Polymorphic Forms of Furosemide Characterized by THz Time Domain Spectroscopy

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Terahertz time domain spectroscopy (THz-TDS) is applied in transmission to identify the five forms of modifications of furosemide and one commercial product from 0.3 THz to 1.6 THz at room temperature. The different absorption spectra of the different forms are sensitive to crystal structures. Density function theory (DFT) calculation was used to understand the vibrational modes of furosemide in the THz region. X-ray powder diffractometry (XRPD) was applied to confirm the different forms of modifications. The results demonstrate that THz-TDS is a potential analytical technique in investigating polymorphic forms in the pharmaceutical fields.

Key Words: Terahertz, Furosemide, DFT, XRPD, Polymorphic forms

Introduction

In a broad sense, polymorphism refers to all solid forms of the same molecule that have the same vapor, liquid, or solution phase,[1] that is, true polymorphs, solvates, desolvates, liquid crystals, as well as the amorphous materials and mixtures. It is well known that many pharmaceutical solids can exist in more than one solid form. Over the last several decades, there has been a sharp rise in the interest in polymorphic systems, especially in pharmaceuticals.[1-3] These polymorphs can lead to the different physiochemical properties of the material. For example, different polymorphs may have different rates of dissolution or bioavailability, and may even affect the stability of the drug.[4] To date, a wide spectrum of analytical techniques has been used extensively to characterize polymorphs including crystallographic, spectroscopic, microscopic, and thermal techniques.[5-7] However, there is not one method that could address the issues of high throughput polymorph screening or could quickly and conveniently confirm the polymorphic state of drugs while in storage or during manufacturing. A quick, simple, and versatile technique for investigating the different polymorphic forms is needed.

Terahertz (THz) spectroscopy, which occupies the portion of the electromagnetic spectrum between mid-infrared and microwave bands, has been utilized in diverse fields.[8] In the fields of pharmaceutical materials, some groups[9-12] have shown that Terahertz Pulse Spectroscopy (TPS) can discriminate between different polymorph forms and examine the mechanism of a polymorphic transition. Some[13] have demonstrated that Terahertz Pulsed Imaging (TPI) can be used to analyze nondestructively tablet coating thicknesses. Liu *et al.*[14] concluded that terahertz time domain spectroscopy (THz-TDS) is an advantageous technique for pseudopolymorph identification and study. These results show the potential future of THz as an effective and alternative technique in the identification of the polymorphic forms of drugs. The advantages of THz-TDS are that THz is not ionizing and is safe, and THz radiation can be transmitted through many materials. These advantages can be successfully used in the nondestructive inspection of mail and packaging, and in pharmaceutical inspections. Therefore, THz technology has a great potential to become a powerful analytical tool in pharmaceutical production and process control.

Furosemide is a loop diuretic used in the treatment of congestive heart failure and edema. It is known to exist in five forms of modifications (three polymorphs and two solvates)[15] aside from one commercial product. Conventional methods such as X-ray powder diffractometry (XRPD), infrared and Fourier transform infrared spectroscopy, and elemental analysis have been used to study the modifications of furosemide. In this work, five forms of modifications of furosemide are prepared and identified by THz-TDS. The characteristic spectral changes make THz-TDS an ideal candidate in investigating the polymorphic forms of furosemide.

Methods and Materials

THz-TDS. The details of the transmission THz-TDS apparatus used in our experiments have been described elsewhere.[16] A mode-locked Ti: sapphire laser was applied to pump and detect the THz wave, with a pulse duration of 100 fs, a central wavelength of 800 nm, and an average power of 700 mW. The pump beam was focused on a THz emitter GaAs crystal to generate THz pulses, wherein the probe beam detected the THz field *via* electro-optical sampling using a 2-mm-thick ZnTe crystal. The usable bandwidth of setup was from 0.3 THz to 1.6 THz, SNR was higher than 1000, and the spectral resolution was better than 40 GHz. The whole system was placed in a closed box purged with dry nitrogen to minimize absorption by water vapor.

Sample preparation. Furosemide was purchased from Acros. The molecular structure of Furosemide is shown in Figure 1. The commercial product was used as received. Methanol and acetone were of analytical grade. Dimethylformamide (DMF), *n*-butanol and dioxane were high performance liquid chromatography (HPLC) reagents. The methods of preparing furosemide modifications and the corresponding types of modifications

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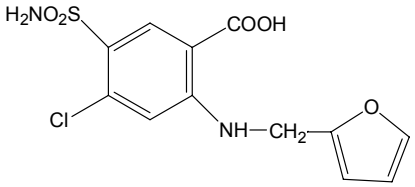

Figure 1. Chemical structure of furosemide.

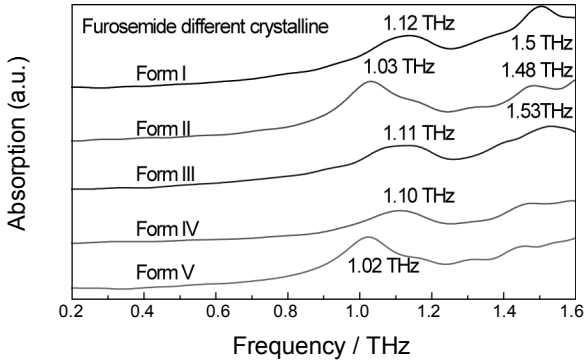

Figure 2. THz absorption spectra of furosemide modifications in the 0.2 ~ 1.6 THz range: Form I (1.12,1.5 THz), Form II (1.03,1.48 THz), Form III (1.11,1.53 THz), Form IV (1.10 THz), and Form V (1.02 THz).

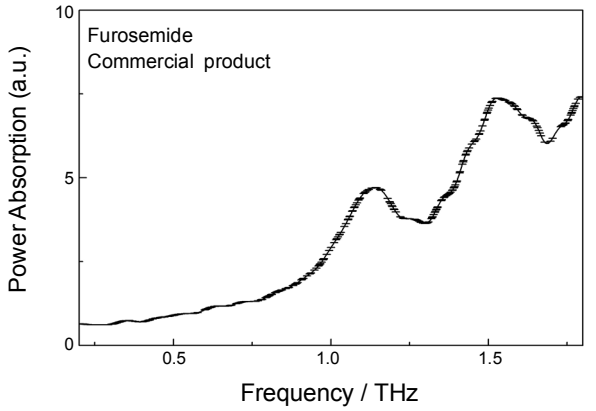

Figure 3. THz absorption spectrum of commercial furosemide (1.14, 1.54 THz) in the 0.3 ~ 1.8 THz range.

are listed in Table 1. Rotary Evaporator (RE-52A) was used to evaporate acetone in the case of Form (III). Y Matsuda[15] gave a more detailed description of the preparation modifications.

Using an agate mortar and pestle, 80 mg of all the samples and 120 mg of polyethylene (PE) were gently ground to reduce particle size and minimize Mie scattering. PE acted as a binder and diluent in the sample preparation due to its negligible absorption in the THz regime. The mixture was then compressed directly with 1 ton load for 10 min into a pellet with a diameter of 13 mm. There were no spectral changes observed for the

Table 1. Methods of preparing furosemide modifications.

Crystalline Form	Solvent	Method
Form I	methanol	Slow recrystallization
Form II	*n*-butanol	Slow recrystallization
Form III	acetone	Fast evaporation
Form IV (DMF solvate)	DMF/water	Addition of a small amount of water to a hot organic solution until the crystals begin to separate
Form V (Dioxane solvate)	1,4-dioxane	Slow recrystallization

samples compressed into tablets using 2 t and 5 t compressions. The thickness of disc was about 1.2 mm.

Results and Discussion

The power absorption coefficient and the index of refraction of the different samples were obtained in the frequency range of 0.3 ~ 1.6 THz. Figure 2 shows the THz absorption spectra for Form I to Form V. The presence of the absorption features is confirmed by the observation of abnormal changes in the refractive index. The distinct absorption peaks of every sample are observed. It is clear that Form I has two absorption peaks observed at 1.12 THz and 1.5 THz. Form II shows two absorption peaks at 1.03 THz and 1.48 THz. There are also two absorption peaks (1.11 THz and 1.53 THz) for Form III. One major absorption peak at 1.10 THz was observed for Form IV. Form V exhibits one prominent absorption feature at 1.02 THz. Table 2 presents the experimental and theoretical THz absorption peaks of furosemide. It is evident from Figure 2 and Table 2 that the differences in the five polymorphic forms of furosemide give rise to marked differences in the THz absorption spectra between 0.3 THz and 1.6 THz. This can be due to the different crystal structures. By comparing the shape (including the position and intensity of the peaks) of the spectra, it is clear that THz can be used in the identification of the five different forms of furosemide. Although the absorption peaks of Form I are close to those of Form III below 1.6 THz, the spectra features are found to be different in the whole spectrum range. It is noted that the position of the peaks (1.14 THz and 1.54 THz) of the commercial product (Figure 3) are similar to that of Form III below 1.6 THz, although the THz absorption spectrum in the commercial product is much lower than the absorption in form III. It is not easy to confirm whether the commercial product belongs to form III. As reported previously,[17] the differences in THz spectra between carbamazepine forms I and III are very clear at low temperatures, allowing the clear identification of these polymorphic forms. Thus, the clear differences in low-frequency vibrations between the five different forms of

Table 2. Theoretical and experimental THz absorption peaks for furosemide.

Form I	Form II	Form III	Form IV	Form V	Commercial product	Calculation
1.12	1.03	1.11	1.10	1.02	1.14	0.97
						1.32
1.5	1.48	1.53			1.54	1.50

furosemide and the commercial product with a wide-bandwidth THz system at a lower temperature should be examined.

It is known that many factors contribute to the low-frequency spectrum and result in obvious absorption, including: intermolecular vibrational modes, crystalline phonon vibrations, hydrogen-bonding stretches, torsion vibrations, and molecular rotations in gases. In previous reports, Fischer *et al*.[18] attempted to assign the THz spectrum of thymine to the hydrogen-bonding vibrations between molecules within the crystal structure. Shen *et al*.[19] reported the temperature-dependent THz spectra of purine and adenine, which were assigned to phonon vibrations. The density functional theory (DFT) has been proven as a theoretical model to predict vibrational information. In this work, Gaussian 03 program package[20] at B3LYP/6-311G level is used to predicat the frequency vibration of the single molecule of furosemide. The optimized conformation of the monomer of furosemide at the B3LYP/6-311G level is presented in Figure 4. Table 3 lists the bond lengths (Å) and bond angles (^{o}) optimized

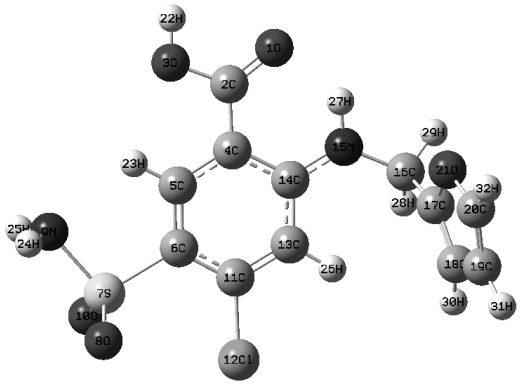

Figure 4. DFT: B3LYP/6-311G calculated structure of furosemide showing the atom numbering.

at the same level. Three vibration modes with very weak intensity are obtained in the THz region. The 0.97 THz mode is associated with the rotation of the furan group and the carboxylic group. The 1.32 THz mode is associated with the rotation of the substituted groups except for the furan part. The 1.50 THz mode is caused by the vibration of the whole molecule. Although the 1.50 THz from the calculation is very close to the 1.50 THz of Form I, 1.48 THz of Form II, 1.53 THz of Form III, and 1.54 THz of the commercial product, ascribing this mode to intramolecular interactions seems unlikely. These calculations would first be attempted to help understand the characteristic THz absorption peaks measured by the experiment. One reason for this is that the calculation is simulated at 0 K, while the experiment is performed at room temperature. Another reason is that the intermolecular interactions are not considered in a single molecule calculation. It would be much better to calculate the solid state vibration spectra using periodic boundary conditions. With regard to furosemide, every polymorphic form has different packing structures. For instance, Form I unit cell was shown to possess an intermolecular hydrogen bond network involving sulphonamide groups.[21] The IR data for the furosemide II crystalline form suggests that there is an alteration in the hydrogen bond sequence within the crystal.[22] From the above results, it can be concluded that the THz spectra differences of the polymorphic forms of furosemide are not intramolecular interactions, possibly due to the crystalline phonon and the hydrogen-bonding vibrations.

The solid-state forms of furosemide are confirmed by XRPD (Ri-gaku D/MAX-2550 VB/PC, Japan). Figure 5 shows the XRPD patterns of all modifications as well as the commercial product in the range of 4 ^{o} ~ 35 ^{o} 2 θ. The comparison of the characteristic angle diffraction and the three main peaks for furosemide modifications together with the commercial product are presented in Table 4. The most significant aspects of our

Table 3. Geometry of the monomer of furosemide optimized at the B3LYP/6-311G level. Bond lengths (R) in Å; bond angles (A) in degrees.

R	Å	R	Å	R	Å
C2-C4	1.458	C5-H23	1.079	C14-N15	1.360
C4-C5	1.403	C13-H26	1.076	C16-N15	1.467
C4-C14	1.437	C16-H28	1.092	N15-H27	1.014
C5-C6	1.380	C16-H29	1.091	N9-H24	1.018
C6-C11	1.400	C18-H30	1.076	N9-H25	1.018
C11-C13	1.377	C19-H31	1.075	C6-S7	1.884
C13-C14	1.421	C20-H32	1.072	S7-O8	1.638
C16-C17	1.493	C2-O1	1.249	S7-O10	1.638
C17-C18	1.364	C2-O3	1.377	C11-Cl12	1.810
C18-C19	1.442	C17-O21	1.400	O3-H22	0.976
C19-C20	1.360	C20-O21	1.395		
A	^{o}	A	^{o}	A	^{o}
C4-C5-C6	120.7	C17-O21-C20	106.9	N15-C16-C17	114.8
C5-C6-C11	120.1	O21-C17-C18	109.1	H28-C16-H29	106.9
C6-C11-C13	120.7	O21-C20-C19	109.7	C17-C18-H30	126.3
C11-C13-C14	121.0	O1-C2-O3	120.2	C18-C19-H31	126.5
C13-C14-C4	117.6	C2-O3-H22	109.9	C19-C20-H32	134.6
C5-C4-C14	119.8	C4-C5-H23	119.9	C6-S7-N9	100.5
C2-C4-C14	120.7	C14-C13-H25	120.2	H24-N9-H25	113.6
C17-C18-C19	107.4	C14-N15-C16	125.9	O8-S7-O10	122.2
C18-C19-C20	107.0	C14-N15-H27	116.0	C6-C11-Cl12	121.3

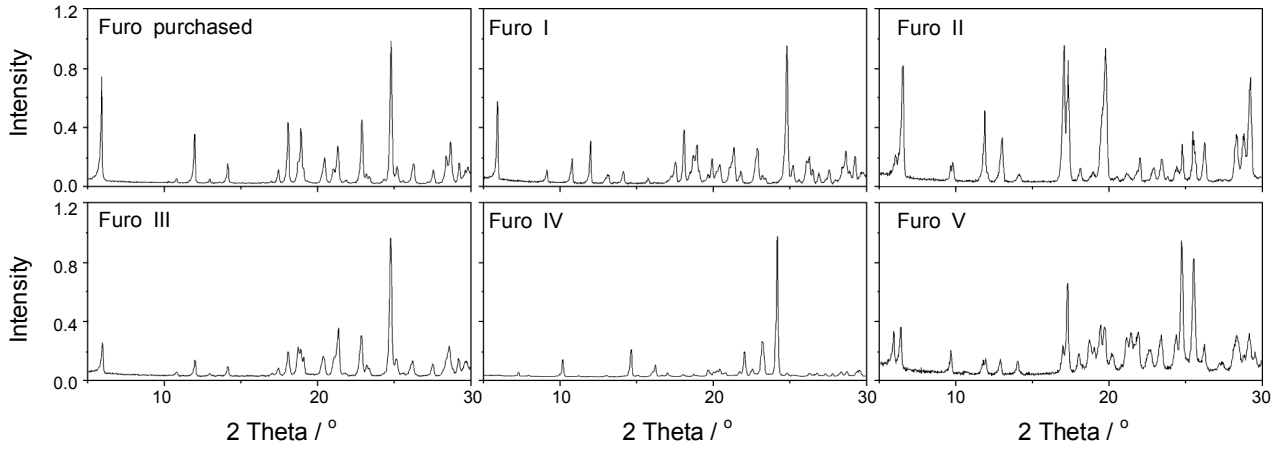

Figure 5. XRPD patterns of furosemide modifications: Form I, Form II, Form III, Form IV, Form V, and the commercial product.

Table 4. Comparison of the three main peaks in the XRPD patterns of furosemide modifications: Form I, Form II, Form III, Form IV (MF solvate) and Form V (Dioxane solvate).

Modification	Experiment		Ref. 15	
	2 θ / °	I/I_0 / × 100	2 θ / °	I/I_0 / × 100
Form I	24.7	100	24.8	100
	6.1	57	21.3	46
	18.1	37	22.9	40
Form II	17.1	100	17.3	100
	19.8	96	20.1	74
	17.3	89	6.7	47
Form III	24.9	100	24.6	100
	21.4	35	26.1	98
	22.9	29	19.8	94
Form IV	24.2	100	23.5	100
	23.2	24	24.5	90
	14.7	20	14.9	56
Form V	24.7	100	8.3	100
	25.5	88	18.7	31
	17.3	72	18.3	29

results are in agreenment with those of Y. Matsuda.[15] The XRD spectra of furosemides recrystallized from different solvents differ from one another through the appearance of new pattern lines and alterations in the relative peak heights of other peaks. The differences in XRD profiles among the modifications of furosemide are distinct, suggesting that the solid-state forms of furosemide we prepared belong to different crystal packing and structures. It is clear that the XRPD pattern of Form I differs significantly from that of Form III. This indicates that THz-TDS is very sensitive to the crystalline structures of Form I and Form III.

Conclusion

THz-TDS is applied to analyze the five forms of modifications of furosemide and one commercial product. Each form shows a distinct absorption feature in the THz range, making it possible to distinguish the five different modifications of furosemide. The computed low frequencies of furosemide by DFT are compared with experimental data using THz-TDS. The different forms of modifications are confirmed by the results of XRPD. The capability to identify the different modifications of furosemide in the THz region is a great advancement towards novel applications in the pharmaceutical fields of scientific research and industry.

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