A Contribution to the Glimepiride Dissociation Constant Determination

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Knowledge of druglike properties, such as dissociation constants (pK_a), is of great importance in drug development and analysis. However, poor aqueous solubility often causes serious limitations in accurate determination of a drug's pK_a. In this study, the apparent dissociation constant (pK_a') of the poorly soluble drug, glimepiride, has been determined by application of the spectrophotometric and solubility methods. Compared to the literature reported glimepiride pK_a' values of 4.99 ± 0.50 and 6.2 ± 0.1, the values obtained in the present study were 8.07 ± 0.02 and 7.26 ± 0.01 determined by the spectrophotometric and solubility method, respectively. In addition, the advantages of these two methods in pK_a' determination of glimepiride are discussed.

Introduction

The acid−base property of a drug molecule is an important physicochemical parameter, and knowledge of a drug's apparent dissociation constant (pK_a') is of fundamental importance in a wide range of applications and research areas. It is a key parameter for drug development because it governs solubility, absorption, distribution, metabolism, and elimination.[1] Since the majority of drugs are weak electrolytes, their ionization state and, consequently, solubility and dissolution are controlled by both solution pH and the dissociation constant (K_a value). In this context, the dissociation constant might indicate whether the drug will be sufficiently soluble in gastrointestinal fluids or how gastric fluids might affect its dissolution rate.

If the drug is poorly water-soluble (Class II according to the Biopharmaceutics Classification System, BCS), its oral absorption would mainly be limited by solubility and, consequently, its dissolution process.[2] However, some of these drugs dissolve almost completely in vivo due to the combined effect of pH and naturally occurring surfactants.[3,4] It is not possible to identify, on an a priori basis, which compound will be efficiently bioavailable,[5] but there are available mathematical models[6] and software computational methods[7] capable of forecasting pH-surfactant mediated solubilization and dissolution. Successful implementation of these techniques implies certain input parameters concerning drug-related properties such as the drug's pK_a'.

For glimepiride (GLI) (Figure 1), a sulfonylurea-type antidiabetic drug with extremely low water solubility (BCS Class II drug, solubility $< 4\ \mu g \cdot mL^{-1}$),[8] there are limited but controversial literature data about its pK_a'. Calculations based on chemical structure gave a value of $pK_a' = 4.99 \pm 0.50$,[9] while another report stated a value of 6.2 ± 0.1.[10] It was, therefore, our intention to re-examine and determine the pK_a' of glimepiride in a more pertinent way.

Glimepiride is used for the treatment of noninsulin-dependent diabetes mellitus. Although glimepiride shares the principal mechanism of action with other drugs of this class (the stimulation of insulin secretion from pancreatic cells), it has several clinical benefits such as lower dose, rapid onset, lower insulin levels, and less-pronounced glucagonotropic, insulin-sensitizing, and insulin-mimetic effects.[11] Because it is poorly soluble in water, it is of great interest to accurately determine its pK_a' to design a reliable model capable of forecasting its biopharmaceutical properties, namely, solubility and dissolution in the in vivo environment.

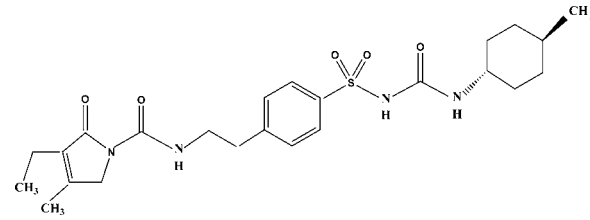

Figure 1. Chemical structure of glimepiride.

There is a large spectrum of pK_a' determination techniques such as potentiometric titration, spectrophotometric determination, software computational prediction, etc.[12,13] Several authors give critical reviews discussing the relative merits and applicability of some of the methods, along with the degree of precision attainable.[12,14] It is noted that existing methods have certain limitations, especially in the case of poorly water-soluble drugs. For insoluble compounds, the recommended procedure for pK_a' determination is UV−vis spectrophotometry.[1,12] Another useful technique to determine the pK_a' of poorly soluble drugs is based on solubility measurements where experimentally determined solubilities at different pHs are used to calculate pK_a'.[13,15]

In the present work, the apparent dissociation constant (pK_a') of glimepiride was investigated by means of the spectrophotometric method introduced by Seok et al.[16] and the solubility method. In addition, the advantages of these two methods in pK_a' determination of glimepiride are discussed.

Experimental Section

Materials and Apparatus. Investigation was carried out with glimepiride produced by Zydus Cadila (India). All other reagents

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were of analytical grade. Double distilled water was prepared in house and used for all experiments.

A WTW inoLab Level 1 pH meter (Weilheim, Germany) was used for pH determination. The pH-electrode (SenTix-HW, pH 0−14/3 mol·L^{-1} KCl) was regularly calibrated using a set of WTW buffers (Weilheim, Germany). The spectra and absorbance readings were recorded on a spectrophotometer model GBC UV/vis 914 (GBC, Australia) with 1 cm quartz cells.

Solubility Determination. Glimepiride equilibrium solubilities were determined by a "shake-flask" method using 0.2 M KH_2PO_4/0.2 M NaOH buffers at pH 4.5, pH 6.8, pH 7.4, pH 7.6, pH 7.8, pH 8.0, and pH 8.2. An excess amount of glimepiride powder was placed into the vials containing 40 mL of tested media and shaken at 250 rpm for 24 h at ambient temperature [(298.15 ± 1) K]. Samples withdrawn were filtered, properly diluted, and assayed for glimepiride spectrophotometrically at the wavelength of maximum absorbance in the range of (226 to 230) nm, depending on the media employed. All measurements were performed in triplicate.

Spectrophotometric Determination. The pH was adjusted to near the desired value by addition of small amounts of acid (0.005 M HCl) or base (0.005 M NaOH) to 0.1 M NaCl solution in water, to adjust the ionic strength. These amounts were high enough to provoke a measurable change in the pH of the tested solutions, while maintaining the sample volume and ionic strength relatively unaffected. Aliquots of each solution were placed into 10 mL tubes and mixed with 1 mL of GLI stock solution in ethanol (98.3 μg·mL^{-1}) to give a final drug concentration of 9.83 μg·mL^{-1}. The solutions were mixed, and after final pH measurements, the absorption spectrum of each solution was recorded, and absorbances over the wavelength range of (210 to 260) nm were obtained from the spectra.

Results and Discussion

In an attempt to estimate the accuracy of the a priori controversial literature data regarding the glimepiride pK_a', the pH-solubility profile was experimentally determined and compared to the theoretical values predicted on the basis of reported pK_a' data.

The total solubility of GLI (C_s) as a function of pH can be expressed as a sum of the individual concentrations of its respective species

$$C_s = [HA] + [A^-] \quad (1)$$

After combining with the Henderson−Hasselbalch equation (eq 2)

$$pH = pK_a' + \log \frac{[A^-]}{[HA]} \quad (2)$$

eq 1 can further be expressed in the following form

$$C_s = [HA] \cdot [1 + 10^{pH - pK_a'}] \quad (3)$$

The intrinsic solubility of glimepiride [HA] was experimentally determined (at pH 4.5) and used for all calculations. Theoretical curves representing glimepiride solubility as a function of different pK_a' values were calculated using eq 3.

Experimentally determined pH-solubility values for glimepiride, along with the corresponding theoretical curves calculated on the basis of different pK_a' values, are presented in Figure 2.

As can be observed, the theoretical curves calculated using the literature reported pK_a' values of 4.99 and 6.20 diverge from the experimentally determined values. The lower solubilities at certain pHs than expected from theoretical curves indicate a noticeable shift of the glimepiride pK_a' to higher values than previously reported. For this reason, the glimepiride pK_a' value was generated using experimentally determined solubilities.

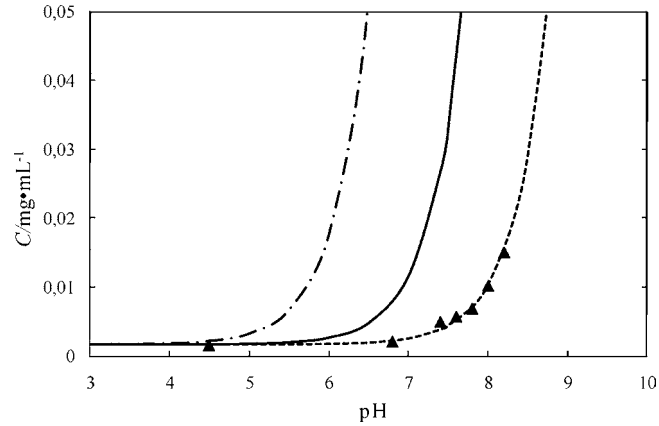

Figure 2. pH-dependent solubility of glimepiride at (298.15 ± 1) K: ▲, experimental values; — · —, theoretical curve (pK_a' 4.99); —, theoretical curve (pK_a' 6.20); — — —, theoretical curve (pK_a' 7.26).

Solubility Method. The principle of the determination of pK_a' on the basis of solubility measurements can be elucidated by several fundamental equations.

In saturated aqueous solutions of GLI, the following equilibria between the solid phase (HA_s) and the solution may be assumed

$$HA_s \leftrightarrow HA, \qquad K_{s0} = [HA] \quad (4)$$

$$HA_s \leftrightarrow H^+ + A^- \qquad K_{s1} = [H^+] \cdot [A^-] \quad (5)$$

(K_{s0}, K_{s1}, solubility equilibrium constants), whereas dissociation in the solution can be described as

$$HA \leftrightarrow H^+ + A^- \qquad K_a = \frac{[H^+] \cdot [A^-]}{[HA]} = \frac{K_{s1}}{K_{s0}} \quad (6)$$

(K_a, dissociation constant).

Total solubility of GLI (C_s) over the investigated pH range (4.5 to 8.2) is the sum of the individual concentrations of its respective species (eq 1).

After combining with eqs 4 and 5, eq 1 can be rearranged into the following form

$$C_s = K_{s0} + \frac{1}{[H^+]} \cdot K_{s1} \quad (7)$$

In acidic solutions, the concentration of the A^- species can be neglected so that eq 7 becomes

$$C_s = [HA] = K_{s0} \quad (8)$$

On the basis of experimentally obtained solubility data and dependencies given by eqs 7 and 8, the corresponding constants (K_{s0} and K_{s1}) for GLI were obtained. Experimentally determined solubility at pH 4.5 was used to determine K_{s0} (eq 8). This value was then used as a fixed parameter in the linear regression analysis where K_{s1} was obtained from the slope (Figure 3).

The results obtained were $3.23 \cdot 10^{-6}$ and $1.77 \cdot 10^{-13}$ for K_{s0} and K_{s1}, respectively. These values were used to calculate the glimepiride dissociation constant (eq 6). The pK_a' value obtained was 7.26 ± 0.01 (the theoretical pH−solubility curve comprising pK_a' = 7.26 is presented in Figure 2).

Spectrophotometric Determination. In addition, the spectrophotometric method, a recommended procedure for pK_a' determination of poorly soluble compounds, was also conducted. The theoretical basis for determination and calculation of the pK_a'

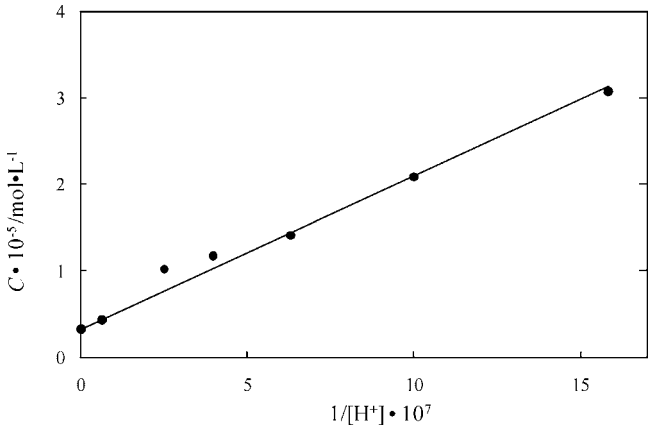

Figure 3. Solubility of glimepiride as a function of reciprocal value of proton's concentration (1/[H^+]): ●, experimental values; —, regression line (defined by the equation: $y = 1.77 \cdot 10^{-13} \cdot x + 3.23 \cdot 10^{-6}$ and R-squared value of 0.9946).

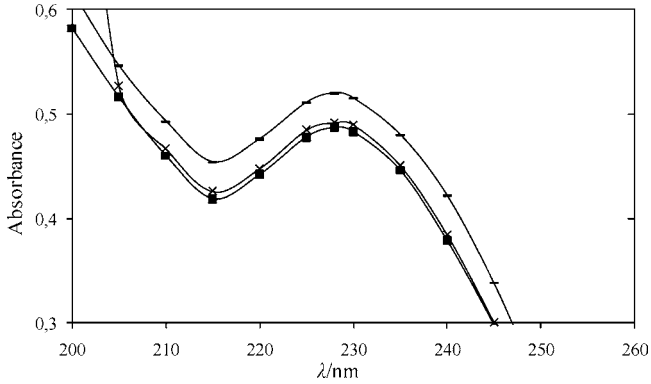

Figure 4. UV-absorption spectra of glimepiride at various pH: ■, pH 7.4; ×, pH 7.8; —, pH 8.0.

by this method has been described by several authors.[16,17] The calculations are based on the following equation (eq 9)

$$pK_a = I - \log \frac{(10^b - 10^a) \cdot A_1 + (1 - 10^b) \cdot A_2 + (10^a - 1) \cdot A_3}{(10^a - 10^b) \cdot A_1 + (10^{a+b} - 10^a) \cdot A_2 + (10^b - 10^{a+b}) \cdot A_3} \quad (9)$$

where A_1, A_2, and A_3 are absorbances measured at three different pH values: I, $I + a$, $I + b$ ($a \neq b \neq 0$), at an appropriate wavelength which is not the isosbestic point.

In eq 9, the ratio of free acid to its conjugate base is solely determined by the relative absorbances of the mixture at a randomly selected wavelength at three randomly selected pH values. It is independent of the concentration and extinction coefficient of each species.

The UV-absorption spectra of GLI solutions as a function of pH are shown in Figure 4.

The absorbances of GLI solutions in the wavelength range of (210 to 260) nm at three different pH values were obtained to calculate pK_a' from eq 9 (Table 1). The glimepiride pK_a' value, calculated from eq 9, was 8.07 ± 0.02.

As can be observed, the results obtained differed from the literature reported data. Also, there were some discrepancies between the pK_a' values obtained by the spectrophotometric and solubility methods. However, it should be highlighted that any comparison of the pK_a' values determined by different methods makes sense only if the experimental conditions were identical during the measurements (temperature, ionic strength, drug concentration).[1] The report[10] citing a GLI pK_a' of 6.2 ± 0.1 does not provide any source of information nor details of the methodology. As for the computational method, SciFinder Scholar[9] enables calculation of pK_a' value(s) at 298.15 K and zero ionic strength in aqueous solution. It uses a structure fragment approach, estimating pK_a' on the basis of equations that relate pK_a' to the molecular structure of the drug. However, owing to the very complicated nature of molecular interactions and the relative simplifications used in the development of this model, the use of additional methods is inevitable for accurate pK_a' determination. The use of the fragmentation method alone may be useful in early discovery stages, when only a rough estimation of pK_a' value(s) is needed.[13]

Table 1. Change in the Absorbance of Glimepiride as a Function of pH and pK_a' Values[a]

	absorbance			
λ/nm	pH 7.4	pH 7.8	pH 8.0	pK_a'
210	0.460	0.467	0.493	8.10
215	0.419	0.426	0.454	8.09
220	0.442	0.448	0.476	8.08
225	0.478	0.485	0.511	8.10
228	0.487	0.492	0.520	8.06
230	0.483	0.490	0.515	8.10
235	0.446	0.451	0.480	8.06
240	0.379	0.385	0.422	8.06
245	0.298	0.301	0.338	8.03
250	0.210	0.213	0.239	8.04
255	0.131	0.136	0.159	8.08
260	0.074	0.079	0.103	8.08

[a] pK_a' (average) = 8.07 ± 0.02.

In the spectrophotometric and solubility measurements applied in this study, the glimepiride concentrations and ionic strength of the solutions were different. Saturated aqueous solutions of glimepiride are necessary for the determination of equilibrium constants by the solubility method. On the other hand, the drug has to be completely dissolved for spectrophotometric determination of its pK_a', meaning that the optimum concentration of glimepiride in the test solutions has to be selected on the basis of previously determined solubility. Additional studies (data not shown) revealed that the presence of NaCl did not significantly affect the pH-induced shift in glimepiride UV absorption spectra and, consequently, the pK_a' value determined by this method. This indicates that changes in ionic strength are unlikely to be the reason for the observed pK_a' discrepancies. However, an important annotation regarding the spectrophotometric method is that only slight differences in the spectra of glimepiride solutions at different pH values were observed. This might be considered as a limitation for reliable spectrophotometric determination of the glimepiride dissociation constant and is likely to be associated with potential errors. Also, it was observed that the spectral curves failed to intersect at sharp isosbestic points, which generally indicated deviation from Beer's law.[16] For these reasons, the value of pK_a' = 7.26 ± 0.01 obtained by the solubility method might be considered as more reliable.

Conclusions

Having in mind the importance of the pK_a' value as an essential parameter for understanding the behavior of weak acids and bases, overestimation or underestimation of a compound's dissociation constant might cause inappropriate development strategies and analytical procedures for that compound. Although a spectrophotometric titration is generally recommended for pK_a' determination of poorly soluble drugs, the present study

demonstrates that the solubility method might be considered as a more reliable technique for determination of the glimepiride pK_a'.

The results of this study can help in reporting a more accurate pK_a' value for glimepiride. Also, it can facilitate a variety of research concerned with drug formulation and analysis using glimepiride.

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