Short communication

Thermodynamic and Kinetic Studies of Glucose Mutarotation by Using a Portable Personal Blood Glucose Meter

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Dedicated to Professor Josef Barthel on the occasion of his 80th birthday

Abstract

A thermodynamic and kinetic study of the mutarotation reaction of D-glucose in aqueous solution was carried out using a portable personal blood glucose meter. This physical chemical experiment is proposed as an alternative to classical polarimetry. The glucose meter allows the indirect monitoring of the mutarotation process in water, by using an enzymatic redox reaction. The test strips of the glucose meter contain glucose dehydrogenase which converts β-D-glucose into D-glucolactone. This reaction selectively converts glucose and generates an electrical current in the glucose meter which is proportional to the glucose concentration.
This experiment allows the teacher to explore the kinetics and thermodynamics of the mutarotation of D-glucose and, moreover, the stereospecificity of enzymatic reactions.

Keywords: Glucose, mutarotation, blood glucose meter

1. Introduction

Glucose is a polyhydroxy aldehyde belonging to the chemical class of carbohydrates. It is the most abundant and most important carbohydrate in nature, since the metabolism of a majority of living species is based on the oxidative decomposition of the glucose molecule obtained from endogenous or exogenous sources.

Industrialy, glucose is produced from the chemical or enzymatic hydrolysis of starch, which is obtained from different plant sources around of the world[1]. The glucose molecule, as well as other polyhydroxy aldehydes and polyhydroxy ketones acquires a cyclic form when in solution[2]. In the case of glucose, the more stable conformation is the chair-like shape, similar to the cyclohexane molecule, as presented in Figure 1[3–5].

In solvents such as water, glucose undergoes a phenomenon known as mutarotation, first observed in 1846[6] but only understood after the discovery that carbohydrates are present in a cyclic form in solution, with a very small amount of the acyclic form (~0.01%)[2, 3]. The mutarotation is a dynamic process resulting from a chemical reaction that promotes the change between the forms α- and β-D-glucose in solution, which has a pseudo-first order reaction kinetics in aqueous solutions. The mutarotation reaction is acid catalyzed and occurs through the formation and hydrolysis of a hemiacetal after passing through an acyclic aldehyde intermediate, as shown in Figure 1[3, 5].

The mutarotation reaction continues until the establishment of a thermodynamic equilibrium between the α-D-glucose and β-D-glucose forms. Strictly speaking, in an aqueous solution of glucose, six forms coexist in equilibrium[7, 8]. However, the two six-membered cyclic forms (α and β) and the acyclic aldose form are the most important forms for the discussion of the mutarotation process, as represented in Figure 1. For practical reasons, the α- and β-D-glucose forms will be the only forms considered in this work.

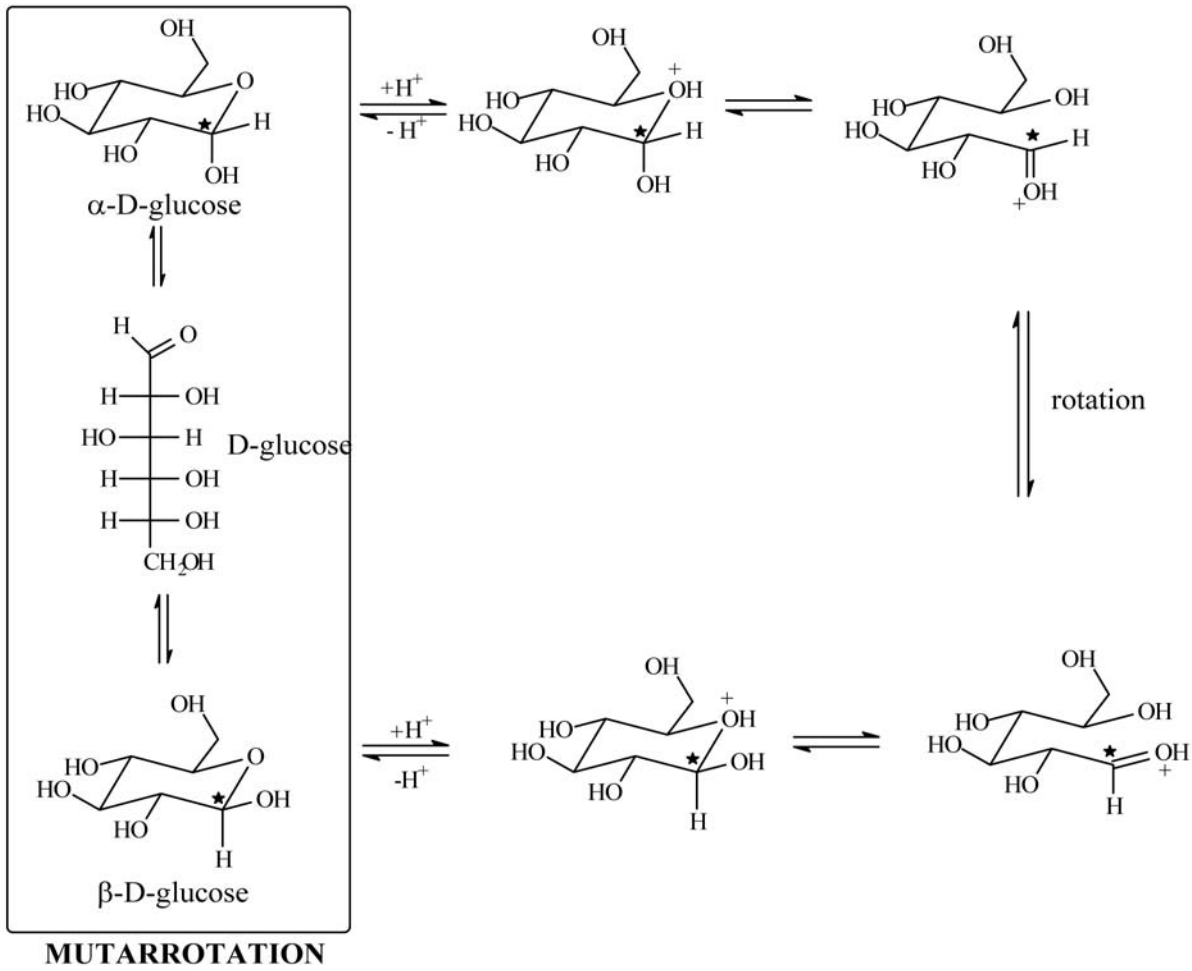

Figure 1. Schematic represention of glucose forms in solution. Right side, the inversion of the anomeric carbon configuration. Left side, the equilibrium of mutarotation.

The α- and β-D-glucose forms differ only in the configuration of the chiral hemiacetal carbon C-1, which is marked by star in the structures presented in Figure 1. They are optical isomers known as anomers, a specific nomenclature for carbohydrates used to designate the carbon atom that undergoes the nucleophilic attack in the cyclization process of glucose. The anomeric carbon differs in reactivity from the other carbon atoms in the molecule.

The very small structural difference of the α and β forms and their respective interactions with the solvent are responsible for the difference in the stability between those anomers in solution. It is known that at equilibrium, the β form prevails over the α form. Some results studies have shown that a larger amount is in the β form due to the more perfect fit of the glucose molecule in the three-dimensional structure of the clusters of water, since all of the hydroxyls are in the equatorial orientation[2]. In the α form, the hydroxyl of the anomeric carbon is in the axial orientation and disturbs the three-dimensional arrangement of water, becoming the less stable form in an aqueous solution. Furthermore, there are studies in the literature which indicate that the hydrophilic/ lipophilic ratio of the β anomer is larger than that of α anomer [3, 4].

Since α- and β-D-glucose are optical isomers, a direct technique for real time monitoring of the kinetic process of mutarotation in solution is polarimetry, whose principles are based on the optical rotation of plane polarized light interacting with each isomeric form. In fact, the "mutarotation" term originated from the observation of the variation of the optical rotation with time.

Recently we proposed that students in general chemistry classes in secondary schools and colleges use a simple inexpensive commercial portable personal blood glucose meter (BGM), commonly used by people with diabetes and by health care professionals, to follow the mutarotation process of the glucose in aqueous solution[9].

The present paper reports the thermodynamics and kinetics of the mutarotation reaction of D-glucose in aqueous solution at two temperatures, 283.2 and 293.2 K, using a portable personal blood glucose meter as an alternative detection method to classical polarimetry.

2. Experimental

2. 1. The Portable Personal Blood Glucose Meter (BGM)

The functioning principle of a BGM is based on a redox enzymatic reaction that generates a small electrical current proportional to the amount of D-glucose in solution[10]. The test strips of the BGM contain the enzyme glucose dehydrogenase (GDH, EC.1.1.1.47), an stereoespecífic enzyme that, together with the coenzyme pyrroloquinoline quinone (PQQ), catalyzes the oxidation of only the β form of D-glucose to D-glucolactone[11], as shown in Figure 2. The limit of detection of the BGM is 2.20 10^{-2} mol L^{-1}. However, the BGM gives the total concentration of glucose (forms α + β), assuming an equilibrium between the two anomeric forms at body temperature (~ 309.2 K).

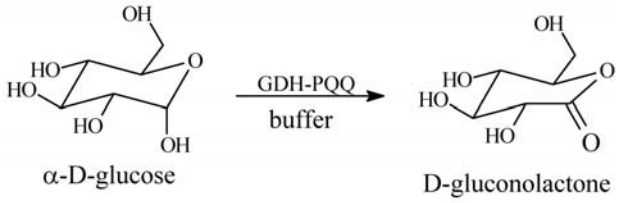

Figure 2. Oxidation of α-D-glucose to D-gluconolactone by glucose dehydrogenase enzyme (GDH) in the presence of a coenzyme pyrroloquinoline quinone (PQQ).

Due to the high selectivity of the enzyme GDH for the β anomer, it is possible to follow the mutarotation kinetics of the glucose in real time in aqueous solution using this type of monitor.

The BGM used in this work (Accu-Check Advantage II) is manufactured by the Roche Pharmaceutical Company and is widely available in pharmacies or in stores specializing in products for clinical diagnoses. It is relatively inexpensive, costing about US$50.00. There are other BGM available on the market were not tested in this work; however, since they possess the same analytical principle as the Accu-Check Adavantage II (amperometric method), they should present similar results.

2. 2. Reagents

Commercially available α-D-glucose monohydrate (Sigma Aldrich) was used to prepare the solutions with distilled water.

2. 3. Monitoring of the Mutarotation Using the BGM

50.00 mL of an aqueous glucose solution at a concentration of 2.00 10^{-2} mol L^{-1} (360 mg dL^{-1}) were prepared. Immediately after dissolution of the glucose, 10 mL of this solution were transferred to a test tube, stoppered and maintained in a thermostated water bath (QUIMIS) with temperature control of 0.1 K. It was not possible to maintain the BGM in the thermostated bath during the time of measurement. However, due to the short time of each measurement (~15 s), the mutarotation equilibrium is not affected during this period at room temperature and the accuracy of the analysis is restricted by the temperature limit of the operation of the BGM (275.2 K to 305.2 K)[11]. The experiment can be performed in a water bath whose temperature control is less rigorous, with considering the larger experimental errors. However, tests are recommended to evaluation the errors when the experiment is performed outside the conditions indicated in this work.

The kinetics of mutarotation was studied by taking samples of the thermostated glucose solution at fixed time intervals using a micropipette. A drop of the solution was immediately deposited on the lateral opening of the test strip, which had previously been introduced into the BGM. The volume necessary for the measurement is, approximately, 4 μL and the drop is absorbed by capillary action. The result is presented in, approximately, 15 seconds. The values of the concentration, shown in mg dL^{-1}, were converted to mol L^{-1}.

2. 4. Monitoring of the Mutarotation Using the Polarimeter

The kinetics of mutarotation of D-glucose was also followed using an automatic polarimeter (PerkinElmer 341) coupled to a microcomputer. Absolute optical rotation data (α) were registered at intervals of 5 minutes by the software POL WINLAB®. The experiments were performed in a thermostated polarimeter cell with a 0.1 dm optical path length at the wavelength of 589 nm (sodium lamp) at two different temperatures, 283.2 or 293.2 ± 0.1 K. The glucose solution concentration was the same as used in the experiment with BGM (360 mg dL^{-1}). After the complete dissolution of D-glucose, the polarimeter cell was carefully filled with the solution to avoid the formation of bubbles. The cell was introduced into the polarimeter and the acquisition of data was started. Care was taken to record the time interval between the preparation of the sample and the data acquisition because the mutarotation reaction begins immediately. The values of the absolute optical rotation (α) were converted to specific optical rotation ($[\alpha]_D$).

3. Results and Discussion

It is important to emphasize that crystalline commercial glucose used in our experiments is in the monohydrate form, as is possible to observe from the polarimetric curve and from the curve obtained using the BGM, shown in Figure 3.

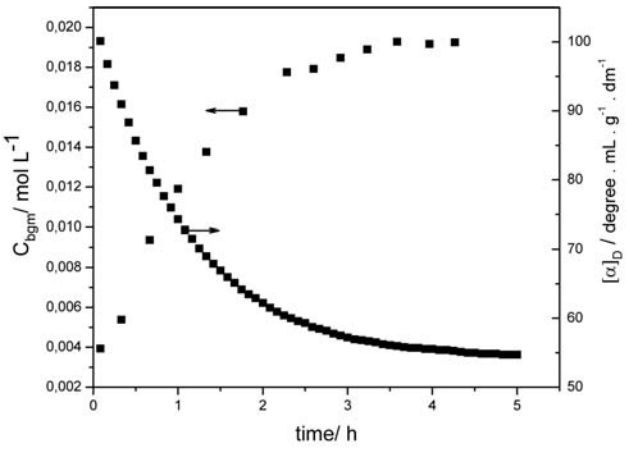

Figure 3. Comparative kinetic curves obtained by the GBM and by the polarimeter.

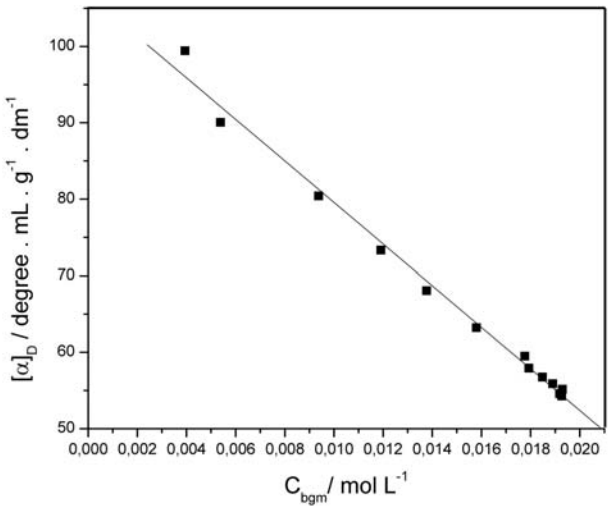

Figure 4. Correlation between the concentration of total D-glucose, obtained by the BGM, and the specific optical rotation $[\alpha]_D$.

Figure 3 shows a comparison between the kinetic curves of mutarotation obtained using the BGM (concentration C_g in mol L^{-1}) and a polarimeter (specific optical rotation, α, in degrees mL g^{-1} dm^{-1}). Both curves show the same kinetic behavior, confirming that the curve obtained by the BGM is the mutarotation reaction.

From the specific optical rotation values for the glucose anomers, $[\alpha_\alpha]^{20}_D$ = +112 and $[\alpha_\beta]^{20}_D$ = + 19[7], and from the experimental mutarotation kinetic curves obtained using the BGM and the polarimeter in Figure 3, we conclude that the commercial D-glucose used in these experiments is in the monohydrate form, with 91% of α-D-glucose and 9% of water.

The fact of the commercially available D-glucose be composed exclusively by α-D-glucose anomer is due to the higher solubility of β anomer. Its larger solubility is a direct consequence of the good adjustment between the conformation of the β anomer with the hydroxyl groups of the glucose molecule disposed in the equatorial orientation and the water cluster structure known as triidimite, in analogy to the structure of silicate.[1, 12,13] In the industrial process of crystallization of glucose, the less soluble α-anomer crystallizes more quickly, yielding only the α-form. For more details see references[1, 3–5].

As previously stated, the functioning principle of the BGM is electrochemical or more exactly, amperometric. During the oxidation of β-D-glucose to D-gluconolactone, the electrical current produced is proportional to the concentration of glucose in solution. This oxidation of β-D-glucose is catalyzed by the enzyme GDH, which is specific only for the β anomer, which is responsible for the high selectivity of the BGM for this anomer and not to the total glucose in solution.[10, 14].

Figure 4 shows an excellent linear correlation between the values of the specific optical rotation ($[\alpha]_D$) obtained by the polarimeter and the concentration values obtained by the BGM. From Figure 4 a linear equation:

$$[\alpha]_D = -2817.5\ C_g + 108.4 \quad (1)$$

was obtained for the conversion of the concentration values obtained by the BGM (C_g in mol L^{-1}) into values of $[\alpha]_D$ (degrees mL g^{-1} dm^{-1}). This equation was obtained from the average of the coefficients A and B of the mutarotation process performed at 283.2 K and 293.2 K. Equation 1 was used to study the thermodynamics and kinetics of the mutarotation reaction.

From the C_g data and the values of the specific optical rotation of each anomer ($[\alpha_\alpha]^{20}_D$ and $[\alpha_\beta]^{20}_D$), the concentrations of each anomer was calculated as a function of time by using the Equations 2 and 3. Equation 2 was derived from the equations of specific optical rotation for each anomer[1, 6]. The concentration of the anomer α was calculated by difference (Equation 3), assuming that the glucose in solution is in only the cyclic α and β forms. It should be emphasized that Equations 2 and 3 were derived considering the concentration of glucose of 2.00 10^{-2} mol L^{-1} and the optical path length of the polarimeter cell of 0.1 dm. These equations are therefore, valid only in these conditions. If necessary, similar equations can be derived for different concentration conditions and polarimeter path length.

$$C_\beta = -\left(\frac{0{,}0386[\alpha]_D - 4{,}323}{180{,}16}\right) \quad (2)$$

$$C_\alpha = 0{,}020 - C_\beta \quad (3)$$

Where $[\alpha]_D$ is the absolute optical rotation and C_α and C_β are the concentrations of the two anomers (mol L^{-1}).

The values of C_g, obtained by the BGM at temperatures 283.2 K and 293.2 K were converted to values of absolute optical rotation ($[\alpha]_D$), by using the equation 1.

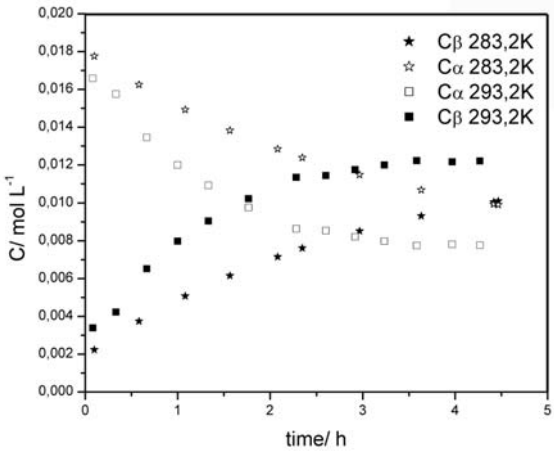

Figure 5. Kinetic curves for the conversion of α to β-D-glucose in aqueous solution at 283.2 and 293.2 ± 0.1K, obtained using the GBM.

From the values of $[\alpha]_D$, the concentrations of β and α forms were calculated by equations 2 and 3 respectively. These values are presented in Figure 5.

3. 1. Kinetic and Thermodynamic Calculations of the Glucose Mutarotation Reaction

The concentrations obtained using the BGM were used to study the kinetics and thermodynamics of the glucose mutarotation reaction. The polarimeter was used in this work only to obtain the mathematical relationship between C_g and the optical rotation of the glucose solution. The concentrations of α- and β-D-glucose in the conditions of thermodynamic equilibrium for each temperature were obtained from the kinetic curves shown in Figure 5. The value of C_g at time t = 0, as well as in the instantaneous t and at equilibrium, can be converted to values of specific optical rotation ($[\alpha]_D$), using Equation 1. It was verified that the anomeric forms of D-glucose, in aqueous solution, only reach equilibrium after 3–4 hours under the studied conditions[12].

The values of the equilibrium constant *K* at each temperature can be used to calculate the Gibbs free energy (ΔG^0) of the mutarotation, according to Equation 4. Table 2 presents the thermodynamic and kinetic data obtained for the glucose mutarotation reaction. These data allow the students to explore concepts associated with the spontaneity of chemical reactions and to observe the effect of temperature on the Gibbs free energy (ΔG^0).

$$\Delta G^0 = -RT \ln K \tag{4}$$

The kinetic rate (k) of the glucose mutarotation reaction for each temperature was obtained using the Equation 5.

$$\ln\left([\alpha_t]_D - [\alpha_{eq}]_D\right) = -kt + \ln\left([\alpha_0]_D - [\alpha_{eq}]\right) \tag{5}$$

The values of the specific optical rotation $[\alpha_t]_D$ and $[\alpha_{eq}]_D$ were calculated from concentration values obtained by the BGM ($\mathbf{C_g}$) at each "**t**" and at equilibrium. The term $\ln[\alpha_o]_D - \ln[\alpha_{eq}]_D$ is a constant and, therefore, the value of $[\alpha_o]_D$ is needed. However, if there is interest in knowing the proportion between the α- and β-D-glucose forms, the value of $[\alpha_o]_D$ can be obtained from the linear coefficient of Equation 5.

The activation energy (E_a) can be calculated by using the Arrhenius equation (Equation 6). It is important to make clear to the students that thermodynamics controls the spontaneity of the reaction, while the kinetics of reaction is controlled by E_a; in other words, by the energy of the transition state between the α and β forms.

$$\ln\left(\frac{k_2}{k_1}\right) = \frac{E_a}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{6}$$

Table 1 shows the thermodynamic and kinetic parameters for the glucose mutarotation reaction from the data obtained by the BGM at the temperatures 283.2 K and 293.2 K. The activation energy, E_a = 67.2 kJ mol^{-1} and the rate constants are in excellent agreement with the values obtained by Panov[3], which are, respectively, E_a = 67.7 kJ mol^{-1} and k = 9.4 10^{-5} s^{-1} at 283.2 K and k = 24.5 10^{-5} s^{-1} at 293.2 K.

The value of the kinetic rate constanat (k) has contributions from the values of the kinetic rate constants of the direct (k_1) and reverse (k_{-1}) reactions. Such kinetic parameters can be calculated by equations 7 and 8 where *K* is the equilibrium constant.

$$k = k_1 + k_{-1} \tag{7}$$

$$K = \frac{k_1}{k_{-1}} \tag{8}$$

Table 1. Thermodynamic and kinetic data for the mutarotation of the D-glucose solution calculated from the concentration values obtained by the BGM at 283.2 K and 293.2 K ± 0.1K.

T/ K	[α-D-glicose] / %	[β-D-glicose] / %	K*	ΔG^0/ kJ mol^{-1}	k/ s^{-1}	Ea / kJ mol^{-1}
283.2	37	63	1.7	–1.3	7.6 . 10^{-5}	67.2
293.2	32	68	2.1	–1.8	20.2 . 10^{-5}	

Substituting Eq.7 in Eq.8, we obtain the following relationship:

$$k_{-1} = \frac{k}{K+1} \quad (9)$$

$$k_1 = \frac{Kk}{K+1} \quad (10)$$

From equations 7 or 8 the kinetic rate constants of the forward reaction can be calculated and then from equation 9 the kinetic rate constant of the reverse reaction can be obtained. Knowing the values of k_1 and k_{-1} for the two temperatures and applying the Arrhenius equation (Eq. 6), the activation energy E_a is obtained for the direct (E_a) and reverse (E_{a-1}) mutarotation reactions. Table 2 shows the kinetic parameters for the direct and reverse glucose mutarotation reactions from the data obtained by the BGM at the temperatures of 283.2 K and 293.2 K.

Table 2. Thermodynamic and kinetic data for the direct and reverse mutarotation reaction of D-glucose calculated from the concentration values obtained by the BGM at 283.2 K and 293.2 K ± 0.1 K.

T/ K	$^{K}_{1}$/ s^{-1}	$^{K}_{-1}$/ s^{-1}	$^{E}_{a1}$/ kJ mol^{-1}	E_{a-1} / kJ mol^{-1}
283.2	4.8 10^{-5}	2.8 10^{-5}	72.4	58.1
293.2	13.7 10^{-5}	6.5 10^{-5}		

4. Conclusions

This approach was proposed as an alternative method for the thermodynamic and kinetic study of the mutarotation reaction of commercial D-glucose in aqueous solution using a personal blood glucose meter.

This method is cheaper than the classical physical chemical method which normally uses polarimetry and therefore, is more accessible for many teaching institutions. Furthermore, the use of the personal glucose meter, whose functioning principle is based on an enzymatic reaction, provides an important didactic advantage in the study of the kinetics and thermodynamics of a mutarotationl reaction since it introduces physical chemical aspects of a biochemical approach to enzymatic selectivity, catalyzed reactions and stereoseletivity.

5. Acknowledgments

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Povzetek

Reakcijo mutarotacije D-glukoze običajno zasledujemo s klasično polarimetrijo. V tem delu smo kot alternativo uporabili prenosni osebni merilnik koncentracije glukoze v krvi in izvedli termodinamsko in kinetično raziskavo reakcije mutarotacije D-glukoze v vodnih raztopinah. Uporabljeni merilnik omogoča indirektno zasledovanje procesa mutarotacije v vodi s spremljanjem encimatske redoks reakcije. Testni lističi namreč vsebujejo glukozno dehidrogenazo, ki pretvori D-glukozo v D-glukonolakton, reakcija pa generira električni tok v merilniku, ki je sorazmeren koncentraciji glukoze v krvi.

V tem delu opisan postopek je primeren zlasti za izvedbo v šolah in omogoča tako obravnavo termodinamike in kinetike mutarotacije kot tudi stereospecifičnost encimatskih reakcij.