Rheological studies of tautomerization kinetics in supercooled glibenclamide drug

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Rheological measurements have been applied to study the tautomerization of the pharmaceutically active compound glibenclamide. The rate constant and activation energy of the imidic-acid-amide transformation have been successfully determined by monitoring the evolution of shear viscosity. The kinetic parameters from rheological measurements agree reasonably well with the data previously obtained from dielectric spectroscopy. The present Brief Report demonstrates that rheology can provide a fast and precise way to characterize the reaction kinetics of tautomerization.

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Tautomerization, a special case of structural isomerism, attracts the attention of scientists from many disciplines, such as physics, organic chemistry, and pharmacology. This class of intramolecular proton transfer reactions between donors and acceptors has been widely used in designing chelating agents, polymer uv stabilizers [1], laser dyes [2], and biological and medical agents [3,4]. On the other hand, tautomerization sometimes brings undesirable consequences. For example, the proton migration in nucleobases causes point mutations in genetic codes [5]. The difference in drug activity for some pharmaceutical compounds is also a result of tautomerization [6,7]. In this context, it is of great importance to understand the proton transfer kinetics and isomer equilibrium at various thermal and chemical conditions. In particular, since the tautomerization often leads to significant changes in physicochemical properties, the pharmaceutical industry has been trying for years to identify an experimental method which would allow rapid and reliable determination of tautomerization kinetics and, subsequently, predict the equilibration time of various chemical substances.

Recently, it was demonstrated that broadband dielectric spectroscopy (BDS), traditionally used to study the relaxation dynamics of glass-forming liquids, can be successfully applied to monitor the kinetics of proton migration in condensed materials [8–11]. This is possible because various isomers of a given organic compound may be characterized by different chemical and physical properties [12]. The variation in tautomer concentration may be reflected in the change in dipole moment or glass transition temperature of the sample, i.e., parameters that can be easily determined from dielectric measurements.

The investigations of proton transfer by means of the BDS technique were recently performed for the glibenclamide (GCM) drug, one of the most frequently prescribed hypoglycemic agents [9]. The GCM molecule is characterized by two tautomerization centers where the spontaneous migration of a proton from a nitrogen to an oxygen atom of the carbonyl group takes place (see Fig. 1). As a result, in a liquid or glassy state, i.e., under conditions where the proton transfer is possible, GCM is a mixture of one amide and two energetically less stable imidic-acid forms coexisting together in equilibrium. Therefore, one of the possibilities to observe the amide-imidic-acid transformation is to prepare the GCM drug in an amorphous state. According to the literature reports, the glassy GCM sample, prepared by rapid cooling of the melt ($T_m = 443$ K), is characterized by 16% of imidic-acid forms [6]. However, if the same material is subsequently annealed above T_g, the isomeric ratio changes due to tautomerization: The population of amide forms slightly increases. At the same time, the glass transition temperature of the sample is getting higher, and structural relaxation time τ_α is getting longer. Thus, by monitoring the time evolution of structural relaxation time τ_α, it is possible to estimate the kinetic parameters of the proton transfer reaction of GCM, i.e., its rate constant and activation energy barrier.

Rheological (RH) measurements can provide a fast and precise way to continuously monitor the change in material properties and are commonly used in a wide variety of applications [13]. For example, viscosity measurement is frequently used to characterize the kinetics of polymerization or cross-linking reactions [14,15]. However, this experimental technique has not been employed to study the tautomerization kinetics in pharmaceutical molecules. Taking into account the fact that the value of T_g can also be easily determined from rheological measurements, one can expect that the kinetics of proton migration within the GCM molecule also affects shear viscosity. To verify this idea, we carried out the rheological measurements to investigate the time scale of the chemical equilibration process of the GCM drug at various thermal conditions. Indeed, the rheological measurements provide a very effective tool in the analysis of the tautomerization. We demonstrate that the obtained results agree well with the kinetic parameters determined previously from dielectric experiments.

To demonstrate that rheological measurements can be used to study tautomerization kinetics, we performed shear viscosity measurements of supercooled GCM at three different temperatures: 357, 365, and 373 K. In each experiment, the sample was first melted at 440 K for 3 min and then was quenched to the desired testing temperature using rapid liquid nitrogen cooling. After temperature stabilization, a stress of 1000 Pa was applied, and the viscosity of the sample was monitored as a function of time. The measurements were

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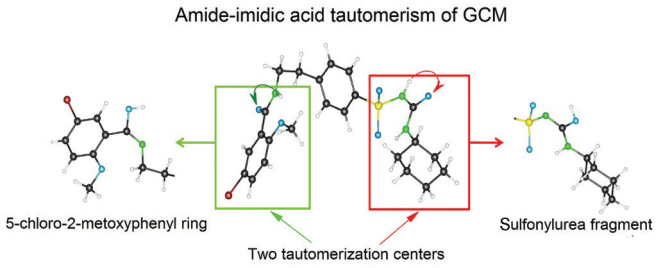

FIG. 1. (Color online) The molecular structure of the GCM drug. Both frames highlight the two centers in the GCM molecule where the amide-imidic-acid tautomerization occurs, i.e., sulphonylurea fragment and 5-chloro-2-metoxyphenyl ring. In both cases, the proton migrates from the nitrogen atom to the oxygen atom of the carbonyl group.

carried out on an AR2000-ex rheometer (TA Instruments) with 8 mm parallel plates. Temperature control was achieved by using an environmental test chamber with nitrogen as the gas source.

According to the existing infrared measurements [6], the intermolecular proton transfer reaction begins at the melting temperature of GCM. If the temperature is kept constant, an equilibrium between the amide and the imidic-acid forms is achieved after a period of time. However, if the sample is quenched immediately after melting, the system has to struggle for a long time until a new equilibrium can be established due to the low molecular mobility near T_g. The rheological measurements essentially monitor the kinetics of the proton transfer reaction from the imidic-acid to the amide forms.

The obtained transient shear viscosity $\eta(t)$'s are presented in Fig. 2 on a double logarithmic scale. As a first observation, we notice that the shear viscosity increases monotonically with time in all cases. The most significant change (more than 50 times the change in η) is found at the lowest examined temperature $T = 357$ K. Under these thermal conditions, the sample equilibration takes the longest time $\sim 10^5$ s. On the other hand, the equilibration time is reduced to $\sim 10^4$ s at 373 K. This is due to the fact that the proton migration is a thermally activated reaction.

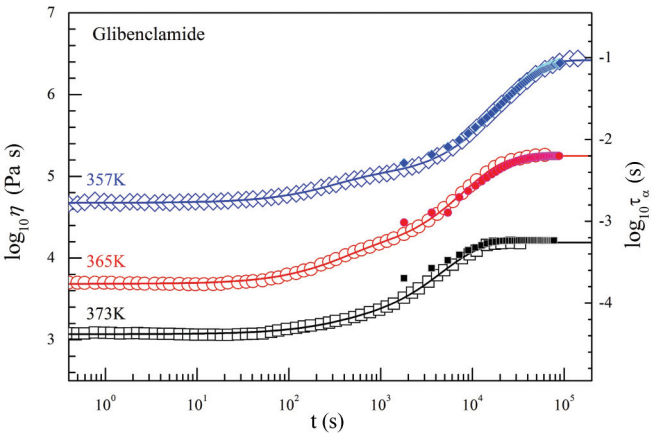

FIG. 2. (Color online) Open symbols: $\eta(t)$ kinetic curves recorded during isothermal rheological measurements. Solid symbols: $\tau_\alpha(t)$ kinetic curves from dielectric measurements. Solid lines: fits of rheological data using Eq. (1).

Now, we would like to turn our attention to the shape of the kinetic curves of the proton transfer reaction. It is easily seen that the transient viscosity data exhibit a two-step growth—the first one, weakly visible and the second one, much more pronounced. Both of them show exponential dependence on time. Since the mechanical relaxation time of all the isomers is much shorter than the characteristic tautomerization time, the evolution of shear viscosity with time is not caused by the mechanical relaxation phenomena but reflects the change in the composition of the isomer mixture. To compare the obtained results with kinetic data determined previously from dielectric measurements, the time evolutions of η are presented in Fig. 2 together with τ_α kinetic curves recorded at similar thermal conditions. At first sight, it is noticeable that, in the long-time range, there is an almost perfect agreement between time dependences of viscosity and structural relaxation time. On the other hand, at shorter times (up to 10^3 s), there is a lack of the dielectric $\tau_\alpha(t)$ experimental points due to an experimental limitation that requires 15 min for temperature stabilization. For this reason, we were not able to monitor the early stage of tautomerization with dielectric spectroscopy. One can only observe the characteristic sigmoidal shape of $\tau_\alpha(t)$ dependences, being a part of the double exponential behavior of the GCM kinetic curves. On the other hand, fast rheological measurements enable us to monitor the onset of the tautomerization reaction and, thereby, to give new insight into the tautomerization phenomenon in the supercooled GCM drug. However, at the same time, a new question arises, i.e., what is the mechanism of proton transfer reaction in the GCM molecule? This issue will be discussed in the next part of this Brief Report.

The presented detailed measurements of kinetics of the transfer (Fig. 2) indicate the existence of the two-step process during the tautomerization. To extract kinetic parameters from the rheological data, the transient viscosity curves are fitted by the superposition of two exponential functions,

$$\log \eta = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t) + C, \qquad (1)$$

where η denotes viscosity, k_1 and k_2 are the rates of reactions, t is the time, A_1 and A_2 are the preexponential factors, and C is the additional constant that characterizes the viscosity of the equilibrated sample. As can be seen in Fig. 2, the fitting curves describe the experimental data very well. The adjusted R^2 coefficient, which defines the fits quality, is higher than 0.999 for each isothermal $\eta(t)$ curve. Thus, one can expect small error bars in the kinetic parameters. The values of the rate constant k, one of the most important kinetic factors characterizing the chemical reaction, are summarized in Table I, together with kinetic parameters determined from earlier broadband dielectric measurements. However, it should be stressed that, in the case of dielectric measurements, the kinetic curves of $\tau_\alpha(t)$ were fitted by a single-exponential function due to the

TABLE I. Kinetics data of the proton transfer reaction in the GCM drug determined from dielectric (BDS) and RH measurements.

T (K)	k_{BDS} (s^{-1})	k_{1-RH} (s^{-1})	k_{2-RH} (s^{-1})	$t_{1/2BDS}$ (min)
373	2.01×10^{-4}	3.64×10^{-3}	2.16×10^{-4}	57.3
365	7.17×10^{-5}	3.13×10^{-3}	1.23×10^{-4}	161
357	3.16×10^{-5}	3.48×10^{-3}	4.75×10^{-5}	366

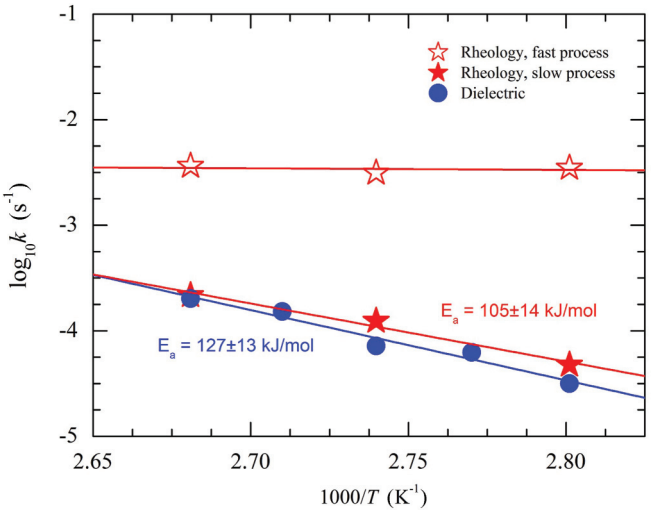

FIG. 3. (Color online) The temperature dependence of the rate constants determined from solid circles: dielectric and open and closed stars: rheological measurements. Solid lines denote the Arrhenius fits of the experimental points determined on the basis of viscosity and structural relaxation times analysis, respectively.

lack of experimental data in the first 15 min. From Table I, one can notice that reasonably good agreement is found between k_{BDS} and $k_{2\text{-RH}}$ determined from the dielectric and rheological measurements. On the other hand, the $k_{1\text{-RH}}$ results are very surprising. It is easily seen that this parameter is almost the same for each kinetic curve. Consequently, the early stage of the GCM isomerization process seems to be only weakly temperature dependent. In addition, the values of $k_{1\text{-RH}}$ are larger than $k_{2\text{-RH}}$ by approximately 1 order of magnitude. This means that the early stage of GCM tautomerization is much faster than the second phase.

To shed more light on the tautomerization phenomenon within the GCM molecule, the values of k determined from the $\eta(t)$ dependences are used to calculate the activation energy barrier of imidic-acid-amide conversion. The fit of the experimental data for the rates k to the Arrhenius equation (Fig. 3) provides an estimation of the activation energy of proton migration in the GCM drug,

$$\log k = \log A - E_a/2.303RT, \tag{2}$$

where E_a is the activation energy, T is the absolute temperature, R denotes the gas constant, and $\log A$ is a preexponential factor. Since the $\log k_{1-\mathrm{RH}} = f(1/T)$ dependence is weakly temperature dependent in the examined T range, we have estimated only E_a of the second slow stage of the tautomerization process observed during rheological measurements. It was found to be equal to $E_{a\mathrm{RH}} = 105 \pm 14$ kJ/mol. For comparison, the value of E_a determined from BDS measurements, using the same fitting procedure, is equal to $E_{a\mathrm{BDS}} = 127 \pm 13$ kJ/mol. Thus, one can see that both values of E_a agree within the error bars.

Finally, we would like to briefly discuss the tautomerization mechanism of the GCM drug in light of the rheological and dielectric measurements. The rheological measurements have revealed that the proton transfer in GCM consists of a fast and a slow process. The slow one has an activation energy ~105 kJ/mol, whereas, the fast one is rather insensitive to temperature.

This result suggests that the first step of the tautomerization of GCM is a rapid conversion of the imidic-acid form to some relatively stable intermediate. The subsequent change from the intermediate to the stable amide form has a much higher activation energy and, therefore, is significantly slower.

To summarize, it has been demonstrated that isothermal rheological measurements can be applied to monitor proton transfer reactions in condensed materials. This fast and precise experimental technique allowed us to identify a two-stage mechanism for tautomerization in the pharmaceutically important glibenclamide molecule. The rate constant and activation energy of proton migration were determined from the time evolution of shear viscosity. These kinetic parameters (k and E_a) are in good agreement with the values obtained from broadband dielectric spectroscopy [9].

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