

Improved detection sensitivity of D-mannitol crystalline phase content using differential spectral phase shift terahertz spectroscopy measurements

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Abstract: We report quantitative measurement of the relative proportion of δ - and β - D-mannitol crystalline phases inserted into polyethylene powder pellets, obtained by time-domain terahertz spectroscopy. Nine absorption bands have been identified from 0.2 THz to 2.2 THz. The best quantification of the δ -phase proportion is made using the 1.01 THz absorption band. Coherent detection allows using the spectral phase shift of the transmitted THz waveform to improve the detection sensitivity of the relative δ -phase proportion. We argue that differential phase shift measurements are less sensitive to samples' defects. Using a linear phase shift compensation for pellets of slightly different thicknesses, we were able to distinguish a 0.5% variation in δ -phase proportion.

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1. Introduction

Process monitoring is used in the fabrication of a pharmaceutical product for quality and reproducibility control. Dehydration is used to increase shelf life-time and to reduce its weight for transportation: it is however a critical part of the overall fabrication process [1]. Freeze drying or lyophilisation is widely used in the industry and D-mannitol is an alcohol sugar that is commonly used as an excipient. However, depending on the freeze drying conditions different crystalline phases of D-mannitol can be produced [2, 3] resulting in potentially different biological activities of the final product. Specific signatures of the different D-mannitol crystalline phases have already been revealed using techniques such as X-Ray powder diffraction (XRPD) [4], Raman spectroscopy [5], nuclear magnetic resonance (NMR) [6] and mid-infrared (MIR) spectroscopy [7]. Each of these techniques has its own drawbacks [8] so the pharmaceutical industry is still looking for a high detection sensitivity technique for quantifying the polymorphic content of D-mannitol compounds. Such a technique can take advantage of the fact that D-mannitol has an extensive inter-molecular H-bond network [9]. Multi-molecular vibrational modes are very sensitive to molecular composition and crystalline phase: the energies and the intensities of these modes represent a precise molecular finger print. With the advent of reliable broad band THz radiation sources, a spectrum of the low-energy vibrational modes can now be measured. Recently, Chakkittakandy qualitatively measured 0.5 - 7 THz spectra of D-mannitol crystallized during freeze drying, using a quasi near-field setup [10]. While the conventional far-field measurement with a semiconductor emitter and a ZnTe electro-optic detection is restricted to a maximum frequency of about 4 THz, its signal/noise ratio is much better than the one of a near-field measurement. On an industrial point of view, it is advantageous to measure the D-mannitol absorbance at 1 THz since this frequency falls below the inter-molecular and intra-molecular vibrational modes [11] of most other excipients or growth ingredients. Far-field measurements are also more interesting for on-line quantification of different crystalline phases of a specific product.

The quantification of different crystalline phases of a molecule in a mixture sample has already been reported using time-domain terahertz spectroscopy [8, 10–14]. The polymorphic content can be extracted from a chemometric analysis [13, 14.] based on a multi-parameters fitting procedure that sets the best calibration model relating each THz absorption feature to one or several crystalline forms of the molecule. By choosing an appropriate reference sample, it is sometime possible to simplify drastically the analysis by obtaining absorbance spectra with well resolved absorption bands over a given spectral range. In these circumstances, one can verify that the amplitude of each chosen absorption band scales almost linearly with the crystalline phase content. The detection sensitivity of one particular crystalline phase depends on the experimental considerations such as the signal to noise ratio of the measurements and the reproducibility of the fabrication process used for making the set of calibration samples. Thickness variations resulting from the inevitable loss of material during the pellet's compression and fluctuations in the density of defects from sample to

sample have a direct impact on the amplitude of a given THz absorbance peak. In this paper, we show that it is advantageous to use the spectral phase shift of the transmitted THz waveform to improve the polymorphic content detection sensitivity. We argue that differential spectral phase shift measurements are less sensitive to samples' defects.

We report room temperature quantitative measurements of relative crystalline phases of a mixture of δ - and β -phase D-mannitol inserted into a polyethylene powder pellet, with far-field Time-Domain THz Spectroscopy (TDS). Multiple absorption bands spectra of a series of D-mannitol samples are obtained and reproduced using Voigt functions that account for homogeneous and inhomogeneous spectral broadening mechanisms. The quantitative measurement of the δ -phase proportion is obtained using specific absorption bands. Differential phase shift spectra of the transmitted THz waveform are also obtained for the different samples. We calculate a series of differential phase shift data curves by subtracting measurements obtained for the x% δ -phase samples with the one obtained for the 100% β -phase sample, and compensate for the linear behavior resulting from any slight variation in thickness between two given pellets. We then compare the quantification methods of D-mannitol crystalline phases using either the amplitude of one particular absorbance band or the maximum variation of the differential phase shift values around the same absorption band.

2. Experiment

The time-domain THz spectroscopy (THz-TDS) setup uses a conventional 4 parabolic mirrors configuration. A photoconductive low-aperture antenna was used for the generation of short THz pulses. The THz emitter was fabricated on a multi energy H-bombarded high resistivity GaAs substrate [15]. A 60 fs mode-locked Ti:sapphire oscillator was used as the excitation laser source. A home-made frequency chirping pulse apparatus allows minimizing the pulse duration at the position of the THz emitter. A 4 mm hemispherical silicon lens was fixed on the backside of the emitter. The terahertz radiation was collected over a large solid angle and refocused on our sample using a pair of off-axis parabolic mirrors. The transmitted THz beam was collected and refocused on our detector using a second set of parabolic mirrors. The THz traces were detected using a 40-ps delay line and electro-optic sampling in a 0.5 mm-thick $\langle 110 \rangle$ ZnTe crystal. The intensity of the pump beam was modulated at a frequency of 3 kHz using a mechanical chopper and a lock-in amplifier was used to retrieve the THz signal. The spectra of the THz traces were then obtained using numerical Fourier transforms. The samples were mounted on a rotating wheel and the entire setup is enclosed in a vacuum chamber. A turbo-molecular pump was used to quickly reduce the pressure ($< 10^{-3}$ Torr) inside the chamber and measurements were carried out after filling the chamber with dry nitrogen.

The ACS Reagent D-mannitol and the ultra high molecular weight 53-75 μ m polyethylene powder were purchased from Sigma-Aldrich. As received, the D-mannitol was in the β -phase and consists in coarse polycrystalline particles. The protocol described by L. Walther –Lévy [16] was followed to crystallize a part of the D-mannitol in its δ -phase and separate each phase in order to obtain pure polycrystalline samples. We have verified the purity of each phase by comparing the X-ray powder diffraction pattern (XRDP) with the International Center Diffraction Data (ICDD) table. To insure a correct interpretation from XRDP data, the polycrystalline D-Mannitol powder were carefully ground with a mortar and pestle until a very fine powder is obtained.

Samples were made from a mixture of polyethylene powder and D-mannitol crystallites in a proportion of 3:1. A set of 14 samples has been prepared with the D-mannitol portion containing a mixture of δ - and β -crystalline phases: the δ -phase content in the D-mannitol portion is respectively 0.5%, 1%, 2.5%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. To insure a good homogeneity, the polyethylene + D-mannitol preparation was mixed thoroughly with the mortar and pestle. Pellets were then made from 500 mg of this powder compressed at 7000 lbs/cm². A 500 mg of polyethylene powder pellet was also made as a reference. Another XRDP analysis on the compressed pellet confirms that humidity or

pressure did not cause any phase transformation. Samples were kept into a desiccators' chamber after each manipulation. All results presented in this article were measured on the same set of samples.

3. Results

$$Absorbance = \frac{\alpha(\omega)d}{2} = -\ln\left(\frac{E(\omega)}{E_0(\omega)}\right). \quad (1)$$

Equation (1) is the absorbance with $\alpha(\omega)$ being the coefficient of absorption at a given wavelength, d is the pellet thickness, $E(\omega)$ and $E_0(\omega)$ are the electric field of the transmitted THz pulses through the sample and reference, respectively. A zero padding procedure with a quenching window is applied to the THz traces. The instrumental spectral resolution is about 0.03 THz. Figure 1(a) shows the absorbance spectra of D-mannitol samples as the δ -phase content is increased from 0% (top) to 100% (bottom) by 10% increments. The reference is the polyethylene only pellet. The β -phase absorbance spectrum (pellet with 0% δ -phase – 100% β -phase) shows two main absorption bands at 1.11 THz (M_1) and 1.49 THz (M_2). Other absorption bands appear as the δ -phase proportion increases: the main one being at 1.01 THz (δ_1). The M_1 and M_2 bands seem to undergo a frequency shift as the δ -phase proportion increases. Fitting the absorbance using specific and non-specific δ -phase absorption bands reveals that this apparent shift results from a change in the relative importance of two closely separated absorption bands when the δ -phase proportion increases.

Fitted curves and their related baseline corrected data points are shown in Fig. 1(b) for the sample with a D-mannitol δ -phase content of 50%. For all samples, a zero baseline is first obtained using a linear correction that removes any offset between data points at 0.9 and 1.2 THz. Two specific δ -phase absorption bands (δ_1 and δ_2) and one absorption band (M_1) common to both the β - and δ - crystalline phases are taken into account in the fitting procedure. Best fits of the corrected absorption spectra are obtained using Voigt functions that consist in a convolution of Lorentzian and Gaussian functions. Homogeneous broadening of an absorption band is related to the finite relaxation time of the excitation mode that is influenced by thermal effects: this broadening mechanism gives rise to a Lorentzian absorption band profile. The Gaussian inhomogeneous broadening mechanism is attributed to the polycrystalline nature of the sample. The M_1 absorption band parameters (amplitude, width and central frequency) were determined using the best fit of the β -phase pellet's THz trace. For the general fitting procedure of other absorption spectra, central frequencies of δ_1 and δ_2 peaks and all M_1 parameters were kept constant. The integrated amplitude corresponding to the area under the fitted absorption peak is plotted in Fig. 1(c) as a function of δ -phase proportion, for the δ_1 and δ_2 bands. The integrated amplitude of each absorption band increases almost linearly as a function of the δ -phase proportion. Error bars in this figure are given by the standard errors on the fits of the absorbance spectra while the errors on the proportions are estimated to be smaller than the data point symbol. Considering the proximity of M_1 , the signal to noise ratio of our measurements is not good enough to obtain a relevant fit for δ -phase contents smaller than 10%.

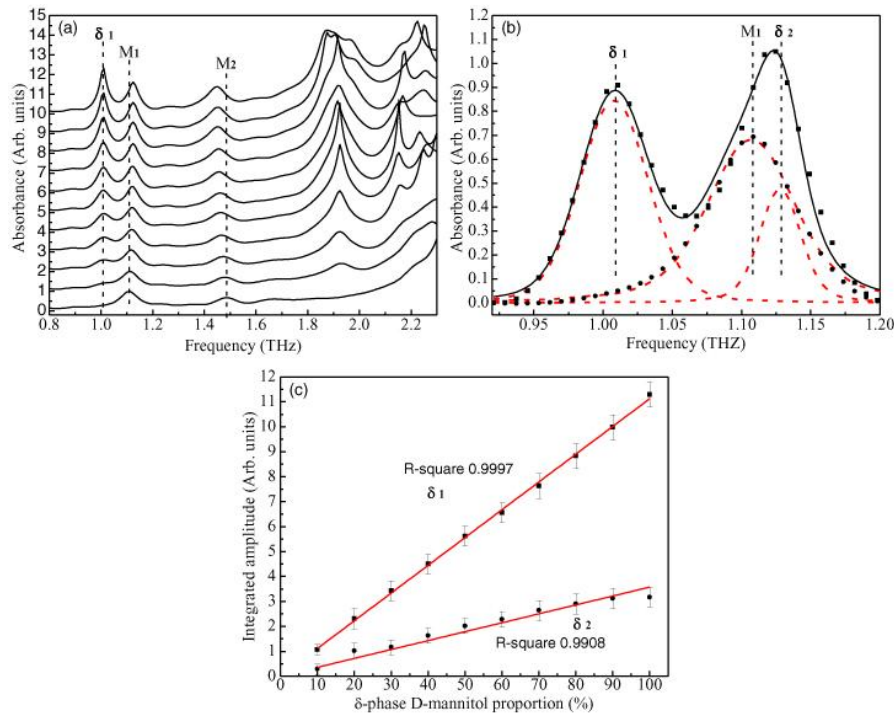


Fig. 1. (color online) (a) Transmitted THz electric field amplitude measured between 0.7 and 2.2 THz for different 4.2 mm thick pellets containing a mixture of δ - and β - crystalline phase D-mannitol combined with polyethylene powder. The reference sample is the polyethylene only pellet. The δ -phase proportion varies from 10% (bottom curve) to 100% (top curve), by increment of 10%. The curves are displaced vertically for clarity. (b) Corrected data points between 0.9 to 1.3 THz for the 50% δ -phase – 50% β -phase mixture pellet (full squares) and 100% β -phase (full circles). The best fit (solid line) of the 50% δ -phase data points is obtained using three Voigt functions (dashed lines) centered at the δ_1 , M_1 and δ_2 peaks. (c) Integrated amplitude corresponding to the area under the fitted absorption peak plotted as a function of the δ -phase proportion, for the δ_1 (full squares) and the δ_2 (full circles) bands. The R-square values of the best linear fits (solid lines) passing through the data points are 0.9997 and 0.9908 for the δ_1 and δ_2 bands, respectively.

It would have been interesting to perform low-temperature measurements in order to reduce the width of the absorption bands. Our setup is not yet equipped for such measurements. However, it is possible to simplify the analysis by choosing a more appropriate reference sample. Figure 2(a) shows a new set of absorbance spectra obtained using the polyethylene + D-mannitol 100% β -phase sample as a reference. The same set of pellets' samples has been used for these measurements. In this figure, the δ -phase proportion varies from 10% (bottom curve) to 100% (top curve), by increment of 10%. The absence of negative valleys in all these spectra at 1.11 THz and 1.49 THz confirms that the amplitude of the M_1 and M_2 bands are the same for both D-mannitol crystalline phases. The choice of a new reference sample allows obtaining well resolved absorption bands. The remaining absorption bands are only related to the D-mannitol δ - phase. The amplitude of each band increases gradually with the δ -phase proportion except for the δ_6 and δ_7 absorption bands where a saturation phenomenon is observed at high δ -phase proportions ($> 40\%$). This phenomenon is explained to the limited sensitivity of our experiment around 2 THz where the transmitted terahertz pulses through the sample is very low due to the presence of strong absorption bands. In Fig. 2(b), the integrated amplitude corresponding to the area under the absorbance peak is plotted as a function of the δ -phase proportion, for the δ_1 , δ_2 and δ_4 bands. The error bars in this plot are determined from the numerical integration uncertainties resulting from taking different baseline for each peak. The integrated amplitude of these

absorption bands increases almost linearly as a function of the δ -phase proportion. The phase quantification data points are well reproduced using a linear regression with R-square values of 0.9953, 0.9978, and 0.9901 for the δ_1 , δ_2 and δ_4 bands, respectively. Considering the error bars on the data points and the signal to noise ratio of our measurements it is still difficult to quantify the δ -phase proportion below 5%. An apparent saturation of the amplitude is observed at high δ -phase proportions ($> 80\%$). The underestimated values calculated for these high δ -phase proportions are probably related to the use of a baseline subtraction technique for each independent peak. The baseline for one particular band tends to get higher as the δ -phase proportion increases due to the presence of neighboring bands.

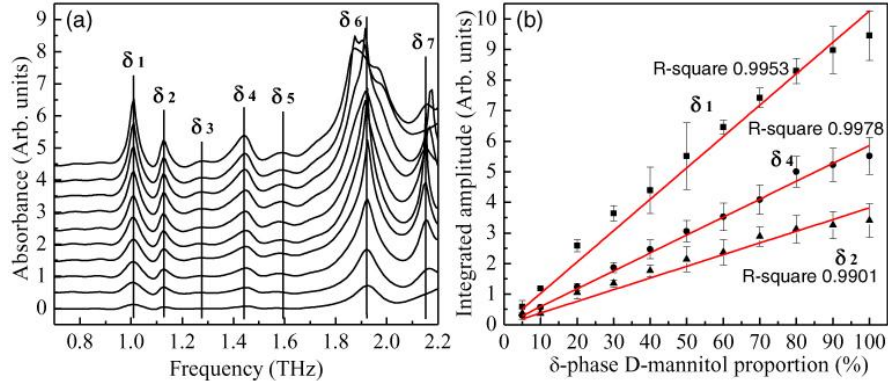


Fig. 2. (color online) (a) Absorbance of the δ -phase D-mannitol pellets plotted between 0.75 and 2.2 THz, for various proportions of δ and β phases. The δ -phase proportion varies from 10% (bottom curve) to 100% (top curve), by increment of 10%. The curves are displaced vertically for clarity. (b) Integrated amplitude corresponding to the area under the absorbance peak plotted as a function of the δ -phase proportion, for the δ_1 (full squares), δ_2 (full triangles) and δ_4 (full circles) bands. The R-square values of the best linear fits (solid lines) passing through the data points are 0.9953, 0.9978 and 0.9901 (full squares) for the δ_1 , δ_2 and δ_4 bands, respectively.

The central frequencies of all absorption bands are listed in Table 1. The frequencies of the δ_1 to δ_5 absorption bands are determined from the fit of the absorbance spectrum of D-mannitol 100% δ -phase sample using the 100% β -phase sample as a reference, see Fig. 2(a). The fitting procedure consists in using one Voigt function per absorption band. The error bars on these frequencies are estimated by comparing the frequency values obtained using the same fitting procedure for at least three other spectra corresponding to samples with different δ -phase contents. The central peak frequencies of δ_6 and δ_7 bands are evaluated using the low δ -phase proportion absorbance curves before the onset of the saturation regime. Finally, the central frequencies of the M_1 and M_2 absorption bands are determined using the 100% β -phase absorbance curve obtained using the polyethylene only sample as a reference.

Table 1. Absorption bands of δ and β phase D-mannitol between 0.2 and 2.2 THz.

Name	Frequency (THz)	Fit from
δ_1	1.01 ± 0.03	Figure 2(a) 100% δ sample
M_1	1.11 ± 0.03	Figure 1(a) 100% β sample
δ_2	1.13 ± 0.03	Figure 2(a) 100% δ sample
δ_3	1.27 ± 0.03	Figure 2(a) 100% δ sample
δ_4	1.44 ± 0.03	Figure 2(a) 100% δ sample
M_2	1.49 ± 0.03	Figure 1(a) 100% β sample
δ_5	1.58 ± 0.03	Figure 2(a) 100% δ sample
δ_6	1.92 ± 0.03	Figure 2(a) 30% δ sample
δ_7	2.15 ± 0.03	Figure 2(a) 30% δ sample

Central frequencies are determined by a reduced chi-square Voigt function fit of the given absorbance bands.

The THz-TDS technique gives also access to the phase information. Each frequency component of a THz pulse suffers a different phase shift as it travels through a sample. The frequency-dependent phase shift is directly related to the complex index of refraction of the sample. At frequencies sufficiently far away from the absorption bands the index of refraction is nearly constant and the phase of the THz wave varies linearly with the sample thickness.

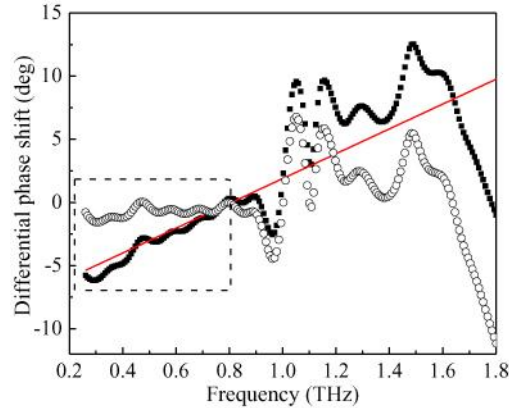


Fig. 3. (color online) Differential phase shift values of the THz waveform transmitted through the β -phase (0% δ -100% β) and the 10% δ -phase (10% δ -90% β) D-mannitol pellets plotted as a function of the frequency (full square symbol). The corrected data points (empty circle symbol) are obtained by subtracting a linear curve fit (solid line) to the experimental data points.

The index of refraction increases with frequency below and above the absorption band (normal dispersion regime) and the variation in the index of refraction suffers a sign change at the resonant frequency (abnormal dispersion regime). The amplitude of the frequency dependent phase shift profile around a specific absorption band depends on the oscillator strength of the vibrational mode; this amplitude is proportional to the crystalline phase content in the linear regime of oscillation. The δ -phase quantification can then be obtained using a differential phase shift measurement which consists in subtracting the THz phase spectrum of a $x\%$ δ -phase sample to the one of a 100% β -phase D-mannitol reference sample. Figure 3 shows the differential phase shift plot of the 10% δ -phase D-mannitol sample.

The experimental data curve (full square symbol) presents several absorption band features superimposed on a linear function of the phase characterized by a positive slope and a negative intercept. This linear frequency-dependent behavior is attributed to a difference in the pellet thickness of the two samples. Corrected data curve (empty circle symbol) is obtained by subtracting a simple linear interpolation that passes through the experimental data points. Since no absorption band is observed between 0.2 and 0.8 THz, we have used this frequency range (dotted box region shown in Fig. 3) to evaluate the standard deviation between the fitted and the experimental data points.

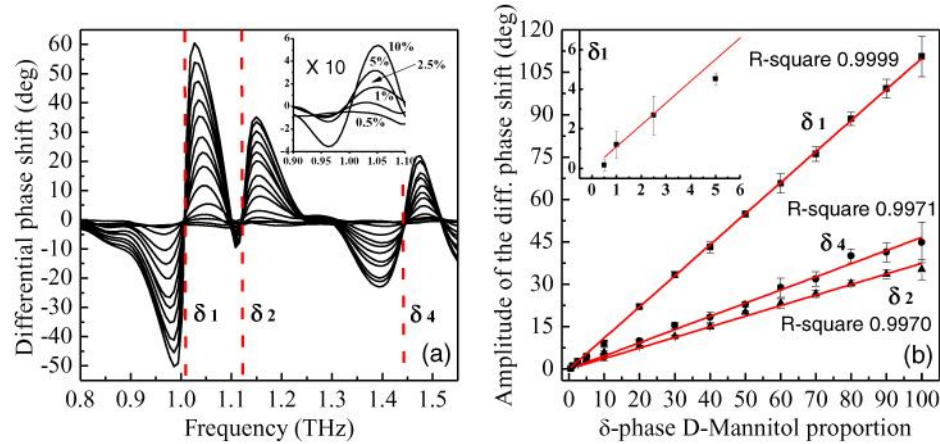


Fig. 4. (color online) (a) Corrected values of the differential phase shift, calculated using data from the 100% β -phase and the x% δ -phase D-mannitol pellets, plotted as a function of the frequency for the different δ -phase proportions from 100% (top curve) to 0.5%. Differential phase shift curves obtained for smaller δ -phase proportions (from 0.5% to 10%) are also plotted in the insert with a magnification factor of 10. Dotted lines indicate the central frequencies of the δ_1 , δ_2 and δ_4 absorption bands. (b) Amplitude of the differential phase shift, obtained for the δ_1 (full squares), δ_2 (full triangles) and δ_4 (full circles) bands, plotted as a function of the D-mannitol δ -phase proportion. The inset shows a zoom of this plot for low δ_1 -phase proportion values.

Figure 4 (a) illustrates the corrected differential phase shift measurements between 0.8 and 1.5 THz. The different curves correspond to different D-mannitol δ -phase proportions, varying from 0.5% to 100%. The inset shows a zoom (x10 magnification) of the curves corresponding to lower δ -phase proportions. Again, we have used the 100% β -phase sample for the reference phase spectrum because it allows removing the contribution of the M_1 band. For each band, we see a gradual rise of the corrected differential phase shift signal as the δ -phase proportion increases. However, the negative lobe of the δ_2 band is almost completely blurred by the presence of the intense positive lobe of the δ_1 band. We have plotted in Fig. 4(b) the amplitude of the differential phase shift as a function of the δ -phase proportion for each band. For the δ_1 and δ_4 bands, each data point is calculated by taking the difference between the maximum and the minimum of the differential phase shift around the central frequency of the band. For the δ_2 band, each data point is calculated by taking only the amplitude of its positive differential phase shift lobe. The inset in Fig. 4(b) shows that error bars are small enough to distinguish the linear behavior even at very low δ -phase proportions. A 0.5% difference in the D-mannitol δ -phase proportion is detectable using these corrected differential phase shift measurements. The phase quantification data points are well reproduced using a linear regression with an R-square value of 0.9999. For the δ_2 and δ_4 bands, the linear fits through the data points are also very good and the phase quantification capability is limited to samples with crystalline phase contents higher than 2.5%. The slopes of the linear fits are affected by the overlap of neighboring bands but the relative quantification of the crystalline phase content is nevertheless possible. The main factor contributing to the improved detection sensitivity of D-mannitol crystalline phases of the proposed method is probably related to the fact that the differential phase shift is unaffected by small defects on the pellets' surfaces while these defects normally cause extra absorption or diffusion losses. Since the density of these defects varies from sample to sample it is a limiting factor for the quantitative crystalline phase method that uses absorbance measurements. The differential phase shift measurements need to be corrected for the slight thickness variation between two samples but this is done at all frequencies using a simple linear extrapolation. This phase correction can also be performed with indirect measurements

of the sample's thicknesses obtained using a THz pulse echoes technique [17]. Notice that we do not correct for the influence of the extra absorbing material but only for the extra phase shift resulting from the different travel distance of the THz pulses.

The phase of the THz signal is very sensitive to the presence of an absorption band and we can see in Fig. 4(a) that the shape of the differential phase shift signal is influenced by one particular band far away from its central frequency. However, one great advantage of using the differential phase shift measurement is related to the extremely constant zero baseline signal observed over a large frequency range. This particularity reduces some arbitrary considerations and should help increasing the precision of a multi-parameters fitting procedure.

4. Conclusion

The quantification of β - and δ - crystalline phases of D-mannitol diluted into polyethylene pellets have been studied between 0.2 and 2.2 THz. We have identified seven absorption bands attributed to the δ -phase while two bands are common to both crystalline phases. Good fits of the baseline corrected absorbance curves are obtained using Voigt functions. Absorbance spectra with well resolved absorption bands have been obtained using an appropriate reference sample. The absorption bands observed at 1.01 THz, 1.13 THz and 1.44 THz are particularly interesting for quantification of the δ -phase proportion. The integrated amplitude of each absorption band increases almost linearly as a function of the δ -phase proportion but quantification is limited to samples having a δ -phase content higher than 5%. We have shown that improved detection sensitivity of D-mannitol crystalline phase content is obtained using differential spectral phase shift measurements. This quantification method is less sensitive to pellets' defects and the quality of the linear fits through the experimental data points are enhanced in comparison to those obtained using absorbance measurements. Variations of the δ -phase proportion as small as 0.5% can be distinguished in our D-mannitol + polyethylene samples. Next step will be to verify if further improvement of this new polymorphic phase quantification method can be obtained using a multivariable chemometric analysis on a spectral range that includes several absorption bands.

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