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# Ionization, lipophilicity and solubility properties of repaglinide

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**Abstract**

Potentiometric and spectrophotometric titrations were used for the determination of ionization behaviour, lipophilicity and solubility profile of repaglinide. Acid–base equilibria were characterized by means of protonation macro- and microconstants using Target Factor Analysis of spectrophotometric data. Lipophilicity profiles were evaluated by determination of partition coefficients of neutral and ionized forms of repaglinide in biphasic octanol/water system. The intrinsic solubilities of repaglinide were determined from the solubility data and temperature dependence of intrinsic solubilities were evaluated using van't Hoff equation. Repaglinide possesses two protonation sites and in aqueous solutions exhibits ampholitic properties. At isoelectric pH the zwitterionic form of the molecule predominates over the uncharged form with the tautomeric ratio, $\log K_z = 1.9$. The difference between calculated and measured $\log P$ values, as well as the difference between $\log P$ values of uncharged form of repaglinide, $HR^0$, and either one of mono-charged forms indicated the significant partition of zwitterion into octanol. Temperature dependence of solubility data revealed exothermic dissolution process with $\Delta_{sol}H = -36\ \text{kJ mol}^{-1}$ and negative entropy of solution of $\Delta_{sol}S = -0.19\ \text{kJ K}^{-1}\ \text{mol}^{-1}$.


*Keywords:* Repaglinide; Acid–base properties; Ionization macro- and microconstants; Lipophilicity; Solubility

## 1. Introduction

Repaglinide, (*S*)-(+)-2 ethoxy–4(2[[3-methyl-1[2-(1-piperidinyl)phenyl]-butyl]amino]-2 oxoethyl] benzoic acid, is a novel blood glucose lowering agent from the class of carbamoylmethylbenzoic acids. It stimulates release of insulin from the pancreatic β-cell by closure of $K_{ATP}$ channels and is rapidly absorbed and eliminated from the body [1]. Repaglinide is developed in attempts to overcome the adverse effects associated with existing antidiabetic compounds. These include hypoglycaemia, secondary failure and cardiovascular side effects [2]. Although repaglinide exhibits some chemical resemblance to sulphonylurea-type antidiabetic drug glibenclamide, it differs from other sulphonylureas in both profile of action and excretion mechanism. Repaglinide binds to different receptor sites from other sulphonylureas [3]. As a result it is three- to five-fold more potent than glibenclamide [4] and in contrast to glibenclamide it does not stimulate release of insulin in the absence of glucose [3]. Since orally and intravenously administered repaglinide is excreted most entirely via bile [5], it is an attractive drug for diabetic patients with impaired kidney function.

In order to better understand its in vivo behaviour, the fundamental physicochemical properties of repaglinide should be evaluated. Ionization pattern can profoundly affect its solubility and biological activity. Lipophilicity and solubility play crucial role in both drug absorption from the gastrointestinal tract and passive diffusion through phospholipid bilayers throughout the body. In addition to deeper understanding of its in vivo behaviour, the knowledge of these physicochemical properties is inevitable for the development of the pharmaceutical dosage forms.

In contrast to glibenclamide that is a weak acid with p*Ka* of 6.8, repaglinide possesses one weakly basic and one weakly acidic group resulting in ampholitic nature of the molecule in the aqueous solutions. The existence of two protonation sites gives rise to the four possible species in equilibrium with each other. At isoelectric pH, two neutral forms of repaglinide (zwitterionic and uncharged form) should exist with their equilibrium ratio defined by tautomeric constant, $K_z$.

In this work, our interest is focused on the characterization of solution properties of repaglinide in terms of protonation macro- and microconstants, lipophilicity and its solubility in wide pH

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range. The usual approach for the determination of protonation microconstants involves combination of at least two experimental techniques. Beside potentiometric titration, one or more selective spectroscopic monitoring of protonation sites should be carried out. Recently, a procedure has been developed at *Sirius* for the evaluation of tautomeric ratio from the multi-wavelength UV/vis spectrophotometric data obtained from several titrations of varying water/co-solvent ratio [6,7]. The method overcomes several problems associated with sometimes incorrect assumptions necessary to extract microconstants by other methods [7].

## 2. Material and methods

### 2.1. Materials

Repaglinide was obtained from Biocon Ltd. with purity of 99.6% and used without further purification.

The solutions of HCl and KOH were prepared from titrivals supplied from Merck. Methanol and 1-octanol were spectroscopic grade (Merck). Ionic strength of the deionized water was adjusted with the reagent grade KCl (Kemika).

### 2.2. Determination of ionization constants

Both potentiometry (Sirius GlpKa) and spectrophotometry (Sirius D-PAS) were employed for the determination of ionization macroconstants. All measurements were carried out at constant temperature of 25 °C, constant ionic strength of 0.15 M KCl and with continuous flow of argon to prevent the absorption of $CO_2$ from the atmosphere. The pH-electrode was standardized using Sirius Four-Plus$^{TM}$ procedure.

Due to poor water solubility of repaglinide, the apparent ionization constants, ps*Ka*, were determined by performing the titrations in the co-solvent solutions of varying ratios of methanol and water. Methanol content ranged from 10 to 60 wt.%. The initial pH was adjusted to 9 with standardized 0.5 M NaOH prior each titration and then the titrations were performed with standardized 0.5 M HCl to pH 2. The apparent ionization constants, ps*Ka*, were obtained at each co-solvent solution by a weighed non-linear least square procedure built in RefinementPro software, ver. 1.0.0.19. The aqueous ionization constants, p$Ka_1$ and p$Ka_2$, were obtained by Yasuda–Shedlovsky extrapolation of ps*Ka*'s to 0% methanol [8].

Ionization microconstants and tautomeric ratio were determined on Sirius GlpKa equipped with D-PAS module. This method, developed by *Sirius* and referred as $K_z$ method [6,7], consisted of a series of spectrophotometric titrations or repaglinide in the co-solvents of varying ratios methanol/water. Methanol content ranged from 10 to 60 wt.%. Spectral data were recorded in the region of 200–800 nm and similarly to the determination of ionization macroconstants, each spectrophotometric titration was carried out from pH 9 (adjusted with 0.5 M NaOH) to pH 2 with standardized 0.5 M HCl. The Target Factor Analysis (TFA) is then applied to resolve the molar absorptivity spectra of species $H_2R^+$, HR and $R^-$ for the each methanol/water solution. The molar absorptivity spectra of HR so obtained are linear combination of molar absorptivity spectra of $HR^{\pm}$ and $HR^0$, from which tautomeric ratio and all microconstants were derived as described by Takács-Novák and Tam [6].

### 2.3. Determination of partition coefficient

The partition coefficient of repaglinide was determined by potentiometric titration in biphasic 1-octanol/water system at *Sirius GLpKa* using the difference of so obtained apparent ionization constants and aqueous ionization constants, p$Ka_1$ and p$Ka_2$. Six separate potentiometric titrations of repaglinide were carried out at 25 °C in the systems of various octanol/water (0.15 M KCl) ratios. The octanol/water ratios ranged from 0.5 to 50% octanol. The initial pH was adjusted to 10 with standardized 0.5 M NaOH prior each titration and then the titrations were performed with standardized 0.5 M HCl to pH 1.8. The partition coefficients, log *P*, were obtained by a weighed non-linear least square procedure built in RefinementPro software, ver. 1.0.0.19.

### 2.4. Solubility-pH determination

The solubility measurements have been carried out by potentiometric titration method on pSOL Model 3 solubility profilier (pION Inc.). The titration pH range was 2.0–9.0. The titrations measurements were performed at constant temperature in 0.15 M KCl solution with either 0.5 M HCl or 0.5 M KOH standard solutions. Mathematical treatment of experimental data was performed in pS software, ver. 1.5 (pION Inc.) from which the intrinsic solubility, $S_0$, was obtained. The solubility of repaglinide is determined at 10,15, 20, 25 and 30 °C in three replicate measurements at each investigated temperature.

## 3. Results and discussion

### 3.1. Ionization behaviour

Repaglinide possesses two proton binding sites and therefore can exist in four protonated forms in solution (Fig. 1). The ionization microconstants, p$k_1$–p$k_4$, and the tautomeric ratio, $K_z$, describe the distribution of various forms as a function of pH. On the other hand, ionization macroconstants, p$Ka_1$ and p$Ka_2$, are composite constants and the following relationships are valid:

$$Ka_1 = k_1 + k_2 \tag{1}$$

$$Ka_1 Ka_2 = k_1 k_3 = k_2 k_4 \tag{2}$$

The equilibrium between zwitterionic and neutral forms is defined by tautomeric ratio, $K_z$:

$$K_z = \frac{k_1}{k_2} = \frac{k_4}{k_3} = \frac{C_{HX^{\pm}}}{C_{HX^0}} \tag{3}$$

In order to determine $K_z$, the knowledge of p*Ka* values and any one of the four microconstants are necessary. On the other hand, if $K_z$ is known then ionization microconstants can easily be calculated from $K_z$ and either one of macroconstants.

Macroconstants, p$Ka_1$ and p$Ka_2$, can be evaluated by potentiometric or spectrophotometric titration. If the compound is sparingly soluble in water, the p*Ka* values could be extracted by

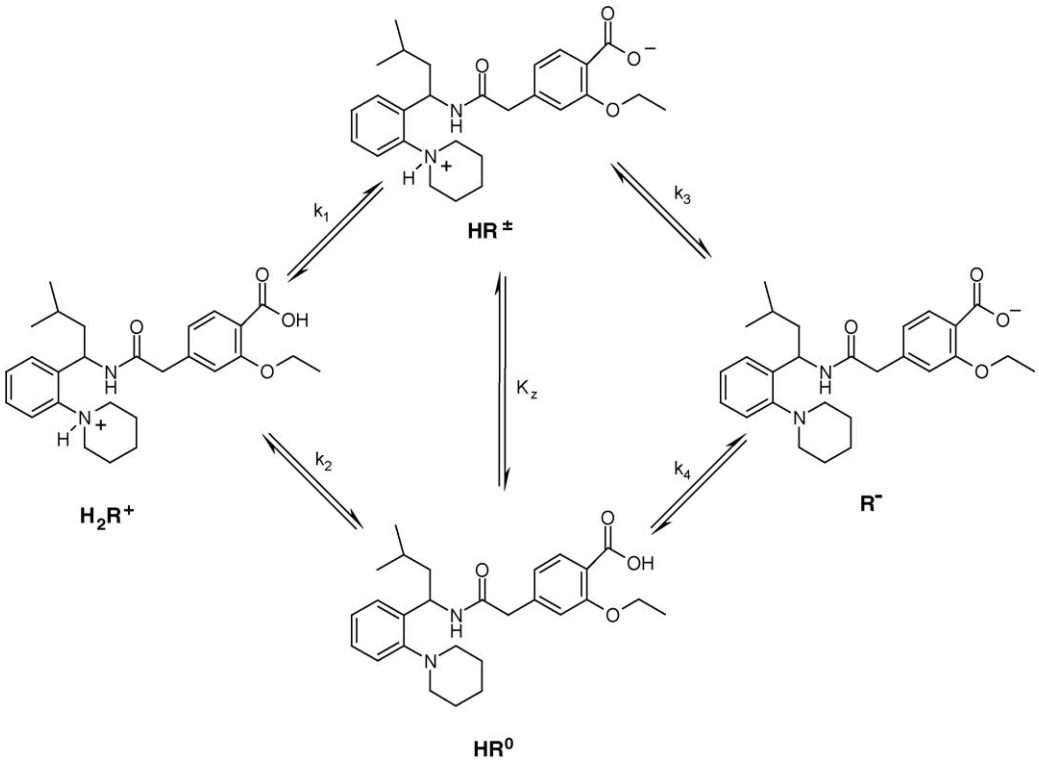


Fig. 1. Ionization scheme of repaglinide.

Yasuda–Shedlovsky extrapolation procedure on the ps*Ka* values obtained in the solutions of several different ratios of co-solvent and water [8].

Fig. 2 shows the absorption spectra of repaglinide in water obtained at different pH values. Principal component analysis (PCA) on this data matrix revealed three independent components ($H_2R^+$, HR and $R^-$) are involved in the ionization process. Since the concentrations of zwitterion, $HR^{\pm}$, and neutral species, $HR^0$, are linearly dependent on each other, PCA could not resolve them in the single spectrophotometric titration and these species degenerate to one principal component, HR. The p*Ka* values of 6.20 and 3.96 were obtained using Target Factor Analysis and are in a good agreement with the p*Ka* values obtained by pH-metric titration using Yasuda–Shedlovsky extrapolation procedure in the solutions of varying water/methanol ratios (6.01 and 4.16). The p*Ka* values so obtained could be assigned to aromatic amino and carboxylic groups, respectively. The lesser extent of delocalization of nitrogen lone electron pair due to different inclination of piperidine amino group to phenyl ring results in higher basicity of aromatic amino group in repaglinide compared to aniline (p*K*a = 4.6). The p*K*a of carboxylic group does not differ markedly from the p*K*a value of the model compound *ortho*-methoxybenzoic acid,[1] since alkyl substitution in aromatic ring does not exert significant conjugation effect upon ionization of acidic centre in the *para* position. Fig. 3 shows linear fits of two p*K*a's obtained in the solutions of varying methanol content. The lower ionization constant has the positive slope characteristic of acidic group, while more basic protonation site has negative slope as expected for the basic group.

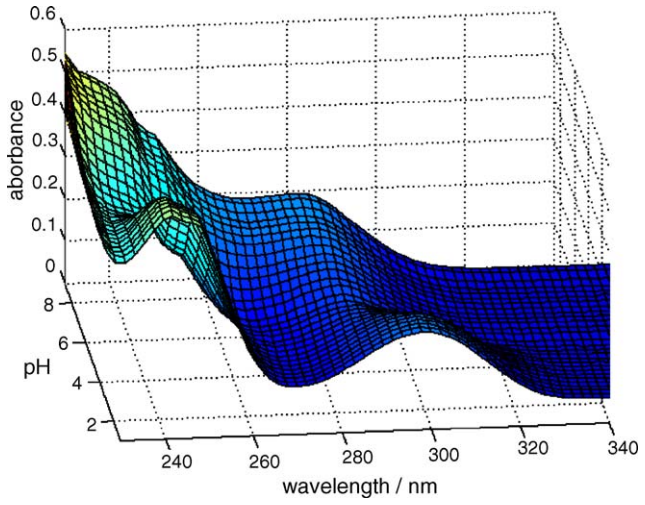


Fig. 2. Absorption spectra of $5.3 \times 10^{-5}$ M repaglinide in water as a function of pH.

The molar absorption spectra of repaglinide undergo hypsochromic and hypochromic shifts upon deprotonation of aromatic acid proton around pH 4 ($H_2R^+ \rightarrow HR$; Fig. 4), since the electrons of carboxylate anion are less capable of conjugation with the aromatic ring than electrons of undissociated carboxyl group [9]. The liberation of the lone electron pair at nitrogen upon deprotonation of aromatic amino group results in the increase of the intensity of the band centred around 280 nm,

[1] p*Ka* of 3.86 for *ortho*-methoxybenzoic acid was determined in our laboratory.

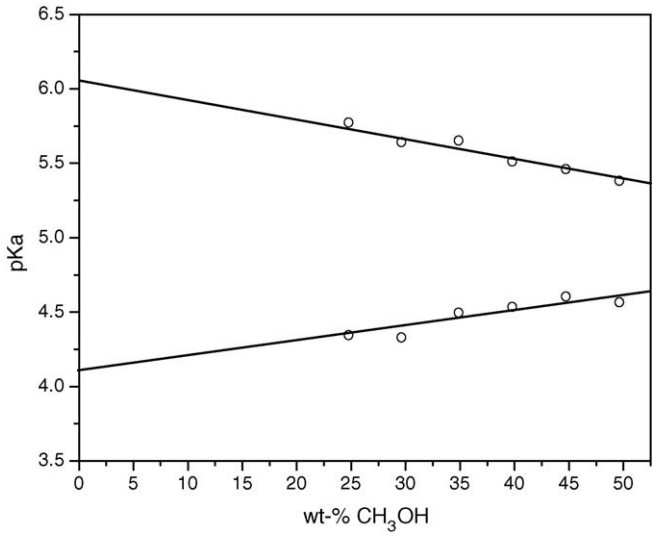


Fig. 3. Dependence of p*Ka*'s on methanol content in solution.

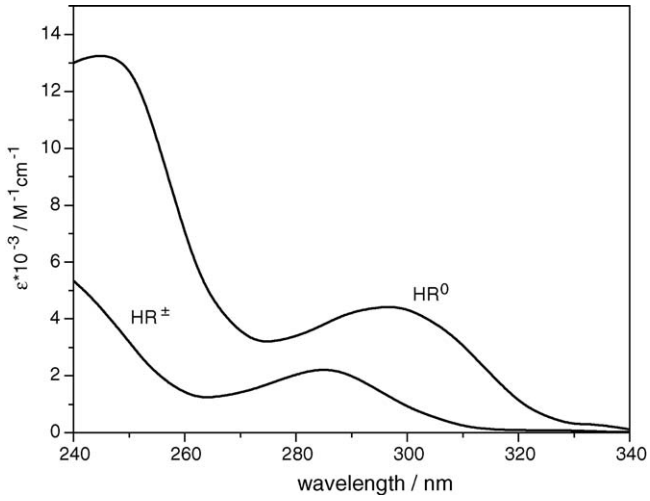


Fig. 5. Molar absorptivity spectra for the zwitterionic and uncharged forms of repaglinide.

due to the effect of the unshared electron pair on the electron system of the aromatic ring.

In order to evaluate tautomeric ratio $K_z$, as well as microconstants $k_1$–$k_4$, multi-wavelength spectrophotometric titration of repaglinide in the solutions of various methanol/water ratios were carried out. For each titration, the TFA method resolves the molar absorptivity spectra of $H_2R^+$, HR and $R^-$. To resolve molar absorptivity spectra of $HR^{\pm}$ and $HR^0$, the slightly different fitting method is applied on molar absorptivity spectra of HR thus obtained [6,7]. Assuming exponential relationship between $K_z$ and wt.% methanol, the aqueous $K_z$ value can be obtained from the intercept of the following equation:

$$\log K_z\,(\%) = WR + S \tag{4}$$

where $R$ stands for wt.% methanol, $W$ the slope and $S$ is the intercept. When the $K_z$ value is obtained by this fitting procedure, all ionization microconstants can be calculated from $K_z$ and p$Ka_1$.

Fig. 5 represents molar absorption spectra of zwitterion and neutral species while Fig. 6 shows the distribution of all species from Fig. 1 as a function of pH. Table 1 gives the results of TFA calculations for $K_z$ tautomeric ratio as well as all micro- and macroconstants.

Since ionization at one center affects the ionization of other, the interactivity parameter which is related to the difference of microionization constants $\Delta pk = pk_4 - pk_1 = pk_3 - pk_2$, is attributed to the magnitude of the ionization effect on the acidity of ionizable groups. The difference of 0.345 pH units ($\Delta pk = 0.345$) indicates weak ionization effect between two protonation sites in the molecule of repaglinide. Considering a long interatomic distance of two protonation sites without possibility of charge delocalization between them, the ionization effect manifests as an electrostatic influence and should predominantly operate through solvent as a field effect. Likewise, the molecule should adopt such conformation in solution to draw two opposite

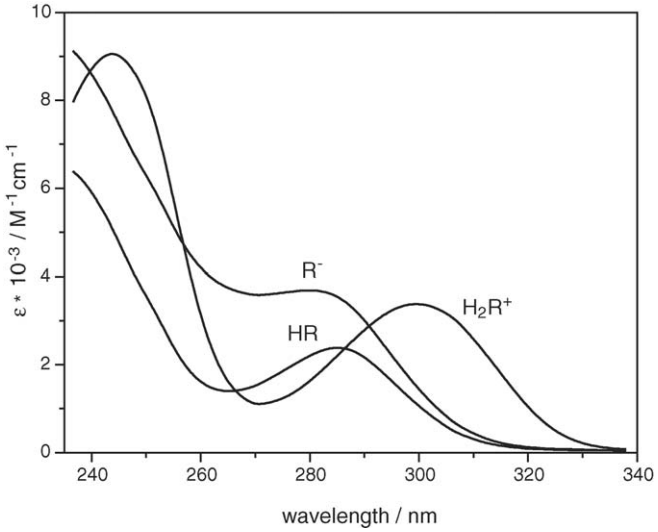


Fig. 4. Molar absorptivity spectra for the ionized and neutral forms of repaglinide.

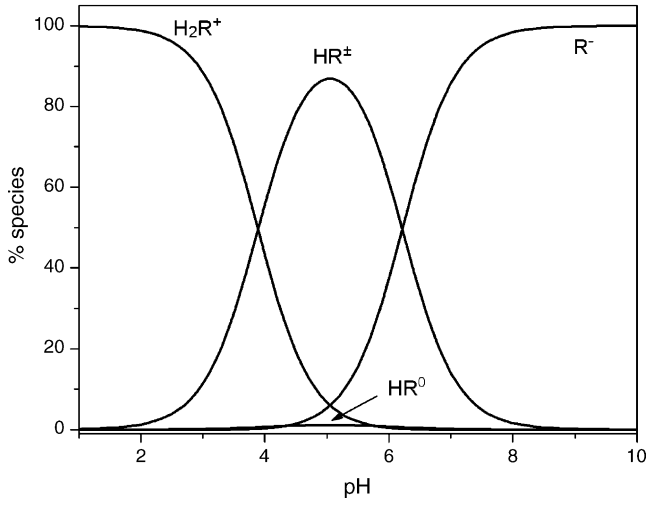


Fig. 6. Distribution of the four possible microspecies of repaglinide in aqueous solutions.

Table 1
Micro- and macroconstants of ionization, tautomeric ratio and log $P$ values of repaglinide

| Parameter | Value |
|---|---|
| p$Ka_1$ | $3.96 \pm 0.11$[a]<br>$4.16 \pm 0.06$[b] |
| p$Ka_2$ | $6.20 \pm 0.05$[a]<br>$6.01 \pm 0.04$[b] |
| log $K_z$ | $1.88 \pm 0.01$ |
| p$k_1$ | 3.97 |
| p$k_2$ | 5.85 |
| p$k_3$ | 6.19 |
| p$k_4$ | 4.32 |
| log $P^{HR}$ | $3.98 \pm 0.02$ |
| log $P^{H_2R^+}$ | $1.62 \pm 0.15$ |
| log $P^{R^-}$ | $1.78 \pm 0.03$ |

[a] Value obtained by UV-spectrophotometric titration.
[b] Value obtained by pH-metric titration.

charges in close proximity.

### 3.2. Lipophilicity

pH-metric titration was used to determine the partition coefficient of repaglinide in octanol/water system. The value of log $P^{HR} = 3.97$ was obtained for the neutral form $HR = HR^{\pm} + HR^0$. If there is no partition of zwitterion into octanol, the following equation would yield the partition coefficient of the neutral species, $HR^0$:

$$\log P^{HR^0} = \log P^{HR} + pk_2 - pKa_1 \quad (5)$$

From Eq. (5), the log $P^{HR^0}$ is found to be 5.89 which differs from the log $P$ value of 4.97 as calculated by *Molinspiration Property Calculation Service* (www.molinspiration.com). This difference could be attributed to the appreciable partition of the zwitterionic form into octanol. The difference of log $P$ values between neutral species and either anionic diff(log $P^{HR^0-R^-}$) or cationic form, diff(log $P^{HR^0-H_2R^+}$) further support this hypothesis. For both charged forms, this difference is above 4 which is significantly higher than 3, the value predicted for the difference between partition coefficients of any pair of neutral and monocharged species in octanol/water system [10]. The enhanced partition of zwitterion form, $HR^{\pm}$, into octanol is most likely due to the intramolecular ion-pairing effect operating in the medium of lower dielectric constants.

Due to high lipophilicity of the uncharged form of repaglinide, $HR^0$, and appreciable partition of zwitterion into octanol, lipophilicity profile of repaglinide shows a bell-shaped curve characteristic for ampholites with small $K_z$ values (Fig. 7).

### 3.3. Solubility

For compounds with two ionizable groups, the solubility at any given pH is the sum of the concentrations of each different species in saturated solution. For the zwitterionic repaglinide the

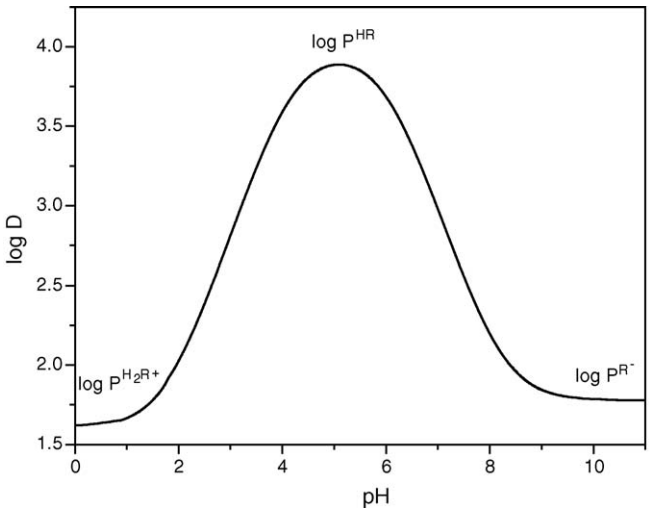


Fig. 7. Lipophilicity profile of repaglinide.

solubility can be expressed by:

$$S = [H_2R^+] + [HR^0] + [HR^{\pm}] + [R^-] \quad (6)$$

It is obvious from Eq. (6) that the solubility of the repaglinide depends on its ionization constants, pH and intrinsic solubility, $S_0$, which is defined as the solubility of the neutral form of the compound, $[HR] = [HR^0] + [HR^{\pm}]$. Therefore, the solubility of repaglinide can be expressed by the following equation:

$$S = S_0(1 + 10^{pKa_1 - pH} + 10^{pH - pKa_2}) \quad (7)$$

Fig. 8 shows the pH-solubility profile of repaglinide at 25 °C. The solubility profile shows U-shape characteristic for zwitterionic compounds and by fitting Eq. (7) to the experimental data the intrinsic solubility, $S_0$, of 68 μg/ml was obtained. Eq. (7) assumes that ionic forms of the compound are freely soluble in water and that the solubility will be dependent on the solubility of the uncharged form, $HR^0$. Thus, the intrinsic solubility will be determined by the solubility of the uncharged form, $HR^0$, and

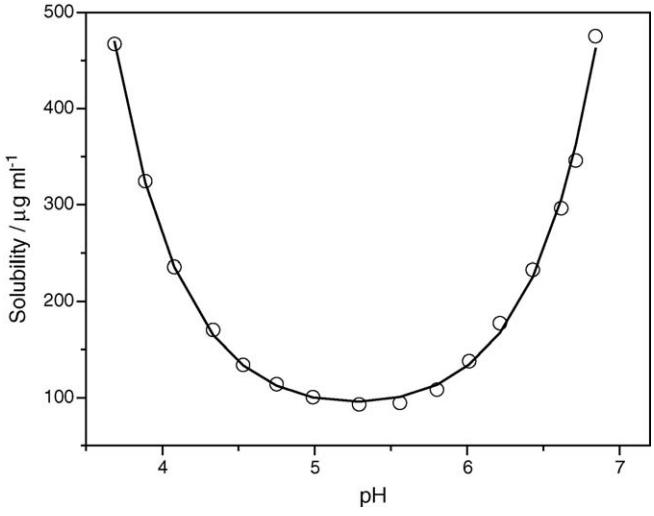


Fig. 8. Solubility of repaglinide as a function of pH at 25 °C. The full line is obtained by non-linear least square regression analysis.

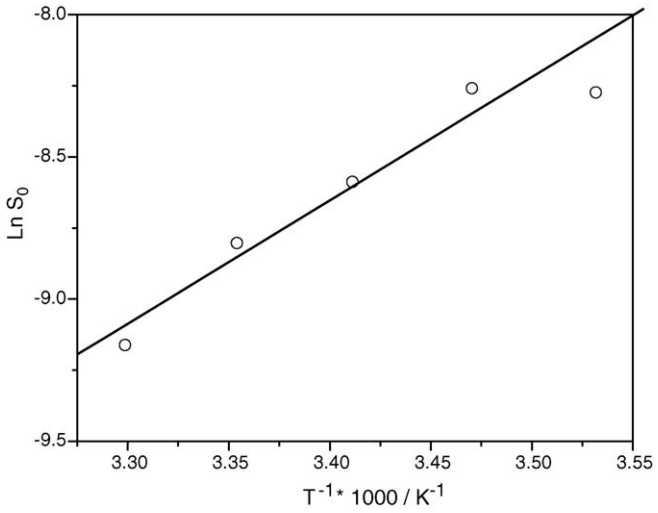


Fig. 9. The van't Hoff plot of $\ln X$ vs. $1/T$ for repaglinide.

tautomeric constant, $K_z$. From the equation:

$$[HR^0] = \frac{S_0}{1 + K_z} \quad (8)$$

the solubility of the neutral form of repaglinide, $S_{HR}$, of 0.85 μg/ml was obtained.

The thermodynamic parameters of solution were obtained by determining the intrinsic solubility of repaglinide at different temperatures and plotting the natural log of the solubility, $S_0$, versus the reciprocal of the absolute temperature (van't Hoff plot; Fig. 9). The change in enthalpy and entropy of solution, $\Delta_{sol}H$ and $\Delta_{sol}S$, respectively, were extracted from the slope and intercept of the linearly fitted experimental data (Eq. (9)).

$$\ln X = \frac{\Delta_{sol}S}{R} - \frac{\Delta_{sol}H}{RT} \quad (9)$$

The change in enthalpy of solution was $-36\,kJ\,mol^{-1}$, a negative value indicating exothermic dissolution of repaglinide as would be expected for molecules having high hydration energies and low lattice energies. This value consists of heat contributions of all processes from initial to final state, i.e. the heat of breakdown of crystal lattice and the heat of hydration of the neutral form of repaglinide as well as the heat of tautomerization reaction in solution. In the tautomerization reaction two charges are created from the uncharged molecule of repaglinide what would be dominating effect in the overall processes due to the high negative enthalpies of hydration of ionic solutes. Thus, the weak intermolecular interaction of the neutral form of repaglinide in the crystal lattice is over-balanced with the energy of hydration of the zwitterionic form of repaglinide and the net result is a release of heat.

The change in entropy of solution was $-0.19\,kJ\,mol^{-1}\,K^{-1}$, and similarly to the enthalpy of dissolution, it consists of the lattice breakdown, hydration and tautomerization entropy terms. The negative value of entropy indicates an overall increase in the system order which is most likely due to the highly ordered water molecules around doubly charged zwitterionic repaglinide.

## 4. Conclusions

Repaglinide possesses two proton binding sites giving it ampholitic nature and its physicochemical and pharmacological behaviour will be mostly determined by the zwitterionic form, which exists predominantly in aqueous solutions. Despite its zwitterionic nature, repaglinide is rather lipophilic as determined by its relatively high $\log P$ value. This is probably due to the presence of intramolecular electrostatic attraction of positively charged aromatic amino group and negatively charged carboxylate anion. Such attraction gives rise to the enhanced partition of repaglinide as an internal ion-pair into octanol.

This physicochemical characteristics will have strong impact on the pharmacokinetic and pharmacodynamic behaviour of repaglinide in vivo, resulting in the unique mechanism of action and excretion mechanism among other antidiabetic drugs.

The $\log P$ value of 3.97 indicates high lipophilicity of repaglinide which together with the intrinsic solubility of 34 μg/ml at 37 °C (obtained by extrapolation of linearly fitted data from Fig. 8) enables its rapid absorption from the gastrointestinal tract.

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## References

[1] S. Oliver, K. Windfeld, V. Hatorp, Diabetes 46 (1997) 331A.
[2] L.C. Groop, in: J.C. Pickup, G. Williams (Eds.), Textbook of Diabetes, vol. 1, Blackwell, Oxford, 1996, pp. 38:1–38:17.
[3] J. Fuhlendorff, P. Rorsman, H. Kofod, C.L. Brand, B. Rolin, P. MacKay, R. Shymko, R.D. Carr, Diabetes 47 (1998) 345–351.
[4] J.A. Balfour, D. Faulds, Drugs Aging 13 (1998) 173–180.
[5] P.N.M. van Heiningen, V. Hatorp, J.J. van Lier, Diabetes 47 (1998) A355.
[6] K. Takács-Novák, K.Y. Tam, J. Pharm. Biomed. Anal. 21 (2000) 1171–1182.
[7] J. Comer, K. Tam, in: B. Testa, H. van de Waterbeemd, G. Folkers, R. Guy (Eds.), Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies, Wiley-VCA, Zurich, 2001, pp. 275–304.
[8] A. Avdeef, J.E.A. Comer, S.J. Thompson, Anal. Chem. 65 (1993) 42–49.
[9] S. Görög, Ultraviolet–Visible Spectrophotometry in Pharmaceutical Analysis, CRC Press, Boca Raton, 1995, pp. 85–88.
[10] F. Reymond, in: A.G. Volkov (Ed.), Liquid Interfaces in Chemical, Biological and Pharmaceutical Applications, Marcel Dekker, New York, 2001, pp. 729–773.