

Using Terahertz Pulse Spectroscopy to Study the Crystalline Structure of a Drug: A Case Study of the Polymorphs of Ranitidine Hydrochloride

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Received 22 July 2002; revised 23 October 2002; accepted 22 November 2002

ABSTRACT: We describe the application of Terahertz pulse spectroscopy to polymorph identification. The particular compounds investigated were the different crystalline Forms 1 and 2 of ranitidine hydrochloride, both in the pure form and also obtained as a marketed pharmaceutical product. Identification was clear. The technique has advantages that excitation is not via a powerful laser source, as used in Raman spectroscopy, so phase changes or photochemical reactions in polymorphs do not occur. Terahertz absorption spectral interpretation and instrumentation are similar to basic Fourier transform infrared (FTIR) spectroscopy and therefore easy to understand. The sample preparation techniques used are the same as those used in FTIR and Raman spectroscopies. The data obtained is complementary to Raman Spectroscopy. As the selection rules are different between the two techniques, we are able to obtain new data set directly related to crystalline structure adding to that obtained by Raman spectroscopy. Terahertz pulse spectroscopy provides information on low-frequency intermolecular vibrational modes; these are difficult to assess in Raman spectroscopy due to the proximity of the laser exciting line. It is concluded that the method has a wide range of applications in pharmaceutical science including formulation, high throughput screening, and inspection in storage. © 2003 Wiley-Liss, Inc. and the American Pharmaceutical Association *J Pharm Sci* 92:831–838, 2003

Keywords: infrared spectroscopy; analytical chemistry; polymorphism; solid dosage form

INTRODUCTION

It has long been known that many pharmaceutical solids can exist in more than one crystalline form.¹ That different forms or polymorphs have the same chemical formula but different crystalline structures that can lead to different physical and chemical properties of the material. These may also impact on pharmaceutical properties of the drug, for example, different polymorphs may

have different rates of dissolution or bioavailability, and may even effect the stability of the drug.²

The formation of different polymorphs can be controlled during crystallization by the solvent used, the rate of cooling, and the degree of supersaturation of the solution. Once in the desired crystalline form the polymorphic state may be changed by incorrect storage or even during tablet preparation. At present, there are no quick and convenient methods for confirming the polymorphic state of drugs while in storage or during manufacture.

To date, many different physical techniques have been used to look at characterization of the solid form of pharmaceuticals, including single-crystal X-ray diffraction, optical spectroscopy, thermal analysis, and microscopy. Single-crystal

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Journal of Pharmaceutical Sciences, Vol. 92, 831–838 (2003)
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X-ray diffraction is still the benchmark method of determining the existence of a polymorph as it gives the crystalline structure directly; however, it does require sufficiently large crystals, and it is severely limited when applied to the polycrystalline or amorphous states. Mid-infrared and Raman spectroscopy are based on the intramolecular vibrations of molecules; these give rise to different peak positions, or peak intensities, for each polymorph the differences in the spectra can be small. Because these techniques frequently probe intramolecular vibrations, they are not direct probes of the intermolecular or collective modes that characterize the crystal structure. Laser Raman spectroscopy can create an extra, serious problem in that the laser irradiation can induce a phase change, or initiate photochemical reactions, in samples being interrogated. In near-infrared (NIR) spectroscopy the situation becomes more complex due to the fact that the spectra obtained will consist of many combination and overtone bands of the fundamental vibrations observed in the mid-infrared, making analysis difficult. There have not been many published reports of the application of NIR to pharmaceutical applications, but a number of spectrometer manufacturers are developing imaging systems for this application. Differences in the electronic environment for different crystalline states also allow the use of solid-state NMR; although the technique is inherently insensitive,³ it has been applied to finished drug products. Thermal properties are sensitive to differences in the crystal structure and reveal the existence of a phase transition at a particular temperature. Optical or electron microscopy can provide secondary or supporting evidence for the existence of a polymorph, but it is only spectroscopic techniques (including single-crystal X-ray diffraction), which can determine the molecular structure. Despite this myriad of techniques, no one method has been able to address the issues of high throughput polymorph screening or been able to quickly and conveniently confirm the polymorphic state of drugs while in storage or during manufacture.

In this article we demonstrate a quick, simple, and versatile technique for investigating different polymorphic forms using the new technique of Terahertz Pulse Spectroscopy (TPS).

There has been very little work reported in the THz-region (0.1 to 3 THz) or far-infrared region of the electromagnetic spectrum due to the considerable experimental difficulties in far-infrared Fourier Transform spectroscopy. These problems

include weak blackbody sources and helium cooled detectors. In other techniques such as FT-Raman spectroscopy, the proximity of the signal wavelength to the laser excitation line gives a requirement for excellent "notch" filters and double or even triple grating spectrometers. TPS uses room temperature sources and detectors; the former are 10^4 times more powerful than conventional incoherent blackbody emitters,⁴ while the latter have noise equivalent powers (NEPs) that can ultimately reach the α W (10^{-18} Watt) level. TPS is a time-gated technique, which minimizes background noise and enhances the signal-to-noise ratio. This allows a spectrum to be acquired in less than 100 ms, and because it is a transform technique there is no requirement for an expensive grating spectrometer. TPS selection rules are the same dipole selection rules found in infrared spectroscopy. The data obtained using this technique therefore complements the results of Raman spectroscopy and allows for the complete description of the vibrational structure of a molecule. Moreover, because coherent detection is used in TPS, one can measure the amplitude and phase of the THz pulse, which allows the absorption coefficient as well as refractive index spectra to be determined. The refractive index provides additional information about the material, but because it is related to absorption (by the Kramers-Kronig dispersion relationship) it only provides confirmation of the transitions observed.

The TPS technique has recently been applied to the study of DNA^{5,6} and low-frequency torsional vibrational modes in long chain carotenoids.⁷ Because very little experimental work has been done in this region, there are correspondingly few theoretical calculations. Hopefully, with recent activity in the field theoretical molecular models will develop and test their models against our results. Walther et al.⁸ have reported the THz-absorption spectra of the isomers of hydroxybenzoic-acid (salicylic acid), showing the power of the technique to determine different conformers of a molecule. To our knowledge, TPS has not been used previously for the detection of polymorphism. The sensitivity of THz spectroscopies such as TPS to the polymorphic state of the crystal follows from the fact that vibrations in this region are due either to large-amplitude torsional vibrations (intramolecular) or intermolecular interactions between nearest neighbour molecules. Provided such vibrations induce a change in the dipole moment of the crystal, absorption should occur. An example of the former would be the vinyl

group torsion in 4-methylstyrene, which has a fundamental mode⁹ at 1.17-THz, while an example of the latter is the hydrogen-bond translation S-band vibration^{10–12} in water at 6 THz.

To test the application of TPS to polymorph detection we investigated ranitidine hydrochloride, (N-[2-[(5-(dimethylaminomethyl)-2-furanyl)methyl]thio]ethyl)-N'-methyl-2-nitro-1,1-ethenediamine·HCl); the molecular structure is shown in Figure 1. This molecule is used in treatment of duodenal-gastric ulceration and Zollinger-Ellison syndrome. Crystalline ranitidine is polymorphic and exists in two crystalline forms known as Form 1 and Form 2, and there are also several pseudopolymorphic forms.¹³ The crystalline form depends on the solvent used for crystallization; for example, Form 1 is obtained by crystallizing from an ethanolic solution after the addition of ethyl acetate,¹⁴ while Form 2 is obtained from a solution of isopropanol-HCl.¹⁵ The two forms have equal solubilities, and there is no difference in bioavailability.^{16,17} As far as the pharmaceuticals companies are concerned, Form 1 is more difficult and expensive to crystallize while Form 2 is cheaper to manufacture, and is used exclusively in GSK products (Zantac). There have been no reports of interconversion between the two forms.^{18,19}

The mid-infrared (500 to 3000 cm^{-1}) and Raman (1000 to 1400 cm^{-1}) spectra of the two forms have

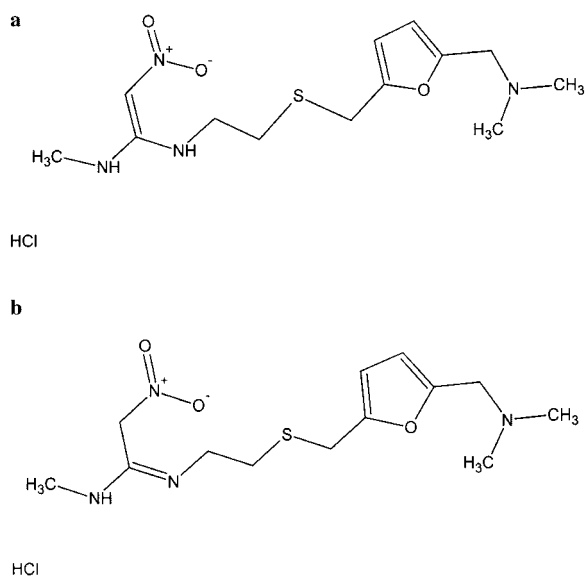


Figure 1. (a) Molecular structure of ranitidine hydrochloride (b) indicates a possible tautomeric form of ranitidine hydrochloride that has been suggested to be involved in difference between the two polymorphic forms.

been reported by Foster et al.²⁰ Taylor and Langkilde²¹ have reported the laser Raman spectrum of the drug in a tablet formulation. It was noted that the Raman beam was sufficiently intense to induce changes in the spectra of some of their samples. The X-ray diffraction data indicates that the orientation of the ranitidine molecules in the two forms is different,^{14,22} and this is thought to be due to a tautomerism in the ranitidine molecule.²²

In this article we report on the technique of obtaining the TPS absorption spectra of the two forms, both as pure samples and in the drug dosage formulation.

EXPERIMENTAL

Ranitidine-HCl forms 1 and 2 were obtained from Neuland Laboratories Limited and used without any further purification. The following dosage forms were also purchased; Apo-ranitidine 75-mg (Apotex Inc), Ranitidine 300-mg (Eastern Pharmaceuticals Ltd), Ranitidine 300-mg (Generics UK Ltd), Zantac (GSK), Ranitidine 300-mg (Noton, Ranbaxy Laboratories), and Ranitil 300-mg (Tillomed). Table 1 summarizes the percentage of the active ingredients in the above formulations.

All tablets and drugs were lightly crushed and mixed with polyethylene (PE) powder at a percentage weight of 25%. In the case of the formulated drug the percentage weight refers to the percentage of tablet, so the amount of ranitidine hydrochloride may vary between samples. The mixture was compressed into disc of thickness about 1 mm with a compression of about 2 tons. PE is transparent in the THz region, thus making it a suitable material as a matrix for spectroscopy in this region. The polyethylene disc mixtures were prepared at different compressions between 1 to 2 tons. The terahertz absorption were recorded for each compression; there was no observed interconversion between the two polymorphic states; this confirms the X-ray diffraction data obtained by Madan and Kakkar,¹³ who reported compressions between 4 to 6 tons and did not also observe any interconversion between the polymorphs.

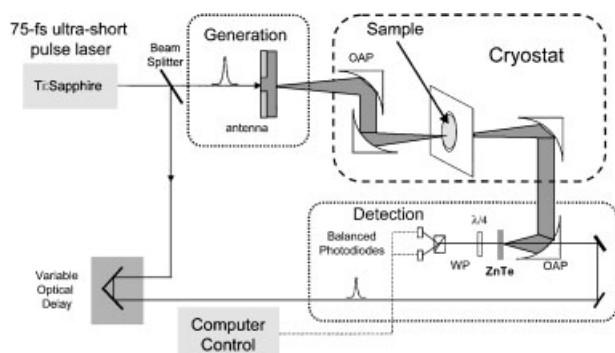
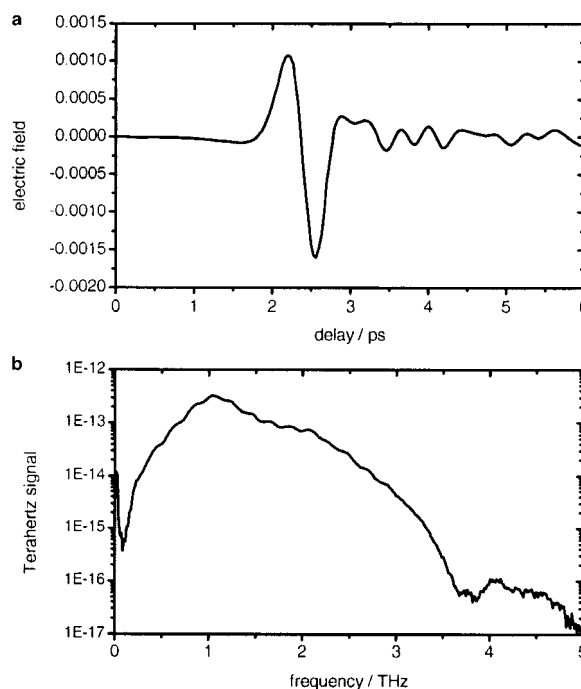
The apparatus employed for TPS is shown in Figure 2. The laser used was a Coherent Vitesse Kerr-lens, mode-locked Ti:Sapphire laser, which produces near-bandwidth limited pulses of 75-fs duration with an average power of 300 mW and a repetition rate of about 80 MHz. The laser beam is split into two; one beam is attenuated by 25% and

Table 1. Summary of the Percentage of the Active Ingredients of the Drugs Investigated

Trade Name	Manufacturer	Approx Weight Percentage of Drug/%
Apo-ranitidine	Apotex Inc	54
Ranitidine 300-mg tablets BP	Eastern Pharmaceuticals Ltd	54
Ranitidine tablets BP	Generics UK Ltd	66
Zantac	GSK	68
Ranitidine tablets BP	Norton, Ranbaxy Laboratories	53
Ranitol 300-mg tablets BP	Tillomed	63

is used to generate the THz radiation. The other beam is used as a probe to detect the THz radiation using electro-optic sampling (EOS).²³ The THz radiation is generated by focusing the laser beam onto a photoconductive switch,²³ which is placed on a 500 μm -thick GaAs substrate. The photoconductive emitter consisting of metal strips on a GaAs substrate, across which a DC bias is applied. The mechanism for THz emission is most simply explained by the device acting as an ultrafast, photoconductive switch. In the presence of a laser pulse, the switch is open and there is a flow of electrons across the gap. The acceleration of the electrons results in the emission of terahertz radiation. A more detailed description of the mechanism is given by Leitenstorfer et al.²⁴ The THz radiation generated by the semiconductor is collimated by an off-axis parabolic (OAP) mirror and is focused by another mirror onto sample with a spot size of about 1 mm. The transmitted beam is collected and collimated. Both THz radiation and the probe beam are then focused onto a zinc telluride (ZnTe) crystal. The probe beam polarization is modified in the presence of the THz radiation (Pockels effect) in the ZnTe. With the use

of a Wollaston prism, which splits the polarization of a beam into its respective components, a pair of balanced photodiodes can monitor the changes in the probe beam. The signal is then amplified by a lock-in amplifier, with a time constant of 100 ms. The time delay is either generated in a rapid-scanning mode by use of a scanning delay line or step-scanned by using a motorized translation stage. A typical time-gated signal is shown in Figure 3(a). Fast Fourier transforming this time-domain signal results in the frequency response of the device as shown in Figure 3(b). The useful bandwidth of detection is from about 100 GHz to

**Figure 2.** Schematic diagram of the TPS spectrometer. WP is a Wollaston prism. $\lambda/4$ is a quarter waveplate at 800 nm. OAP is an off-axis parabolic mirror.**Figure 3.** (a) Representative time-domain interferogram obtained from the system used. (b) Frequency-domain spectrum of the time-domain interferogram shown in Figure 3(a).

about 3 THz (6–100 cm^{-1}). In a step-scan mode a spectrum can be taken in less than 2 min with a spectral resolution of about 1 cm^{-1} . In the rapid-scan mode with slightly less spectral resolution, a few cm^{-1} , spectra can be obtained in less than 100 ms.

RESULTS

The THz field transmitted through a sample is modified by dispersion and the absorption of the media under examination. The ratio of the electric field strength before $E_r(\omega)$ and after transmission, $E_s(\omega)$, is given by

$$\frac{E_s(\omega)}{E_r(\omega)} = T(n(\omega)) \exp\left(-\alpha(\omega)d + \frac{\text{in}(\omega)\omega d}{c}\right) \quad (1)$$

where d is the thickness of the sample, ω the frequency of the radiation, c the speed of light in vacuum and $T(n(\omega))$ is the reflection loss at the sample surface. Both the refractive index $n(\omega)$ and the absorption coefficient $\alpha(\omega)$ can be determined from the ratios of the measured THz fields.

Figure 4 shows the THz absorption spectra for Form 1 and Form 2 of ranitidine HCl. The insert in Figure 4 highlights the region around 1.10 THz where there are considerable differences in the THz spectrum between the two polymorphs; this can be used in the identification of the two structures. This region selected has been highlighted because it shows the most obvious differences between the polymorphs. The data can be fitted to

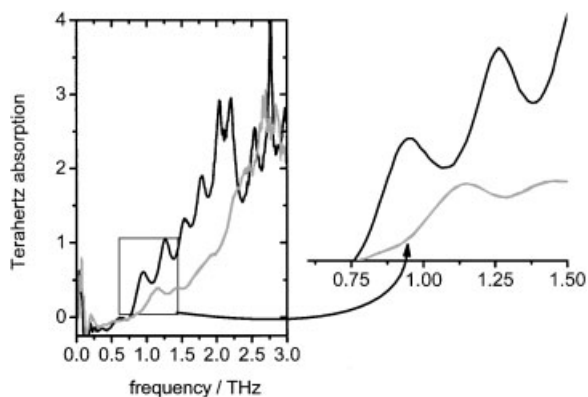


Figure 4. Comparison of Form I (black) and Form II (gray) at 300 K. The insert highlights the region around 1.10 THz where there are considerable changes in the THz-spectrum between the two polymorphs. Terahertz absorption is defined as $\log(\text{transmission of reference pulse}/\text{transmission of sample pulse})$.

Table 2. Active Bands in the Ranitidine Hydrochloride Forms 1 and 2 at 300 K

Form 1 300 K Frequency/THz	Form 2 300 K Frequency/THz
2.76	2.76
2.55	2.39
2.20	2.26
2.04	1.85
1.78	1.40
1.53	1.13
1.26	
0.95	

a series of Lorentzian lineshape functions to obtain the peak positions; these are given in Table 2. Temperature-dependent THz absorption spectra of the two forms of ranitidine have been obtained.²⁵ These will aid spectroscopic assignment of the bands. Because the molecular structures of two polymorphs are the same, the main changes in the absorption spectra must be due to the different local crystalline environment induced by nearest neighbour interactions. Form 1 has a series of vibrations spaced at about 250 GHz stretching from 0.95 THz to 2.04 THz, which could be a torsional motion in one of the functional groups in ranitidine. It is noted that in Form 2, the position and the intensity of the peaks are different, and in particular, the almost harmonic character seen in Form 1 vanishes. This could be due to the steric hindrance preventing rotation of the group, which

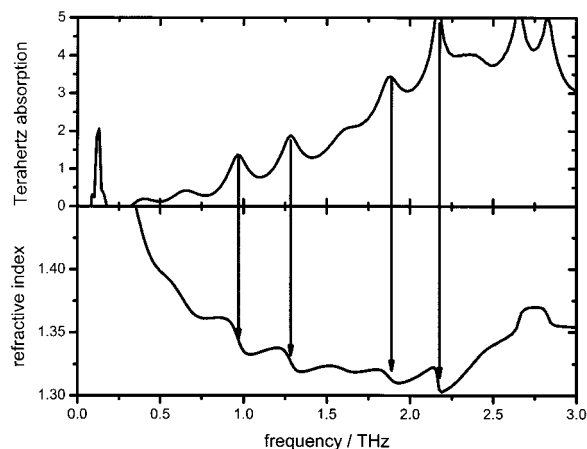


Figure 5. The terahertz absorption spectrum of drug formulated Apo-ranitidine (ApoTex) upper graph. The lower graph shows the change in refractive index. The arrows indicate the correlation between the refractive index and the absorption coefficient of the material.

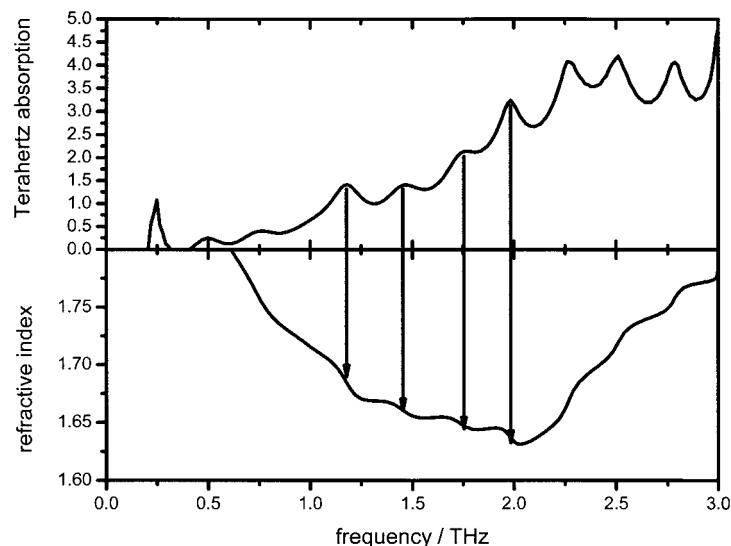


Figure 6. The terahertz absorption spectrum of drug formulated Zantac (Glaxo) upper graph. The lower graph shows the change in refractive index.

causes the observed bands in the Form 1 spectrum. Assignments of these vibrations are difficult due to the lack of theoretical data available and the number of “free-rotators” available in the ranitidine molecule. Understanding of the crystalline phonon bands could also lead to the assignment of these spectra.

To highlight the power of the technique Figures 5 and 6 shows the absorption spectra and the change in refractive index of the formulated version of the drugs, namely, Zantac and Aporanitidine, respectively, in the THz region. By comparing the spectra in Figure 4 with the two formulations in Figures 5 and 6, it is clear that Zantac is based on form 2 while Aporanitidine contains form 1. Figure 7 shows comparison of

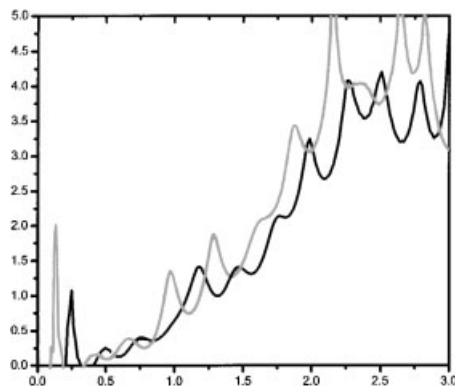


Figure 7. Comparison of the absorption curves shown in Figures 5 and 6. Aporanitidine is the black curve and Zantac is the gray curve.

the two over the range 300 GHz to 3 THz (Apo-ranitidine is the black curve and Zantac is the gray curve). Figure 8 shows the THz absorption spectra of other four ranitidine generic formulations outlined in Table 1; these all contain Form 1. It is noted that there are considerable differences between the spectra in Figure 8 above 1.5 THz. These differences could be due to different excipients used in the preparation of the formulated tablet. We are in the process of studying the different excipients and their effect on the terahertz absorption spectra.

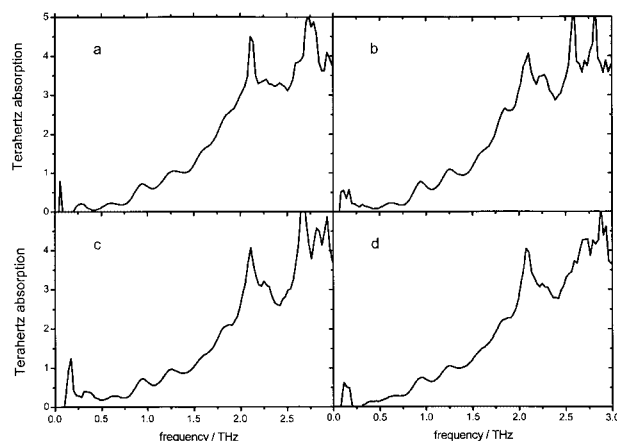


Figure 8. Comparison of four different ranitidine drug dosage tablets at room temperature from the following manufactures (a) Eastern Pharmaceuticals Ltd, (b) Generics UK Ltd, (c) Tillomed, and (d) Norton Ranbaxy Laboratories.

CONCLUSIONS

No normal mode calculations have been carried out for the two forms of ranitidine hydrochloride; thus, assignments of the transitions observed in the THz region are currently not possible. But what the spectra do show is that using TPS it is possible to rapidly (<100 ms) distinguish between the two polymorphic forms in drug formulations. Because the spectrum in the THz region is very sensitive to intermolecular interactions, we expect to resolve the polymorphs of other molecules of pharmaceutical significance.

We should also point out that because most plastics in which drugs are stored are transparent to THz radiation, it is possible to use the technique to examine substances while in storage. The system can also be configured to work in reflection resulting in an instrument that can monitor a compound while in production, allowing for quality assessment and quality control of processes.

TPS, unlike many other optical techniques, uses radiation of sufficiently long wavelength such that it does not induce any phase changes or photochemical reactions. In addition, because the technique utilizes very low-power densities, in the THz beam is not sufficient to heat the sample. We also note that the potential for using the TPS technique in a rapid-mode scan mode, that is obtaining spectra in less than 100 ms, makes possible applications in high-throughput environments.

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