ELSEVIER

International Journal of Pharmaceutics 190 (1999) 41–47

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Solubility profiles of some isoxazolyl–naphthoquinone derivatives

Gladys Granero, María M. de Bertorello *, Margarita C. Briñón

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina

Received 10 March 1999; received in revised form 30 June 1999; accepted 15 July 1999

Abstract

Water, ethanol and *n*-hexane solubility and pH–solubility behavior of a homologous series of isoxazolyl–naphthoquinone derivatives, which exhibit important biological activity, were studied. Their pK_a values were determined spectrophotometrically as well as from their pH–solubility profiles. Also, the molar aqueous solubility of these compounds was estimated with the equations developed by Yalkowsky and Valvani using the data of their melting points and octanol/water partition coefficients.

Keywords: 1,2-Naphthoquinones; Solubility study; Dissolution profile; Acid dissociation constant; Intrinsic aqueous solubility

1. Introduction

The importance of the physicochemical properties of drugs in determining their biological and pharmaceutical characteristics has long been recognized. For this reason, these properties should be preliminary evaluated in the selection of a new drug in a series of drug candidates. Of particular importance are the aqueous solubility and the partition coefficient which are the features determining a drug's dissolution, distribution, and availability.

Since aqueous solubility is also a key factor in controlling drug efficacy, before an orally administered drug can become available to its receptor, it must dissolve in the gastrointestinal fluid. The dissolution rate and the maximum amount of drug that can be dissolved are governed by the solubility of the drug in the biological medium (Yalkowsky and Valvani, 1980); therefore the basis for reliable formulations development being the accurate determination of the solubility. Traditional methodology for solubility determination is the 'equilibrium method', where excess of drug is added to the solvent system and some means of stirring is employed under constant temperature. Samples are then removed, filtered, and analyzed for drug concentration (Higuchi et al., 1979).

On the other hand, in the biological sciences, many solutes of interest can act as acids or bases. In an ionizing medium such as water, they may dissociate into ions which are usually highly water

* Corresponding author. Fax: + 54-351-4334127.

E-mail address: marcor@dqo.fcq.unc.edu.ar (M.M. de Bertorello)

PII: S0378-5173(99)00245-8

soluble. The extent that a molecule is ionized in an aqueous solution is largely dependent on its pK_a and the pH of the medium. For this reason, often a useful approach to increase the aqueous solubility of an ionizable drug is pH adjustment. The pH at which a drug substance is formulated is usually determined from its pH–solubility profile (Sweetana and Akers, 1996). When the aqueous solubility is inadequate, it is also useful to formulate the drug using water-miscible solvents (like ethanol) as cosolvents.

The isoxazolyl–naphthoquinone derivatives have demonstrated to exhibit important biological activity against the causative agent of Chagas' disease (Schwarcz de Tarlovsky et al., 1988; Amuchastegui et al., 1990) and against *Staphylococcus aureus* (Albesa et al., 1995).

The purpose of the present work is to determine water, ethanol and *n*-hexane solubility as well as the pH–solubility behavior of a homologous series of isoxazolyl–naphthoquinone derivatives with an amino group in the four-position of the isoxazole ring (Fig. 1).

The intrinsic aqueous solubility and pK_a values were obtained from the pH–solubility profiles of these compounds. The pK_a values were determined spectrophotometrically and the molar aqueous solubility was calculated using equations from the literature based on melting points and partition coefficients.

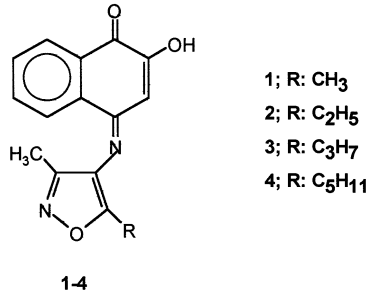

Fig. 1. Structure of the isoxazolyl–naphthoquinones used in this work. **1**, 2-hydroxy-*N*-(3,5-dimethyl-4-isoxazolyl)-1,4-naphthoquinone-4-imine; **2**, 2-hydroxy-*N*-(3-methyl-5-ethyl-4-isoxazolyl)-1,4-naphthoquinone-4-imine; **3**, 2-hydroxy-*N*-(3-methyl-5-propyl-4-isoxazolyl)-1,4-naphthoquinone-4-imine; **4**, 2 - hydroxy - *N* - (3 - methyl - 5 - pentyl - 4 - isoxazolyl) - 1,4 3-naphthoquinone-4-imine.

2. Materials and Methods

2.1. Materials

The isoxazolyl–naphthoquinone derivatives **1**–**4** were prepared as previously described (Férnandez et al., 1982; Granero et al., 1999a). All other materials and solvents were of analytical reagent grade. Water reagent grade was generated by a Millipore Milli-Q water purification system.

2.2. Buffer solutions

Buffer solutions consisted of suitable mixtures of analytical grade citric acid, sodium hydrogen phosphate, sodium bicarbonate and sodium hydroxide (Elving et al., 1956).

2.3. Solubility studies

Equilibrium solubilities of **1**–**4** were determined at room temperature (25 ± 0.1°C) in a constant-temperature bath by adding an excess of the solid to 5 ml of aqueous buffer (or water or ethanol or *n*-hexane) in a series of 10 ml vials. The mixtures were shaken several times on a vortex apparatus. After equilibration (48 h) an aliquot was filtered through a 0.45 μm membrane filter. The first 1 ml of the filtrate was discarded to eliminate any adsorptive effect by the membrane. An aliquot of the remaining filtrate was diluted with the appropriate buffer solutions or solvents and analyzed by an UV-spectrophotometric method (Shimadzu UV 260 UV/visible spectrophotometer) and the remainder of the filtrate was employed for pH determination (ORION SA520 pH-meter).

2.4. pK_a Determination by UV-spectrophotometry

The pK_a values of **1**–**4** were determined by an UV-spectrophotometric method (Agarwal and Blake, 1968) by diluting 2 ml of a stock solution (0.0200 g in 25 ml of methanol) of each compound in ethanol with the appropriate buffer solutions. The absorbance of each solution was measured at the wavelength where the maximum difference in absorption for the ionized and unionized species occurs on a recording spec-

Table 1
Solubility data of **1**–**4** at 25°C in water, ethanol and *n*-hexane

Compound	Solubilities (mg/ml) ± S.D.		
	Water	Ethanol	*n*-Hexane
1	0.0338 ± 0.0001	1.3866 ± 0.0406	Insoluble
2	0.0659 ± 0.0070	2.8866 ± 0.0576	0.0227 ± 0.0010
3	0.0578 ± 0.0004	3.6184 ± 0.0535	0.1030 ± 0.0068
4	0.1449 ± 0.0021	1.7798 ± 0.0398	0.0358 ± 0.0007

Table 2
Solubility of **1**–**4** in aqueous solutions at different pHs at 25°C

Compound	pH	Solubility (mg/ml) ± S.D.
1	3.45	0.0140 ± 0.0013
	6.65	0.0180 ± 0.0006
	7.64	0.0251 ± 0.0017
	9.45	0.2710 ± 0.0262
	9.89	0.4747 ± 0.0131
	10.38	1.4445 ± 0.0117
2	3.72	0.0391 ± 0.0011
	6.44	0.0436 ± 0.0060
	7.01	0.0632 ± 0.0012
	7.62	0.1088 ± 0.0024
	8.81	0.5285 ± 0.0131
	9.16	0.5412 ± 0.0057
3	3.45	0.0640 ± 0.0016
	6.47	0.0561 ± 0.0008
	7.52	0.0944 ± 0.0028
	7.96	0.1252 ± 0.0037
	8.85	0.6392 ± 0.1875
	9.62	0.7404 ± 0.0712
	10.39	0.8370 ± 0.0613
4	4.86	0.0924 ± 0.0003
	6.81	0.0974 ± 0.0020
	7.02	0.1449 ± 0.0021
	8.23	0.2151 ± 0.0014
	9.89	1.9620 ± 0.0056

trophotometer (Shimadzu UV 260 UV/visible spectrophotometer).

2.5. Melting points determination

The melting points of all compounds were determined by the capillary method on a Büchi 510 melting point apparatus and were uncorrected (Table 4).

3. Results and discussion

3.1. Water, ethanol and n-hexane solubility

The solubility data for **1**–**4** in different solvents are listed in Table 1. We report the average of duplicate determinations.

The aqueous solubility of compounds **1**–**4** in general increases with chain length on the isoxazole ring, presumably by weakening the crystal lattice since solubility requires the disruption of crystal structure to allow molecular dispersion in the solvent.

The solubilities of **1**–**4** in *n*-hexane and ethanol show some distinctive behavior. As long as compound **1** remains insoluble in *n*-hexane, the solubilities of **2** and **3** increase with alkyl chain length up to **3** and then a definite break in the solubility is observed (compound **4**).

In ethanol the solubility has orders of magnitude higher than in *n*-hexane and water. This solubility enhancement may be partially ascribed to the capacity of ethanol to form hydrogen bonds. On the other hand, the solubility behavior of **1**–**4** in ethanol resembles that in *n*-hexane in the sense that the solubility increases with chain length of the hydrocarbon groups up to **3** and then a significant decrease is observed.

3.2. pH–Solubility behavior

The solubility data of **1**–**4** in different aqueous buffer solutions are listed in Table 2. We report the average of duplicate determinations.

From these values and the pH–solubility profile (Fig. 2) we can see that the aqueous solubility of **1**–**4** at 25°C is 103-, 14-, 13- and 21-folds

higher at pH ≅ 10 than at pH ≅ 3, respectively. These solubilities increase with increasing pH are in accordance with Eq. (1),

$$C_s = C_o[1 \pm K_a/H^+] \quad (1)$$

where C_s is the buffer aqueous solubility, C_o is the intrinsic aqueous solubility of the substrate and K_a is the dissociation constant (Wells, 1988).

Non-linear regression analysis of the data generated from the pH-solubility profiles according to Eq. (1) yielded the estimated intrinsic aqueous solubility. From the logarithmic form of Eq. (1), the pK_a values for **1**–**4** were calculated (Eq. (2)) and are listed in Table 3, along with those obtained spectrophotometrically.

$$pK_a = pH + \log[(C_s - C_o)/C_o] \quad (2)$$

According to these data, the intrinsic aqueous solubility as well as the pK_a values obtained from pH–solubility profile and by conventional methods are in good agreement. Consequently, the pH–solubility profiles can be used to determine the aqueous solubility and pK_a values of these isoxazolyl–naphthoquinones. In addition, the

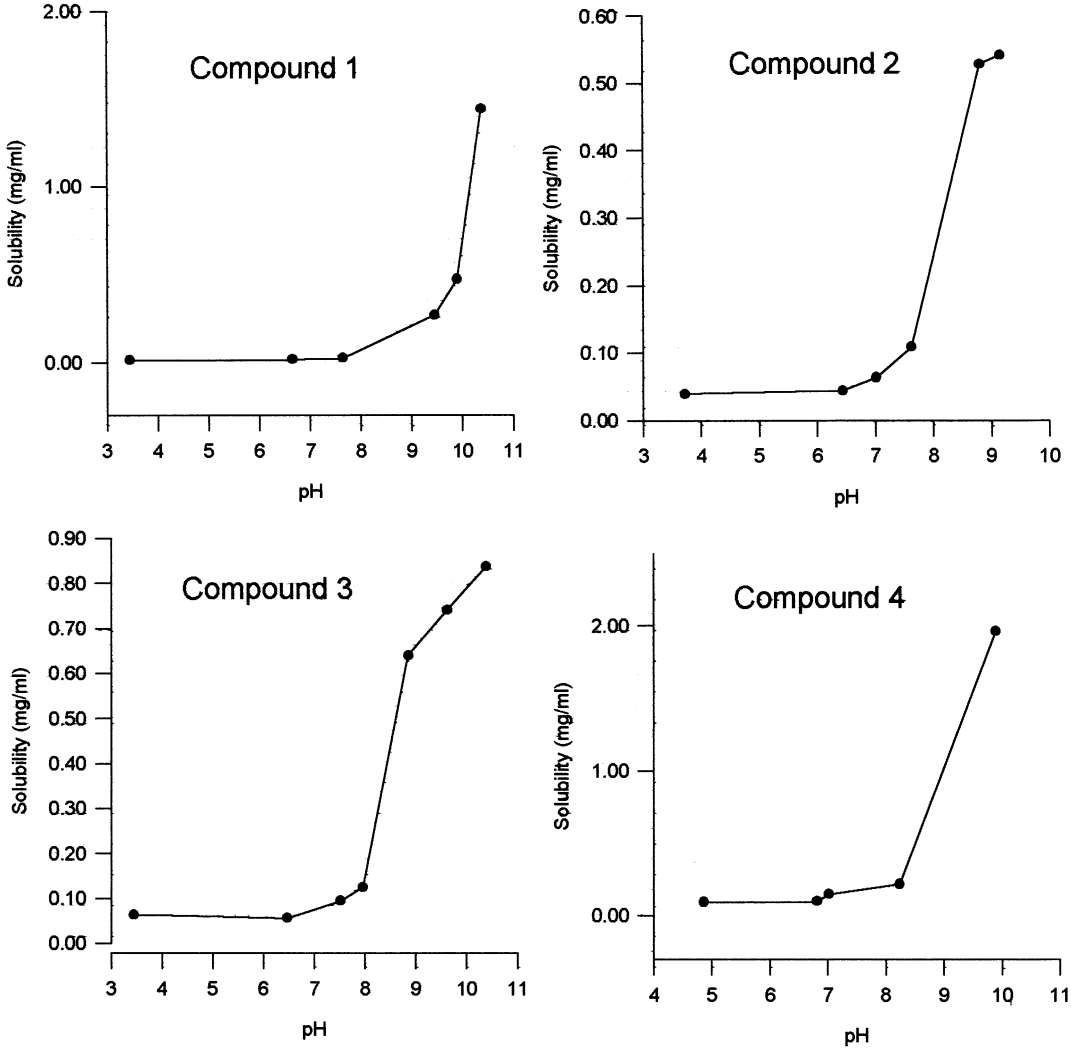

Fig. 2. pH–Solubility profiles of **1**–**4** at 25°C.

Table 3
Intrinsic solubility and pK_a values of **1–4**

Compound	$pK_a \pm$ S.D. [a]	C_o (mg/ml) $\pm$ S.D. [b]	$pK_a \pm$ S.D. [c]	C_o (mg/ml) $\pm$ S.D. [d]
1	8.97 ± 0.74	0.0338 ± 0.0001	8.77 ± 0.25	0.0349 ± 0.0192
2	8.19 ± 0.88	0.0659 ± 0.0070	8.26 ± 0.29	0.0804 ± 0.0464
3	7.29 ± 0.55	0.0578 ± 0.0004	7.86 ± 0.04	0.0591 ± 0.0042
4	7.39 ± 0.17	0.1449 ± 0.0021	7.72 ± 0.07	0.1261 ± 0.0199

[a] pK_a determined by spectrophotometry.
[b] C_o determined by a traditional method.
[c] pK_a calculated from the pH–solubility profile.
[d] C_o calculated from the pH–solubility profile.

strategy of pH-adjustment, routinely employed to increase the solubility of ionizable drugs, can be applied to achieve an enhancement of the aqueous solubilities for the studied compounds.

3.3. Molar aqueous solubility: calculation based on melting point and octanol/water partition coefficient

The molar aqueous solubilities for isoxazolyl–naphthoquinones **1–4** were estimated by Eqs. (3) and (4) developed by Yalkowsky and Valvani using the data for partition coefficient and melting temperature (Yalkowsky and Valvani, 1980).

$$\log S_w \cong 0.80 - \log K_{ow} - 0.01\ (MP - 25) \quad (3)$$

$$\log S_w \cong 0.54 - 8.16 \times 10^{-4}\ \Delta S_f\ (MP - 25) - \log K_{ow} \quad (4)$$

where S_w is the molar aqueous solubility of an uncharged solute, K_{ow} is the octanol/water partition coefficient, MP is the melting point and ΔS_f is the entropy of fusion. A ΔS_f of 13.5 cal mol^{-1} K^{-1} for rigid molecules was assumed in these estimates. The ability of these equations to predict the $\log S_w$ values for **1–4** are listed in Table 4.

Comparison of the estimated $\log S_w$ values with those obtained with the experimental methods, shows that Eq. (4) gives values near the experimental ones, whereas Eq. (3) underestimates the $\log S_w$ of **1–3** and overestimates the $\log S_w$ of **4** (Table 4).

For a crystalline nonelectrolyte, the critical factors involved in the solubilization processes are the solid-phase activity of the solute–solute and molecular interactions between solute and solvent. Due to the polyfunctional character of drug molecules, a wide variety of molecular interactions are often simultaneously operative. Current solubility theories are limited to specific types of intermolecular interactions (Yalkowsky et al., 1983). Since isoxazolyl–naphthoquinone derivatives have one ketone moiety and one hydroxyl group, these compounds are capable to form hydrogen bonds with water around these centers favouring its solubility. Taking into account that experimental aqueous solubilities of **1–4** are higher than it would be expected from the use of regular solution theory, the aqueous solubility values could not be estimated adequately with Eqs. (3) and (4) (Hagen and Flynn, 1983).

The lack of correlation between experimental and calculated values was also observed by other authors (Hagen and Flynn, 1983; Krist, 1999). Hence, these equations appear inadequate for aqueous solubility estimation for these isoxazolyl–naphthoquinones.

Acknowledgements

The authors thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) and the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECYT-UNC) of Argentina for financial support.

Table 4
Calculation of the molar aqueous solubility (log S_w) of **1**–**4**

Compound	Log S_w								
	MP (°C)	Log K_{ow} [a]	Experimental method	pH–Solubility profile	Δ [b]	Eq. (3)	Δ [c]	Eq. (4)	Δ [d]
1	234	1.04	−3.8992	−3.8854	−0.0138	−2.33	−1.5692	−2.8023	−1.0969
2	225	1.57	−3.6314	−3.5450	−0.0864	−2.77	−0.8614	−3.2332	−0.3994
3	173	1.80	−3.7094	−3.6970	−0.0124	−2.48	−1.2294	−2.8904	−0.8190
4	145	3.42	−3.3495	−3.4098	+0.0603	−3.82	+0.4705	−4.2019	+0.8524

[a] The log K_{ow} of **1**–**3** were determined by the shake flash method and for **4** by a TLC method (Granero et al., 1999b).
[b] Difference between log S_w determined by the experimental method and estimated from the pH–solubility profile.
[c] Difference between log S_w determined by the experimental method and estimated from Eq. (3).
[d] Difference between log S_w determined by the experimental method and estimated from Eq. (4).

References

Agarwal, S.P., Blake, M.I., 1968. Determination of the p*K*a value for 5,5-diphenylhydantoin. J. Pharm. Sci. 57 (8), 1434–1435.

Albesa, I., Bogdanov, P., Eraso, A., Sperandeo, N.R., de Bertorello, M.M., 1995. Antibiotic activity of isoxazolylnaphthoquinone imines on mice infected with *Staphylococcus aureus*. J. Appl. Bacteriol. 78, 373–377.

Amuchastegui, P.I., Moretti, E.R.A., Basso, B., Sperandeo, N.R., de Bertorello, M.M., 1990. Isoxazolylnaphthoquinone effects on the growth of *Trypanosoma cruzi*. Rev. Argent. Microbiol. 22, 199–207.

Elving, P., Markowitz, J., Rosenthal, I., 1956. Preparation of buffers systems of constant ionic strength. Anal. Chem. 28, 1179–1180.

Férnandez, A., de Bertorello, M.M., Manzo, R., 1982. Síntesis y propiedades espectroscópicas de 1,2-naftoquinona-4-aminoisoxazoles. Anal. Asoc. Quím. Argent. 70, 49–55.

Granero, G.E., de Bertorello, M.M., Briñón, M.C., 1999a. Synthesis of new isoxazolylnaphthoquinones as potential trypanocidal and antibacterial agents. J. Chem. Res. 2, 110–111.

Granero, G.E., de Bertorello, M.M., Briñón, M.C., 1999b. Hydrophobicity parameters determined by reversed-phase liquid chromatography of some new isoxazolyl–naphthoquinones. J. Liq. Chrom. Rel. Technol. 22 (2), 229–240.

Hagen, T.A., Flynn, G.L., 1983. Solubility of hidrocortisone in organic and aqueous media: evidence for regular solution behavior in apolar solvents. J. Pharm. Sci. 72 (4), 409–414.

Higuchi, T., Shih, F.L., Kimura, T., Rytting, J.H., 1979. Solubility determination of barely aqueous-soluble organic solids. J. Pharm. Sci. 68, 1267–1272.

Krist, A., 1999. Estimation of aqueous solubility for some guanine derivatives using partition coefficient and melting temperature. J. Pharm. Sci. 88, 109–110.

Schwarcz de Tarlovsky, M.N., Goijman, S.G., Stoppani, A.O.M., 1988. Inhibición del crecimiento y la síntesis de DNA y formación del superóxido por quinonas-iminas en *Trypanosoma cruzi*. Rev. Argent. Microbiol. 20, 183–194.

Sweetana, S., Akers, M.J., 1996. Solubility principles and practices for parenteral drug dosage form development. PDA J. Pharm. Sci. Technol. 50 (5), 330–342.

Wells, J.I., 1988. Pharmaceutical Preformulation. The Physicochemical Properties of Drugs Substance. Ellis Horwood, Chichester, pp. 25–28.

Yalkowsky, S.H., Valvani, S.C., 1980. Solubility and partitioning I: solubility of nonelectrolytes in water. J. Pharm. Sci. 69 (8), 912–922.

Yalkowsky, S.H., Valvani, S.C., Roseman, T.J., 1983. Solubility and partition VI: octanol and octanol–water partition coefficients. J. Pharm. Sci. 72 (8), 866–870.