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Using terahertz pulsed spectroscopy to study crystallinity of pharmaceutical materials

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Abstract

The application of terahertz pulsed spectroscopy to polymorphic, liquid crystalline and amorphous forms of pharmaceutical compounds has been investigated. The different polymorphic forms of carbamazepine and enalapril maleate exhibit distinct terahertz absorbance spectra. In contrast to crystalline indomethacin and fenoprofen calcium, amorphous indomethacin and liquid crystalline fenoprofen calcium show no absorption modes, which is likely to be due to a lack of order. These findings suggest that the modes observed are due to crystalline phonon and possibly hydrogen-bonding vibrations. The large spectral differences between different forms of the compounds studied is evidence that terahertz pulsed spectroscopy is well-suited to distinguishing crystallinity differences in pharmaceutical compounds.

1. Introduction

Polymorphism and crystallinity changes are enduring issues in the pharmaceutical industry. Eight out of the ten most commonly prescribed drugs in the United States in 2001 are known to exhibit polymorphism or hydrate formation. Crystallinity variations in a pharmaceutical substance may exhibit physicochemical differences that impact at therapeutic, manufacturing, commercial and legal levels [1–3]. While polymorphism is usually undesired, a metastable polymorphic form or an amorphous form of a drug may sometimes be used advantageously, for example to increase solubility of a poorly soluble compound or to improve flow properties, important in tablet or capsule manufacturing [4,5]. In both situations variation in drug crystallinity must be investigated and monitored.

Terahertz radiation (~0.1–3 THz, corresponding to 3.3–100 cm^{-1}) can induce low frequency bond vibrations, crystalline phonon vibrations, hydrogen-bonding stretches, torsion vibrations and in gases molecular rotations [6]. Detection of these modes is likely to yield rich information when characterising materials. However, until recently experimental difficulties, especially with regard to sources and detectors, inhibited the use of the terahertz regime to investigate material properties. Recent advances are now allowing terahertz technology to be applied to many fields such as the semiconductor, medical, defence and space industries [7,8]. In particular terahertz pulsed spectroscopy (TPS), which produces broadband pulses on the femtosecond time scale, shows several application advantages. Its coherent nature provides high sensitivity with room temperature sources and detectors over a relatively broad frequency range. TPS measurements allow both the absorption coefficient and refractive index of a material to be calculated, and

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time-resolved studies on the sub-picosecond time scale potentially allow insight into dynamic systems [7,8]. In addition the low energy of terahertz radiation minimises the risk of sample degradation.

TPS has been applied to biologically active compounds, e.g. to illustrate low frequency vibrational modes of amino acids, proteins and DNA [9–11], and to differentiate isomeric configurations of retinal chromophores [12]. Benzoic acid and some monosubstituted derivatives, including salicylic acid and acetylsalicylic acid (aspirin) have also been differentiated using TPS [13].

The ability of TPS to probe lattice and hydrogen-bonding vibrations [6] makes it an ideal technique to investigate pharmaceutical polymorphism and crystallinity. TPS has been used to probe such vibrations in polycrystalline and amorphous saccharides [14]. In a recent study by Taday et al. [15], two polymorphs of ranitidine hydrochloride were shown to give distinct terahertz spectra, with only one peak at the same frequency between 0 and 3 THz. To our knowledge, no other studies using TPS to investigate pharmaceutical crystallinity and polymorphism have been published.

Carbamazepine (CBZ), enalapril maleate (EM), indomethacin (IM) and fenoprofen calcium (FC) (Fig. 1) are pharmaceutical examples that collectively exist in polymorphic, liquid crystalline and amorphous states. CBZ is known to exist in four [16] and EM in two anhydrous crystalline forms [17,18]. IM can be produced in both a crystalline and amorphous form [19,20], and FC may form a crystalline dihydrate or a supercooled thermotropic liquid crystalline state [21]. This Letter extends the work of Taday et al. [15] by investigating the ability of TPS to differentiate crystalline, amorphous and supercooled liquid crystalline forms of these four pharmaceutical compounds, demonstrating the technique's applicably to a broad range of solid-state forms of pharmaceutical compounds.

2. Experimental

2.1. Materials

Carbamazepine (5*H*-dibenz[*b, f*]azepine-5-carboxamide, purity >99%) and enalapril maleate (N-N-[(S)-1-ethoxycarbonyl-3-phenyl-propyl]-L-alanyl-L-proline hydrogen maleate, purity >99%) were obtained from Salutas Pharma GmbH (Barleben, Germany). Fenoprofen calcium dihydrate (calcium methyl-3-phenoxybenzeneacetate) was purchased from Spectrum Chemical Manufacturing Corp. (New Jersey, USA). Indomethacin (1-[19]-5-methoxy-2-methylindole-3-acetic acid) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyethylene (PE) powder (Inducos 13/1, particle size <80 μm) was obtained from Induchem AG (Volketswil, Switzerland).

2.2. Sample preparation

CBZ was supplied as form III (*P*-monoclinic), and this form was used without further purification. Form I (triclinic) was obtained by heating form III to 170 °C for 2 h, as described by Lefebvre et al. [22] and McMahon et al. [23]. EM designated as form II was used as received. Form I of EM was prepared by crystallisation from ethyl acetate in the presence of methanol (3.5% w/w) as described by Ip et al. [24]. Crystalline FC dihydrate was used as received. The supercooled thermotropic liquid crystalline form of FC was prepared by heating the

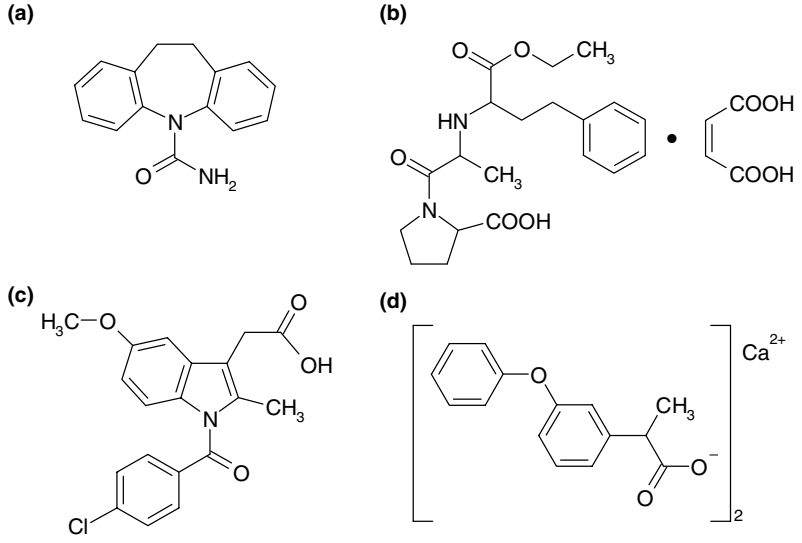

Fig. 1. Molecular structures of: (a) carbamazepine; (b) enalapril maleate; (c) indomethacin; (d) fenoprofen calcium.

crystalline powder in beakers open to air in a preheated oven for 45 min at 125 °C to remove the water of crystallization [25]. The samples were then brought back to room temperature over silica gel. Crystalline IM (γ-form) was used as received. Amorphous IM was produced by melting the crystalline form at 165 °C, followed by quench cooling in liquid nitrogen and subsequent warming back to room temperature in a dessicator over silica gel [20]. All samples were gently ground using a pestle and mortar to reduce particle size as much as possible and therefore minimize Mie scattering, and their solid-state form was confirmed by X-ray powder diffraction. Samples were stored at 4 °C over silica gel.

Sample tablets were prepared by mixing the pharmaceutical solid powder with PE powder (EM 25% w/w; CBZ 50% w/w or FC and IM 75% w/w in PE) with a pestle and mortar using geometric dilution. PE is a tablet binder and diluent with negligible absorption in the terahertz regime. Circular tablets (300 mg, 13 mm diameter) were formed with a hydraulic press using 1 ton compression (Specac Ltd., UK). Samples were also compressed into tablets using 2 and 5 ton compression, with no spectral changes observed for any of the compounds. Samples were prepared and measured in triplicate.

2.3. Measurements

All measurements were made using a TPI^{TM} spectra 1000 transmission spectrometer (TeraView Limited, Cambridge, UK). Samples were measured at an instrument resolution of 2–3 cm^{-1} over the range from 2 to 75 cm^{-1}. Data was acquired and processed using OPUS^{TM} (Bruker Optics, Germany) software.

3. Results and discussion

Figs. 2–5 show the terahertz absorption spectra for the different solid-state forms of CBZ, EM, IM, and FC respectively. It is evident from the figures that differences in the solid-state forms for all four compounds give rise to marked differences in the terahertz absorption spectra between 2 and 75 cm^{-1}.

Comparison of the spectra of CBZ forms III and I (Fig. 2) show peaks that are polymorph-distinct. The spectrum of form III exhibits major peaks at 41, 60 and 68 cm^{-1}, and a smaller peak at 47 cm^{-1} while the form I spectrum has prominent peaks at 31, 44, 52 and 70 cm^{-1}, with a low intensity peak at 23 cm^{-1}. The mid-infrared (IR) and Raman spectra of forms III and I are similar. Polymorph sensitive absorptions however, can be observed at 1390 and 1680 cm^{-1} in the IR spectra [16]. Computational studies have shown that these modes are associated with the $CONH_2$ moiety. Both forms exhibit dimer formation with hydrogen-bonding

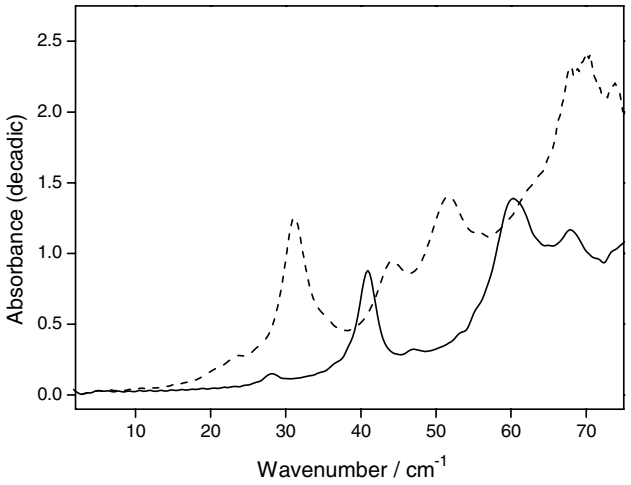

Fig. 2. Absorbance spectra of CBZ form III (solid line) and form I (dashed line) 50% in PE.

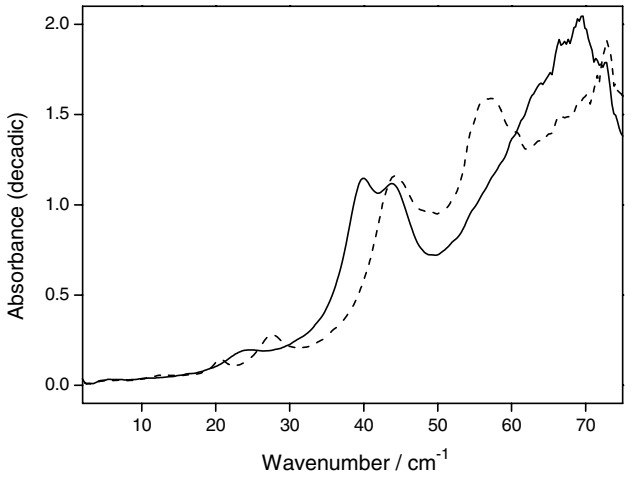

Fig. 3. Absorbance spectra of EM form I (solid line) and form II (dashed line) 25% in PE.

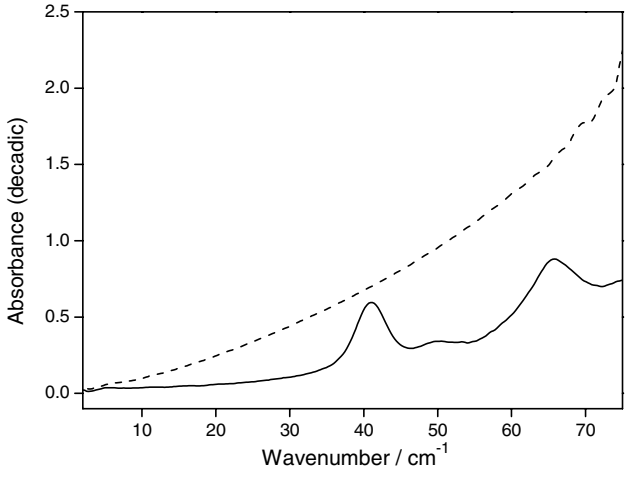

Fig. 4. Absorbance spectra of IM crystalline (solid line) and amorphous (dashed line) 75% in PE.

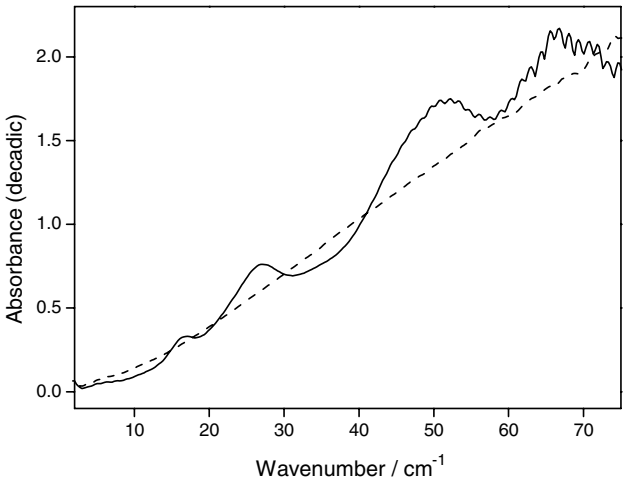

Fig. 5. Absorbance spectra of FC crystalline hydrate (solid line) and liquid crystalline anhydrate (dashed) 75% in PE.

between the $CONH_2$ groups. Differences in the crystal structure associated with this hydrogen-bonding are likely to be responsible for the differences observed in the terahertz region [26].

EM forms I and II also show pronounced spectral differences (Fig. 3). Form I has peaks which occur at 23, 39 and 69 cm^{-1}, with form II exhibiting peaks at 20, 27, and 57 cm^{-1}. In addition both forms have a common mode at approximately 44 cm^{-1}. Forms I and II of EM exhibit nearly identical mid-IR and Raman spectra. This has been attributed to very similar crystal packing, hydrogen-bonding patterns and conformations [17]. However, differences have been observed between the two forms of EM in the far-IR Raman spectra of forms I and II between 25 and 100 cm^{-1} [27]. The TPS results presented in this Letter suggest that the terahertz regime is better suited than the mid-IR region to differentiate polymorphs when dealing with organic crystals showing similar molecular conformations.

IM was studied in both the γ-crystalline and amorphous forms. The crystalline form shows peaks at 41, 50 and 66 cm^{-1}. There are no distinct peaks in the spectrum of the amorphous form. Diffuse, unstructured absorption was observed in the amorphous from. A similar observation was made by Walther et al. [14] when comparing polycrystalline and amorphous forms of the saccharides glucose, fructose and sucrose. Bertie et al. [28] observed a broad featureless spectrum of amorphous ice when comparing crystalline and amorphous forms, however the spectrum only extended down to 130 cm^{-1}. Such spectra suggest that the observed signals with the crystalline IM are due to intermolecular vibration modes of long-range order. If the modes present in the crystalline sample were due to intramolecular vibrations, one would also expect to see these modes in the amorphous state.

It is interesting to note the increasing absorbance for amorphous indomethacin with increasing frequency. Previous experimental work on both PE and lactose with various particle sizes (data not shown) has demonstrated that as particle size approaches the incident radiation wavelength, attenuation of the terahertz radiation occurs, as would be expected for Mie scattering. The average particle size of the crystalline IM sample was very small (<80 μm). As the amorphous IM was formed by quench cooling of the melt, with a solid mass resulting from the process it was necessary to create particles by grinding this mass with a pestle and mortar. Care was taken in this process to avoid recrystallisation of the amorphous drug making it difficult to reduce the particle size to the same size range as that of the crystalline form. It is thus likely that some particles of the amorphous form of the drug remained sufficiently large to induce Mie scattering, causing the absorbance of the sample to increase with wavenumber. However, with longer wavelengths, larger particle sizes are required for Mie scattering than is the case for shorter wavelength techniques, and thus it is likely that TPS can tolerate much larger particles before particle size influences the spectra obtained than for example, near-IR spectroscopy.

FC was chosen as a further pharmaceutical example due to its ability to exist in a thermotropic liquid crystalline state that can be cooled to room temperature. The liquid crystalline state has previously been identified as hexagonal [21]. The crystalline hydrate exhibited spectral peaks at 17, 27, 52 and approximately 66 cm^{-1}. The liquid crystalline form, however, lacked any distinct peaks in the spectral region studied. Comparison of the crystalline and liquid crystalline samples suggests that all modes present in the crystalline sample are due to long-range order resulting in phonon modes and/or hydrogen-bonding between the water and FC molecules. The liquid crystalline form exists as a hexagonal close-packed thermotropic phase formed by 1.7 nm diameter rods [25]. The absence of any terahertz signature in the liquid crystalline form would suggest that either the 1.7 nm order is insufficient to sustain a phonon mode in the spectral region studied or the modes observed in the crystalline form are a consequence of interactions with the water molecules in the crystal as no solvent molecules are present in the thermotropic mesophase.

4. Conclusions

The pharmaceutical examples investigated in this study show that a variety of different crystalline and amorphous forms of organic molecules are readily differentiated by their terahertz absorption spectra. The absence of distinct modes in the amorphous and liquid crystalline samples suggests that the absorbances in

more ordered samples are due to crystalline phonon vibrations. Terahertz spectroscopy easily differentiates different crystalline polymorphs, even when the crystalline structures of polymorphs are very similar. It also differentiates crystalline forms from liquid crystalline and amorphous forms of drugs. These results demonstrate the utility of TPS to differentiate solid-state forms of organic molecules in the pharmaceutical setting.

References

[1] J. Haleblian, W. McCrone, J. Pharmaceut. Sci. 58 (1969) 911.
[2] H.G. Brittain, Polymorphism in Pharmaceutical Solids, Marcel Dekker Inc., New York, 2000.
[3] J. Bernstein, Polymorphism in Molecular Crystals, International Union of Crystallography Monographs on Crystallography, Oxford University Press, Oxford, 2002.
[4] B.C. Hancock, G. Zografi, J. Pharmaceut. Sci. 86 (1997) 1.
[5] J.T. Carstensen, Pharmaceutics of Solids and Solid Dosage Forms, Wiley, New York, 1977.
[6] G.W. Chantry, Submillimetre Spectroscopy: A Guide to the Theoretical and Experimental Physics of the Far Infrared, Academic Press Inc. Ltd., London, 1971.
[7] B. Ferguson, X. Zhang, Nat. Mater. 1 (2002) 26.
[8] M.C. Beard, G.M. Turner, C.A. Schmuttenmaer, J. Phys. Chem. B 106 (2002) 7146.
[9] A. Markelz, S. Whitmire, J. Hillebrecht, R. Birge, Phys. Med. Biol. 47 (2002) 3797.
[10] A.G. Markelz, A. Roitberg, E.J. Heilweil, Chem. Phys. Lett. 320 (2000) 42.
[11] P.F. Taday, I.V. Bradley, D.D. Arnone, J. Biol. Phys. 29 (2003) 109.
[12] M. Walther, B. Fischer, M. Schall, H. Helm, P. Uhd Jepsen, Chem. Phys. Lett. 332 (2000) 389.
[13] M. Walther, P. Plochoka, B. Fischer, H. Helm, P. Uhd Jepsen, Biopolymers (Biospectroscopy) 67 (2002) 310.
[14] M. Walther, B.M. Fischer, P.U. Jepsen, Chem. Phys. 288 (2003) 261.
[15] P.F. Taday, I.V. Bradley, D.D. Arnone, M. Pepper, J. Pharmaceut. Sci. 92 (2003) 831.
[16] A.L. Grzesiak, M. Lang, K. Kim, A.J. Matzger, J. Pharmaceut. Sci. 92 (2003) 2260.
[17] Y.-H. Kiang, A. Huq, P.W. Stephens, W. Xu, J. Pharmaceut. Sci. 92 (2003) 1844.
[18] G. Précigoux, S. Geoffre, F. Leroy, Acta Crystallogr. C42 (1986) 1022.
[19] T.J. Kirstenmacher, R.E. Marsh, J. Am. Chem. Soc. 94 (1972) 1340.
[20] V. Andronis, G. Zografi, J. Non-Cryst. Solids 271 (2000) 236.
[21] T. Rades, Y. Padmadisastra, C.C. Mueller-Goymann, Pharmazie 51 (1996) 846.
[22] C. Lefebvre, A.M. Guyot-Hermann, M. Draguet-Brughmans, R. Bouché, J.C. Guyot, Drug Dev. Ind. Pharm. 12 (1986) 1913.
[23] L. McMahon, P. Timmins, A. Williams, P. York, J. Pharmaceut. Sci. 85 (1996) 1064.
[24] D.P. Ip, G.S. Brenner, in: K. Florey (Ed.), Enalapril Maleate, Harcourt Brace Jovanovich, New Brunswick, NJ, 1987.
[25] J. Patterson, A. Bary, T. Rades, Int. J. Pharmaceut. 247 (2002) 147.
[26] C.J. Strachan, S.L. Howell, T. Rades, K.C. Gordon, J. Raman Spectrosc. 35 (2004) (in press).
[27] D.P. Ip, G.S. Brenner, J.M. Stevenson, S. Lindenbaum, A.W. Douglas, S.D. Klein, J.A. McCauley, Int. J. Pharmaceut. 28 (1986) 183.
[28] J.E. Bertie, E. Whalley, J. Chem. Phys. 46 (1967) 1271.