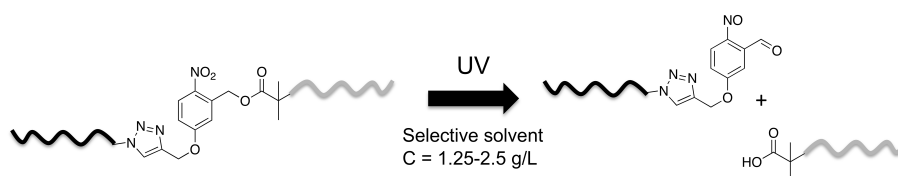


Figure 8.2: Cleavage of linear block copolymers into two dissociated blocks upon light irradiation.

Figure 8.3 presents the optimization of the photocleavage conditions. The photocleavage was carried out with lamps having their maximum emission wavelength at 365 (a-d) and at 300 nm (e-f). The effect of the irradiation time was studied. The photocleavage was followed by UV-Vis spectroscopy by recording the evolution of the absorption spectra of the copolymer with irradiation time (a, c and e). At the end of the photo-

cleavage, the irradiated solutions were analyzed by GPC (b, d and f) to check the dissociation of the copolymers into two homopolymers.

The UV-Vis spectra of the block copolymer solutions display the typical behavior of irradiated *o*-nitrobenzyl ester functions (Figures 8.3a, c and e). At the beginning of the photocleavage, the UV-Vis spectra display a band at $\lambda_{max} = 309$ nm, corresponding to the *o*-nitrobenzyl ester functions. Upon irradiation, the intensity of this band decreases since the ester bonds are cleaved and a band centered at $\lambda_{max} = 345$ nm appears due to the formation of the nitrosobenzaldehyde functions in solution. When irradiation time is further increased, the intensity of the nitrosobenzaldehyde band also starts to decrease (Figure 8.3a). This decrease is most probably the consequence of the dimerization of the nitrosobenzaldehyde groups [4, 5].

Concerning the irradiation wavelength, one can note that the photocleavage is more efficient at 300 nm than at 365 nm (Figure 8.3 c and e). Indeed, respectively 4 or 40 min were needed to obtain similar UV-Vis spectra when the irradiation was carried out at 300 nm (e) or 365 nm (b). This can be rationalized by three factors, the first is the fact that the photosensitive moiety absorbs more light at 300 nm since the λ_{max} is located at 309 nm. The second factor is the irradiation intensity, indeed the 300 nm lamps used for the cleavage are almost 8 times more powerful than the 365 nm ones.¹ The last factor explaining the increased efficiency is the presence in the medium of nitrosobenzaldehydes produced during the photocleavage, which are also chromophores that absorb light ($\lambda_{max} = 345$ nm) and thus act as competitors with *o*-nitrobenzyl moieties for light absorption at 350 nm.

Figures 8.3b, d and f present overlays of the GPC chromatograms of the copolymer before (dashed curve), after (full curve) irradiation and of the homo-PtBA precursor (dotted curve) for the photocleavage of PtBA₁₁₈-*hv*-PDMAEMA₁₄₁. Figure 8.3b displays the GPC curve of the PtBA₁₁₈ and PDMAEMA₁₄₁ irradiated 3h at 365nm. The photocleav-

¹Light intensity at 300 nm = 41.6 mW/cm²; light intensity at 365 nm = 5.10 mW/cm².

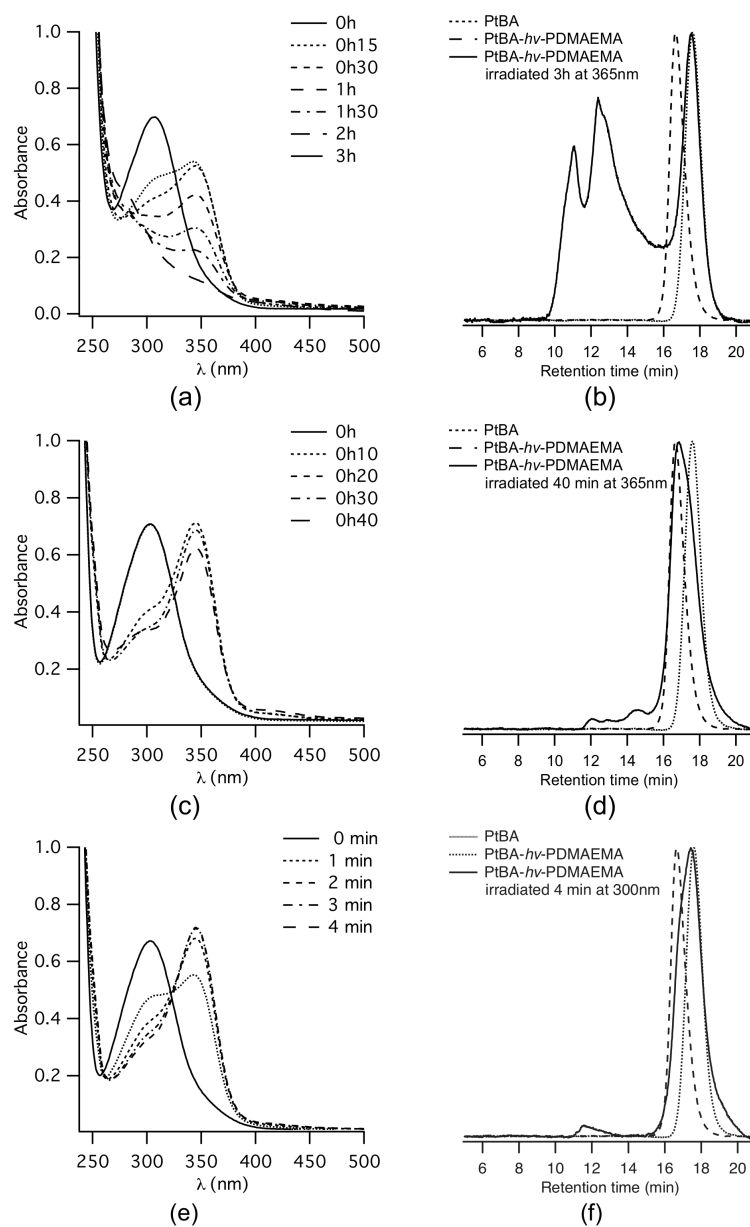


Figure 8.3: Optimization of the photocleavage conditions for PtBA₁₁₈-*hν*-PDMAEMA₁₄₁ in EtOH (C = 2.5g/L): evolution of the UV-Vis spectra (a,c,e), GPC curves of the precursors and of the irradiated copolymer (b,d,f).

age under these conditions is quantitative as the peak associated to the block copolymer is no longer observed and that a peak corresponding to the PtBA appeared. However, these conditions are not suited for the photocleavage since high molar mass populations are observed indicating side reactions during the photocleavage, possibly a cross-linking of the PDMAEMA.

When irradiation time was reduced (Figure 8.3d), the side reactions and the cleavage degree decreased. Indeed, the peak centered at 16.8 min displays a bimodal distribution corresponding to the contribution of PtBA, PDMAEMA and of PtBA-*hv*-PDMAEMA with a major contribution from the block copolymer. It should be noted that the signal corresponding to the dissociated PtBA₁₁₈ and PDMAEMA₁₄₁ are superimposed in the same peak. This was confirmed by the GPC analysis of an equimolar mixture of PtBA₁₁₈ and PDMAEMA₁₄₄ homopolymers.

The photocleavage of the block copolymer was also realized at 300 nm (Figure 8.3f). In the GPC curve, we can see that the side reactions are less pronounced than in the case of the irradiation at 365 nm. However, four minutes of irradiation are not sufficient to quantitatively dissociate the block copolymer into two homopolymers. The major contribution to the bimodal peak is due to the PtBA and PDMAEMA homopolymers but traces of the block copolymer are still visible.

The photocleavage of the PtBA-*hv*-PDMAEMA-*b*-POEGMA and PS-*hv*-PAA copolymers were realized under these conditions. Figure 8.4a presents the evolution of the UV-Vis spectrum during the irradiation of a solution of PtBA₁₄₅-*hv*-PDMAEMA₅₇-*b*-POEGMA₁₀₄ in EtOH at $C = 2.5$ g/L and the GPC chromatogram of the polymer before and after irradiation. The UV-Vis spectrum also displays the typical behavior of the *o*-nitrobenzyl function under irradiation. The GPC² shows that the irradiation of the triblock copolymer leads to the dissociation of the

²On the GPC chromatograms of the irradiated block copolymers, the difference in intensity between the two peaks associated to the cleaved blocks is due to the fact that chemically different polymer blocks have different refractive index increments (dn/dc) in solution, resulting in different magnitudes for the signal of the RI detector.

copolymers into PtBA homopolymer and into PDMAEMA-*b*-POEGMA copolymer. Moreover, the side reactions are here almost absent.

The photocleavage of PS₄₆-*hν*-PAA₅₀ was carried out in THF (*C* = 1.25 g/L). In this case, four minutes of irradiation were not sufficient to quantitatively cleave the *o*-nitrobenzyl ester junction (Figure 8.4b). This is most probably the consequence of the absorption of light by THF, which thus acts as a competitor for *o*-nitrobenzyl moieties.

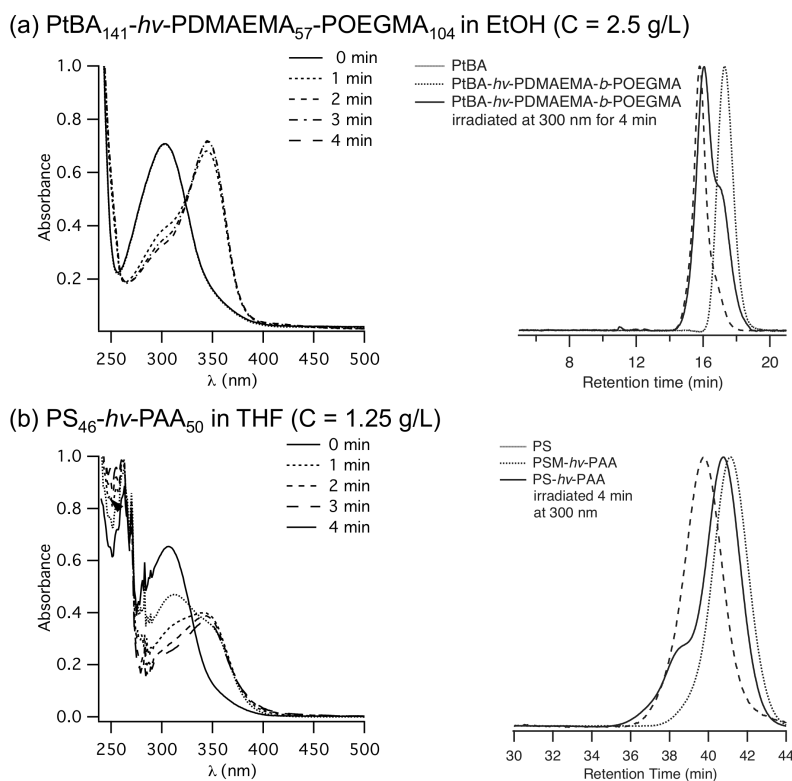


Figure 8.4: Photocleavage in non-selective solvents followed by UV-Vis spectroscopy and GPC: (a) PtBA₁₄₅-*hν*-PDMAEMA₅₇-*b*-POEGMA₁₀₄ in EtOH (*C* = 2.5g/L), (d) PS₄₆-*hν*-PAA₅₀ in THF(*C* = 1.25g/L).

The conclusion of those experiments is that the photocleavage of the block copolymers should be carried out at 300 nm to observe an efficient

dissociation. In the case of the PS-*hv*-PAA copolymer, the irradiation duration should be longer than 5 min to ensure the quantitative cleavage of the *o*-nitrobenzyl junction. In the case of copolymers composed of a PDMAEMA block, the irradiation duration should be limited to less than 5 min to avoid side reactions. The irradiation of the shell cross-linked micelles will thus be realized in several irradiation-extraction steps.

8.3 Formation of PAA nanocage

The first step for the formation of nanocages is the micellization of the PS-*hv*-PAA in water. Two polymers were selected to demonstrate the viability of our approach: PS₄₆-*hv*-PAA₅₀ and PS₄₆-*hv*-PAA₁₃₅. The experiments described below were realized within the framework of the Master thesis of Roland Duchêne [6]. The micellization of the copolymers was carried out by the cosolvent method. The copolymers are first dissolved in a non-selective solvent (DMF, $C_{pol} = 10$ g/L), the selective solvent (H₂O at pH = 8) is then added dropwise in the solution to induce the precipitation of the PS blocks. DMF is finally removed from the solution by dialysis ($C_{pol,final} = 2$ g/L). The micelles are then analyzed by DLS, AFM, TEM and cryoTEM. Figure 8.5 presents the characterization of the PS₄₆-*hv*-PAA₁₃₅ micelles in water.

The first DLS results for the micellization of PS-*hv*-PAA copolymers in water (pH = 8) demonstrated that the micelles formed under these conditions were characterized by a polyelectrolyte effect. The population of the isolated micelles even disappeared in some samples. To solve this issue, the micellizations were conducted at pH = 8 and the micelles solution was dialyzed against water at pH = 5. The use of slightly acidic water for the dialysis ensures the partial protonation of the carboxylic acid functions of the PAA corona and thus decreases the magnitude of the polyelectrolyte effect. In figure 8.5a (dotted curve), the CONTIN size distribution of PS₄₆-*hv*-PAA₁₃₅ micelles ($C = 2$ g/L, pH = 5) is presented. The sample is still characterized by a polyelectrolyte effect with a population centered at 380 nm, which is odd considering the composition of the investigated copolymer.

To decrease the polyelectrolyte effect, we studied the effect of the copolymer concentration in the micellar solution (Figure 8.5b) and the effect of the addition of a monovalent salt (Figure 8.5b, NaCl) in the solution. Indeed, the decrease of the polymer concentration induces the dilution of the charged micelles in solution and thus reduces the electrostatic interactions between them. Moreover, the addition of salt in the solution induces a screening of the charges of the PAA corona.

In figure 8.5a, we observed that the decrease of the polymer concentration in solution leads to the decrease in of the polyelectrolyte effect. At a concentration of 0.01 g/L, only one peak is observed in the CONTIN size distribution diagram with a tailing at high $R_{h,app}$. This peak corresponds to the contribution of two unresolved populations, isolated micelles and "polyelectrolyte micelles" with similar sizes. The dilution of the solution to lower concentrations should lead to the disappearance of the polyelectrolyte effect. However, the measurements realized at 0.01 g/L were already close to the detection limit of the DLS and thus this experiment was not realized.

Figure 8.5b presents the effect of the addition of NaCl in the solution studied by DLS. In the CONTIN size distribution diagram, we can conclude that the addition of salt in the solution does not lead to the disappearance of the polyelectrolyte effect.

Finally the micelles were characterized by AFM, TEM and cryoTEM (repectively Figures 8.5c, d and e). In the microscopy images we can see that the PS₄₆-*h*v-PAA₁₃₅ micelles adopt a spherical morphology. The micellar radii were respectively of 6.8, 8 and 9 nm for AFM, TEM and cryoTEM measurements.

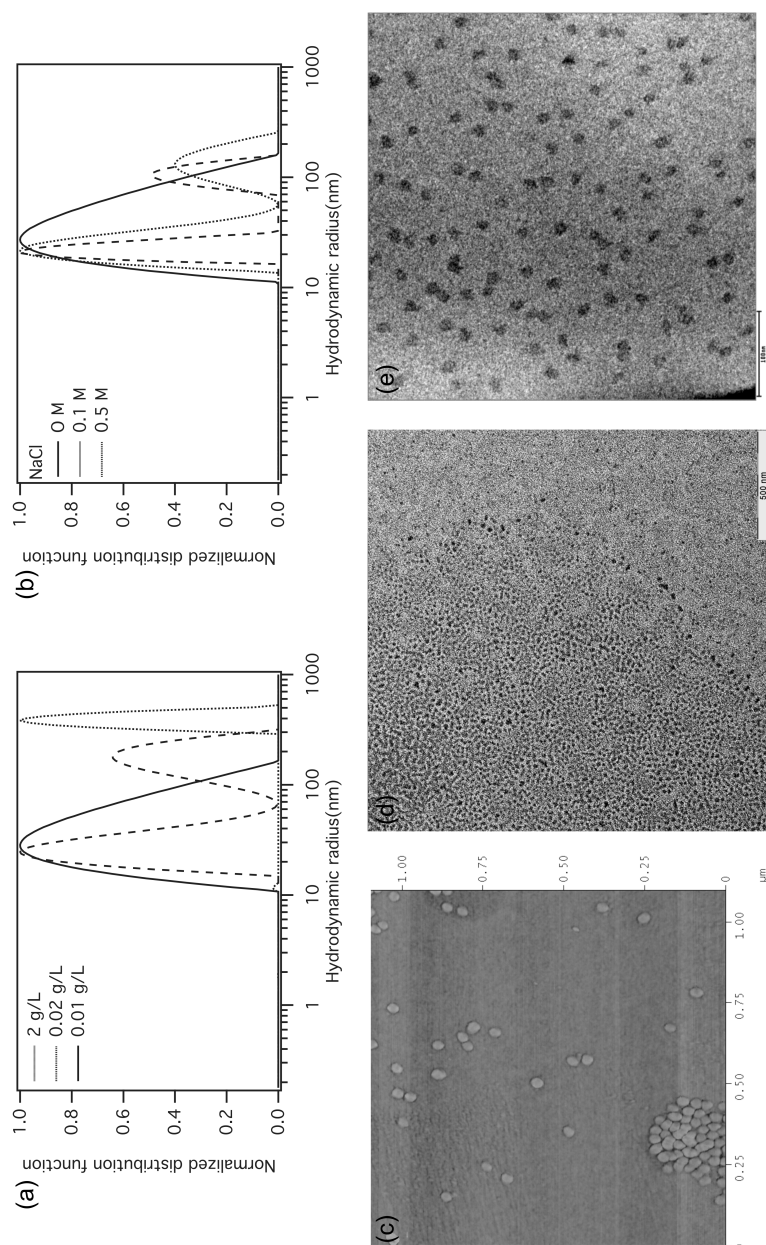


Figure 8.5: Micellization of PS₄₆-hv-PAA₁₃₅ in water: (a) evolution of the CONTIN size distribution with the polymer concentration, (b) evolution of the CONTIN size distribution with the NaCl concentration, (c) AFM, (d) TEM and (e) CryoTEM images of the micelles.

Concerning the comparison of the size of the micelles determined by the different techniques, several explanations should be given. The first comment on these measurements is that the micellar environment is not the same in the different analysis. Indeed, DLS and cryoTEM measurements are realized in solution, whereas AFM (Figure 8.5c) and TEM (Figure 8.5d) are realized in the dry state. This changes in the environment have two major effects. First, aggregates of micelles that are formed during the drying process are observed in the dry state. Secondly, the discrepancy observed between the radii measured by DLS and by TEM-AFM is the consequence of a collapse of the micellar corona during the drying of the samples. Moreover, the AFM radius is determined by the height of the micelles on the substrate, the measure thus represents mainly the radius of the micellar core and not really the radius of the micelles. The second comment is on the cryoTEM image (Figure 8.5e), which was recorded without staining. The micelles observed here thus represent the core of the micelles with a part of the corona. Indeed, the contrast in TEM is the consequence of the absorption of electrons by the polymeric material. Dense area of the sample (the micellar core) will thus stop more electrons than the less densely packed part of the sample (micellar corona).

Table 8.1 presents the summary of the sizes for the PS₄₆-*hv*-PAA₅₀ and PS₄₆-*hv*-PAA₁₃₅ micelles, as determined by DLS, AFM, TEM and cryoTEM.

Polymer	$R_{h,app}$ (nm)	PDI	R_{AFM}^a (nm)	R_{TEM} (nm)	$R_{cryoTEM}$ (nm)
PS ₄₆ - <i>hv</i> -PAA ₅₀	12	ND ^b	5.5 ± 0.5	7 ± 1.1	8 ± 0.7
PS ₄₆ - <i>hv</i> -PAA ₁₃₅	28	0.23	6.8 ± 1.3	8 ± 1.3	9 ± 0.9

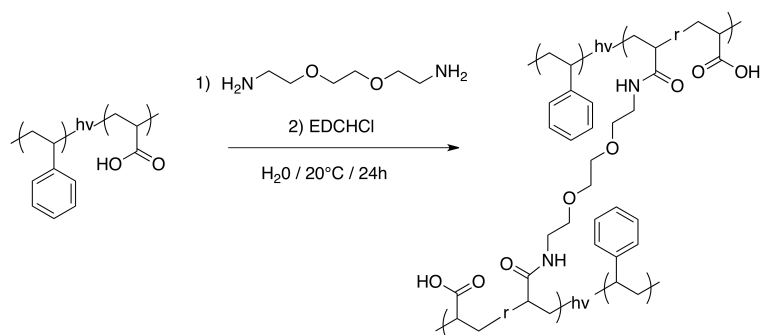
^a The radius represents here the height/2 of the micelles determined by AFM.

^b The PDI was not determined because the sample was characterized by a polyelectrolyte effect and two populations were observed.

Table 8.1: Typical sizes for the PS-*hv*-PAA micelles.

8.3.1 Shell cross-linking

The second step for the PAA nanocages formation is the chemical cross-linking of the PAA corona. This cross-linking was realized by carbodiimide coupling, under similar conditions than the ones developed by Wooley's group [7–10]. The cross-linking reaction was carried out with 2,2'-(ethylenedioxy)-bis(ethylamine) and N-(3-dimethylamino- propyl)-N'-ethylcarbodiimide hydrochloride in water (Scheme 8.1).



Scheme 8.1: Conditions for PS-*hv*-PAA cross-linking.

We decided to maintain the cross-linking degree to rather low values since too high cross-linking may totally prevent the extraction of the PS forming the micellar core in the next step [8]. On the other hand, the cross-linking degree should be sufficient to maintain the integrity of the PAA nanocage after the cleavage of the junction [11]. With this in mind, the cross-linking reaction was realized with 0.05 equivalents of 2,2'-(ethylenedioxy)-bis(ethylamine) compared to the carboxylic functions of the PAA block. These conditions should ensure 10% of cross-linking, *i.e.* 10% of the carboxylic acid functions would react with the cross-linker.

The cross-linking of the micelles was then carried out by the addition of 2,2'-(ethylenedioxy)-bis(ethylamine) in the solution. The solution was stirred for 30 min to have homogeneous mixing of the diamine in the solution. The carbodiimide was then added dropwise into the solution. The solution was then stirred at 20°C for 24h and the micelles were purified by 3 days of dialysis against water to remove small molecular mass

by-products. The success of the cross-linking reaction was monitored by FTIR and ^1H NMR. In FTIR, bands corresponding to amide functions are observed at 1560 cm^{-1} and 1650 cm^{-1} . Figure 8.6 presents the ^1H NMR spectrum of the cross-linked $\text{PS}_{46}\text{-}hv\text{-PAA}_{135}$ micelles in D_2O .

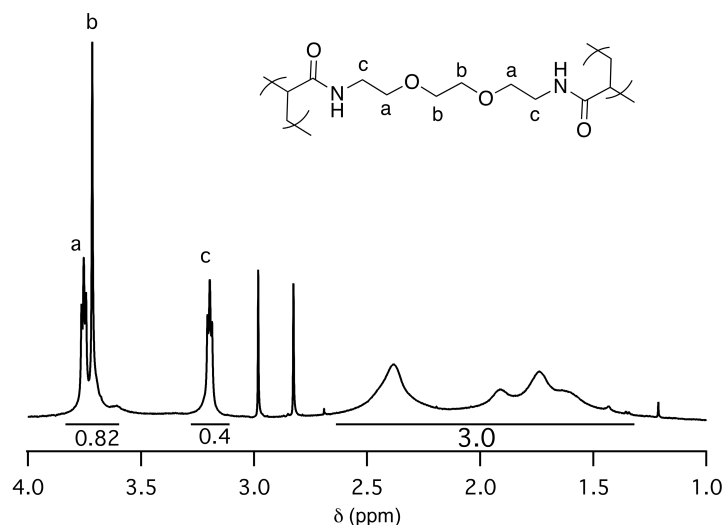


Figure 8.6: ^1H NMR spectrum of the cross-linked $\text{PS}_{46}\text{-}hv\text{-PAA}_{135}$ micelles in D_2O .

The cross-linking degree was determined by the integration of the PAA backbone and of the cross-linker signals (a, b and c). The PAA backbone integration was set to 3 and the cross-linking degree was determined by the integration of the proton in α of the amide function. Figure 8.6 demonstrates that the cross-linking reaction is quantitative, since the targeted 10% of cross-linking were obtained.

Finally, DLS measurements were realized to ensure that the cross-linking reaction led to intra-micellar cross-linking and not inter-micellar cross-linking [11]. Figure 8.7 presents an overlay of the CONTIN size distribution of $\text{PS}_{46}\text{-}hv\text{-PAA}_{135}$ micelles and $\text{PS}_{46}\text{-}hv\text{-PAA}_{135}$ cross-linked micelles. In the CONTIN size distribution diagram of the cross-linked micelle, no inter-micellar cross-linking was obtained during the cross-

linking reaction. Indeed, only one population centered at $R_{h,app} = 27$ nm is observed. The cross-linking of PS₄₆-*hv*-PAA₅₀ micelles was also quantitative. However, the DLS results did not allow to clearly determine if the cross-linking was quantitatively intra-micellar since two populations are still observed after cross-linking ($R_{h,app} = 15$ and 130 nm).

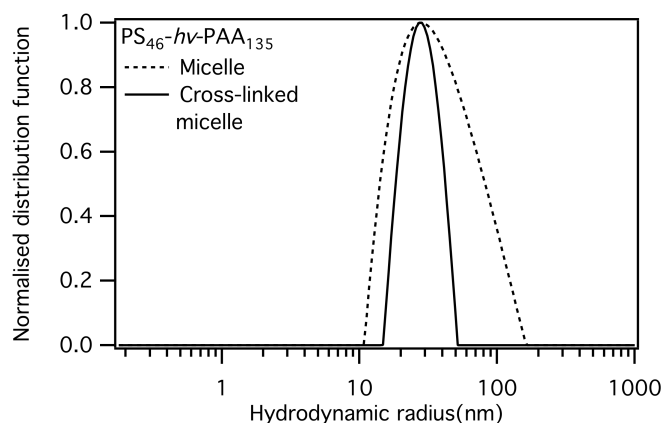


Figure 8.7: CONTIN size distribution diagrams of PS₄₆-*hv*-PAA₁₃₅ micelles and PS₄₆-*hv*-PAA₁₃₅ cross-linked micelles in water (pH = 5, C = 0.01 g/L).

8.3.2 Photocleavage

The photocleavage of the copolymer junctions was realized in water at $\lambda = 300$ nm according to the conditions determined in the photocleavage optimization (Section 8.2). The irradiation of the cross-linked micelles in water led to the formation of PAA nanocages loaded with PS.

The irradiation was monitored by UV-Vis spectroscopy. Figure 8.8 presents the evolution of the UV-Vis spectra during irradiation of PS₄₆-*hv*-PAA₁₃₅ cross-linked micelles in water (pH = 6, C = 1.5 g/L). In the UV-Vis spectra, the typical behavior of *o*-nitrobenzyl esters under irradiation is observed. The band at $\lambda_{max} = 309$ nm, corresponding to the *o*-nitrobenzyl ester functions decreases with irradiation time whereas the band centered at $\lambda_{max} = 345$ nm appears with the formation of ni-

trosobenzaldehyde in solution. In comparison to the results obtained in non-selective solvents (Figure 8.4b), where sharp transitions were observed, the variations in the UV-Vis spectra are here subtle. This is most probably the consequence of the scattered light by the micelles in solution³, similarly to the PMPMA-*b*-POEGMA micelles in solution (cfr. Section 7.3, Chapter 7).

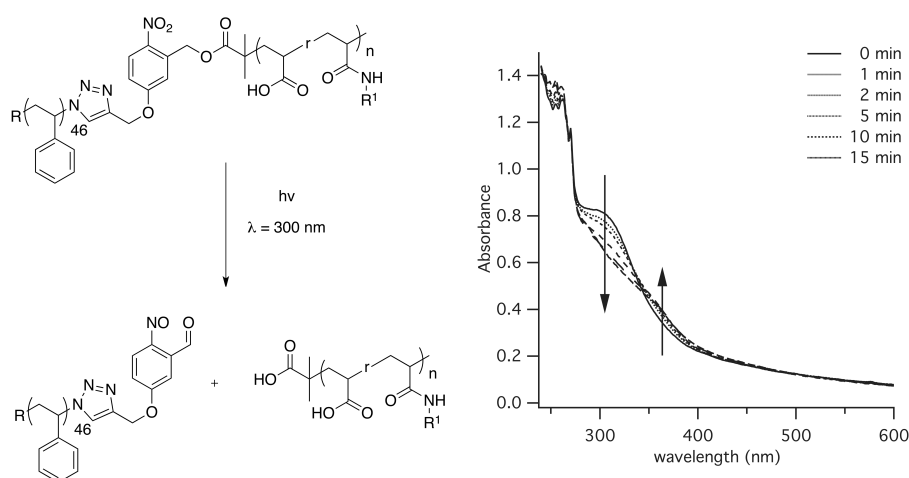


Figure 8.8: Evolution of the UV-Vis spectra during irradiation of PS₄₆-*hν*-PAA₁₃₅ cross-linked micelles in water (pH = 6, C = 1.5 g/L).

8.3.3 Core extraction

The last step for the PAA nanocage formation is the extraction of the PS forming the micellar core. The first investigated technique for the extraction is the dialysis of the loaded nanocages in a THF/H₂O mixture. This approach was successfully employed by O'Reilly's group to produce PAA nanocages [1]. In our case, the dialysis was not successful for the extraction of the PS. Indeed, ¹H NMR analysis of the PAA nanocages still revealed the presence of a large quantity of PS after more than 10 days of dialysis. In a practical way, we have asked ourselves how the

³The scattered intensity scales inversely with the wavelength of the incident light to the power of four [12].

authors manage to extract PS chains from the nanocages in a mixture of THF/H₂O as the PS₄₆ is already insoluble in THF with a small added volume of H₂O (volume fraction of THF/H₂O = 100/1, $C_{PS} = 1\text{g/L}$).

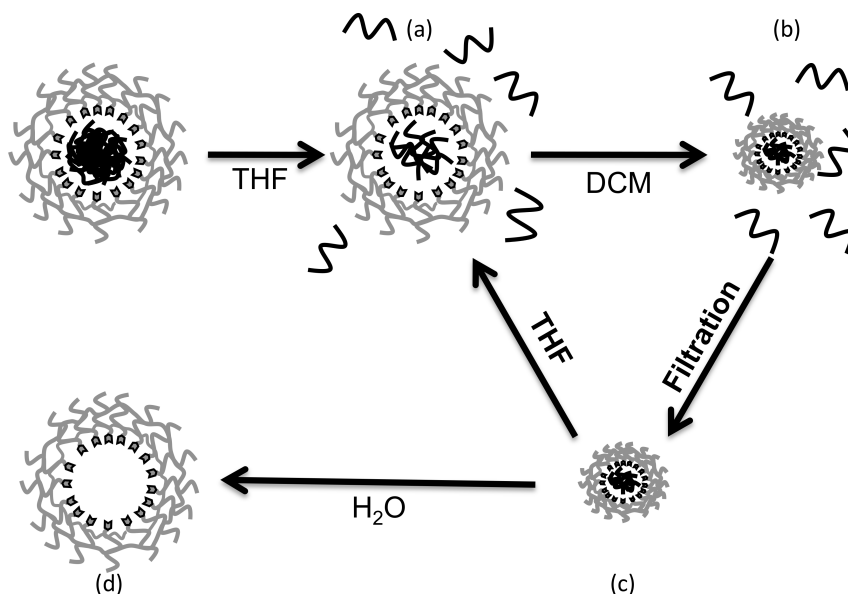


Figure 8.9: Schematic representation of the selective/non-selective solvent extraction process.

Another strategy was thus adopted for the extraction of the PS from the PAA nanocages: the selective/non-selective solvent extraction cycles (latter on referred as extraction cycles). The principle of this technique is illustrated in figure 8.9. The PS loaded nanocages are first dissolved in THF, which is a non-selective solvent of the system. The nanocages are thus in their swollen state and the PS chains can diffuse through the nanocage corona (a). After 24h of stirring, the THF is removed under vacuum. The solid mixture of PS and nanocage is then redispersed in DCM, which is a selective solvent of the system. The PAA nanocages remain in the collapsed state whereas the PS chains are dissolved (b). The solution is then stirred for 24h to solubilize the maximum of the extracted PS. The solution is afterward filtered (c) and the collapsed nanocages are redispersed in THF to restart an extraction cycle. This

procedure is repeated several times, until PS is totally extracted (d).

This methodology was applied on the PAA nanocages. After five extraction cycles respectively more than 93% and 97% of the PS was extracted from the PAA₅₀ and PAA₁₃₅ nanocages. Figure 8.10 presents an overlay of the ¹H NMR spectra of PS₄₆-*hν*-PAA₁₃₅ diblock copolymer (dashed curve) and of extracted PAA₁₃₅ nanocages (full curve) in d7-DMF. In this figure, the decrease of the PS aromatic signals demonstrate that a large quantity of the PS chains was extracted from the PAA nanocages, the integral of the aromatic zone reveals that 97% of the PS was extracted. We were unable to remove the small amount of remaining PS with additional selective/non-selective solvent cycles.

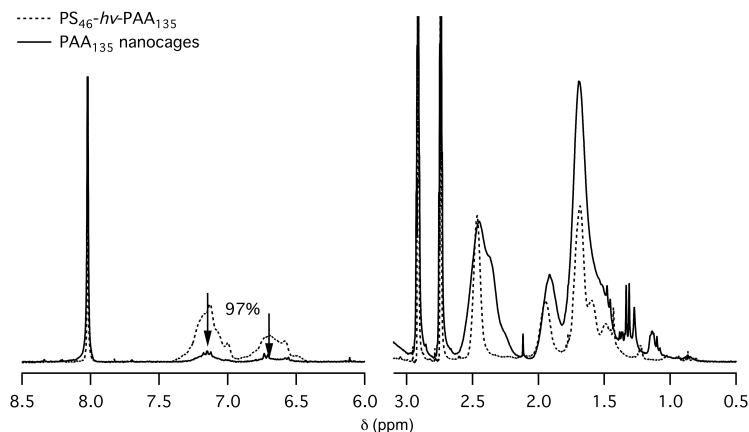


Figure 8.10: Overlay of the ¹H NMR spectra of PS₄₆-*hν*-PAA₁₃₅ diblock copolymer (dashed curve) and of extracted PAA₁₃₅ nanocages (full curve) in d7-DMF.

The nanocages were analyzed in DLS. Figure 8.11 presents the CONTIN size distribution diagram of PS₄₆-*hv*-PAA₁₃₅ cross-linked micelles and PAA₁₃₅ nanocages in water (pH = 5, C = 0.01 g/L). In the size distribution diagram, the population of the nanocages is slightly shifted to higher hydrodynamic radius, respectively $R_{h,app} = 27$ and 34 nm for the cross-linked micelles and the nanocages. This increase in the hydrodynamic radius was also monitored for the PAA₅₀ nanocages with a variation from 12 to 14 nm. The increase of the apparent hydrodynamic radius was expected since the core extraction removes the constraint of the core on the PAA corona leading to the expansion of the corona [1, 13]. It should be noted that the size variation between the PS-*hv*-PAA micelles and the PAA nanocages is less pronounced than in the examples reported by O'Reilly and Wooley, where a variation from tens to hundreds of nanometers was observed [1, 13]. The fact that a slight increase of the $R_{h,app}$ is observed whereas a large increase was expected is probably the consequence of the PS chains remaining in the PAA nanocages which are hypothetically still bonded to the nanocages by the junction.

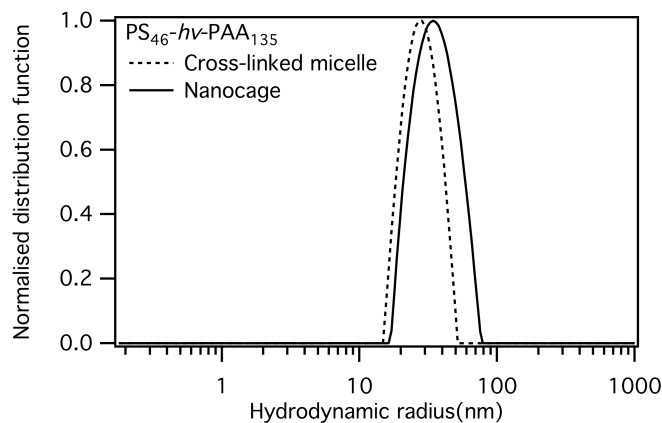


Figure 8.11: CONTIN size distribution diagram of PS₄₆-*hv*-PAA₁₃₅ cross-linked micelles and PAA₁₃₅ nanocages in water (pH = 5, C = 0.01 g/L).

8.4 Formation of PDMAEMA and PDMAEMA-POEGMA nanocages

The formation of PDMAEMA nanocages is realized with PtBA-*hv*-PDMAEMA diblock copolymers whereas the PDMAEMA-*b*-POEGMA nanocages are built from PtBA-*hv*-PDMAEMA-*b*-POEGMA triblock copolymers. The synthesis of the investigated copolymers is presented in chapter 5. Among all the di- and triblock copolymers available for the formation of nanocages (Tables 5.4 and 5.5), we selected PtBA₁₁₈-*hv*-PDMAEMA₁₄₁ and PtBA₁₄₅-*hv*-PDMAEMA₅₇-*b*-POEGMA₁₀₄ because the composition of these copolymers theoretically leads to spherical micelles when self-assembled in water. To simplify the discussion, the block copolymers are later on referred as PtBA-*hv*-PDMAEMA and PtBA-*hv*-PDMAEMA-*b*-POEGMA.

The micellization of the di- and triblock copolymers was realized by the cosolvent method. The copolymers are first dissolved in a non-selective solvent (DMF, $C_{pol} = 12.5$ g/L), the selective solvent (H₂O at pH = 6) is then added dropwise in the solution to induce the precipitation of the PtBA block. DMF is finally removed from the solution by dialysis ($C_{pol,final} = 2.5$ g/L). The micelles are then analyzed by DLS and AFM. Figure 8.12 presents the characterization of the PtBA-*hv*-PDMAEMA and PtBA-*hv*-PDMAEMA-*b*-POEGMA micelles in water.

Figure 8.12a displays the CONTIN size distribution diagram of PtBA-*hv*-PDMAEMA in water (pH = 6, $C = 2.5$ and 0.1 g/L). Since PDMAEMA is a polybase, a polyelectrolyte effect was dreaded in these conditions. From the measurement at $C = 2.5$ g/L, the possibility of a polyelectrolyte effect can not be excluded since the broad size distribution of the micelles may indicate the presence of several populations. To solve this issue, the concentration of micelles in the medium was reduced to decrease the electrostatic interactions ($C = 0.1$ g/L). The decrease in the micellar concentration led to the narrowing of the size distribution. The apparent hydrodynamic radius of the micelles did not change when the micellar concentration was decreased ($R_h = 28$ nm, PDI from cumulant analysis: 0.153).

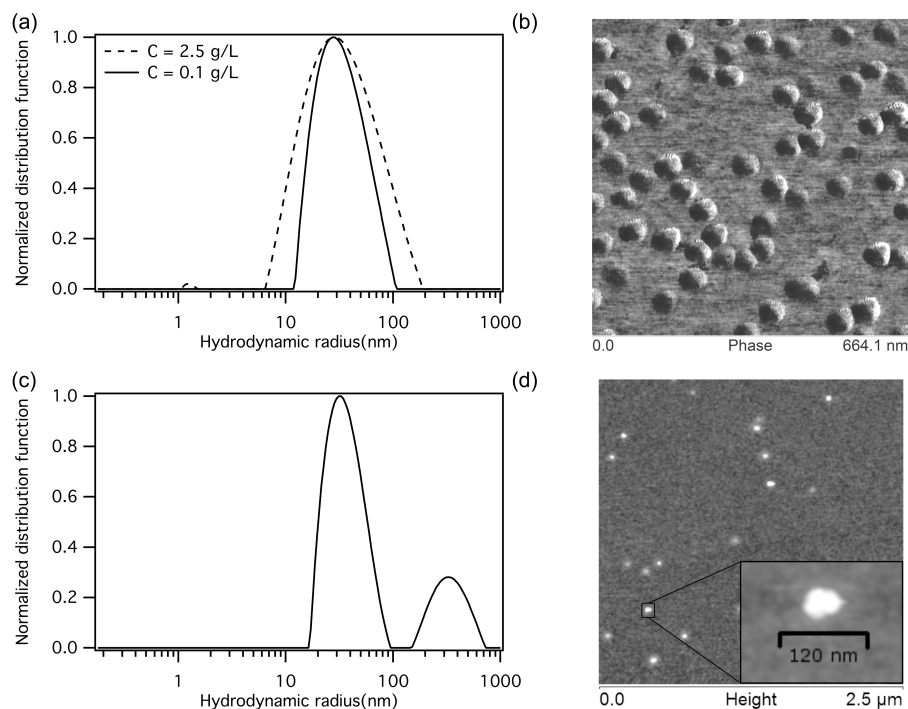


Figure 8.12: (a) CONTIN size distribution diagram, (b) AFM image of PtBA₁₁₈-*hv*-PDMAEMA₁₄₁ micelles in water ($C = 0.1$ g/L, pH = 6), (c) CONTIN size distribution diagram of PtBA₁₄₅-*hv*-PDMAEMA₅₇-*b*-POEGMA₁₀₄ micelles in water $C = 0.1$ g/L, pH = 6) and (d) AFM image of PtBA₁₄₅-*hv*-PDMAEMA₅₇-*b*-POEGMA₁₀₄ micelles in water ($C = 0.025$ g/L, pH = 6).

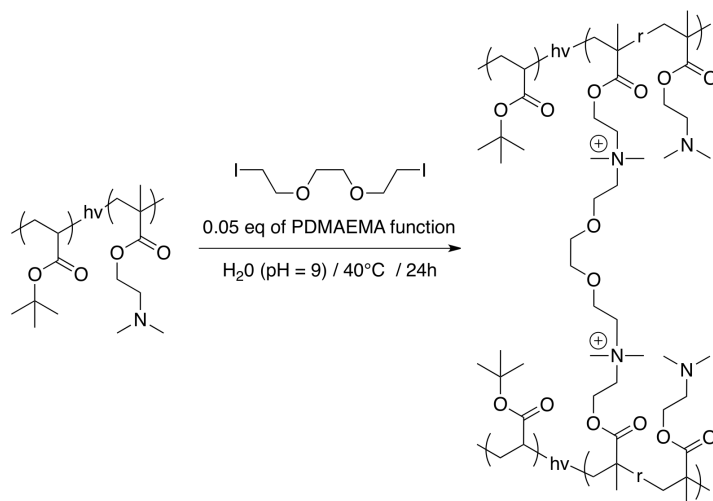
Figure 8.12b presents the AFM image of the micelles ($C = 0.1$ g/L) spin-coated on a silicium substrate. In this image, spherical micelles are observed for the PtBA-*hv*-PDMAEMA sample in water. From the AFM image, the size of the micellar core can be measured. The height of the objects measured by AFM can be ascribed to the size of the micellar core with contributions of the collapsed coronal chains ($R_c = 5.8 \pm 0.7$ nm). On the other hand, the lateral dimension of the micelles in the AFM image is not used since this size is most of the time overestimated due to the tip/sample convolution. The discrepancy observed between the sizes measured by DLS and AFM was expected. Indeed the AFM size

corresponds to the collapsed core of the micelles whereas the hydrodynamic radius represents the radius of the micelles in solution, where the coronal chains are extended due to the electrostatic repulsion between the charged DMAEMA units.

Figure 8.12c presents the CONTIN size distribution diagram of PtBA-*hv*-PDMAEMA-*b*-POEGMA micelles in water (pH = 6). Two populations are observed in the CONTIN size distribution diagram ($R_h = 31$ and 320 nm). The population at high hydrodynamic radius could be the consequence of a polyelectrolyte effect or could be due to a small amount of aggregated micelles. Figure 8.12d presents the AFM images of the PtBA-*hv*-PDMAEMA-*b*-POEGMA micelles ($C = 0.025$ g/L) spin-coated on a silicon substrate. The micelles also adopt a spherical morphology in water ($R_c = 2.3 \pm 1.1$ nm) as shown on the AFM image (Figure 8.12d). In comparison to the PtBA-*hv*-PDMAEMA system, the size of the micelles in the dry state is surprisingly small. This could be the consequence of the collapse of the shell-corona into the core. Moreover, the AFM image of the PtBA-*hv*-PDMAEMA-*b*-POEGMA micelle in water also revealed the presence of aggregates of micelles (inset in the AFM image), which give new insight to the CONTIN size distribution. Indeed, the population at 320 nm is probably not the consequence of a polyelectrolyte effect but to the presence of cluster of micelles in the solution.

8.4.1 Shell cross-linking

The second step of the nanocage formation is the cross-linking of the PDMAEMA micellar shell. The cross-linking was here realized with conditions developed by Armes and coworkers using bis(2-iodoethoxy)ethane (BIEE) [11,14]. This bifunctional reagent selectively quaternizes the tertiary amine groups of the PDMAEMA block by a nucleophilic substitution of the iodine atoms by the amine functions of the polymer units (Scheme 8.2).



Scheme 8.2: Conditions for the PtBA-*hν*-PDMAEMA cross-linking.

Similarly to the PAA nanocages, the cross-linking degree of the shell was maintained low to facilitate PtBA extraction in the next step. The cross-linking was carried out by the addition of a solution of BIEE (0.05 eq. of amine functions, targeted cross-linking degree = 10%) in MeOH to a solution of the PtBA-*hν*-PDMAEMA micelles in water at pH = 9. The solution was allowed to stir for 24h at 40 °C. The micelles were purified by 3 days of dialysis against methanol to remove small molar mass by-products. The success of the cross-linking reaction was monitored by ^1H NMR. Figure 8.13 presents the ^1H NMR spectrum of the cross-linked PtBA-*hν*-PDMAEMA micelles in MeOD.

The cross-linking degree was determined by the integration of the $\text{CH}_2\text{-OC=O}$ of the PDMAEMA block and of the cross-linker signals (a and b). The $\text{CH}_2\text{-OC=O}$ of the PDMAEMA block integration was set to 2 and the cross-linking degree was determined by the integration of the cross-linker protons. Figure 8.13 demonstrates that the cross-linking reaction is quantitative, since the targeted 10% of cross-linking were obtained.

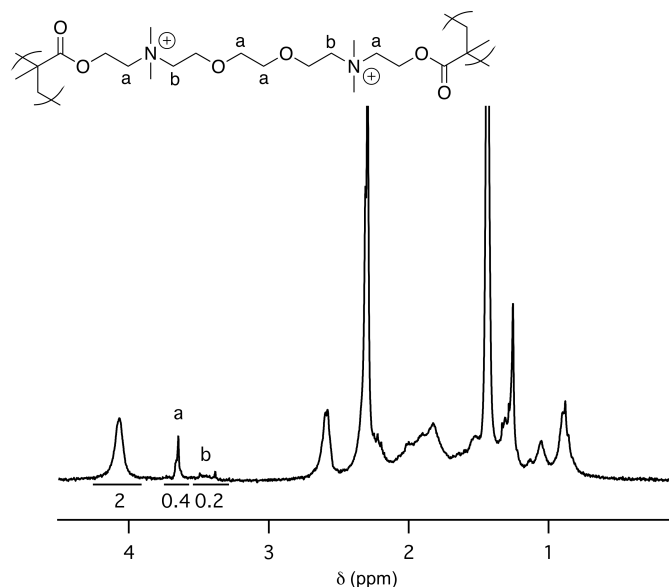


Figure 8.13: ^1H NMR spectrum of the cross-linked $\text{PtBA}_{118}\text{-}h\nu\text{-PDMAEMA}_{141}$ micelles in MeOD.

Finally, DLS measurements in selective and non-selective solvents were realized to ensure that the cross-linking was sufficient to maintain the integrity of the micelles in a non-selective solvent and that inter-micellar cross-linking did not occur during the reaction. Figure 8.14a presents an overlay of the CONTIN size distributions of $\text{PtBA-}h\nu\text{-PDMAEMA}$ shell cross-linked micelles in water and MeOH ($C = 0.1$ g/L). Only one population is observed in the non-selective solvent (methanol), which confirms that 10% of cross-linking are sufficient to avoid the disruption of the micelles. Moreover, the hydrodynamic ra-

dius of the shell cross-linked micelles in methanol is larger than in water ($R_{h,\text{water}} = 28 \text{ nm}$ and $R_{h,\text{MeOH}} = 43 \text{ nm}$). The increase in R_h between selective and non-selective solvents was expected since the PtBA is no longer collapsed and the micelles adopt their expanded form in methanol. This experiment also gives a good idea about the hydrodynamic radius of the nanocages after the extraction step. Since the broad size distribution observed in DLS analysis of the nanocages in MeOH does not allow to monitor the inter-micellar cross-linking, a CryoTEM experiment was realized. In figure 8.14b, one can see that the shell cross-linked micelles are isolated one from another and thus that the cross-linking was essentially intra-micellar.

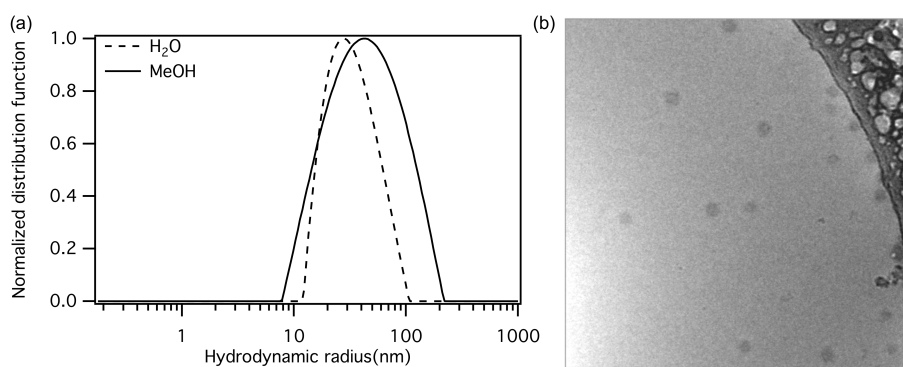


Figure 8.14: (a) CONTIN size distribution diagram PtBA- $h\nu$ -PDMAEMA shell cross-linked micelles in water and MeOH ($C = 0.1 \text{ g/L}$) and (b) CryoTEM image of the shell cross-linked micelles.

8.4.2 Photocleavage

The photocleavage of the copolymer junction was realized in methanol at $\lambda = 300 \text{ nm}$ according to the conditions determined by the photocleavage optimization (Section 8.2). The irradiation of the cross-linked micelles leads to the formation of PDMAEMA nanocages loaded with PtBA.

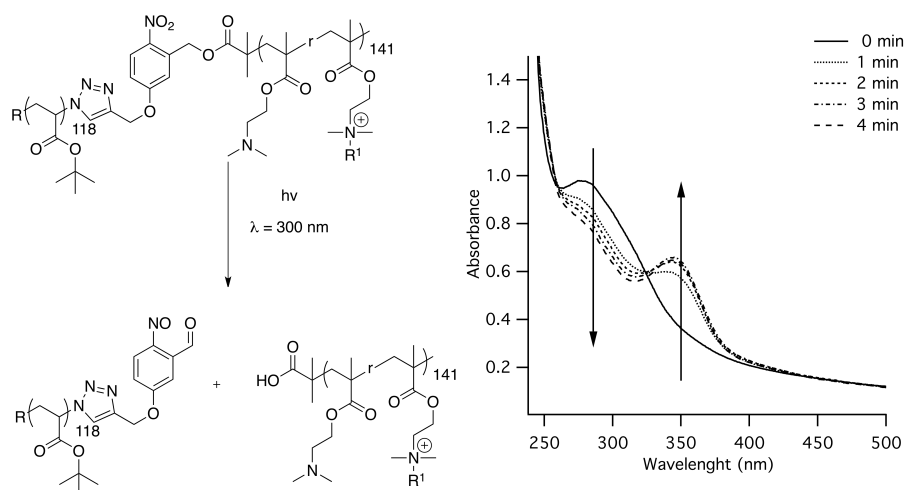


Figure 8.15: Evolution of the UV-Vis spectra during irradiation of PtBA₁₁₈-*hν*-PDMAEMA₁₄₁ cross-linked micelles in methanol ($C = 2.5$ g/L).

The irradiation was monitored by UV-Vis spectroscopy. Figure 8.15 presents the evolution of the UV-Vis spectra during irradiation of PtBA-*hν*-PDMAEMA cross-linked micelles in methanol ($C = 2.5$ g/L). In the UV-Vis spectra, the typical behavior of *o*-nitrobenzyl esters under irradiation is observed. The band at $\lambda_{max} = 280$ nm, corresponding to the *o*-nitrobenzyl ester functions, decreases with irradiation time whereas the band centered at $\lambda_{max} = 345$ nm appears with the formation of the nitrosobenzaldehyde functions in solution. As presented in section 8.2, 4 minutes of irradiation at $\lambda = 300$ nm are not sufficient to have a quantitative cleavage of the *o*-nitrobenzyl esters at this concentration. This feature is clearly visualized in the absorbance spectra, where the nitrobenzyl ester band is still present after 4 minutes of irradiation. In order to try to reach a quantitative cleavage of the junctions, we decided to start the extraction and to reiterate the irradiation when a big part of the PtBA chains are extracted from the nanocages.

8.4.3 Core extraction

Similarly to the PS-*hν*-PAA system, two extraction methods were tested: the dialysis against a non-selective solvent and the selective/non-selective solvent cycles. The dialysis of the loaded nanocages was realized in methanol. Unfortunately this technique did not allowed to separate the PtBA chains from the PDMAEMA nanocages.

The selective/non-selective solvent cycles were realized with water and methanol. In this case, the PtBA loaded nanocages are first dissolved in methanol, which is a non-selective solvent of the system. The nanocages are thus in the swollen state and the PtBA chains can diffuse through the nanocage corona. After 24h of stirring, MeOH is removed under vacuum. The solid mixture of PtBA and nanocages is then re-dispersed in water, which is a selective solvent of the nanocages. The PDMAEMA and PDMAEMA-*b*-POEGMA nanocages are thus dissolved whereas the PtBA chains are precipitated. The solution is then stirred for 24h to ensure the total dissolution of the nanocages. The solution is afterwards filtered using syringe filters (1.2 μ) and water is removed under vacuum. The nanocages are finally redispersed in MeOH to restart an extraction cycle. This procedure was repeated several times, until the whole PtBA is extracted.

The PtBA extraction was followed by recording ^1H NMR of the PtBA-PDMAEMA nanocage mixture after each extraction cycle. In figure 8.16, the evolution of the ^1H NMR spectrum of the PDMAEMA nanocages with the number of extraction cycle is plotted. The decrease of the *tert*-butyl signal clearly demonstrates the extraction of the PtBA chains from the nanocages. The extraction degree was determined by the comparison between the integral of the $\text{CH}_2\text{-OC=O}$ of the PDMAEMA (black curve) and of the *tert*-butyl signal (red curve) of the PtBA block. Figure 8.16b plots the extraction degree after each extraction cycle. After six extraction cycles, 97% of the PtBA was extracted from the PDMAEMA nanocages. It should be noted that the sample was irradiated a second time after three extraction cycles. The irradiation was carried out at 300 nm for 1 min in methanol. The extraction of PtBA

from the PDMAEMA-*b*-POEGMA nanocages was successfully realized by the same technique. In the case of the triblock copolymer, 95% of the PtBA was extracted from the nanocages.

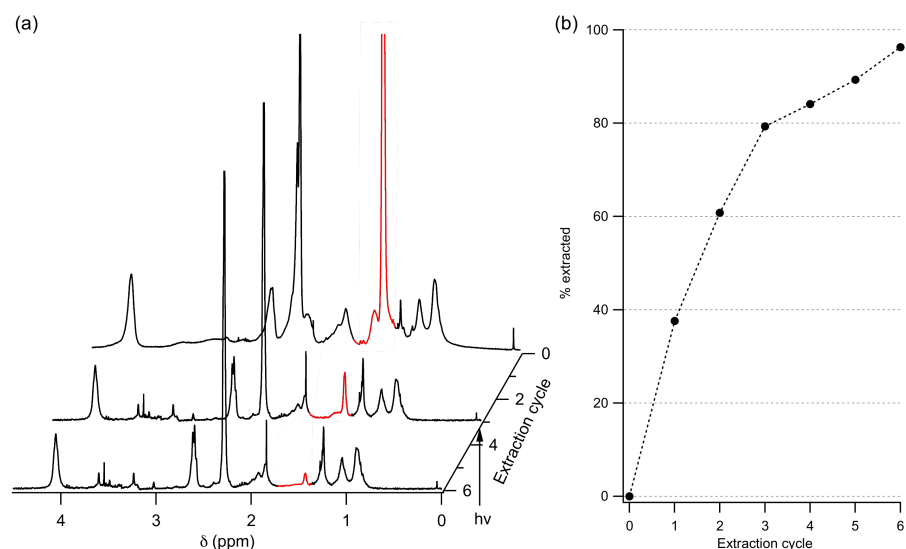


Figure 8.16: Evolution of the ^1H NMR spectrum of the PtBA-PDMAEMA nanocage mixture (a) and of the extraction degree (b) with the number of extraction cycle.

Since the PtBA extraction was almost quantitative, the nanocages were analyzed by DLS and AFM. Figure 8.17a presents the CONTIN size distribution diagram of PtBA-*h*v-PDMAEMA cross-linked micelles and PDMAEMA nanocages in water ($\text{pH} = 6$, $C = 0.1$ g/L). In the size distribution diagram, the population of the nanocages is shifted to higher hydrodynamic radius, respectively $R_h = 28$ and 48 nm for the cross-linked micelles and the nanocages. The hydrodynamic radius of the nanocages in water matches with the size of the shell cross-linked micelles in non-selective solvents ($R_{h,\text{MeOH}} = 43$ nm in section 8.4.1).

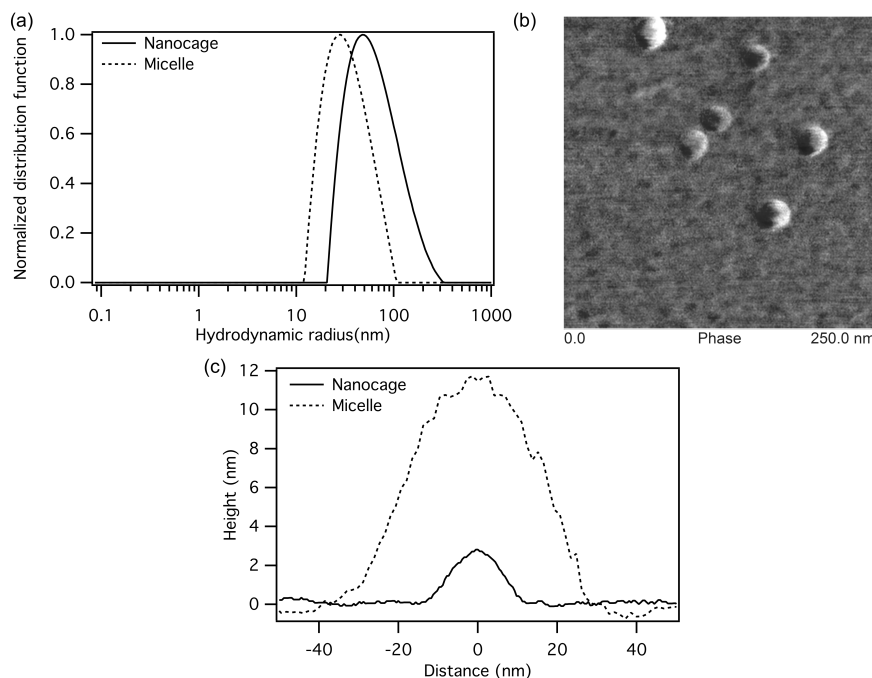


Figure 8.17: Characterization of the PDMAEMA nanocages: (a) CONTIN size distribution diagram of PtBA-*hν*-PDMAEMA cross-linked micelles and PDMAEMA nanocages in water (pH = 6, C = 0.1 g/L), (b) AFM image of the nanocages and (c) comparison of the PtBA-*hν*-PDMAEMA micelles and PDMAEMA nanocage profile.

Figure 8.17b presents the AFM image of the PDMAEMA nanocages. In this figure, spherical objects are observed after the collapse of the nanocage on the substrate ($R_c = 1.8 \pm 0.5$ nm). The height of the nanocages is much smaller in comparison to the PtBA-*hν*-PDMAEMA micelles (Figure 8.17c). The discrepancy observed between the micelles and the nanocages demonstrates the hollowed structure of the nanocages. Indeed, large spherical structures are observed when the micellar shell collapse on the micellar core during the drying process (Figure 8.18 left). On the other hand, the empty core of the nanocages forces the shell to collapse on itself and structures with smaller height are observed in AFM.

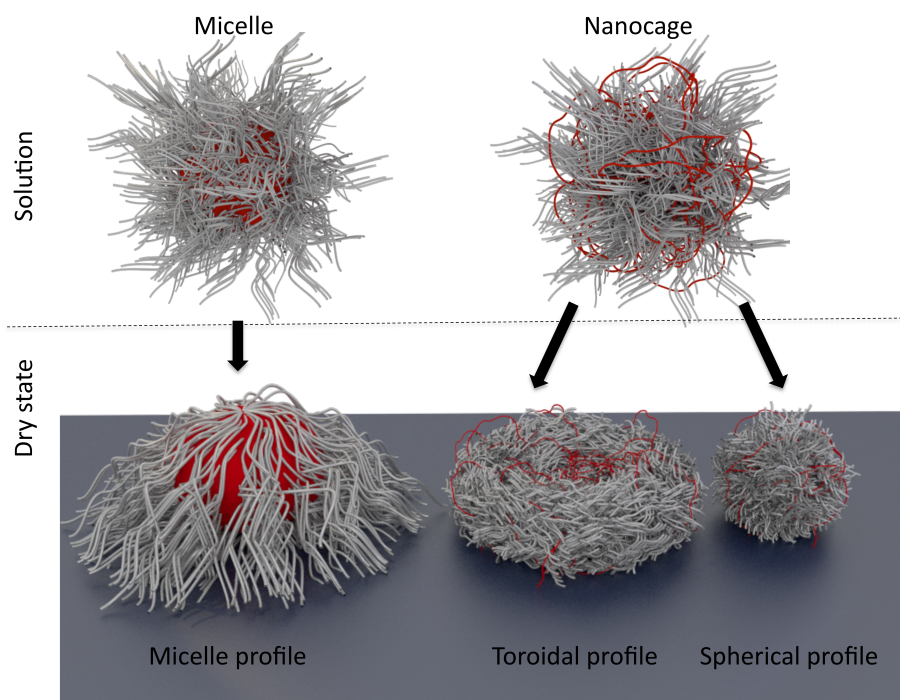


Figure 8.18: Schematic representation of the objects profile obtained after the drying process of a micelle and a nanocage.

Figure 8.18 displays the schematic representation of the profile of nano-objects obtained after the drying of a micelle and a nanocage on a substrate. The collapse of nanocages may lead to two distinctive types of object. The first one presents a toroidal profile and the second one a spherical profile. The toroidal profile is sometime observed for nano-objects with an empty core domain (nanocages and vesicles) [2,15]. This profile may be obtained as a function of several experimental factors: the cross-linking degree of the nanocage shell, the thickness of the shell, the size of the empty core and the existence of preferential interaction between the polymer and the substrate.

Figure 8.19 presents the DLS and AFM analysis of the PDMAEMA-*b*-POEGMA nanocages. The CONTIN size distribution diagram presents the same behavior than the PDMAEMA nanocages. The increase of the hydrodynamic radius of the PDMAEMA-*b*-POEGMA nanocages compared to the PtBA-*hν*-PDMAEMA-*b*-POEGMA micelles is observed (respectively $R_h = 74$ and 31 nm in Figure 8.19a). Figure 8.19b presents the AFM image of the PDMAEMA-POEGMA nanocages. Two types of structures may be observed in the AFM image, big structures with a $R_c = 20.6 \pm 0.5$ nm and smaller structures with a $R_c = 1.2 \pm 0.4$ nm. Since the size of the micellar core determined by AFM equals to 2.3 ± 1.1 nm, the big structures cannot correspond to the nanocages. However, the big structures may correspond to aggregated PDMAEMA-*b*-POEGMA nanocages, obtained after the redispersion of the nanocages in water. Similarly to the PDMAEMA nanocages, the PDMAEMA-*b*-POEGMA nanocages do not exhibit a toroidal profile but a spherical profile. The decrease of the nanocage height in comparison to the starting micelles also indicates their hollowed (Figure 8.19c).

Although the characterization of the nanocages by AFM does not present a toroidal profile, which would be an irrefutable proof of the hollowed structure of the nanocages, spectroscopic analysis of the PDMAEMA and PDMAEMA-*b*-POEGMA demonstrated that the core forming PtBA chains were extracted from the nanocages, thus proving their successful formation via a photocleavable approach.

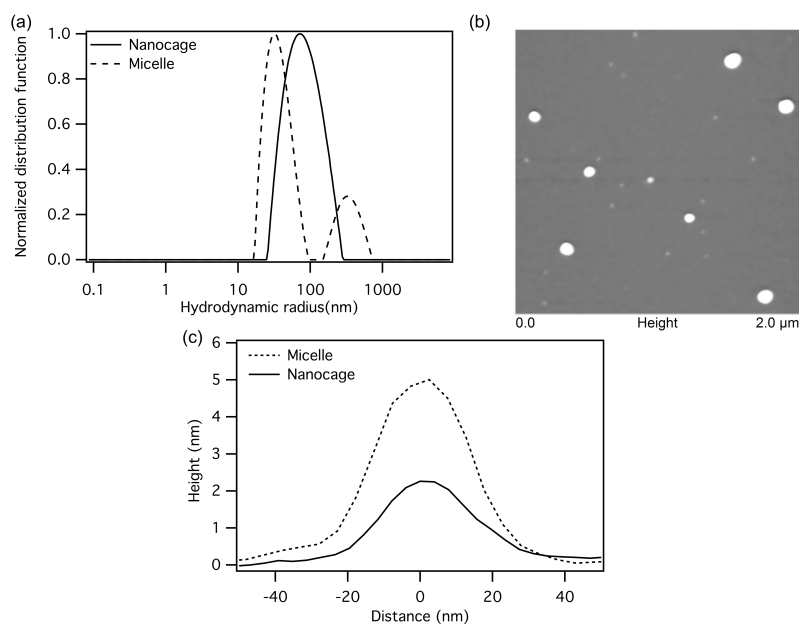


Figure 8.19: Characterization of the PDMAEMA-*b*-POEGMA nanocages: (a) CONTIN size distribution diagram of PtBA-*h**v*-PDMAEMA-*b*-POEGMA cross-linked micelles and PDMAEMA-*b*-POEGMA nanocages in water (pH = 6, C = 0.1 g/L), (b) AFM image of the nanocages and (c) PDMAEMA-*b*-POEGMA nanocage profile.

8.5 From nanocages to "smart" nanoreactors?

Following the successful formation of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages, some interesting features of the system should be highlighted: the ability of the nanocages to respond to external stimuli and the possibility to functionalize the internal cavity of the nanocages. Indeed, our approach allows the formation of nanocages in which the internal face is covered with carboxylic acid functions. These carboxylic acid groups may thus be chemically functionalized to add any desired properties to the nanocages (catalytic, fluorescent, *etc.*). Moreover the selected polymers are able to change their behavior in water in response to pH and temperature.

8.5.1 Functionalization of the nanocages

The availability of the internal carboxylic acids of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages was evidenced by fluorescence spectroscopy. The selective functionalization of the nanocages was carried out by the grafting of amine bearing chromophores (1-aminopyrene) via carbodiimide coupling. Figure 8.20 presents the functionalization conditions. A solution containing 1-aminopyrene and N,N'-dicyclohexylcarbodiimide (DCC) was added to a solution of the PDMAEMA nanocages. The mixed solution was allowed to stir for 24h at 20°C. The grafted nanocages were purified by dialysis against methanol to remove all traces of unreacted 1-aminopyrene. Finally, the fluorescence emission spectrum of the solution was recorded.

1-aminopyrene was selected for the nanocage functionalization because of the reactivity of its amine function toward peptidic coupling with carboxylic acids. We decided to select an amine with a low reactivity to demonstrate the potential and limitation of the system. We thus selected 1-aminopyrene since aromatic amines are less reactive than aliphatic amines toward peptidic coupling. Figure 8.21a presents the fluorescence emission spectra of 1-aminopyrene (PyNH₂, dashed curve) and N-(pyren-1-yl)acetamide (PyNHCOMe, full curve) in methanol (C = 1.05 × 10⁻⁴ mol/L, λ_{ex} = 328 nm). N-(pyren-1-yl)acetamide was ob-

tained by the amidation of acetic acid with 1-aminopyrene via carbodiimide coupling. It is worth to mention that the fluorescence emission spectrum of N-(pyren-1-yl)acetamide was recorded on the crude mixture. This explains the presence of unreacted 1-aminopyrene on the emission spectrum (band at $\lambda_{\text{ex}} = 428 \text{ nm}$). 1-aminopyrene has another advantage: since the fluorescence emission spectra before and after the coupling are significantly different. It is thus possible to qualitatively monitor the efficiency of the coupling reaction on the nanocage before the dialysis step.

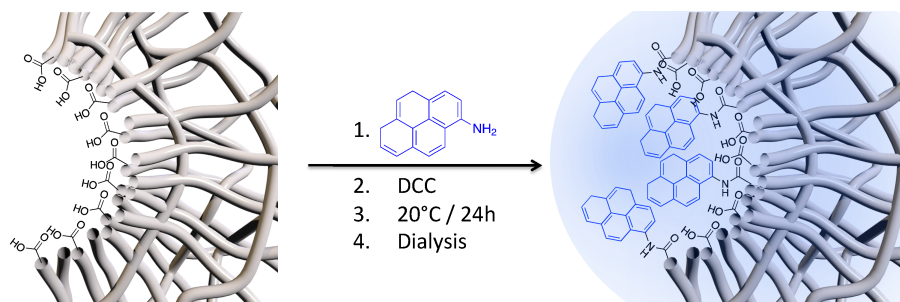


Figure 8.20: Schematic representation of the nanocage functionalization.

Figure 8.21b and c present the fluorescence emission spectra of PDMAEMA (b) and PDMAEMA-*b*-POEGMA (c) nanocages grafted with 1-aminopyrene (full curves). To ensure that the recorded fluorescence emission spectra are not the signature of encapsulated 1-aminopyrene in the nanocages, the same grafting experiment was realized with shell cross-linked micelles (dashed curves). The signal of the chromophores in the case of the nanocages is similar to the fluorescence signal of N-(pyren-1-yl)acetamide in MeOH, which proves that the chromophores are grafted onto the carboxylic acid functions of the nanocages. The experiments on the shell cross-linked micelles demonstrate that the dye cannot be grafted on the micelles since the carboxylic acid function are masked. These experiments also demonstrate that the fluorescence emission observed in the case of the nanocages is not the consequence of an encapsulation of 1-aminopyrene into the nanocage cavity as the "encapsulated" 1-aminopyrene was removed from the shell cross-linked micelles during the dialysis.

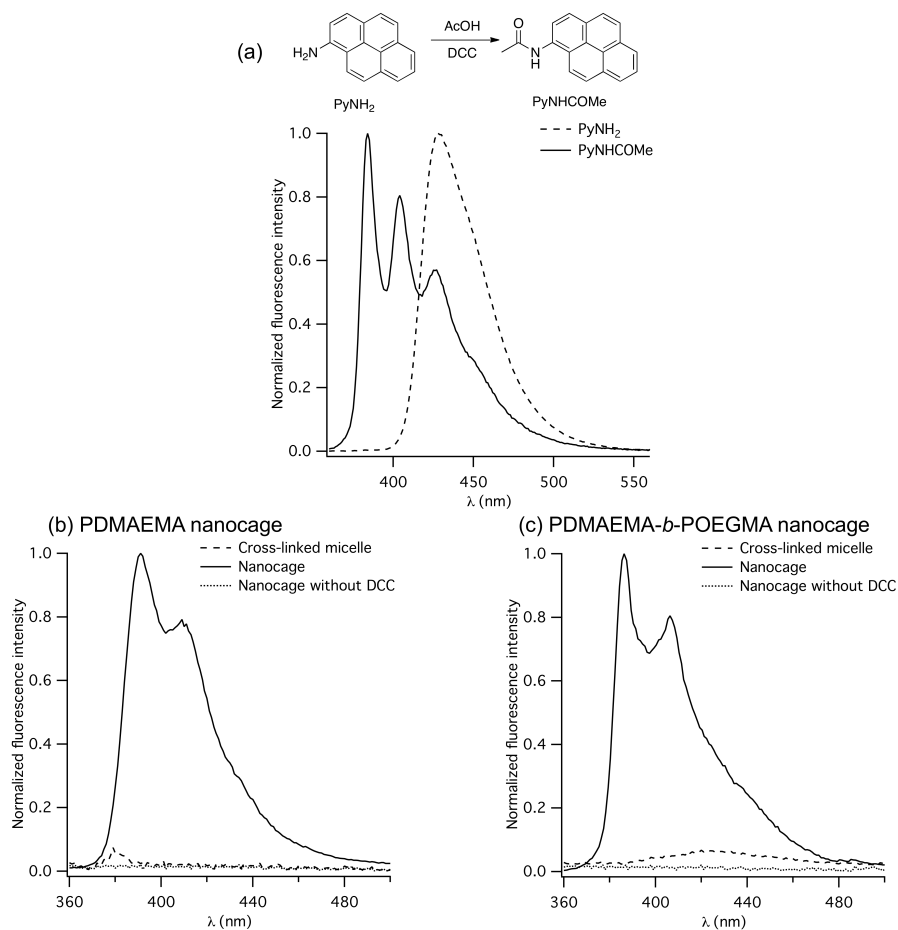


Figure 8.21: Normalized fluorescence emission spectra of 1-aminopyrene in methanol ($C = 1.05 \times 10^{-4}$ mol/L, $\lambda_{\text{ex}} = 328$ nm): (a) before (dashed curve) and after (full curve) carbodiimide coupling with acetic acid, (b) after grafting experiment on PDMAEMA nanocages (full curve) and on PtBA-*hv*-PDEAEMA shell cross-linked micelles (dashed curve), (c) after grafting experiment on PDMAEMA-*b*-POEGMA nanocages (full curve) and on PtBA-*hv*-PDEAEMA-*b*-POEGMA shell cross-linked micelles (dashed curve).

8.5.2 Stimuli-responsive behavior of the nanocages

Since the nanocages are composed of stimuli responsive polymers, it is possible to alter their solubility and thus the size of the nanocages in solution. As mentioned in chapter 2, POEGMA exhibits a response to temperature whereas PDMAEMA is a thermo- and pH sensitive polymer. The effects of pH and temperature were first studied on PDMAEMA nanocages (Figure 8.22). In this case, the thermo-responsive properties of the nanocages are drastically influenced by the solution pH [16]. Indeed, the pH directly influences the degree of neutralization (α) of the DMAEMA units of the nanocages and thus the organization of the surrounding water molecules. With this in mind, we carefully selected the investigated pH in order to ensure a total protonation ($\alpha = 0$ at pH = 3) or deprotonation ($\alpha = 1$ at pH = 9) of the amine functions of the DMAEMA units. We also investigated an intermediate situation, where half of the DMAEMA units are protonated ($\alpha = 0.5$ around the pH = 6) [16].

In the CONTIN size distribution diagram (Figure 8.22a), two effects of the pH on the nanocage size distribution are observed. The first effect is the increase of the nanocage hydrodynamic radius concomitant to the acidification of the solution. R_h of the isolated PDMAEMA nanocages equals to 43 nm, 48 nm and 76 nm at respectively pH = 9, 6 and 3. The expansion of the nanocage size upon acidification is the consequence of the increase of the electrostatic repulsion between the positively charged DMAEMA units. We can note that the size of the nanocages at pH = 6 and 9 are rather similar whereas a sharp decrease of the R_h was expected, since the neutralization degree of the PDMAEMA theoretically increases from 0.5 to 1. This could be the consequence of the cross-linking of PDMAEMA. Indeed, the cross-linking induced the presence of permanent charges on the PDMAEMA nanocage (amine quaternization) and thus does not allow the complete removal of the electrostatic interactions at pH = 9. The second effect of the pH on the size distribution of the PDMAEMA nanocages is the appearance of a polyelectrolyte effect when the pH is decreased from 9 to 3. Indeed, the CONTIN size distribution diagram of PDMAEMA nanocages at pH = 3 presents a bimodal

distribution, where the population centered at 76 nm corresponds to isolated nanocages and the population centered at 467 nm corresponds to a polyelectrolyte effect. The polyelectrolyte effect is here also the consequence of the presence of charged DMAEMA units in the nanocage membrane.

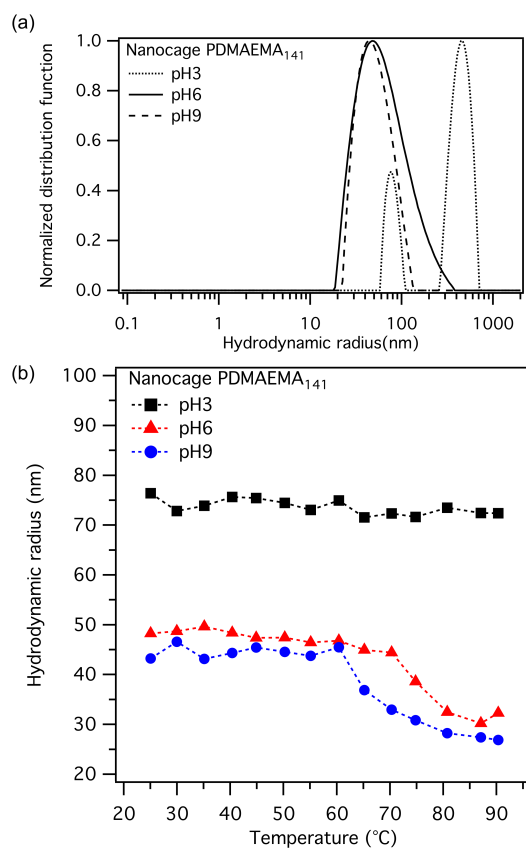


Figure 8.22: Response of PDMAEMA nanocages to pH and temperature: (a) CONTIN size distribution diagram of the PDMAEMA in water at different pH ($C = 0.1\text{g/L}$, $T = 25^\circ\text{C}$), (b) evolution of the nanocage hydrodynamic radius with temperature.

The response of the PDMAEMA nanocages to temperature was then studied at the different investigated pH. Figure 8.22b presents the evolution of the nanocage hydrodynamic radius with variations of temperature at pH = 3, 6 and 9. In the plot of the nanocage size versus temperature, two behaviors can be observed as a function of the pH. Indeed, a reduction of the nanocage size is observed at high temperature in neutral and basic media ($\blacktriangle$ and $\bullet$ curves respectively represent the variations of size at pH = 6 and 9) whereas no variation is observed when a solution of nanocages at pH = 3 is heated ($\blacksquare$ curve). The absence of variation of the nanocage size at pH = 3 may be due to the large quantity of positive charge of the nanocage, which prevents the disruption of the hydrogen bond between the DMAEMA units and the surrounding water molecules at high temperature, *e.g.* the polymer chains do not show a LCST behavior.

At pH = 6, the collapse of the nanocages was observed at a cloud point $\simeq 72^\circ\text{C}$ with the decrease of the hydrodynamic radius from 48 nm to 30 nm. In comparison to the behavior of linear PDMAEMA at pH = 6, the collapse of the nanocages at 72°C is rather odd since the cloud point of linear PDMAEMA is superior to 90°C . The collapse of the nanocages observed here is the consequence of the mobility reduction of the PDMAEMA chains due to the cross-linking and of the increase of the local chain concentration. The decrease of the LCST of thermoresponsive polymers upon a reduction of chain mobility was indeed observed on POEGMA brushes on silica surfaces [17] and on POEGMA and PNIPAm brushes grafted on polystyrene latex particles [18,19]. To confirm that the appearance of a cloud point at pH = 6 is due to the decrease of the chain mobility, the response of the PtBA-*h*v-PDMAEMA micelles to an increase of the temperature was studied.

Figure 8.23 shows the evolution of the size of PtBA-*h*v-PDMAEMA micelles with temperature at pH = 6 ($\blacktriangle$) and 9 ($\bullet$). The pH = 6 curve displays a decrease of the micelle hydrodynamic radius from 26 nm to 13 nm at a cloud point $\simeq 62^\circ\text{C}$. This reduction of the micelle size confirms that the collapse of the nanocages observed around 72°C at pH = 6 is the consequence of the decrease of the chain mobility. When the cloud

points of the nanocages and micelles at the same pH are compared, we can see that the cloud point of nanocages in solution is approximately 10 to 15°C higher than the cloud points of the micelles. This increment may be attributed to the increase of the hydrophilic character of the nanocage compared to the micelles (disappearance of the PtBA block and permanent charges due to the cross-linking).

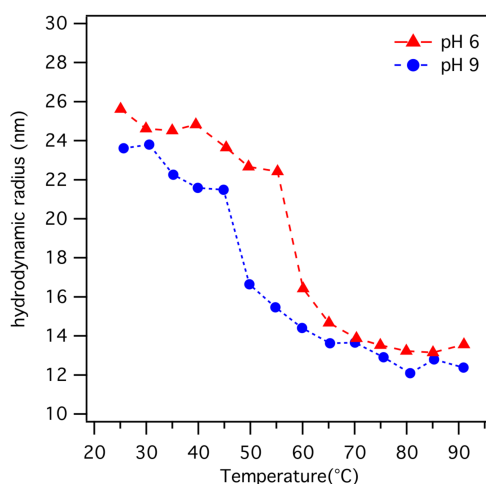


Figure 8.23: Evolution of the PtBA-*hν*-PDMAEMA micelles hydrodynamic radius with temperature.

In the case of the PDMAEMA nanocages at pH = 9, the collapse of the system is observed at a cloud point $\simeq 62^\circ\text{C}$ with the decrease of the hydrodynamic radius from 43 nm to 27 nm. The cloud point observed here is higher than the LCST of the PDMAEMA unimers at this pH, which is located around 45-50 °C. The increase of the cloud point of our system in comparison to linear PDMAEMA can be explained by the presence of the permanent positive charges due to the cross-linking of the PDMAEMA.

The stimuli responsive behavior of the PDMAEMA-*b*-POEGMA nanocages was also studied by DLS. Figure 8.24a presents the CONTIN size distribution diagram of PDMAEMA-*b*-POEGMA nanocages at pH = 3,

6 and 9. In this case, the size of the nanocages is not influenced by the solution pH, even if the shell of the nanocage is composed of a pH sensitive polymer ($R_h = 74$ nm). The absence of size versus pH variations may be explained by the brush structure of the POEGMA corona. Indeed, the steric hindrance between the PEO side groups of the POEGMA forces the PDMAEMA shell of the nanocage to adopt its expanded form and thus the electrostatic interactions between the charged PDMAEMA units do not drastically influences the micellar size.

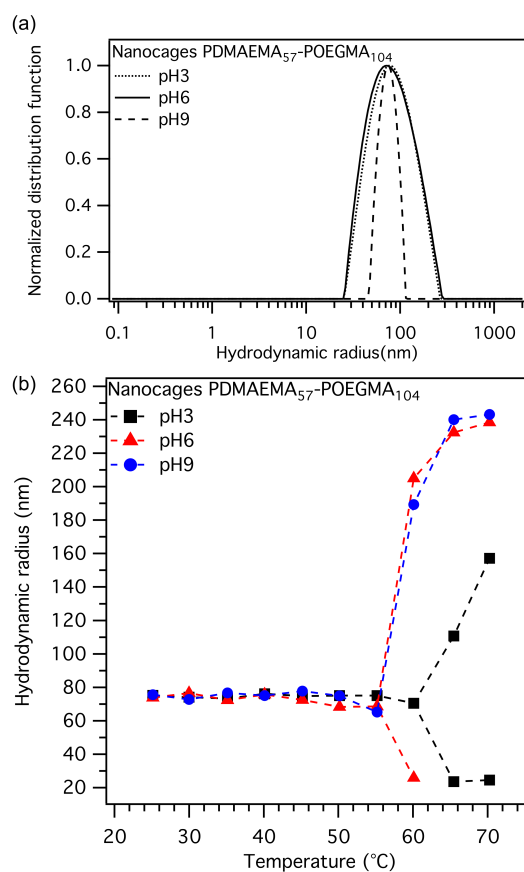


Figure 8.24: Response of PDMAEMA-*b*-POEGMA nanocages to pH and temperature.

Similarly to the PDMAEMA system, the PDMAEMA-*b*-POEGMA nanocages were submitted to a thermal stimulus at the different investigated pH. The increase of the temperature at around 60°C should induce the collapse of the POEGMA chains, which are thermo-responsive. Figure 8.24b plots the evolution of the nanocage size versus the temperature. At pH = 6 and 9 (▲ and ● in Figure 8.24b), the collapse of the nanocages is observed around 57°C. This cloud point is in agreement with the LCST of the POEGMA reported in literature and with the cloud point of the PMAA-*b*-POEGMA system determined in chapter 7. In this case, the collapse of the nanocage is not only characterized by the reduction of the nanocage size ($R_h = 26$ nm at 60°C pH = 6) but also by the appearance of a large size population corresponding to the aggregation of the collapsed nanocages ($R_h = 205$ nm). Moreover, the disappearance of the small size population is observed when the temperature is further increased. It should be noted that heating the system for long periods in basic medium leads to the hydrolysis of the POEGMA side groups and thus to the disappearance of the thermoresponsive properties of the PDMAEMA-*b*-POEGMA nanocages.

When the nanocage solution is heated at pH = 3 (■ in Figure 8.24b), the cloud point of the system is shifted towards higher temperatures ($T_{cp} = 62$ °C). The increase of the nanocage cloud point at pH = 3 is the consequence of the protonation of the PDMAEMA shell, which increases the hydrophilic character of the system.

8.6 Conclusions

This chapter focused on the formation of hollow nanocapsules from the self-assembly of linear photocleavable copolymers. In the first section of the chapter, the cleavage of linear photocleavable copolymers by light was studied in non-selective solvents by UV-Vis spectroscopy and GPC. The effects of the irradiation time and wavelength were studied on PtBA-*hv*-PDMAEMA diblock copolymers. We demonstrated that the photocleavage should be carried out at $\lambda = 300$ nm to ensure an efficient cleavage of the nitrobenzyl esters. The GPC of the copolymers after irradiation proved the detrimental effect of a too long irradiation time since high molar mass populations were observed after 15 min of irradiation at 350 nm. The optimization of the irradiation conditions allowed us to determine the best photocleavage conditions (4 minutes of irradiation at $\lambda = 300$ nm). These conditions were finally tested with the different investigated di- and triblock copolymers.

The second section dealt with the formation of PAA nanocages from PS-*hv*-PAA diblock copolymers. The micellization of PS-*hv*-PAA was first studied in water. DLS and microscopy analysis demonstrate that the micelles were characterized by a polyelectrolyte effect. To avoid this polyelectrolyte effect, the effect of the micellar concentration and of the addition of monovalent cation was studied. The second step of the nanocage formation was the chemical cross-linking of the PAA corona. This cross-linking was carried out by a carbodiimide coupling between a diamine compound and the carboxylic acids of the PAA corona. DLS and ^1H NMR studies demonstrated that a quantitative cross-linking was obtained with the selected conditions and that no inter-micellar cross-linking was observed. The photocleavage was then realized in water at 300 nm for 15 minutes. The last part of the nanocage formation, was the PS extraction. Two techniques were studied to extract the PS chains from the PAA nanocages, the dialysis and the selective/non-selective solvent cycles. The dialysis of the loaded nanocages in THF/H₂O mixture was not successful. On the other hand, the selective/non-selective solvent extraction was successfully employed to extract more than 95% of the PS chains from the PAA nanocages.

In the third section, the formation of PDMAEMA and PDMAEMA-*b*-POEGMA nanocages from PtBA-*hv*-PDMAEMA and PtBA-*hv*-PDMAEMA-*b*-POEGMA copolymers was detailed. The diblock and triblock copolymers were micellized in water via the cosolvent method. The micelles were characterized by DLS and AFM. The PDMAEMA micellar shell was cross-linked with bis(2-iodoethoxy)ethane. The shell cross-linked micelles were characterized by ^1H NMR, DLS and CryoTEM. To demonstrate that 10% of cross-linking are sufficient to maintain the integrity of the micelles, the systems were analyzed by DLS in a non-selective solvent (MeOH). The photocleavage of the junction was realized in methanol at $\lambda = 300$ nm and the irradiation was followed by UV-Vis spectroscopy. The core extraction was realized with selective/non-selective solvent cycles and around 95% of the PtBA chains were extracted from the nanocages.

The last section of this chapter focused on the ability of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages to respond to stimuli and the possibility to functionalize the cavity of the nanocages. The internal wall of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages was functionalized by a chromophore (1-aminopyrene) via a carbodiimide coupling. The grafting of the chromophore was monitored by fluorescence spectroscopy. Moreover, the grafting reaction was realized on cross-linked micelles to ensure that the recorded fluorescence emission spectrum is not the consequence of the 1-aminopyrene "encapsulated" in the nanocage. The response of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages to variation of pH and temperature was monitored by DLS. We demonstrated that the size of the PDMAEMA nanocages can be tuned by the variation of pH and that the increase of the solution temperature at pH = 6 and 9 lead to the collapse of the nanocages. In the case of the PDMAEMA-*b*-POEGMA nanocages, we observed that the size cannot be varied by a pH stimulus. On the other hand, the increase of the temperature induces the collapse of the POEGMA chains and the precipitation of the nanocages. Moreover, the study of the thermal stimulus at different pH allowed to determine the limitations of the system. Indeed, an increase of the POEGMA cloud point was monitored

when the nanocages were heated for a long period at $\text{pH} = 9$ due to the partial hydrolysis of the POEGMA block.

In this chapter, we presented the concepts of the formation of nanoreactors via the self-assembly of photocleavable block copolymers. A novel approach to produce polymeric hollow nanocapsules via the photocleavage of linear light responsive copolymers was detailed. The use of photocleavable junctions has the advantage to avoid chemical reagents and to control the cleavage from outside of the system. Moreover, the photocleavable approach allows the control of the chemical functions obtained after irradiation, which is interesting for the functionalization of the nanocages. Finally, the selected polymeric systems (PDMAEMA and POEGMA) enable, by their stimuli responsive behavior, to control the nanocage size and thus the accessibility of molecules to the internal cavity of the nanocage.

Experimental part

Materials and instrumentation. N-(3-dimethylamino-propyl)-N'-ethyl-carbodiimide hydrochloride (EDCHCl, Acros, 98%), 2,2'-(ethylenedioxy)-bis(ethylamine) (Aldrish, 98%), bis(2-iodoethoxy)ethane (BIEE, Aldrish, 96%), N,N'-dicyclohexylcarbodiimide (DCC, Acros, 99%), 1-aminopyrene (Aldrish, 97%) and all other chemicals were used as received.

Proton nuclear magnetic resonance (^1H NMR) spectra were acquired on a 300 and 500 MHz Bruker Avance II. Irradiations were performed with 3 Rayonet photochemical reactor lamps (maximum emission wavelength at $\lambda = 300$ and 365 nm). The intensity received by the sample was respectively equal to 41.6 and 5.1 mW/cm^2 and was measured with a radiometer RM12 from Dr Gröbel UV-elektronik GmbH equipped with a UVA (315-400 nm) and UVB (280-315 nm) sensors. UV-visible spectra were recorded on a Varian Cary 50 Conc. spectrophotometer. GPC chromatograms the polymers were measured on an Agilent gel permeation chromatography (GPC) system equipped with an Agilent 1100/1200 pump (35°C; eluent: DMF; flow rate of 1 mL/min), an Agilent differential refractometer and two PSS GRAM columns (Beads 10 μ ; porosity of column 1: 1000 Å; porosity of column 2: 100 Å) and on a Waters SEC system equipped with a Waters 510 pump and a 410 differential refractometer (35°C ; eluent: DMF with NH_4PF_6 (2.5 mM)); flow rate of 0.5 mL/min). The calibration was performed using polystyrene standards. Dynamic light scattering (DLS) measurements were performed at an angle of 90° on a Malvern CGS-3 apparatus equipped with a He-Ne laser with a wavelength of emission at $\lambda = 632.8$ nm. Atomic force microscopy was performed on a Digital Instruments Nanoscope V scanning force microscope in tapping mode using NCL cantilevers (Si, 48 N/m, 330kHz, Nanosensors). Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer.

Section 8.2

Typical procedure for the photocleavage in non-selective solvent (UV-Vis and GPC):

A solution of $\text{PtBA}_{118}\text{-}hv\text{-PDMAEMA}_{141}$ in EtOH ($C = 2.5$ g/L) was irradiated at 300 nm. The photocleavage was followed by UV-Vis spectroscopy until no change was observed in the shape of the spectra ($t(\text{min}) = 0, 1, 2, 3, 4$). Ethanol was then removed under vacuum. The crude mixture was redispersed in DMF and analysed in GPC.

Section 8.3

Typical procedure for the micellization of PS-*hv*-PAA:

PS₄₆-*hv*-PAA₁₃₅ was dissolved in DMF at a concentration of 10 g/L. A volume of water equal to 3 times the DMF volume was then added dropwise to induce aggregation of the insoluble PS block, followed by the addition of a volume of water equal to the DMF volume in one shot to "freeze" the micelles ($C_{\text{final}} = 2 \text{ g/L}$). Subsequently, the DMF/water solution was dialyzed against water (pH = 8) for 24 hours replacing the water at least three times (Spectra-Por dialysis bags, cutoff 14-16 kDa). The final concentration of the copolymer in water was set to 2 g/L. The micelles were characterized by DLS, AFM and TEM.

Typical procedure for the preparation of AFM sample:

Silicon substrates were cleaned by a piranha solution (H₂SO₄ 98%/H₂O₂ 30% 3/1) during 30 minutes before being rinsed in ultra-pure water. The substrates were then dried with the spin-coater at a velocity of 4000 rpm for 20 s. Solutions of micelles in were then spin coated onto these substrates at 4000 rpm during 30 s.

Typical procedure for the cross-linking of the corona the of PS-*hv*-PAA micelles:

To a stirred solution of micelles (19.0 mL, 38.0 mg, 2.52×10^{-3} mmol (PS₄₆-*hv*-PAA₁₃₅), 0.34 mmol (acid residues)), a solution of 2,2'-(ethylenedioxy)-bis(ethylamine) (25.2 μL , $C = 50 \text{ mg/mL}$ 8.50×10^{-3} mmol, 0.025 eq. of PAA units) in water was added. The resultant solution was allowed to stir for 1 h at ambient temperature. A solution of N-(3-dimethylamino-propyl)-N'-ethylcarbodiimide hydrochloride (32.6 μL , $C = 100 \text{ mg/mL}$ 1.70×10^{-2} mmol, 0.05 eq. of PAA units) in water was subsequently added dropwise. The resultant solution was allowed to stir overnight under ambient conditions. Removal of low molar mass contaminants was achieved through dialysis through a Spectra-Por dialysis bag (cutoff 14-16 kDa) against deionized water for three days replacing the water at least three times. The micelles were characterized by DLS, ¹H NMR and FTIR.

Typical procedure for the photocleavage (UV-Vis):

A solution of PS₄₆-*hv*-PAA₁₃₅ micelles in water ($C = 2 \text{ g/L}$) was irradiated at 300 nm. The photocleavage was followed by UV-visible spectroscopy until no change was observed in the shape of the spectra ($t(\text{min}) = 0, 1, 2, 5, 10, 15$).

Typical procedure for the PS extraction:

The solution of PS₄₆ loaded PAA₁₃₅ nanocages in water was first acidified with HCl to protonate the carboxylic function of the PAA nanocages. Water was removed under vacuum and the yellow powder was redispersed in THF. The solution was allowed to stir at 50°C for 24h, THF was afterwards removed under vacuum. DCM was added to the crude mixture of PAA nanocage and PS, the solution was stirred at 20°C for 24 h. The stirring was stopped to let the PAA nanocages sediment and DCM was removed with a syringe. The remaining power was finally redispersed in THF to restart an extraction cycle. After six extraction cycles, the PAA nanocages were analyzed by DLS and ¹H NMR.

Section 8.4**Typical procedure for the micellization of PtBA-*hv*-PDMAEMA:**

PtBA_{118-*hv*}-PDMAEMA₁₄₁ was dissolved in DMF at a concentration of 12.5 g/L. A volume of water equal to 3 times the DMF volume was then added dropwise to induce aggregation of the insoluble PtBA block, followed by the addition of a volume of water equal to the DMF volume in one shot to "freeze" the micelles ($C_{\text{final}} = 2.5 \text{ g/L}$). Subsequently, the DMF/water solution was dialyzed against water (pH = 6) for 24 hours replacing the water at least three times (Spectra-Por dialysis bags, cutoff 14-16 kDa). The final concentration of the copolymer in water (pH = 6) was set to 2.5 g/L. The micelles were characterized by DLS, AFM and TEM.

Typical procedure for the cross-linking of the corona the of PtBA-*hv*-PDMAEMA micelles:

To a stirred solution of micelles (12.0 mL, 30.0 mg, $7.90 \times 10^{-4} \text{ mmol}$ (PtBA_{118-*hv*}-PDMAEMA₁₄₁), 0.11 mmol (amine residues)) in water (pH = 9), a solution of bis(2-iodoethoxy)ethane (20.7 μL , $C = 100 \text{ mg/mL}$ $5.60 \times 10^{-3} \text{ mmol}$, 0.05 eq. of DMAEMA units) in methanol was added. The resultant solution was allowed to stir for 16 h at 40°C. Removal of low molar mass contaminants was achieved through dialysis through a Spectra-Por dialysis bags (cutoff 14-16 kDa) against methanol. The micelles were characterized by DLS and ¹H NMR.

Typical procedure for the photocleavage (UV-Vis):

A solution of PtBA_{118-*hv*}-PDMAEMA₁₄₁ micelles in water ($C = 2.5 \text{ g/L}$) was irradiated at 300 nm. The photocleavage was followed by UV-visible spectroscopy ($t(\text{min}) = 0, 1, 2, 3, 4$).

Typical procedure for the PtBA extraction:

The solution of PtBA₁₁₈ loaded PDMAEMA₁₄₁ nanocages in water was first basified with NaOH to obtain uncharged PDMAEMA. Water was removed under vacuum and the yellow powder was redispersed in methanol. The solution was allowed to stir at 20°C for 24h, methanol was afterwards removed under vacuum. basic water (pH = 9) was added to the crude mixture of PDMAEMA nanocages and PtBA, the solution was stirred at 20°C for 24 h. The stirring was stopped to let PtBA sediment. The separation of the nanocages from the precipitated PtBA was realized by filtration using syringe filters (1.2 and 0.2 μ) and water was removed under vacuum. The extraction cycle was afterwards restarted. The extraction was monitored with ¹H NMR, After six extraction cycles, the PDMAEMA nanocages were analyzed by DLS.

Section 8.5**Typical procedure for the grafting of 1-aminopyrene on the PDMAEMA nanocages:**

To a solution of PDMAEMA nanocages (1.00 mL, C = 1.48 g/L, 6.5×10^{-5} mmol (carboxylic acid residues)) in DCM, a solution of 1-aminopyrene (28.2 μ L, C = 5 g/L, 6.5×10^{-4} mmol, 10 eq. (carboxylic acid residues)) in DCM was added. The solution was allowed to stir for 1h at 25°C. A solution of N,N'-dicyclohexylcarbodiimide (28.2 μ L, C = 5 g/L, 6.5×10^{-4} mmol, 10 eq. (carboxylic acid residues)) was then added to the mixture. The solution was stirred at 25 25°C for 24h. The grafted nanocages were purified by dialysis against methanol for 24 hours replacing the MeOH at least three times (Spectra-Por dialysis bags, cutoff 14-16 kDa).

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CONCLUSIONS AND PERSPECTIVES

This work was devoted to the production of functional nanostructures in solution and more particularly on how to combine light and photo-cleavable block copolymers to control the formation of these nanostructures.

In the first chapter of this thesis, an overview of the characteristic features of block copolymers was given. Some tools commonly used for the synthesis of well-defined block copolymers were presented. The basics of controlled radical polymerization techniques and of "click" coupling reactions were developed. The self-assembly properties of block copolymers were then presented. Generalities on the self-assembly in bulk and in thin films were developed. The micellization of block copolymers in solution was then briefly reviewed and the factors influencing the size and morphology of the self-assembled structures were detailed. Finally, the questions of "what is light?" and "how does light interact with matter?" were answered.

The second chapter focused on functional nanostructures in solution. An overview of stimuli-responsive polymers was presented with some details on the response of polymers to pH, temperature and light stimuli. The second part of this chapter presented several multistimuli-responsive block copolymer systems. Several examples of the so-called schizophrenic copolymers were discussed. The second type of nanostructure presented

in the chapter was the hollow nanocapsule (or nanocage). This nanostructure has similar characteristic features as vesicles. Various strategies to produce nanocages were finally detailed here.

Light is an interesting stimulus for the production of nanostructured materials as this stimulus can be triggered from outside the system, it does not need additional reagents, and generates a limited amount of by-products. With that in mind, we decided to use this stimulus to control the formation of nanostructures in solution. As presented in chapter 3, light was used to induce the micellization of block copolymers in organic media and to disrupt micelles in water. Moreover, when the photocleavable moiety was introduced in the main chain of the polymer, light was used to trigger the formation of nanocages.

Attention was first focused on the synthesis of the photocleavable copolymers. The first family of copolymers is composed of a sequence bearing photocleavable side groups and of another sequence, with either a hydrophobic or a hydrophilic character (Chapter 4). Two types of photocleavable moieties were introduced as side groups, *o*-nitrobenzyl esters or *p*-methoxyphenacyl esters. The synthetic strategy for the production of copolymers bearing *o*-nitrobenzyl esters relies on the post modification of PAA-*b*-PS precursor copolymers. Well-defined PNBA-*b*-PS and PDMNBA-*b*-PS copolymer, with a nearly quantitative grafting of the photocleavable side groups, were synthesized by this method. However, if this methodology is well adapted to synthesize block copolymers bearing different types of photocleavable side groups, the synthesis of copolymers where the nature of the non-photocleavable block is varied can be harsh. Moreover, it was previously demonstrated that the grafting efficiency is largely influenced by the non-photocleavable block length. These two drawbacks led us to investigate the synthesis of new types of block copolymers bearing photocleavable side groups. The main advantage of copolymers bearing *p*-methoxyphenacyl esters is that they can be synthesized by controlled radical polymerization. The controlled atom transfer radical polymerization (ATRP) of *p*-methoxyphenacyl methacrylate was optimized by screening different experimental parameters. The conclusion of this optimization is that the polymerization of MPMA

should be conducted with the TsCl/CuCl/dNbipy system. The controlled behavior of the polymerization of MPMA under these conditions was demonstrated by the kinetic study of the polymerization. The synthesis of diblock copolymers composed of one PMPMA block and another block was then studied. We demonstrated that block copolymers with a PMPMA block and a block composed of styrenic, acrylate or methacrylate monomers can be synthesized by ATRP. As demonstrated at the end of chapter 4, a wide range of well-defined block copolymers bearing *p*-methoxyphenacyl side groups may be easily synthesized in two steps by ATRP, which is an undeniable advantage compared to copolymers bearing *o*-nitrobenzyl side groups.

The synthesis of block copolymers with a photocleavable *o*-nitrobenzyl ester junction was presented in chapter 5. The synthetic strategy relies on a "one pot" simultaneous CuAAC-ATRP process where the photocleavable junction is grafted on an azide terminated polymer by CuAAC and the second block is grown by ATRP. This strategy revealed itself to be efficient and versatile, as PS-*hv*-PAA, PtBA-*hv*-PDMAEMA and PtBA-*hv*-PDMAEMA-*b*-POEGMA were synthesized by this method. However, if the synthesis is efficient for copolymers with a PtBA block, the synthesis of copolymers with a PS block is problematic since we were unable to synthesize PS-*hv*-PtBA diblock copolymers without traces of homo-polystyrene. The presence of homo-PS was the consequence of chain-end losses in the first synthetic step. Fortunately, the homo-PS was easily removed from the PS-*hv*-PAA copolymer. To improve this synthesis, it is worth testing some polymerization parameters of styrene to limit the chain-end loss and thus produce PS-*hv*-PtBA diblock copolymer without traces of homo-polystyrene.

Once the photocleavable block copolymers were synthesized, we were able to study their behavior in solution and their response to light. The light induced micellization in organic medium was first studied with copolymers composed of a polystyrene block and of a block bearing *o*-nitrobenzyl derivatives and *p*-methoxyphenacyl esters (Chapter 6). The combination of DLS and TEM measurements allowed the characterization of the micelles formed during the irradiation. Moreover, in this

chapter we demonstrated the ability of the system to act as a trapping agent. The encapsulation of coumarin 343 into the micellar core produced during the irradiation of the block copolymer in solution was realized. This encapsulation method revealed itself to be a fast and reliable process compared to the encapsulation by direct micellization. However the system must be studied in depth to evaluate its capacity for encapsulation. Moreover, it is also worth to perform some selective encapsulation tests with a mixture of molecules to evaluate if the system is adapted for applications such as *in situ* product removal or product separation. Although the light induced micellization of PNBA-*b*-PS and PDMNBA-*b*-PS copolymers works well in chlorinated solvents, these polymeric systems do not show a solvent versatility. Indeed, the solubility of the PS, PNBA and accordingly obtained PAA does not allow the light induced micellization in most commonly used solvents. The same problems occurs for the light induced micellization of copolymers bearing *p*-methoxyphenacyl side groups, since the PMPMA is only soluble in a limited number of solvents.

Chapter 7 focused on the use of light to induce the disruption of micelles. Micelles consisting of a PMPMA core and a POEGMA corona were characterized by DLS and TEM. UV irradiation induced the disruption of the micelles. The optimization of the cleavage conditions revealed that a buffer at $\text{pH} = 8$ was needed to produce unimers in solution after the irradiation. The multi-responsive behavior of the accordingly obtained PMAA-*b*-POEGMA double hydrophilic copolymer was studied by the application of various stimuli including temperature, pH, calcium cations, and phosphate anions. The schizophrenic behavior of this copolymer was demonstrated since all the stimuli-induced micellization processes were reversible. Although the light induced release of drugs was demonstrated in this chapter, several possible applications of this photocleavable copolymer need to be further studied. Such kind of systems may be used for the one step *in-situ* production of metallic nanoparticles. As represented in Figure 9.1, the irradiation of PMPMA-*b*-POEGMA copolymer in the presence of multivalent cations should produce micelles with PMAA/ M^+ core and a POEGMA corona. The reduction of the metal cations would thus lead to the formation of metallic

nanoparticles. The advantages of this strategy are that the size and the shape of the nanoparticles would be controlled by the size and morphology of the core of the starting micelles and that the nanoparticles would be stabilized by an thermo-responsive POEGMA corona. An other possible application is the selective encapsulation, where the schizophrenic behavior of the PMAA-*b*-POEGMA copolymer would be used to encapsulate selectively a molecule depending on the stimulus used for the micellization (Figure 9.1).

The last chapter of this thesis presented the results for the formation of nanocages from linear photocleavable block copolymers. In the first part of the chapter, the photocleavage of the block copolymers was realized in a non-selective solvent in order to determine the optimal irradiation conditions. The formation of PAA nanocages from a PS-*hv*-PAA diblock copolymer was afterwards presented. The obtained PS-*hv*-PAA micelles were characterized by a polyelectrolyte effect. DLS, TEM and AFM were used to characterize these micelles. Shell cross-linking of the PAA chains was carried out with a diamine. After irradiation of the micelles, the extraction of the PS chains was realized by selective solvent separation (THF/DCM) and more than 95% of the PS chains were extracted from the PAA nanocages. The same strategy was then applied on the PtBA-*hv*-PDMAEMA and on the PtBA-*hv*-PDMAEMA-*b*-POEGMA copolymers. Shell cross-linking of the PDMAEMA block was in this case carried out with a diiodo compound. Core extraction was also realized by selective solvent extraction (MeOH/H₂O) and more than 95% of the PtBA chains were extracted from the nanocages. Moreover, the access to the carboxylic functions formed after photocleavage was demonstrated by the functionalization of the inner wall of the nanocages with 1-aminopyrene. We also showed by DLS that the size of the PDMAEMA and PDMAEMA-*b*-POEGMA nanocages can be tuned by the combination of pH and temperature stimuli. The size of the PDMAEMA nanocages is dramatically influenced by the pH of the solution. Indeed, due to the electrostatic interactions between the charged DMAEMA units, the PDMAEMA nanocages adopt their expanded form at low pH. On the other hand, the nanocages can shrink at high temperature when the solution is slightly acidic or basic. In the case of

the PDMAEMA-*b*-POEGMA nanocages, an increase of the temperature above the LCST of POEGMA induces the collapse of the nanocage corona.

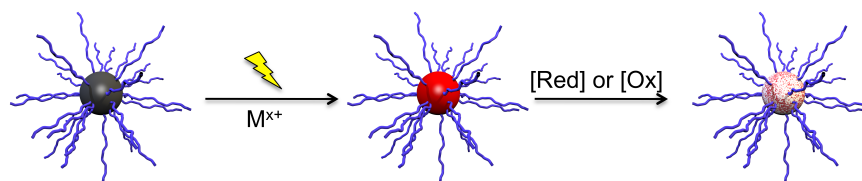
The major difficulty encountered during the production of the nanocages occurred during the extraction of the core forming polymer. Indeed, the extraction of the core required several solvent switches, which was really time consuming and is not viable from an economic point of view. Our strategy could be improved by designing a photocleavable block copolymer with another hydrophobic block. The use of thermo-responsive polymers with low LCST could be considered, such as PNIPAm. In this case, the totality of the nanocage formation could be carried out in water, the micellization would occur by the increase of the temperature around 40°C and the extraction of the core would be realized by a dialysis against water. We could also rely on a totally photocleavable approach, where the core forming polymer is composed of several photocleavable units. The irradiation of the shell cross-linked micelles would thus induce the detachment of the corona from the core and the degradation of the core.

The stimuli responsive behavior of these nanocages is interesting for the production of nanoreactors and drug nanocarriers. Indeed, the ability to functionalize the internal cavity of nanocages allows the production of stimuli-responsive nanoreactors. The nanoreactors might be produced after the grafting of a selected catalyst onto the carboxylic acid functions of the nanocages. Moreover, the stimuli responsive behavior of the nanocages potentially enables to control the activity of the nanoreactor and the catalyst recovery. Indeed, the ability to control the size of the nanoreactor enables, via the variation of electrostatic interactions and of the chain conformation, to control the diffusion of the reactants into the internal cavity of the nanoreactor (Figure 9.2). The collapse of the nanocages at high temperature also facilitates catalyst recovery, since the reactant-nanocage mixture may be separated by filtration. The stimuli responsive behavior of the nanocages is also valuable for drug delivery applications, where the encapsulation of active molecules into the internal cavity of the nanocages is possible upon an increase of temperature.

Inversely, the release of drugs would be observed when the temperature is decreased.

As a general conclusion, I want to point the major advantages and drawback of light-responsive materials. The advantages of light are that the reactions are triggered from outside the system, the reactions are realized without the addition of chemical reagents and finally that the irradiation is a "on-off" process, which can be stopped at any moments. the mains drawback of light to this point is the wavelength used for the materials formation. Indeed, the use of UV light for long irradiation periods, which is detrimental to the user and sometime to the material itself, is right now the major point that slows down the use of light-responsive materials for everyday applications. The positive point is that several research groups investigate this subject and try to produce new photosensitive materials that do not present this drawback. As an example, I would like to point the beautiful work of Zhao and coworkers on NIR responsive materials such as coumarin based copolymers and more recently the use of upconverting nanoparticle (Figure 2.14 in Chapter 2) to bypass the wavelength issue. Finally, I hope that my humble contribution on the subject of photocleavable block copolymers has inspired and enthused the reader and has helped to the development of the knowledge on photosensitive materials.

In situ fabrication of metallic nanoparticles of precise size and shape



Selective encapsulation system

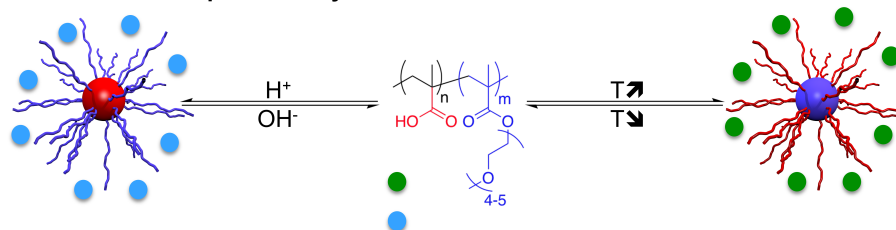


Figure 9.1: Schematic representation for the formation of metallic nanoparticles from PMPMA-*b*-POEGMA micelles and for the selective encapsulation in PMAA-*b*-POEGMA micelles.

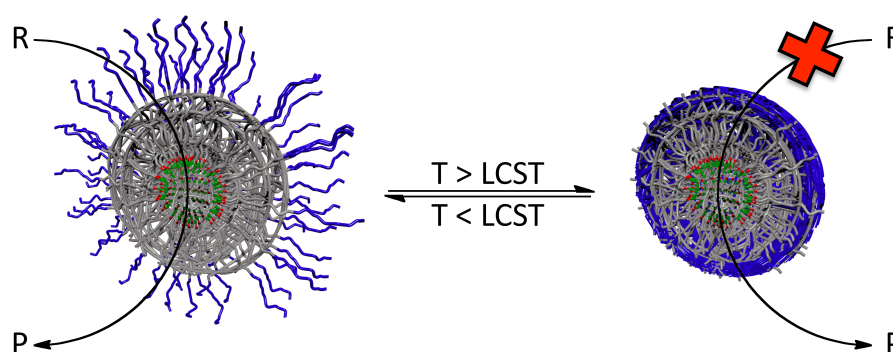


Figure 9.2: Schematic representation for the formation of a stimuli-responsive nanoreactor from a PDMAEMA-*b*-POEGMA nanocage.

CURRICULUM VITAE

Refereed Publications

Bertrand, O.; Schumers, J.-M.; Kuppan, C.; Marchand-Brynaert, J.; Fustin, C.-A.; Gohy, J.-F. Photo-induced micellization of block copolymers bearing 4,5-dimethoxy-2-nitrobenzyl side groups. *Soft Matter* **2011**, 7, 6891-6896. [DOI: [10.1039/c1sm05631j](https://doi.org/10.1039/c1sm05631j)]

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Brassinne, J.; Mugemana, C.; Guillet, P.; **Bertrand, O.**; Auhl, D.; Bailly, C.; Fustin, C.-A.; Gohy, J.-F. Tuning micellar morphology and rheological behaviour of metallo-supramolecular micellar gels. *Soft Matter* **2012**, 8, 4499-4506. [DOI: [10.1039/c2sm07442g](https://doi.org/10.1039/c2sm07442g)]

Gamys, C. G.; Vlad, A.; **Bertrand, O.**; Gohy, J.-F. Functionalized Nanoporous Thin Films From Blends of Block Copolymers and Homopolymers Interacting via Hydrogen Bonding. *Macromol. Chem. Phys.* **2012**, 213, 2075-2080. [DOI: [10.1002/macp.201200255](https://doi.org/10.1002/macp.201200255)]

Oral Presentations

Bertrand, O.; Duchêne, R.; Fustin, C.-A.; Gohy, J.-F. *Photocleavable block copolymers as precursors of responsive nanocapsules*. **2012 Belgian-German (Macro)Molecular Meeting**, Houffalize, Belgium, December 2012.

Bertrand, O.; Fustin, C.-A.; Gohy, J.-F. *Development of novel photocleavable block copolymers for the design of multi-responsive micellar systems*, **2012 Belgian Polymer Group Meeting**, Blankenberg, Belgium, May 2012.

Bertrand, O.; Fustin, C.-A.; Gohy, J.-F. *Synthesis and self-assembly in water of a light and thermo sensitive pseudo-schizophrenic diblock copolymer*, **2012 ACS Spring National Meeting**, San Diego, USA, March 2012.

Bertrand, O.; Fustin, C.-A.; Gohy, J.-F. *Synthesis and light induced micellization of diblock copolymers bearing o-nitrobenzyl side groups*, **2012 ACS Spring National Meeting**, San Diego, USA, March 2012.

Bertrand, O.; Schumers, J.-M.; Kuppan, C.; Marchand-Brynaert, J.; Fustin, C.-A.; Gohy, J.-F. *Block copolymers containing photocleavable side groups : Formation and disruption of micelles by light*, **2011 European Polymer Congress**, Granada, Spain, June 2011.

Honors & Awards

BELGIAN POLYMER GROUP best poster prize, *Formation of nanocages with photocleavable block copolymers*, **2013 Belgian Polymer Group Meeting**, Houffalize, Belgium, May 2013.

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