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On the kinetics of tautomerism in drugs: New application of broadband dielectric spectroscopy

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There are a number of chemical compounds that readily convert to other isomers when their crystalline structure is lost (e.g., during melting or dissolution). This phenomenon, commonly known as tautomerism, is a subject of intense research. It is an important problem especially in pharmaceutical industry because various isomers of a drug may have different pharmacological activity. Therefore, it is important to find appropriate experimental technique which enables the determination of the isomerization ability of compounds. In this communication, we demonstrate that broadband dielectric spectroscopy (BDS) method has the potential of detection and monitoring of tautomerism of drugs. To investigate the tautomerism phenomenon we have chosen one of the hypoglycemic agents that belong to the class II of sulfonylurea drugs. Based on density functional theory (DFT) calculations we have analyzed two possible tautomerization pathways of glibenclamide. By using BDS as a tool, we show it can detect the conversion between the isomeric forms through time dependence in the dielectric properties. The activation energy (E_a) of this process is in good agreement with that obtained from DFT analysis. Finally, we discuss the possible effects of tautomerism on basic pharmaceutical parameters such as biological activity or bioavailability in the case of the glibenclamide drug. © *2010 American Institute of Physics*. [doi:10.1063/1.3475688]

I. INTRODUCTION

Tautomerism is an important area of chemical study that has long been a challenge computationally, experimentally, and intellectually. According to the commonly accepted definition, tautomers are structural isomers of organic compound that differ only in the position of a hydrogen atom or proton.[1,2] Tautomerism may occur mainly in solutions, in gas phase, or in the amorphous state, because proton transfer is possible only in such cases. In the crystal state tautomerism is also allowed but one should remember that in this case the special conditions (e.g., high temperature) are required to observe this effect.[3,4] Moreover, the tautomerism rate is much smaller than in the case of supercooled liquid, solution, or gas phase. Unlike other classes of isomers, tautomeric compounds exist in dynamic equilibrium with each other, so that attempts to prepare the separate substances usually result in the formation of a mixture that shows all the chemical and physical properties to be expected on the basis of the structures of the components.[1] It is important to note that the exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. Thus, by using different solvents one can obtain different tautomers in equilibrium.[1,5] Just as there are different sorts of tautomers, there are different kinds of tautomerization. The most commonly observed tautomeric pairs are ketone-enol, amide-imidic acid, or lactam-lactim.

It is well known that there are many biologically and pharmaceutically important chemical substances which can undergo isomerization. In the previous century many scientists focused their attention on understanding the mutarotation process, being the most well known phenomena occurring in saccharides.[6,7] The other extensively studied isomers were the tautomeric forms of the DNA and RNA bases.[8] It has been recognized that mismatching of purine and pyrimidine bases could lead to errors in replication and consequently causing the point mutations.[9–11] A number of papers are also dedicated to the keto-enol tautomerism of p-hydroxyphenylpyruvic acid, whose level in blood and urine is used to diagnose a congenital metabolic defect known as tyrosinemia.[12]

Tautomerism phenomenon is also the subject of considerable interest in pharmaceutical industry. In the recent years an essential part of the current pharmaceutical research has benefited from the advance made in the physics of glasses.[13,14] It is commonly recognized that preparation of pharmaceuticals in amorphous state gives a lot of benefits. One can add that amorphous solids characterize with better solubility and chemical reactivity. However, one should not forget that there are pharmaceutically important molecules which can undergo tautomerization. Such reaction can be observed in the liquid and glassy state, but it does not occur in crystalline state. Consequently, if tautomerization phenomenon takes place, preparation of the amorphous drug can lead to the state which is chemically different than its crystalline counterpart. This phenomenon can have positive as well as negative impacts on the quality of the amorphous drug. On the one hand, the existence of the tautomerization

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products may enhance bioavailability of the active substance and reduce the tendency to crystallization of the amorphous system. On the other hand, biological activity of the given tautomers may be completely different. The best example which illustrates very well different mechanism of action of the given isomers is thalidomide drug which has two optical isomers. The first one works properly for treatment, while the second one was found to be a cause of birth defects if the drug was taken during pregnancy. The thalidomide tragedy led to much stricter testing being required for drugs before they can be licensed for distribution.[15] It should be noted that optical isomers cannot transform directly into each other. Moreover, they can be separated at the drug production stage. Far more complicated situation is observed in the case of tautomerism, where there is always equilibrium between two or more forms.

To investigate the tautomerism phenomenon we have chosen glibenclamide (GCM), which is one of the long-acting and orally taken pharmaceutical used to treat type II of diabetes mellitus [noninsulin-dependent diabetes mellitus (NIDDM)].[16] It belongs to the class II of sulfonylureas drugs which have been one of the mainstays of oral antidiabetic therapy for many years. Compounds that belong to the first generation of drugs for treatment of NIDDM such as acetoxamide, chlorpropamide, or tolbutamide are still in use, but are less potent than the more recently introduced drugs of the second generation such as GCM.[17] However, it is important to add that all mentioned substances have the same backbone structure as GCM. Therefore tautomerism also should be observed in all these other pharmaceuticals.

The occurrence of tautomerization in GCM was postulated for the first time by Hassan *et al.*[18] in 1991. In the next years this phenomenon was also observed by other researchers in glibenclamide; however, it has been not thoroughly analyzed.[19,20] For many years the only one experimental technique used to analyze the glibenclamide tautomerization was infrared spectroscopy. The reason why this phenomenon has not been studied sufficiently may be the lack of attention paid to the influence of tautomer on the biological activity of this drug. In our previous work[21] on glibenclamide drug we have used infrared spectroscopy [FT-IR (Fourier Transform Infrared)], ultraperformance liquid chromatography, and NMR measurements to characterize the glassy GCM samples. All the methods applied confirmed tautomerism in the glassy material. However, it is worth noting that the chromatograms as well as IR and NMR spectra can be misinterpreted. For example the additional peaks visible in the chromatogram of glassy sample can be considered as due to sample contamination or products of material decomposition. This kind of incorrect analysis was presented in the work of Ref. 22. In the same paper, also NMR spectra have been interpreted incorrectly. Based on these wrong results, Patterson *et al.* suggested that GCM undergo thermal decomposition at the melting temperature. In our recent work, we have shown that glibenclamide drug is stable chemically, and the observed differences in the chromatograms and NMR spectrum between crystalline and amorphous samples are definitely connected to formation of tautomers. Moreover, our investigations have also shown that the interpretation of IR measurements can be ambiguous. It is well known that the crystalline GCM consists of amide form while in its amorphous counterpart one can distinguish both amide and imidic acid forms. Many times it was shown that the sample recrystallization is connected with the imidic acid-amide conversion. However we have found that the same process is observed during the drug tautomerization. Thus, the changes of the IR spectrum in the region attributed to N–H stretch as well as in the carbonyl stretching region should not be always treated as a sign of crystallization. These mentioned facts all show how easily tautomerization can be overlooked and wrongly interpreted. Therefore, it is important to find appropriate experimental technique which unambiguously enables us to determine the ability of isomerization in pharmaceuticals and other compounds.

Herein we use broadband dielectric spectroscopy (BDS) as the experimental tool to shed more light on the tautomerism phenomenon in GCM. This technique is most commonly used for studying the molecular dynamics in supercooled liquids and glasses.[23] However in this paper we demonstrate that BDS is also the technique of choice for the detecting and monitoring tautomerism of drugs. It is important to notice that the BDS method does not involve the use of expensive solvents, what is necessary, e.g., in the case of modern NMR methods. The next advantage of the BDS technique is that the tautomerization process can be observed over a wide temperature range. Moreover, BDS enables us to control the temperature conditions automatically and very precisely. It is worth noting that the analysis under different temperature conditions is also available in optical and NMR spectroscopy, but in these methods a specialized instrumentation is required. Additionally, an advantage of BDS is that it can be easily extended to the study at elevated pressure.

It is well known that when a liquid is cooled rapidly below its melting point, it can avoid crystallization to form an equilibrium supercooled liquid at which molecular rearrangements are observed as the structural α-relaxations dielectrically. On cooling, viscosity of the liquid increases and the supercooled liquid eventually is vitrified into the glassy state. As a consequence, the α-process shifts toward lower frequencies. From the present knowledge it is known that every tautomer may have its unique physical properties (such as the glass transition temperature). Thus, tautomerism should lead to fluctuations of viscosity in the system, and accompanying changes of the dielectric relaxation phenomena such as the α-relaxation time, dc-conductivity, or static permittivity at constant temperature while equilibrating of the sample. The changes of the dielectric spectrum observed during tautomerization process can be distinguished from that due to crystallization, which is manifested by the decrease of the structural relaxation intensity due to a reduction of the number of mobile dipoles. Consequently, using minimal volume of sample and carrying out simple isothermal time dependent measurements, not only one can confirm or exclude the tautomerization of the compound analyzed, but also can observe the kinetics of this process. Moreover, dielectric data enabled us to determine activation energy of this reaction as well as recognize the real time scale of tautomerism of GCM drug at various temperature conditions. This

knowledge, impossible to achieve using the other experimental methods, could be crucial in the pharmaceutical industry because it is relevant for determining the expiration date as well as the best storage conditions of GCM drug.

The next interesting problem is to check the influence of GCM isomers on bioavailability of the drug and consequently on the human health. The literature reports that the appearance of new tautomers of GCM is connected with the conversion to the glassy state. However one should note that it is more a universal problem because tautomerization starts after administrating oral dosage in the crystalline form, when molecules are being dissolved. Because the exact ratio of the structural isomers depends on several factors including solvent, temperature, or pH, the equilibrium between tautomers is dynamically changed in human gastro-intestinal track. Thus, it is important to understand the consequences that this phenomenon can have. Therefore, in the final part of this work, we will present how the tautomerization of GCM may affect biological docking procedure in the living organisms and explain why it is so crucial.

II. MATERIAL AND METHODS

Glibenclamide drug (99% purity) was purchased from Polpharma and was used as received. The amorphous GCM drug was obtained by quench-cooling of the melt (T_m =440 K). X-ray diffraction of the final material did not show evidence of any crystalline phases.

A. Experiments

Isothermal dielectric measurements on GCM at five different temperatures of 357, 361, 365, 369, and 373 K were carried out using a Novo-Control GMBH Alpha dielectric spectrometer (10^2–10^7 Hz). The temperature was controlled by the Quattro system, employing a nitrogen-gas cryostat, and temperature stability of the sample was achieved better than 0.1 K/s. Additionally, the dielectric loss spectra of both fresh prepared and equilibrated sample in a wide temperature range have been collected.

The standard differential scanning calorimetry measurements of glassy glibenclamide were carried out using Perkin Elmer Pyris 1 DSC instrument with liquid nitrogen cooling. The samples were analyzed under helium purge (20 ml/min) in the hermetically sealed aluminum pans. The instrument was calibrated for temperature and heat flow using high-purity standards of indium. The thermal analysis was carried out between 253 and 463 K at a heating rate of 10 K/min.

The x-ray diffraction experiment was performed at ambient temperature on Rigaku-Denki D/MAX RAPID II-R diffractometer (Rigaku Corporation, Tokyo, Japan) with a rotating anode Ag Kα tube (λ=0.5608 Å), an incident beam (002) graphite monochromator, and an image plate in the Debye–Scherrer geometry.

B. Simulation details

All calculations were performed with the use of density functional theory in the ORCA package.[24] In the first step we have performed geometry optimizations of dozen of random GCM structures with use of gradient BLYP functional and 6-31G basis set. Five most stable structures were reoptimized in the hybrid B3LYP functional and with use of the same basis set. Final energy was evaluated by performing single point calculations on optimized structures on the B3LYP/6-311++G(2d,2p) level. All transition states as well as local minima were checked by performing vibrational analysis. Activation energies were corrected by the zero point energy, calculated on the B3LYP/6-31G level of theory. The electrostatic potential on the molecular surface was calculated by means of GAUSSIAN 03 package.[25]

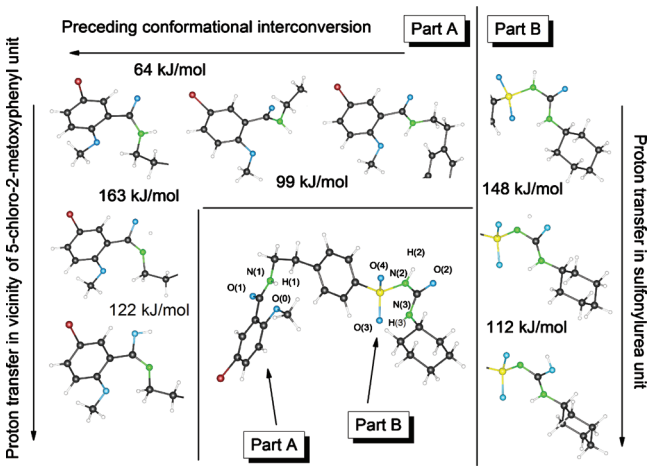

FIG. 1. The presented, most energetically stable structure was divided into two parts, i.e., A and B. In part A one can monitor tautomerization process in the vicinity of 5-chloro-2-metoxyphenyl. As one can see, in the most stable structure in part A, oxygen from carbonyl group O(1) is in the opposite site than the hydrogen attached to neighboring nitrogen atom H(1). In such situation there is a stabilizing effect of attractive interaction between hydrogen H(1) and the lone electron pair on the oxygen from metoxy group O(0). (It could be treated as very week hydrogen bond.) On the other hand, this structure is especially preferred because of unfavorable repulsive interactions between lone pairs on carbonyl oxygen O(1) and metoxy oxygen O(0). However, in such stable conformation proton cannot be transferred directly from nitrogen to oxygen. Preceding conformational interconversion is necessary for the reaction. In the sulfonylurea fragment (part B), the most stabilizing effect has strong attractive interaction between oxygen attached to the sulfur O(3) and the hydrogen H(3). In the case of sulfonylurea site, proton can be transferred directly. Energy barriers in both fragments are comparable.

III. RESULTS AND DISCUSSION

A. The molecular mechanism of tautomerization process

At the beginning we present the possible mechanism of tautomerization process in analyzed drug. It was shown that there are two centers in GCM molecule at which tautomerization may occur.[22] The first one is sulfonylurea fragment which is characteristic for the wide group of antidiabetic drugs. The second one is located in the vicinity of 5-chloro-2-metoxyphenyl ring (see Fig. 1 for details). We have studied two tautomerization pathways in both mentioned centers. In the case of GCM, tautomerization is an amide-imidic acid transformation, which is a proton transfer reaction from the nitrogen to the oxygen atom of carbonyl group. Consequently, there is a double bond shift into the carbon-nitrogen position and the fragment becomes more rigid. The calculated activation energies at center 1 (part A in Fig. 1) is

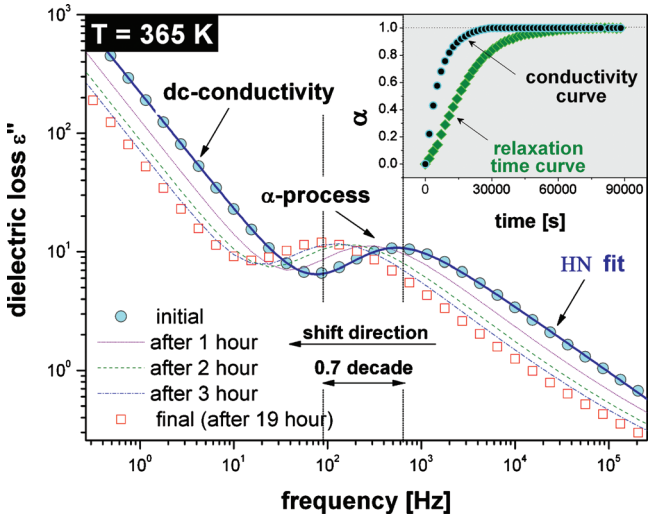

FIG. 2. The time evolution of dielectric loss spectra collected at 365 K. Dispersion curve depicted as solid blue squares is the first reported dielectric spectrum after temperature stabilization (~10 min after melting) while the curve depicted as open red squares was recorded after equilibrating of the sample by 19 h. The solid blue line denotes the Havriliak–Negami fit to the experimental data. In the inset panel the rescaled 0–1 scale kinetic curves representing relaxation time and dc-conductivity dependence on time at 365 K are depicted. Parameter α denotes the degree of reaction, being in this case a measure of tautomerism reaction in GCM drug.

163 kJ/mol for forward and 122 kJ/mol for reversed reactions, while at center 2 (part B in Fig. 1) they are 148 and 112 kJ/mol, respectively. In the case of transformation at center 1, the transition state for the studied reaction connects unfavorable amidic conformation, which has O(1) and H(1) on the same side of molecule. Interconversion between this conformation and the favorable one has energy barrier of 99 kJ/mol. From this point of view, tautomerization at the sulfonylurea center is more probable than that in center 1. There is a lower amount of accessible conformations for tautomerization in center 1 in the sample.

Before we start to present the kinetics part, we will try to explain the processes that occur during the preparation of amorphous glibenclamide. In order to prepare supercooled glibenclamide, we have melted the sample, and next we have quenched the melt to the desired temperature. In the freshly prepared melt, when the temperature is very high, tautomerization process is very fast. However after quenching, one can observe re-equilibration of the sample. It results from the fact that the created equilibrium is temperature dependent. Due to the Boltzmann distribution, one can suppose at the lower temperature that the population of more energetically stable forms needs to be improved. Therefore we probably observe reverse reactions, i.e., from imidic acid to the amide form.

B. Tautomerization kinetics of the supercooled GCM

To investigate the tautomerism phenomenon of supercooled GCM drug we performed time dependent dielectric measurements at five different temperatures in the temperature range of 357–373 K. In the main panel of Fig. 2 we demonstrate the representative dielectric loss spectra evolving in time at 365 K. Two characteristic relaxation processes

TABLE I. Kinetics data from the first-order fits.

Temperature (K)	Measured rate constant k (s^{-1})	Half-life time (min)	Shift of α-process (decade)
373	2.0148×10^{-4}	57.33	0.37
369	1.524×10^{-4}	75.79	0.71
365	7.167×10^{-5}	161.17	0.82
361	6.249×10^{-5}	184.85	0.86
357	3.155×10^{-5}	366.13	1.1

can be clearly identified in all collected spectra. In the low-frequency region one can observe a very strong increase of ε'', which is related to translational motions of ions. This upward trend in dielectric loss is known as the dc-conductivity relaxation process. The prominent peak occurring in the high-frequency region is associated with the rotational motions of electric dipole of entire molecule and is designed as the α-structural relaxation process. As can be seen in Fig. 2 the dielectric spectra evolve with time in accordance with our previous expectation, i.e., α-relaxation time increases while an opposite trend was found for dc-conductivity. Initial and final states were plotted as solid circles and open squares, respectively, while the selected interim states are depicted by dashed lines. Because the viscosity is increasing in the time scale at constant temperature one can find that the rate of change of α-process corresponds to the rate of tautomerism. One can see that at 365 K the maximum of α-relaxation peak shifts toward lower frequencies by about 0.7 decade. We have found out that the time required for sample equilibration is getting longer with decreasing temperature. As can be seen in Table I this frequency distance is the longest at 357 K.

To establish the rate of the tautomerization process at different temperature conditions we have analyzed the evolution in time of two parameters, i.e., relaxation time and dc-conductivity. In order to determine both these values from the dielectric permittivity spectra, we employed the standard procedure based on numerically fitting the experimental data by means of the Havriliak–Negami function additively with the dc-conductivity term,[26]

$$\varepsilon^*(\varpi) = \varepsilon_\infty + \frac{\Delta\varepsilon}{[1+(i\omega\tau_{\mathrm{HN}})^{\alpha}]^{\beta}} + \frac{\sigma}{\varepsilon_0 i\omega}, \tag{1}$$

where $\omega=2\pi f$, ε_∞ is the high-frequency limit permittivity, ε_0 denotes the permittivity of vacuum, $\Delta\varepsilon$ is the relaxation strength, and τ_{HN} denotes a characteristic relaxation time; however, α and β parameters characterize the shape of the dielectric loss curve. As can be observed in Fig. 2 there is a very good agreement between fitting curve and experimental points in the whole fitted frequency range.

By plotting relaxation time of α-process (τ_α) (defined as $\tau_\alpha=1/2\pi f_{\max}$, where $f_{\max}$ is the frequency of the maximum peak position) and dc-conductivity (σ) as a function of time, one can construct kinetic curves for each of analyzed temperatures. However, to compare $\tau_\alpha(t)$ and $\sigma(t)$ curves in the one plot, degree of reaction (α) has to be used. To calculate α, being in this case a measure of tautomerization process treated as a degree of conversion of imidic acid to amide

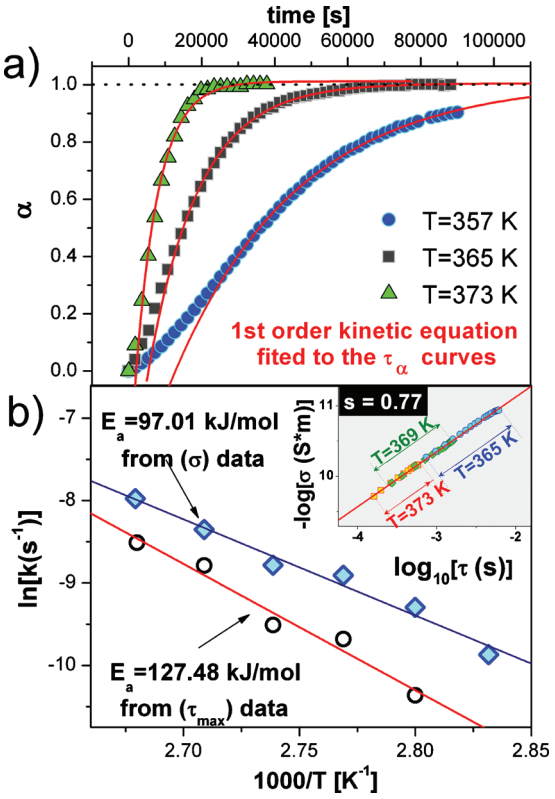

FIG. 3. In panel (a) the three selected kinetic curves for different temperatures representing $\tau_\alpha(t)$ dependence fitted to the first-order kinetic equation (solid red lines) are depicted. In panel (b) the temperature dependence of the rate constant is presented. Solid lines, red and blue, respectively, denote the fits of the Arrhenius equation [Eq. (3)] to the experimental points determined on the basis of α-relaxation time and dc-conductivity analysis. In the inset panel dc-conductivity vs relaxation time in double logarithmic scale for three representative time dependent isothermal measurements is depicted.

form, the procedure presented in work[27] was applied. The representative kinetic curves obtained by rescaling of τ_α and σ determined on the basis of time evolution of dielectric spectra observed at 365 K are depicted in the inset of Fig. 2. As can be seen, both curves have the exponential character. It means that the product concentration is raised to the first power. Thus, the experimentally observed time dependences of τ_α and σ for all studied temperatures can be satisfactory described to the first-order kinetic equation,[28]

$$\alpha = A\ \exp(-kt) + C. \tag{2}$$

Representative kinetic curves, determined from the time changes of τ_α and fitted to the first-order kinetic equation, are presented in the upper panel of Fig. 3. As can be seen in the case of kinetic curve measured at the temperature close to T_g, the exponential function is not able to describe the early stage of tautomerization. Thus, the fitting procedure has to be applied in the limited range of time (see the upper panel of Fig. 3). Although relatively short time is needed to achieve sample stabilization before first measurement, sigmoid was observed only in the narrow temperature range, i.e., 357–365 K. It follows that at the lower temperature the isomerization is relatively slow and that is why it is fairly easy to detect the sigmoidal shape. On the other hand, at higher temperature in which tautomerization is very fast, it is impossible to monitor this early stage. In the work by Wlodarczyk *et al.*,[29] the authors try to connect the sigmoidal character of kinetic curve in supercooled D-fructose with the series of subsequent reactions. However sugars are far more complicated than the GCM tautomers, where tautomerization process can be catalyzed by OH^- or H_3O^+ ions. According to this mechanism, at the first stage the H_3O^+ protonates the carbonyl oxygen, or the OH^- group grabs the hydrogen from the neighboring nitrogen atom. This can be visible as a fast first stage of the tautomerization process. Thus the existence of small amount of free ions could not be excluded. In the other scenario, sigmoidal shape can be caused by the coupling of conformational re-equilibration to the tautomerization. By analyzing the tautomerization pathway in Fig. 1, one can see that the conformational changes that precede tautomerization reaction can have surprisingly high activation energies. Therefore the mechanism could be the same as in the case of subsequent reactions.

Using Eq. (2) one can determine the rate constant of tautomerization for different temperatures and consequently estimate the half-life of the examined process. Knowledge of these kinetic parameters is one of the keys to determine the reaction mechanisms in solid phases. In the pharmaceutical industry, the rate constant has more practical meaning. It is a particularly important physicochemical parameter used to study the degradation of the medication.[30] On the other hand, in the case of drugs which reveal tautomerism, measurements of k and $t_{1/2}$ parameters are needed for the determination of its accurate treatment conditions. It follows from the fact that change in temperature means different tautomer equilibrium and consequently different bioavailability of amorphous drug. In the case of GCM the rate constant can be viewed as a rate of amide concentration in the whole time interval. The values of k determined for different temperatures are summarized in Table I. In the next step, using these parameters we have determined the half-life of the GCM tautomerization defined as $t_{1/2}=\ln(2)/k$. By means of the values of k determined experimentally in the temperature range of 357–373 K, one can also estimate the half-life at room temperature condition. At 298 K, it is equal to almost three years. It means that one can expect that the amide and imidic acid form coexist in the amorphous GCM by more than five years. The literature reports that this kind of equilibrium detected in the glassy state reduces strongly the tendency to the drug crystallization. Consequently, one can expect that in the appropriate conditions the amorphous GCM may be stored for five years without any sign of crystallization. Since our experimental results indicate that GCM seems to be physically stable for a typical shelf-life, it could be produced for commercial use in the amorphous form.

The values of the rate constant determined previously also could be used to calculate the activation energy of the imidic acid-amide tautomeric conversion. As shown in the lower panel of Fig. 3 the logarithm of the rate constant is linearly related to 1/T. Thus, from the fit of Arrhenius equation [Eq. (3)] to the experimental data the activation energy barrier of tautomerization in anhydrous GCM drug has been determined as follows:[28]

$$\ln k = \ln A - \frac{E_a}{RT}. \tag{3}$$

Fit of Arrhenius equation to the data determined on the basis of $\tau_\alpha(t)$ dependences is presented by a red line in Fig. 3(b). The value of E_a in this case is equal to 127.48 kJ/mol. On the other hand, if the time dependence of dc-conductivity was applied to determine the rate constants the value of activation energy was found to be equal to 97 kJ/mol [see the blue line in Fig. 3(b)]. As can be seen, the obtained values of E_a are in good agreement with the computational simulation results presented in the previous part of this paper. However, it is interesting to inquire into what causes the discrepancy in the activation energy from the two different analyses of dielectric data. To solve this problem, at the beginning we would like to recall the relationship between the structural relaxation time and dc-conductivity. Such a correlation is usually discussed in terms of the Debye–Stokes–Einstein (DSE) relation, i.e., $\sigma\tau \cong$ const. However a lot of examples in the literature clearly demonstrate that this equation is valid at high temperatures but it is not always obeyed in deeply supercooled liquid state.[31] In such cases, experimentally it has been found that the relationship between the dc-conductivity and relaxation time can be well described by means of the phenomenological fractional DSE relation $\sigma\tau^s \cong$ const, where s is commonly less than unity. Moreover in Ref. 32 it was shown that the fractional exponent could be related to the ratio $\Delta V_\sigma / \Delta V_\tau$ in which the activation volumes were calculated from the pressure dependence of σ and τ, respectively. However, in the present communication we try to establish the relationship between the parameter s and the activation energy for the two relaxation processes in GCM drug. At the beginning we would like to propose the equation, analogous to the fractional DSE law, which relates the rate constant determined on the basis of $\tau(t)$ and $\sigma(t)$ curves with the parameter s: $k_\sigma k_\tau^s \cong$ const. In the next step, by differentiating the above equation, the following relation is obtained:

$$-R\frac{\partial \ln k_\sigma}{\partial(1/T)} + sR\frac{\partial \ln k_\tau}{\partial(1/T)} = 0. \tag{4}$$

Since the temperature dependence of the rate constant may be easily related to the activation energy of the analyzed process,

$$E_a = -R\frac{\partial \ln k}{\partial(1/T)}\bigg|_P,$$

we arrive at the following relation:

$$E_{a(\sigma)} = sE_{a(\tau)}. \tag{5}$$

To test this new dependence in the case of GCM drug, first we have to determine the fractional exponent s. In order to do that, we plotted dc-conductivity versus relaxation time for three representative time dependent isothermal measurements. The plot in double logarithmic scale is depicted in the inset of Fig. 3(b). As can be seen by inspection, all the experimental data fall on the straight line. From the linear regression we have found that the slope of obtained curve is equal to $s=0.77$. Thus, one can see that our new relation between activation energy and parameter s is valid in the case of GCM drug. Therefore the decoupling phenomenon between the values of E_a determined from the analysis of relaxation times and dc-conductivity can be easily explained.

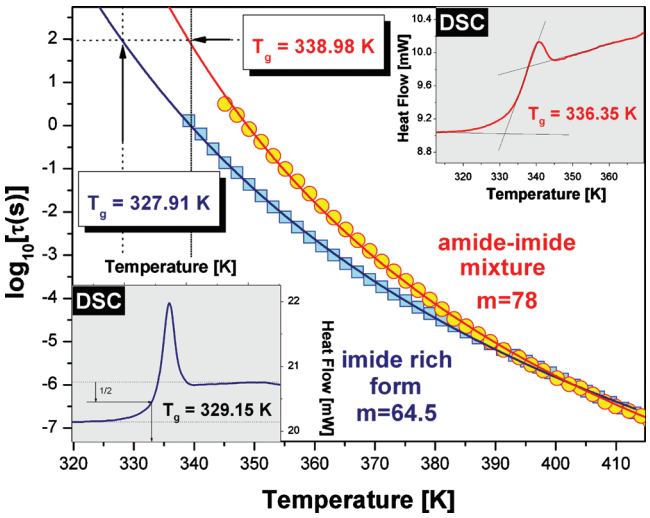

FIG. 4. Fits to the VFT equation describing two GCM systems: imidic acid rich form and amide-imide mixture. In the inset panels standard DSC measurements of GCM are presented.

C. The tautomer's influence on both the glass transition temperature and fragility of the amorphous GCM

One of the characteristic properties of amorphous compound is its glass transition temperature T_g. It is a useful material parameter due to its dependence on structural and thermodynamical properties. Since it is commonly known that every tautomer has its unique physical properties, the vitrification temperature of the drug should vary according to the change of tautomer concentration. In order to estimate the magnitude of T_g variation between equilibrated and imide rich samples, the dielectric measurements in a wide temperature and frequency range have been performed. As a result two temperature dependences of τ_α were obtained (see Fig. 4). The data represented by solid squares refer to the sample quickly quenched after melting, i.e., the GCM drug with large concentration of imidic acid form, whereas the solid circle curve refers to the equilibrated sample. As can be seen in Fig. 4, both log $\tau_\alpha(T)$ dependences for GCM can be satisfactorily described over the entire measured range of temperatures by means of Vogel–Fulcher–Tammann equation,[33–35]

$$\tau_\alpha = \tau_\infty \exp\left(\frac{D_T T_0}{T - T_0}\right). \tag{6}$$

Based on the Vogel–Fulcher–Tammann (VFT) fits and assumption that in both cases T_g can be defined as the temperature at which dielectric relaxation time τ_α reaches 100 s, the glass transition temperatures estimated are 327.91 and 338.98 K for imide rich and equilibrated sample, respectively. It is visible that there is over 10 K difference in the value of T_g between the two materials. Almost the same

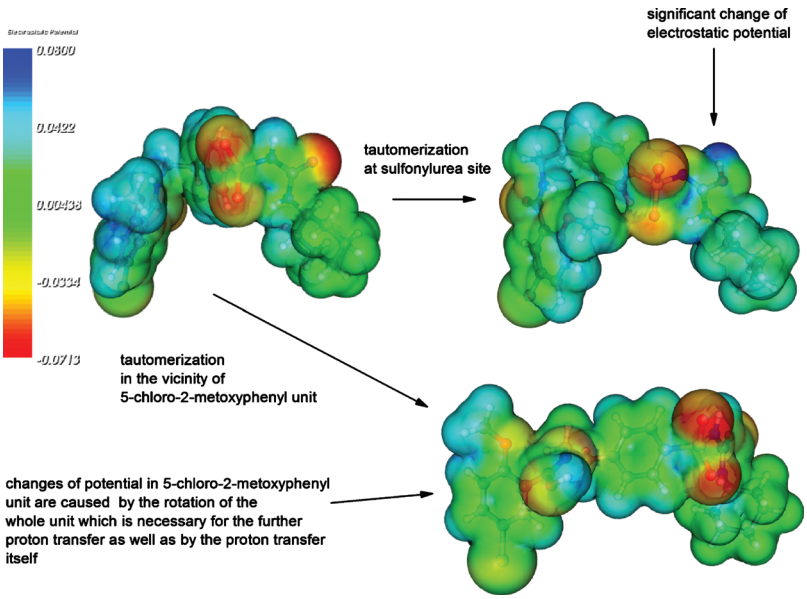

FIG. 5. Electrostatic potentials for amide and imidic acid forms are presented. The significant change in electrostatic potential value can be easily noticed in the sulfonylurea unit. The ability to form hydrogen bonds is changed from one structure to another. In consequence, docking stage of drug is affected, as it is dependent on hydrogen bond pattern and van der Waals weak interactions (see Ref. 38 for more information).

change in T_g has been achieved from the differential scanning calorimetry experiments (see the insets of Fig. 4). Literature data showing this relatively large difference in T_g, actually caused by tautomerism of the GCM drug, had been wrongly interpreted. Patterson *et al.*[22] suggested that it is due to the chemical degradation occurring during the melting process. However, in our previous work which concerns the glibenclamide drug, we have unambiguously shown that chemical decomposition does not occur.

Based on the values of T_g determined previously, we have also calculated the fragility or steepness index m of the GCM samples,[13]

$$m_P = \left. \frac{\partial \log_{10}(\tau)}{\partial (T_g/T)} \right|_{P=\text{const},T=T_g}. \quad (7)$$

This quantity is believed to play a crucial role in the case of amorphous drugs, because it can be used for establishing the appropriate storage conditions. It is commonly known that amorphous substances can be classified as "strong" and "fragile" glass formers based on the temperature dependence of structural relaxation time (or viscosity) above T_g. It is worth noting that fragile glasses reveal a greater tendency to devitrification over the shelf-life.[36] In the case of GCM drug there is distinct difference in fragility depending on the imide concentration. For the imide rich sample the value of steepness index is equal to 64.6, while for the amide-imidic acid mixture it is almost 14 units higher. Thus one can suppose that this change in fragility during tautomerization process could be connected with the different tautomer concentration in the examined sample, and consequently it may reflect the increasing tendency to crystallization. This assumption seems to be justified since in the equilibrated material the amount of imidic acid form is much smaller. Furthermore, we would like to remind the reader that based on the kinetic data, we have found that the equilibrated sample of GCM should remain totally amorphous for a minimum period of five years.

D. Biological importance of tautomers

Last but not least, we will try to show the impact of the studied process on the biological activity of the glibenclamide drug. There are few different schemes of drug working in living organisms. The GCM works by interfering with the proteins in the ionic channels of pancreatic beta cells.[37,38] Its molecule is docked in the active site of SUR-1 protein which is a gate-controlling part of the potassium channel. Ionic activity in the pancreas beta cells is connected with the insulin secretion. To study the affinity of the drug molecule to the protein active cell, the so-called QSAR studies are performed. These methods are based on molecular modeling. The interactions between drug and the protein can have dipole-dipole, hydrogen bonding, or other weak interaction character. The molecule cannot be covalently bonded because protein functions would be permanently disabled. Some of the drugs need to penetrate the cell membrane, and then the docking is performed in the cell interior. In the case of GCM the docking is performed on the external part of the cell. Almost every functional group in this drug is important in the docking procedure; however, the most significant is interaction of the sulfonylurea fragment. The isomerization process causes change of electrostatic potential on the molecule surface. Consequently the imidic acid forms have different affinity to the SUR-1 protein than the amidic forms. Moreover the imidic acid forms are more rigid due to the double bond created between the nitrogen and carbon. To understand this phenomenon we have prepared a figure in which electrostatic potential was mapped on the GCM surface (Fig. 5). There are significant differences between the imidic acid and amidic forms. Moreover the hydrogen bonding sites are changed.

IV. CONCLUSIONS AND FUTURE PROSPECTS

The experimental work in conjunction with the computational simulations reported in this paper provides fresh insights into the kinetics of tautomerism in drug compounds and is essential for understanding this phenomenon. Our

findings strongly suggest that the BDS technique can have the significant importance in the analysis of tautomerism phenomenon of large group of organic molecules such as amorphous pharmaceuticals.

Using BDS technique, one considers the following:

- Tautomerism can be monitored in liquid and deeply supercooled state.
- A small amount of sample is required.
- One can study the tautomerization phenomenon in a wide temperature as well as pressure range.
- Measurements are completely automated and therefore the kinetics of tautomerization can be easily controlled for a long time (several days).
- The measurements of tautomerism process are relatively cheap. We do not need to use any expensive solvents which are necessary, e.g., in the modern NMR measurement. One should remember that the exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. Thus, using different solvents one can obtain different tautomer equilibrium. Since in the case of dielectric measurements we examine the pure sample, based on the kinetic data one can estimate, for example, the expiry date of the analyzed drug.
- It is possible to monitor both changes of the viscosity as well as the dipole moment which occur during the sample equilibration. Thus, one can determine the glass transition temperature and fragility of the samples with different tautomer concentration.
- Tautomerization phenomenon can be easily distinguished from the crystallization process.
- The activation energy of the tautomerization reaction can be simply determined, which is more complicated when the other experimental methods are used.

Admittedly, there are some limitations of the BDS method. For example, we are not able to provide very specific assignments to tautomerization states, one can only confirm or exclude the tautomerization of analyzed compound,

Finally, we would like to draw the attention of the reader to the tautomerism phenomenon and show how important it is to understand the biological consequences that this process can have. It is well known that tautomerization in DNA bases is directly connected with the genome mutations, therefore similar reactions in pharmaceuticals should not be neglected. These related problems worth extensive studies to be carried out in the future.

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