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SELECTED ASPECTS OF TERAHERTZ SPECTROSCOPY IN PHARMACEUTICAL SCIENCES

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Abstract: THz-TDS techniques are applied to investigate selected pharmaceutical samples. Investigations were performed on selected pharmaceutical samples with active pharmaceutical ingredients (API) - famotidine, ranitidine, fenofibrate, lovastatin, simvastatin, aspirin, ketoconazole, acyclovir (hydrated and non-hydrated), on excipients - lactose, glucose (hydrated and non-hydrated), Pluronic 127, and on mixtures of selected compounds. Pseudo-polymorphism effects are considered as well. Examples of the terahertz imaging technique are also given. APIs and excipients can be easily recognized in the terahertz band by their specific "fingerprints" as individual components and in mixtures. The hydration process as a variety of polymorphism can also be easily monitored using the THz technique. Moreover, terahertz light can be useful for the penetration of tablets, giving clear pictures of possible defects in tablet coatings.

Keywords: terahertz technique, pharmaceutical sciences, time-domain spectroscopy, pseudo-polymorphism, hydration, coating, imaging, THz, far-infrared absorption

Nowadays, terahertz technology is definitely not a "dead land", as it was long ago described (1). Terahertz techniques are setting new trends in research methodology used in many domains of biomedicine, including the pharmaceutical sciences (2-7). Waves from the band of the electromagnetic spectrum called THz radiation (10-333 cm^{-1} = 0.3-10 THz) are being successfully harnessed for investigations on pharmaceutical media by utilizing significant spectral information. Terahertz spectroscopy contains information about crystal lattice vibrations that are associated directly with the structure of the crystal. In contrast, so called vibrational spectroscopy - the domain of the MIR and NIR spectrum - focuses its interest on the structure and dynamics of molecules in gases, liquids and at interfaces. In other words, it deals with vibrational motions including chemical reaction dynamics. Spectra extracted from a THz region are very sensitive to structural properties of media including pharmaceutical ingredients (8). In precisely this spectral region "heavy" molecules, which are predominantly used in the pharmaceutical domain, leave so called "fingerprints" - specific distributions of absorption lines, or in other words, spectral details (the "fingerprints"). It spans approximately between 10 and 200 cm^{-1}. The bandwidth is rather fluid and is still being modified by results appearing in scientific journals, even from 8 to 220 cm^{-1} (6, 7, 9-12). The THz technique allows the monitoring of pharmaceutical products throughout the development process, recognizing the specific spectra of the drug components. Identification of the polymorphs or hydrates in drug forms is becoming more and more complicated with

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the development of contemporary pharmacology and drug form technology (13). There are also many methods of the analysis: dissolution testing, thermoanalytical methods, calorimetric analysis, microscopic methods (14, 15), X-ray diffraction (XRD) (13), differential scanning calorimetry (DSC) and FTIR spectroscopy (16). Apart from spectroscopic methods (IR, FTIR, Raman), THz spectroscopy can be used for this purposes, especially to investigate solid dosage forms to demonstrate e.g., pseudopolymorphism (hydration effects) (17). Terahertz waves can substitute for dangerous and not "comfortable" X-radiation for investigations of cross sections of the drugs without destruction of the sample (13).

MEASUREMENT SETUP

In this paper we utilize pulsed THz generators used in the arrangement of terahertz time domain spectroscopy (THz-TDS) (18) to measure the spectral properties of investigated media. In order to verify our results we used typical substances of known data. To illustrate the problem, we present the results of measurements of the following: APIs such as famotidine, ranitidine, fenofibrate, lovastatin, simvastatin, aspirin, ketoconazole, acyclovir hydrated, acyclovir non-hydrated; excipients such as lactose, glucose hydrated, glucose non-hydrated, Pluronic 127; mixtures such as fenofibrate with aspirin = 44/56, lovastatin with aspirin = 27.8/72.2, lovastatin with aspirin = 83.6/16.4, pluronic with ketoconazole = 50/50.

Additionally, an example of terahertz imaging was performed on a ketonal tablet with coating in THz-TDS reflective arrangement.

Terahertz TDS spectrometer arrangement

A standard terahertz time domain spectroscopy measurement system is shown in Figure 1. The system contains a femtosecond laser which drives the emitter Tx and receiver Rx antennas. The pellet is placed in the focus of the THz beam collimated by parabolic off-axis mirrors PM. The beam of the laser is being split with a cubic beam splitter BS into beams "1" and "3" and next, focused with microscopy lenses on Tx and Rx antennas. The coherent homodyne detection method is used for the investigations (1). The THz beam path "2" is purged with dry air to avoid absorption by water vapor. The samples are used in polycrystalline form and mixed with polyethylene (PE) powder. PE powder is used because it is nearly transparent in the terahertz region (19).

THEORY

When a sample is inserted in the arm - see Figure 2 - the test material, the Rx antenna measures the signal E_{med}^{Thz} with the presence of some medium in the arm. The result is a convolution of two functions - terahertz E_{ref}^{Thz} and optical E_{prob} signals - without the sample, and terahertz E_{med}^{Thz} and optical E_{prob} signals - with the sample. The idea of the measurements and calculations is illustrated in Figure 2. The method is called a coherent homodyne detection of the signal. In fact, it is a homodyne setup, where the same optical beam (an exciting beam) creates the terahertz wave in phase with the optical beam, and simultaneously the same optical beam is used as a probing one.

As is known, the conversion of such signals from time domain to frequency domain makes the

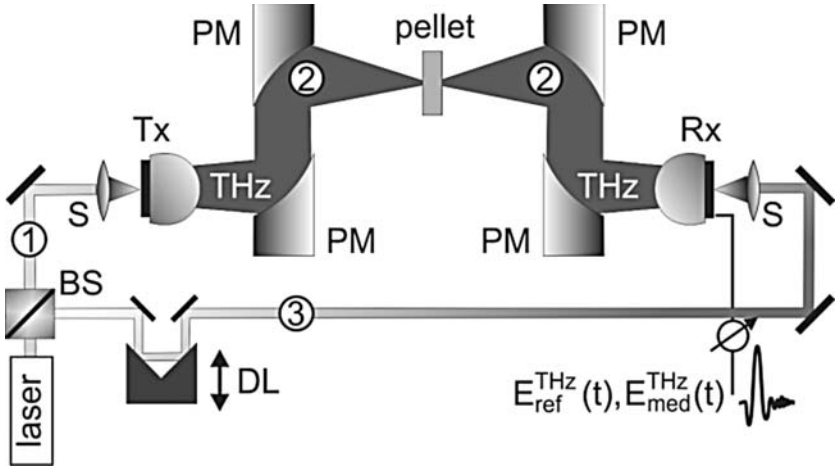

Figure 1. Schematic illustration of the THz-TDS spectrometer. 1 + 2 – measurement arm of the spectrometer, 3 – probing arm, Tx – THz transmitter, Rx – THz receiver, BS – cubic beam-splitter, DL – optical delay-line, PM – parabolic off-axis mirrors, S – microscopy lenses

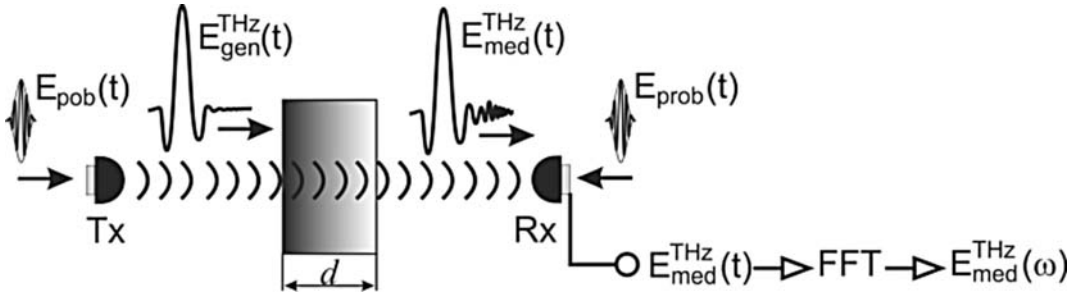

Figure 2. Coherent homodyne detection – the measuring and calculations of the signal with the sample, where d – thickness of the sample. FFT – Fast Fourier Transform operation on the measured signal

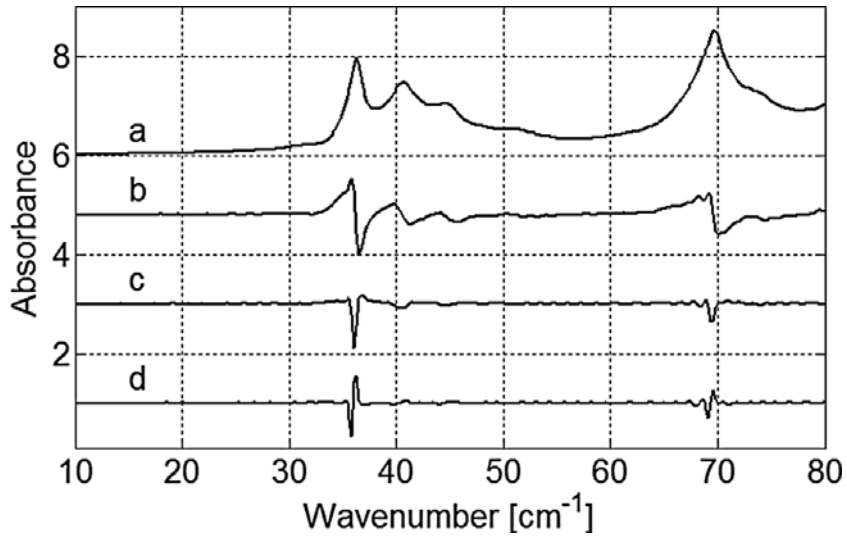

Figure 3. Famotidine spectrum (a), first (b), second (c), and third (d) derivatives of the spectrum

calculations easier. The signals are converted from time domain to frequency domain using the Fourier transform. Then, the convolution function can be reduced to their ordinary multiplication.

The spectrum of the investigated sample is expressed as an absorption coefficient *versus* frequency :

$$\alpha(\omega): -\frac{2}{d} \cdot \ln \frac{E_{med}^{Thz}(\omega)}{E_{ref}^{Thz}(\omega)} \qquad (1)$$

where: d - difference between sample and reference tablet thickness; E_{ref}^{Thz} and E_{med}^{Thz} amplitudes of the reference signal and the signal with the sample medium, respectively.

Improving spectrum visual analysis

The investigation methodology is explained on the example of the famotidine. For better identification of the absorption lines, first, second and third derivative of the spectrum can be used - see Figure 3. All of them have some advantages. Odd derivatives make sense for correct estimation of the central frequency of the absorption lines, because it is possible to recognize a clear transition of the characteristic through the zero level of the measured signal, which indicates the position of the absorption peak. The third derivative method has another advantage - it eliminates the background. On the other hand, the second derivative method is the most comfortable, and was used in our experiment.

Measurements and materials

The investigations are divided into four measurement groups:

- terahertz recognition of drugs commonly used in pharmaceutics - API,
- typical pharmaceutical excipients in the THz range,

- recognition of compounds in mixtures,
- visualization of pseudo-polymorphism or, in other words, hydration effects.
- additionally to spectroscopy we show results of the tablet coating imaging.

Terahertz recognition of drugs

We investigated APIs commonly used in pharmacy. In our studies APIs from different pharmacological groups such as histamine H2 receptor blockers and hypolipidemic drugs of the statin and fibrate groups were analyzed.

- Famotidine (3-[({2-[(diaminomethylidene)amino] -1,3-thiazol-4-yl}methyl)sulfanyl]-N'-sulfamoylpropanimidamide) is a histamine H2 receptor antagonist. It is widely used for the treatment of stomach ulcers and gastroesophageal reflux disease. Famotidine has been intensively investigated in the terahertz region (20). Famotidine was provided as a gift by Polfa S.A. (Kutno, Poland).
- Ranitidine (N-(2-[(5-[(dimethylamino)methyl] furan-2-yl)methylthio]ethyl)-N'-methyl-2-nitroethene-1,1-diamine) is, like famotidine, a histamine H2-receptor antagonist that inhibits stomach acid production. It is commonly used in the treatment of peptic ulcers and gastroesophageal reflux disease. Ranitidine was purchased from Farchemia S. r. l. (Bergamo, Italy).
- Fenofibrate (propan-2-yl 2-{4-[(4-chlorophenyl) carbonyl]phenoxy}-2-methylpropanoate) helps reduce cholesterol, triglicerydes and both low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels in the blood. Fenofibrate was purchased from Sigma-Aldrich (Steinheim, Germany).
- Lovastatin and simvastatin are members of a pharmaceutical class of drugs called HMG-CoA reductase inhibitors, which have pleiotropic effects manifested by protective effects on vascular endothelium, plaque stabilization, anti-inflammatory effect. Lovastatin ((1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2S)-2-methylbutanoate), belongs to class of statins, is used like fenofibrate to reduce cholesterol level in the blood. Lovastatin was obtained from Polpharma S.A. (Starogard Gdański, Poland).
- Simvastatin ((1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate) is the methylated form of lovastatin and has a similar clinical application. Simvastatin was obtained from Polpharma S.A. (Starogard Gdański, Poland).
- Acetylsalicylic acid (2-acetyloxybenzoic acid), also known as aspirin, has been used as an analgesic, antipyretic and anti-inflammatory nonsteroidal drug. A low-dose of acetylsalicylic acid has an inhibiting effect on platelet aggregation

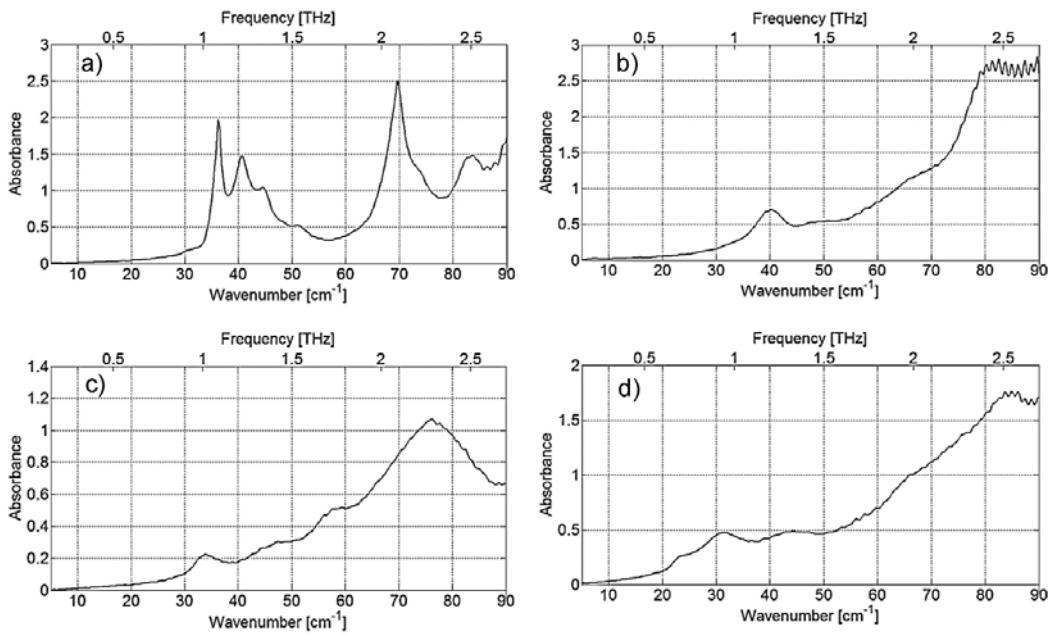

Figure 4. a) spectrum of famotidine in the terahertz range, b) terahertz spectrum of ranitidine, c) THz spectrum of fenofibrate, d) THz spectrum of lovastatin

and is applied to prevent atherosclerotic cardiovascular disease. Aspirin has been intensively investigated in the terahertz region (21). Acetylsalicylic acid was purchased from Sigma-Aldrich (Steinheim, Germany).

- Ketoconazole (1-[4-(4-{[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenyl)piperazin-1-yl]ethan-1-one) is one of the azole-based antifungal drugs used to treat infections caused by a fungus or yeast. Ketoconazole was acquired from Hasco-Lek S.A. (Wrocław, Poland).
- Acyclovir (2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one; chemical name - acycloguanosine) is one of the most commonly used antiviral drugs - primarily used for the treatment of herpes simplex virus infections. Acyclovir was provided as a gift by Sanitas Group (Jelenia Góra, Poland).

Pharmaceutical excipients in the THz range

An excipient is generally a pharmacologically inactive substance used as a carrier for the APIs. The US Pharmacopeia-National Formulary (USP-NF) categorizes excipients as binders, disintegrants, diluents, lubricants, glidants, emulsifying-solubilizing agents, sweetening agents, coating agents, antimicrobial preservatives, and so forth (22). In pharmaceutical technology these substances are widely used in direct compression tableting applications, and as a tablet and capsule filler and binder. Fillers are inert ingredients that can significantly affect the chemical and physical properties of the final tablet thus affecting the biopharmaceutical profile (23). Binders, in turn, are added to tablet formulations to add cohesiveness to powders thereby providing the necessary bonding to form granules which, under compaction, form a compact mass as tablet. In other words, binders are essential to achieve the "hardness" of the tablet (24).

- Lactose in the drug form technology is a basic filler used in the manufacture of granules and tablets. This substance is a hydrophilizing agent used in the process of direct compression. Lactose monohydrate was purchased from Sigma-Aldrich (Steinheim, Germany). Lactose has been investigated in the FIR region (25).
- Glucose is the filler substance in granules, chewable tablets and vaginal tablets. It is also used as a binder in the wet granulation process. The granules obtained are difficult to dry due to the hygroscopic properties of glucose. Tablets with the addition of glucose harden during storage what extends the time of their disintegration. Glucose anhydrous pure p. a. was purchased from Chempur (Piekary Śląskie, Poland). Glucose has been intensively investigated with DSC methods (26).

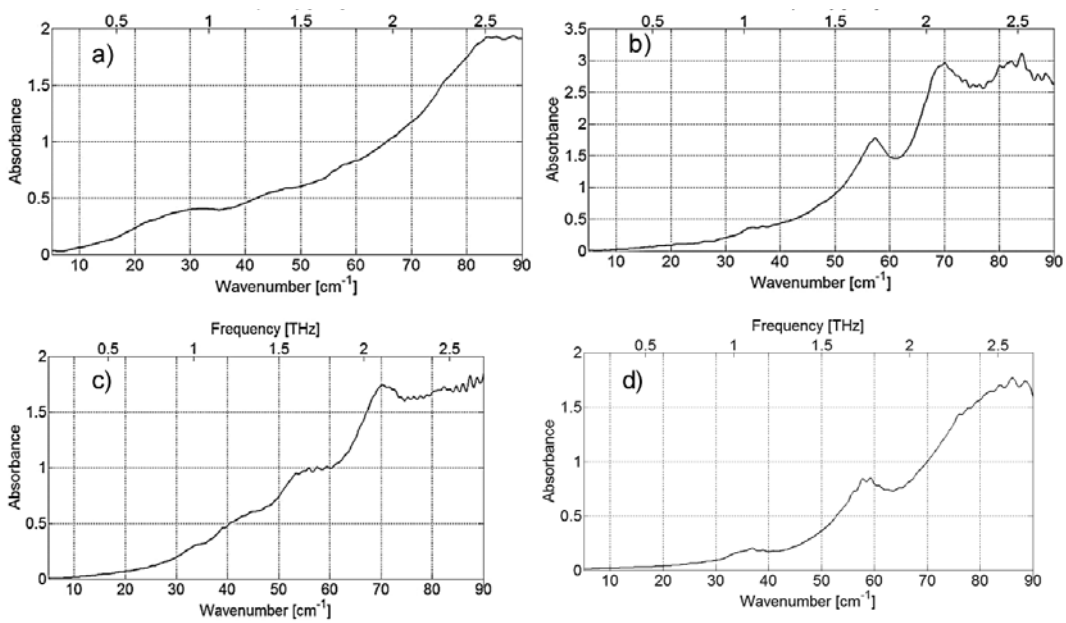

Figure 5. a) THz spectrum of simvastatin, b) THz spectrum of acetylsalicylic acid (aspirin), c) ketoconazole terahertz spectrum, d) acyclovir hydrated spectrum

- Pluronic F127 is a polymer of polyoxyethylene (PEO) and polyoxypropylene (PPO) with two 96-unit hydrophilic PEO chains surrounding one 69-unit hydrophobic PPO chain. Pluronic is exploited in pharmaceutical formulations as an emulsifier, wetting agent and solubilizer for poor water soluble drugs. The use of this surfactant in formulations containing a polymeric carrier may help prevent precipitation and/or protect a fine crystalline precipitate from agglomeration into much larger hydrophobic particles. The inclusion of pluronic as a carrier, was shown to be effective in enhanced in vivo substance bioavailability (27). Pluronic® F-127 was purchased from Sigma-Aldrich (Steinheim, Germany).

RESULTS

The results of investigations are divided into three groups:

- terahertz recognition of APIs commonly used in pharmaceutics,
- typical pharmaceutical excipients in the THz range,

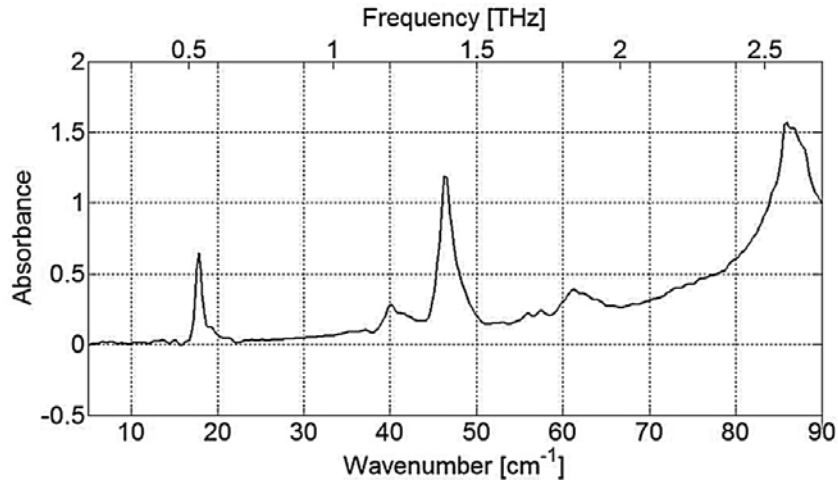

Figure 6. Terahertz spectrum of lactose monohydrate

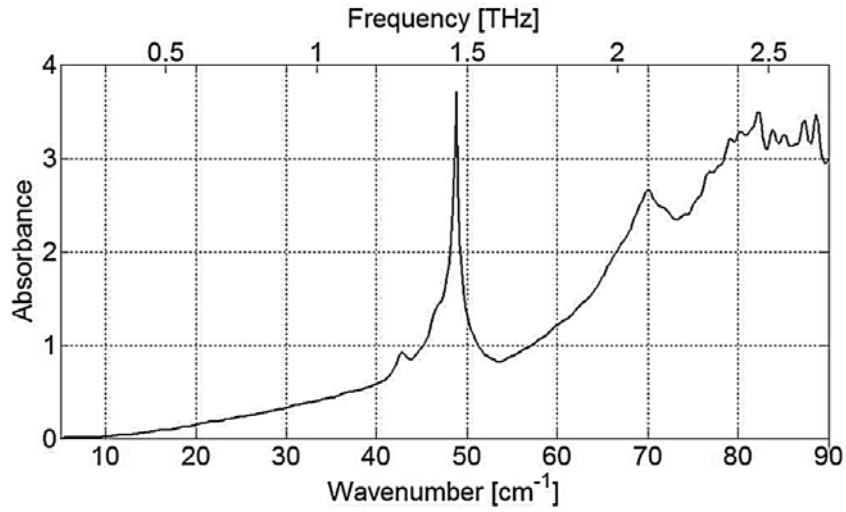

Figure 7. Terahertz spectrum of glucose (non-hydrated)

Table 1. Collected results of the drug spectral measurements in terahertz region, and comparison with the literature. (A) and (B) – polymorphic forms of famotidine (20, 29).

Compound	Absorption line (cm^{-1})
Drugs	
Famotidine	36.3, 40.5, 44.5, 51.0, 69.7, 74.0, 83.6
(29) experimental	30.0 (A), 32.3 (A), 35.0 (B), 35.3 (B), 39.6 (B), 40.0 (B), 44.6 (B), 51.1 (B), 54.0 (A), 55.0 (A), 62.2 (A), 70.7 (A), 80.8 (A), 85.2 (A)
(29) calculated	32.21, 37.89, 44.85, 55.33, 59.83, 72.74, 83.43, 92.1, 97.17
(20)	30.0 (A), 40.0 (B), 46.6 (B), 53.3 (A)
(20)	36.0, 40.0, 43.0, 53.0, 68.0, 73.0, 82.0, 93.0
Ranitidine	40.0, 50.0, 66.8
(30)	Form 1: 31.6, 41.9, 50.9, 59.0, 67.9, 73.3, 84.9, 91.9 Form 2: 37.6, 46.6, 61.6, 75.3, 79.6, 91.9
Fenofibrate	33.9, 46.5, 57.5, 76.0
Lovastatin	22.4, 32.3, 47.0, 57.9, 85.5
Simvastatin	22.5, 29.9, 47.0, 57.8, 76.0, 85.5
Aspirin	34.5, 36.7, 47.0, 57.4, 69.5, 81.9
(21)	37.0, 62.3, 72.3, 78.3, 82.9, 95.6, 111.2, 122.9
(21)	55.9, 71.3 (31) 18, 38.5, 53.5, 60.0, 62.5, 72.5, 76.0, 78.5, 82.5, 88.0, 93.0
(32)	10, 18, 34, 37, 46, 59, 66, 73, 81, 91, 100
Ketoconazole	33.7, 45.0, 55.5, 70.2
Acyclovir hydrated	36.5, 58.5, 77.5
Acyclovir non-hydrated	49.0, 70.4

- tablet coating imaging in terahertz light.

The measurements were performed in the 0.06 - 3 THz (approx.. 2 cm^{-1} - 90 cm^{-1}). The samples were mixed with polyethylene (PE) powder which is transparent to terahertz radiation. Mixtures consisted of 360 mg of PE powder and 40 mg of medium. As reference tablet was used 360 mg PE (HDPE) only (28). Medium was carefully mixed and then pressed into pellets with a Stempel Specac hydraulic press equipped with a 13-mm stainless steel die under the 2 ton pressure for 2 min.

Active pharmaceutical ingredients

The spectrum of famotidine is shown in Figure 4a. Seven absorption lines are indicated: 36.3, 40.5, 44.5, 51.0, 69.7, 74.0 and 83.6 cm^{-1}.

The spectrum of ranitidine in the terahertz region is shown in Figure 4b. Three absorption lines are indicated: 40.0, 50.0 and 66.8 cm^{-1}. The terahertz spectrum of fenofibrate is shown in Figure 4c. Four absorption lines are specified: 33.9, 46.5, 57.5, and 76.0 cm^{-1}. The THz spectrum of lovastatin is shown in Figure 4d. Five lines are visible: 22.4, 32.3, 47.0, 57.9 and 85.5 cm^{-1}.

The THz spectrum of simvastatin is shown in Figure 5a. Six spectral details may be specified: 22.5, 29.9, 47.0 and 57.8, 76.0, and 85.5 cm^{-1}.

The spectrum of aspirin is shown in Figure 5b. Six absorption lines are indicated: 34.5, 36.7, 47.0, 57.4, 69.5, and 81.9 cm^{-1}. The terahertz spectrum for ketoconazole (Figure 5c) is specified for: 33.7, 45.0, 55.5, and 70.2 cm^{-1}. Figure 5d shows the spectrum obtained for acyclovir in non-hydrated form. Two absorption lines are identified: 36.5 and 58.5 cm^{-1}.

Excipients

For the investigations, three known, and typical pharmaceutical excipients - lactose, glucose, and Pluronic F127 were selected. The terahertz spectrum of lactose is shown in Figure 6. Five lines are visible: 17.8, 40.0, 46.5, 61.5 and 86.5 cm^{-1}. The terahertz spectrum of glucose is shown in Figures 7 and 15.

The terahertz spectrum of glucose is shown in Figure 7. Four visible lines are identified for non-hydrated glucose: 42.7, 48.8, 70.0, and 84.7 cm^{-1}.

The terahertz spectrum of Pluronic F127 is shown in Figure 8. Pluronic has been intensively investigated in the terahertz region (7, 12, 21).

It may be observed that, as in the case of APIs, it is easy to recognize the spectra of lactose and glucose. Both show easily observable absorption peaks. The picture is quite different in the case of Pluronic F127. Only one very weak absorption line can be observed at 49.9 cm^{-1} (see Table 2).

DISCUSSION AND COMPARISION WITH THE LITERATURE

The results of spectroscopy measurements of APIs, excipients and their mixtures are discussed in this section. Processes of dehydration effects of glucose and acyclovir are discussed as well. In addition problems of drug homogeneity are also discussed based on the results of terahertz imaging of the tablets.

APIs

The results confirm the possibilities of the terahertz technique in spectroscopy of active pharmaceutical ingredients. Observable absorption peaks in the terahertz region for the investigated ingredients (famotidine, ranitidine, fenofibrate, lovastatin, simvastatin, aspirin, ketoconazole, and acyclovir) give characteristic spectra which are easy to recognize. The collection of absorption lines given in Table 1 creates "fingerprints" which can be easily assigned to suitable ingredients.

As can be seen in Figure 4a, famotidine shows an easily recognized spectrum ("fingerprint") with three well-developed absorption lines at 36.3, 40.5, and 69.7 cm^{-1}, and four weak lines at 44.5, 51.0, 74.0, and 83.6 cm^{-1}. The lines 44.5 and 74.0 cm^{-1} are set on the slope of the characteristic, and this is why estimation of their strength can be confusing. The table contains the results of other authors as well. All lines which are observable in our experiment remain in good agreement with experimentally obtained and calculated lines in (20, 29). Other authors indicated also 30.0, 32.3, 54.0, 62.0, 80.8, 85.2 cm^{-1} and calculated 92.0, and 97.0 cm^{-1} (29); 46.6, 53.3, cm^{-1} (20); 53.0, 93.0 cm^{-1} (33). The lines given in (20, 29) are obtained from two polymorphic forms: (A) - by recrystallization with hot water, and (B) - by recrystallization with hot methanol aqueous solution.

Figure 4b shows the spectrum of ranitidine. We indicated a strong line at 40.0 cm^{-1} and weak lines at 50.0 and 66.9 cm^{-1}. All three lines remain in acceptable agreement with the results of other authors (30). In one paper (30) two polymorphic forms are investigated: form 1 - obtained by crystallization from ethanolic solution after addition of ethyl acetate, and form 2 - obtained from a solution of isopropanol-HCl. The authors also found other lines at 31.6,

Table 2. Collected results of the excipients spectral measurements in terahertz region, and comparison with the literature.

Compound	Absorption line (cm^{-1})
Excipients	
Lactose	17.8, 40.0, 46.5, 61.5, 86.5
(33)	18.0, 40.0, 46.0, 61.0
(35) non-hydrated	30.0, 36.0, 47.0, 54.0, 62.0, 76.0, 87.0, 94.0, 104.0
(35) hydrated	18.0, 40.0, 46.0, 60.0, 85.0, 96.0, 108.0
(34)	18.0, 46.0
Glucose hydrated	61.3, 66.5, 81.9
Glucose non-hydrated	42.7, 48.8, 70.0, 84.7
(36) D anhydrous	43.0, 48.0, 69.9, 86.6
(36) D hydrated	60.6, 65.9, 81.9
(37)	42.3, 47.3, 59.3, 68.3, 83.6, 87.9, 96.9, 110.5, 115.9, 124.5
(38) D-glucose	42.0, 48.3, 69.9
(38) L-glucose	48.3, 70.6
Pluronic	127 49.9

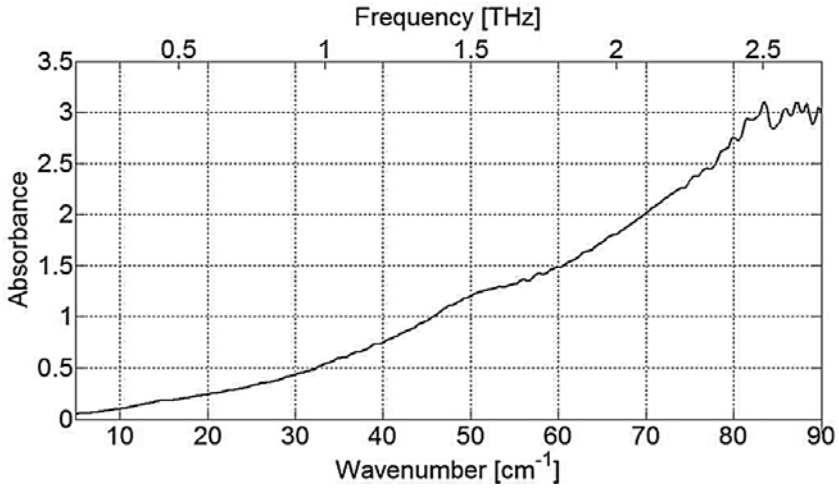

Figure 8. Terahertz spectrum of Pluronic F127

59.0, 73.3, 84.9, 91.9 for form 1, and 37.6, 46.6, 61.6, 75.3, 79.6, 91.9 for form 2. It is very likely that the higher number of lines is a result of the higher amount of the ingredient in the PE tablet (25%) comparing to our experiment (5%).

Fenofibrate (Fig. 4c), lovastatin (Fig. 4d), simvastatin (Fig. 5a), and ketoconazole (Fig. 5c) can be characterized by easily recognized spectra, but they do not show significant absorption lines. The lines for these compounds are collected in Table 1. There are no results of other authors for these investigated molecules.

The aspirin spectrum is presented in Figure 5b. Our experiment reveals five absorption lines - two strong at 57.4 and 69.5 cm^{-1}, and three weak at 34.5, 36.7, 47.0 cm^{-1}. Our spectrum incompletely overlaps with the experimental data of other authors. In one paper (21) aspirin was investigated with two methods - as a tablet and in a waveguide. One of them, at 71.3 cm^{-1}, is in good agreement with our result at 69.5 cm^{-1}, while using the waveguide the authors indicated six lines at 37.0, 62.3, 72.3, 78.3, 82.9, 95.6, 111.2, 122.9 cm^{-1}, where one of them, at 37 cm^{-1} is in a good agreement with our result at 36.7 cm^{-1}. But it is necessary to emphasize that the experiment in this paper (21) was performed at a temperature of 77 K.

The results given in another paper (31) we roughly determined from Figure 4 cited therein. Our results for some selected lines at 36.7, 57.4, and 69.5 cm^{-1} can be approximated to the lines given in this paper (31). The difference may be due to different conditions of both experiments - our experiments were performed at room temperature while the above-mentioned was at 10 K. More spectral features are usually visible at cryogenic temperatures. As we have shown, our results are in very good agreement with theoretical simulations performed in paper (32).

The discussion about acyclovir spectra for both hydrated and non-hydrated samples is to be found below. Figure 5d shows a spectrum for the hydrated form only. As is seen, only two spectral features are visible at 36.5 and 58.5 cm^{-1}.

Excipients

The spectral features of excipients (lactose hydrated, glucose hydrated and non-hydrated, Pluronic 127) are collected in Table 2. Figure 6 shows our results for lactose monohydrate. A comparison with Table 2 shows that our investigations fit the results obtained by other authors (33-35) for the hydrated form.

Glucose results for the non-hydrated form are shown in Figure 7. The results for a non-hydrated form are shown in Table 2. Our results are in excellent agreement with the results in (36), and in acceptable agreement with the results in (37) and (38). In the literature, additional lines at 59.3, 96.9, 110.5, 115.9, and 124.5 cm^{-1} are reported (37).

Lactose and glucose show their "fingerprints" without any doubts, but Pluronic F127 shows a different picture. The spectrum is very smooth, and it is difficult to describe it as distinctive of Pluronic. Many polymers exhibit a similar character of the spectrum (19). Only a very weak absorption line at 49.9 cm^{-1} was recognized in our experiment.

Mixtures - additive process

Our results show that spectra of pharmacological mixtures are formed additively. It means that the

spectra of the mixtures can be easily predicted by numeric summation of the results obtained separately for the constituent substances. Figure 9 shows the results for selected compounds (glucose and lactose). Figure 9a and b show spectra for glucose and lactose measured separately. Figure 9c presents the result of measurements for the tablet with 10% percent of glucose and lactose (together) with the weight ratio of 50/50. The rest of the tablet, 90%, consists of PTE powder. As can be seen, a few specific absorption lines for considered compounds can be easily recognized in glucose and lactose and they can be recognized in the mixture. Morever, the numerical summation process gives almost the same result. The positions of the absorption lines occupy exactly the same places on the wavenumber axis. Only the value of absorbance is different. This is a consequence of differing compositions of

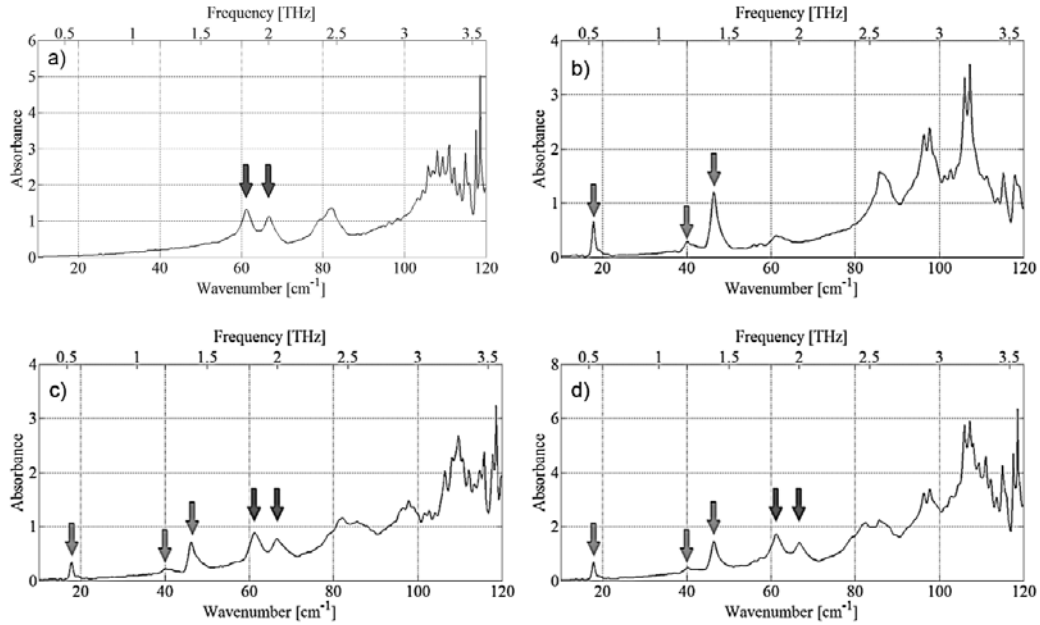

Figure 9. a) glucose (hydrated), b) lactose, c) glucose and lactose mixed in one tablet with PTE powder (90%) in the weight ratio 50/50, d) numerical sum of two signals – glucose and lactose (a + b)

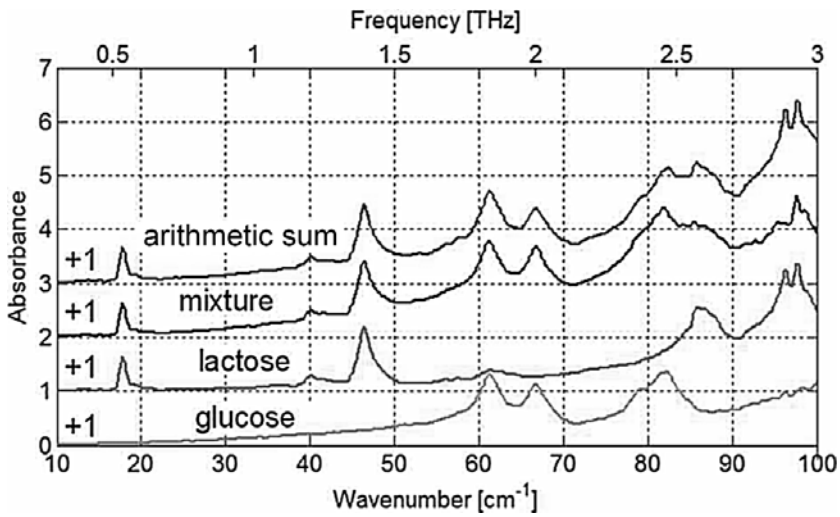

Figure 10. Summarized results in one figure. From bottom to the top: glucose (hydrated), lactose, glucose and lactose mixed in one tablet with PE powder (90%) in the weight ratio 50/50, numerical sum of two signals – glucose and lactose. Spectra are vertically offset for clarity

glucose/lactose in tablets a), b) and c). The result presented in Figure 9d is promising. As can be seen, the summation process does not involve any distortion in the positions of the absorption lines. The summarized results are shown in Figure 10.

Recognition of compounds in mixtures

The idea of combining two or more drugs with complementary modes of action is to produce additivity of the desired therapeutic effect, but not of the side effects. A mixture of fenofibrate (or statin) with aspirin plays an important role in the development of an improved drug delivery system for primary and secondary prevention of coronary heart disease. It also influences the prevention of complications in diabetic patients. In our research, we prepared suitable physical mixtures with aspirin and ketoconazole used in pharmaceutics. Figures 11-13 present the terahertz spectra for physical mixtures of fenofibrate with aspirin and two mixtures of lovastatin with aspirin obtained in different weight ratios.

Figure 11 shows the spectrum of the aspirin with fenofibrate mixture in the ratio 44 : 56, respectively. As can be seen, the aspirin and fenofibrate spectral lines mutually merge. Comparing absorbance of fenofibrate (Fig. 4c) and aspirin (Fig.

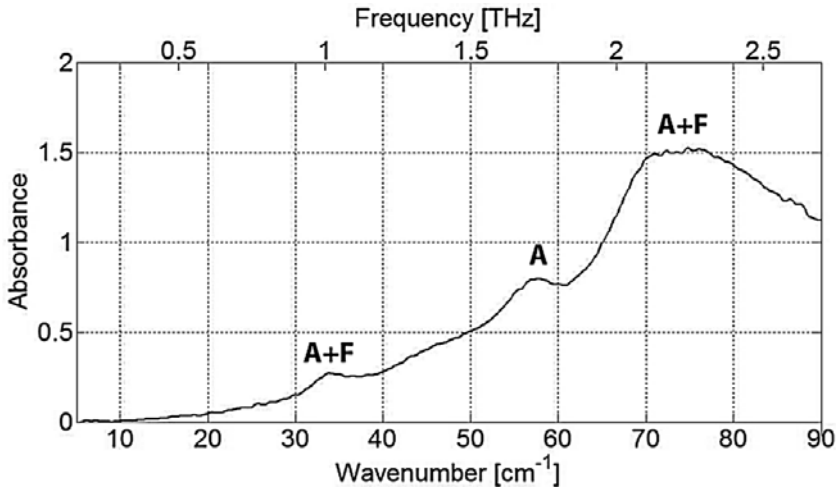

Figure 11. Terahertz spectrum for physical mixture of fenofibrate and aspirin in the weight ratio 0.44/0.56. A – contribution of aspirin, F – contribution of fenofibrate

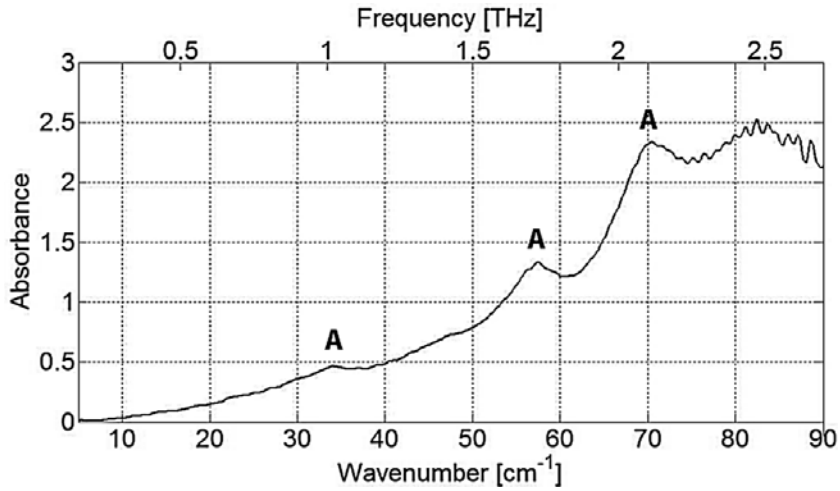

Figure 12. Terahertz spectrum for physical mixture of lovastatin and aspirin in the weight ratio 27.8/72.2. A – contribuiton of aspirin, contribution of lovastatin is overlapped due to higher concentration of the aspirin (cf. Fig. 13)

5b) we have concluded that aspirin has a decisive influence on the absorption peak at 33.9 cm^{-1} (with some influence of fenofibrate). A similar situation can be noted for the second peak at 57.4 cm^{-1}. The last absorption detail around 72.5 cm^{-1} is broadened due to the convolution of two absorption lines: 69.5 cm^{-1} (aspirin) and 76.0 cm^{-1} (fenofibrate).

Another example is shown in Figure 12. The aspirin spectral lines (A) definitely overlap the lovastatin spectrum. In this case the aspirin spectrum is much stronger. The spectrum of aspirin reaches the 3.0 value of absorbance comparing to lovastatin - only 1.7 (see Figs. 5b and 4d), but in this case, the percentage of aspirin in the mixture is much higher. The last absorption feature around 82.6 cm^{-1} is broadened due to the convolution of two absorption lines: 81.9 cm^{-1} (aspirin) and 85.5 cm^{-1} (lovastatin).

A better situation for lovastatin is observed in Figure 13. But in this case there is as much as 83.6% of lovastatin in the mixture with aspirin. The last absorption feature around 84.2 cm^{-1} is broadened due to the convolution of two absorption lines: 81.9 cm^{-1} (aspirin) and 85.5 cm^{-1} (lovastatin).

In our studies, a mixture of ketoconazole with Pluronic F127 was analyzed as well - see Figure 14.

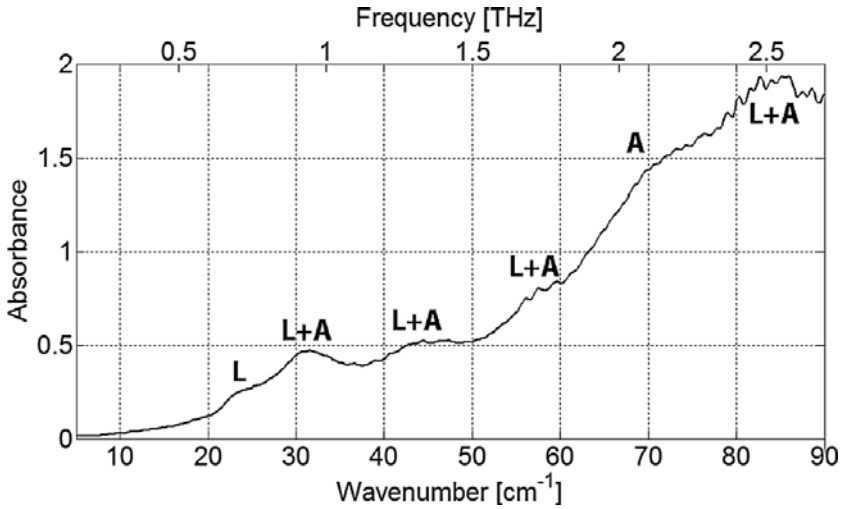

Figure 13. Terahertz spectrum for physical mixture of lovastatin and aspirin in the weight ratio 83.6/16.4. A - contribuiton of aspirin, L - contribuiton of lovastatin

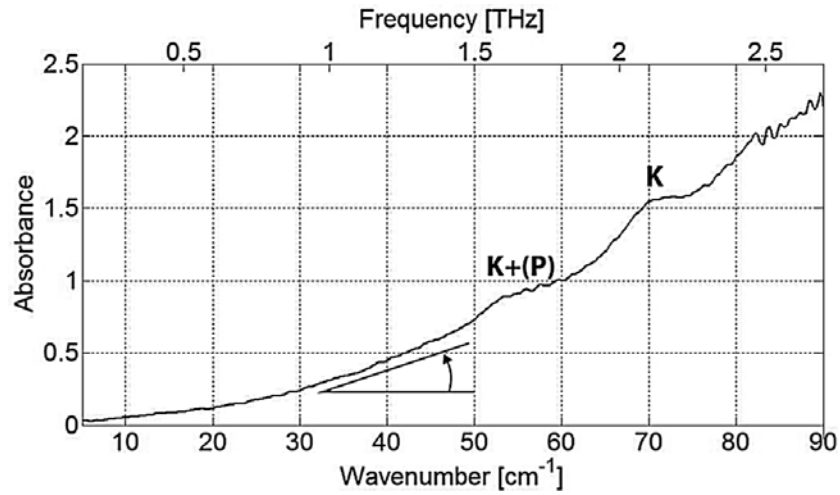

Figure 14. Terahertz spectrum for mixture of Pluronic F127 and ketoconazole in the weight ratio of 50/50. K – contribution of ketoconazole, (P) – weak contribution of Pluronic. The angle of the slope is indicated (explanation in the text)

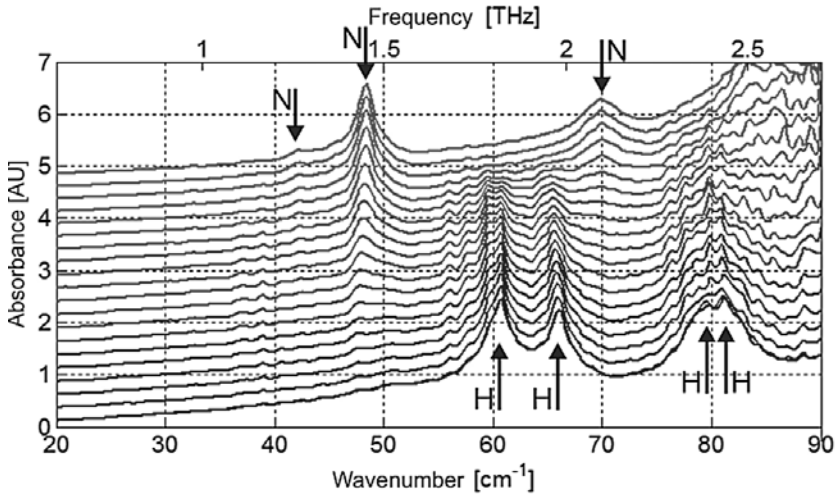

Figure 15. Hydration effect in glucose. Absorption peaks are indicated for both hydrated and non-hydrated forms: H – hydration effect, N – non-hydrated molecule absorption lines. Spectra are vertically offset at 0.25 (AU) for clarity

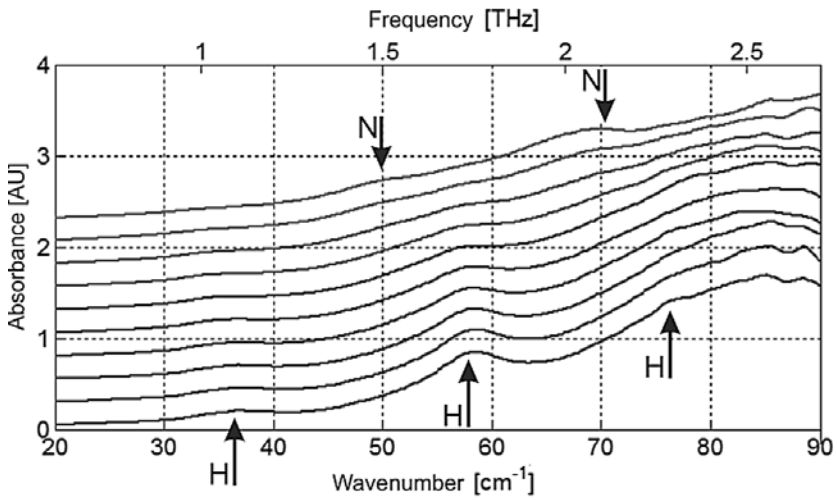

Figure 16. Effect of the drying on hydrated acyclovir (low characteristics). The spectrum of the dry compound is visible at the top of the figure. Spectra are vertically

There is observed an advantage of ketoconazole absorption comparing to Pluronic F127 in the mixture of 50/50. Comparison of both separate spectra (see Figure 5c and Figure 8) reveals that ketoconazole is characterized by much more recognized spectral details than Pluronic. Perhaps in the case of the spectral detail at 53.2 cm^{-1} it can be observed some weak influence of Pluronic on the mixture spectrum indicated in Figure 14 with K + (P). Despite the relatively lower amount of Pluronic in the sample (50% compared to the result presented in Fig. 8) the slope of the characteristic is higher than that in Figure 5c (pure ketoconazole). The influence of Pluronic is obvious.

The spectral details of the mixture spectra are collected in Table 3. All values of the absorption peaks were determined using the second derivative method.

Polymorphism

Many bio-components when exposed to water may form hydrates and, on the contrary, hydrates may lose their water under high temperature or low humidity. The possibility of hydrate formation and dehydration can occur during manufacturing, for example, during several unit operations, such as drying, milling, tableting or during storage. It is important to understand the dehydration behavior of an

Table 3. Collected results of the mixture spectral measurements in terahertz region.

Compound	Absorption line (cm^{-1})
Mixtures	
Fenofibrate/Aspirin 44/56	33.9, 57.4, 72.5
Lovastatin/Aspirin 27.8/72.2	33.9, 57.4, 70.0, 82.6
Lovastatin/Aspirin 83.6/16.4	23.5, 31.0, 44.4, 57.6, 70.4, 84.2
Pluronic/Ketoconazole 50/50	53.2, 70.2

API to get an insight into the dehydration mechanisms encountered during processing, which makes it possible to monitor and control the production process (17, 32, 37, 39).

To show how the effect of polymorphism can influence the shape of the spectrum characteristic in the terahertz region, we chose two compounds: glucose and acyclovir. The results of the hydration process are shown for both compounds. This is also referred as a pseudo-polymorphism. Figure 15 shows the effect on the glucose. The hydrated form is heated from room temperature to 120°C. As can be seen, some absorption lines (indicated as "H") disappear at the higher temperature, when the compounds are dried. Instead, two lines (indicated as "N") appear but in different parts of the observed spectral band.

Figure 15 shows, how clear and easy it is to recognize the pictures of hydrated and non-hydrate forms of chemical compounds - here hydrated and non-hydrated glucoses. The absorption lines appear in completely different places of the frequency axis: in the case of hydrated glucose at 61.3, 66.5, and 81.9 cm^{-1}; in the case of non-hydrated one at 42.7, 48.8, 70.0, and 84.7 cm^{-1} (see Table 2). The presence or absence of water molecules in the molecular structure of the crystaline glucose influence strongly the spectral characteristics. Both components, hydrated and non-hydrated, leave clear traces ("fingerprints") in the spectral characteristics.

Another example of the hydration effect is shown on acyclovir. Figure 16 shows the process of drying. Family of the characteristics at the bottom of the figure shows the beginning of the process from room temperature to approximately 120°C.

Absorptions lines 36.5, 58.5, and 77.5 cm^{-1} create the "fingerprints" for hydrated acyclovir, and two lines 49.0, 70.4, cm^{-1} create "fingerprints" for the non-hydrated form. As in the case of a hydration effect in glucose, the spectral picture for acyclovir is easy to recognize too.

CONCLUSIONS

As has been shown, the pharmaceutical compounds and molecules under investigation have specific "fingerprints" in the terahertz spectroscopy band. The compounds can be easily recognized in the THz region. We used a terahertz time-domain spectroscopy technique to achieve our goal. To improve the measuring methodology, we indicated the absorption lines using the second derivative method applied to the signals obtained. This method can be used to enhance absorption lines e.g., for automatic absorption lines recognition.

We investigated different active pharmaceutical ingredients: (famotidine, ranitidine, fenofibrate, lovastatin, simvastatin, aspirin, ketoconazole) and excipients (lactose, glucose, Pluronic). All measured compounds showed clear spectral details. In this way, we have shown that the spectral images can be easily assigned to individual components. The details of most of the components are so easy to recognize that they are visible even in mixtures of the compounds.

We showed the spectra of typical mixtures used as drugs. We investigated mixtures of fenofibrate/aspirin, and lovastatin/aspirin - (excipient + API) in typical proportions such as 27.8/72.2 and 83.6/16.4, respectively. A Pluronic plus ketoconazole mixture in weight proportion of 50/50 was investigated as well. All individual absorption lines were preserved in the spectra of mixtures. Moreover, when the spectra of separately measured compounds were numerically added, then the same spectra were obtained as for real mixtures. As it was shown, the spectra exhibit additive properties.

The terahertz technique is a useful tool for detection of the parasitic process of drug hydration, which is a typical problem in the pharmaceutical industry occurring during the manufacturing process. We examined two substances - glucose (a

typical excipient in many forms of drugs), and acyclovir (API). In our investigations we have shown the process of water release with the temperature increase.

We have shown in this paper that the terahertz technique can be applied for the monitoring and controlling of the technological process not only by spectral investigations of the drugs, but also by terahertz technique of imaging. A tablet of ketanol was selected as a test sample for the THz imaging. The pictures of cross-sections of the tablet for different penetration depths were obtained. As was shown, the defects of the tablet were easily recognized.

Development of the terahertz technique and its diverse range of applications in recent years, makes it very probable that both terahertz spectroscopy and terahertz imaging will become commonly established techniques in pharmaceutical research, providing excellent opportunities for the pharmaceutical sciences and industry.

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REFERENCES

1. Zhang X.-C., Xu J.:, Introduction to THz Wave Photonics, Springer, New York 2010.
2. Jarzab P.P., Nowak K., Plinski E.F.: Opt. Commun. 285, 1308 (2011).
3. Kim J.-Y., Boenawan R., Ueno Y., Ajito K.: J. Lightwave Technol. 32, 3768 (2014).
4. May R.K., Su K., Han L., Zhong S., Elliott J.A. et al.: J. Pharm. Sci. 102, 2179 (2013).
5. Plinski E.F.: B. Pol. Acad. Sci.-Tech. 58, 463 (2010).
6. Shen Y.-C.:, Int. J. Pharm. 417, 48 (2011).
7. Taday P.F.: Philos. T. Roy. Soc A 362 (1815), 351 (2004).
8. Kroll J., Darmo J., Unterrainer K.: Vib. Spectrosc. 43, 324 (2007).
9. Brandt N.N., Chikishev A.Y., Kargovsky A.V., Nazarov M.M., Parashchuk O.D. et al.: Vib. Spectrosc. 47, 53 (2008).
10. Kikuchi N., Tanno T., Watanabe M., Kurabayashi T.: Anal. Sci. 25 457 (2009).
11. Matei A., Drichko N., Gompf B., Dressel M.:, Chem. Phys. 316, 61 (2005).
12. Zeitler J.A., Taday P.F., Newnham D.A., Pepper M., K.C. Gordon T.R.: J. Pharm. Pharmacol. 59, 209 (2007).
13. Chaulang G., Patel P., Hardikar S., Kelkar M., Bhosale A., Bhise S.: Tropical J. Pharm. Res. 8, 43 (2009).
14. Armas H.N.D., Peeters O.M., Blaton N., Gyseghem E.V., Martens, J. et al.: J. Pharm. Sci. 98, 146 (2009).
15. Dhirendra K., Lewis S., Udupa N., Atin K.: Pak. J. Pharm. Sci. 2, 234 (2009).
16. Liu H.-B., Zhang X.-C.: in Terahertz Frequency Detection and Identification of Materials and Objects. Miles R., Zhang X.-C., Eisele H., Krotkus A. Eds., pp. 251-323, Springer, Netherlands 2007.
17. Brittain H.G., Ed.: Polymorphism in pharmaceutical solids, Drugs and the pharmaceutical sciences, Informa Healthcare, USA 2009.
18. Pupeza I., Wilk R., Koch M.: Opt. Express 15, 4335 (2007).
19. Jarzab P.P., Nowak K., Walczakowski M.J., Augustyn L., Mikulics M. et al.: Opto. Electron. Rev. 20, 335 (2012).
20. Ajito K., Ueno Y., Song H.-J., Tamechika E., Kukutsu, N.: Mol. Cryst. Liq. Cryst. 538, 33 (2011).
21. Laman N., Harsha S.S.., Grischkowsky D.: Appl. Spectrosc. 62, 319 (2008).
22. Smith C.G., O'Donnell J.T., Eds.:, The process of new drug discovery and development, Informa Healthcare, New York 2006.
23. Chang D., Chang R.-K.: Pharm. Technol. 31, 56 (2007).
24. Fischer B., Hoffmann M., Helm H., Modjesch G., Jepsen P.: Semicond. Sci. Technol. 20, 246 (2005).
25. Fischer B.M.: Albert-Ludwigs-Universität Freiburg im Breisgau (2005).
26. Vasconcelos T., Sarmento B., Costa P.: Drug Discov. Today 12, 1068 (2007).
27. England J.L.: J. Undergrad. Sci. 5, 17 (2001).
28. Tirnakiz E., Pamukcu B., Oflaz H., Nisanci Y.: J. Thromb. Thrombolysis 27, 24 (2009).
29. Ajito K., Ueno Y., Song H.-J., Tamechika E., Kukutsu N.: ECS Trans. 35 (7), 157 (2011).
30. Taday P.F., Bradley I.V., Arnone D.D.., Pepper M.: J. Pharm. Sci. 92, 831 (2003).
31. Walther M., Plochocka P., Fischer B., Helm H., Uhd Jepsen P.: Biopolymers 67, 310 (2002).
32. Boczar M., Wójcik M.J., Szczeponek K., Jamróz D., Zięba A.., Kawałek B.: Chem. Phys. 286, 63 (2003).
33. Nishizawa S., Suzuki Y., Iwamoto T., Takeda M.W., Tani M.: 35th International Conference on Infrared Millimeter and Terahertz Waves (IRMMW-THz2010), Th-P.18, Angelicum, Rome, Italy 2010.

34. Shen Y.C., Taday P.F., Newnham D.A., Pepper M.: Semicond. Sci. Technol. 20, 254 (2005).
35. Zeitler J.A., Kogermann K., Rantanen J., Rades T., Taday P.F. et al.: Int. J. Pharm. 334, 78 (2007).
36. Liu H.-B., Zhang X.-C.: Chem. Phys. Lett. 429, 229 (2006).
37. Zheng Z.-P., Fan W.-H., Liang Y.-Q., Yan H.: Opt. Commun. 285, 1868 (2012).
38. Upadhya P.C., Shen Y.C., Davies A.G., Linfield E.H.: J. Biol. Phys. 29, 117 (2003).
39. Strachan C.J., Taday P.F., Newnham D.A., Gordon K.C., Zeitler J.A. et al.: J. Pharm. Sci. 94, 837 (2005).

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