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The effect of salts on the distribution and solubility of an acidic drug

Nivedita K. Pandit, Jill M. Strykowski and Liudoslava Shtohryn *

Pharmaceutics Department, Research and Development, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT 06877 (U.S.A.)

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Summary

The distribution coefficient and solubility of *N*-[4-(1,4-benzodioxan-6-yl)-2-thiazolyl]oxamic acid were determined as a function of added salts. It was found that sodium, potassium and calcium ions decreased the solubility and increased the distribution coefficients significantly, even at low concentrations. The decrease in solubility was shown to be due to the formation of less soluble salts. The increase in distribution coefficients was explained based on ion pairing and/or complexation. It was demonstrated that the effects due to changing activity coefficients of the drug in the presence of the added electrolytes would be negligible under the conditions of the experiments, and could not account for the increases in distribution coefficients.

Introduction

Partition coefficients of drugs are determined routinely in preformulation and structure–activity studies. However, most workers continue to pay little attention to buffer concentrations and ionic strengths in such studies, although the importance of these parameters has been pointed out in some literature reports (Murthy and Zografi, 1970; Dearden and George, 1978; Wang and Lien, 1980; Cools and Janssen, 1983; Davis et al., 1984; Kojima and Davis, 1984; Olejnik and Davis, 1986), and references therein. It is clear that the presence

of other ions can alter the observed distribution coefficient, but the reason for this remains a subject for debate. Some authors claim that the change in the distribution coefficient of drugs in the presence of salts is merely a reflection of salting-in/salting-out behavior (Kojima and Davis, 1984; Olejnik and Davis, 1986). Others invoke the formation of ion pairs between the drug and added salt to explain increases in distribution coefficients (Murthy and Zografi, 1970; Dearden and George, 1978; Cools and Janssen, 1983; Davis et al., 1984).

We report here the effect of salt concentration on the solubility and 1-octanol/water distribution behavior of an anti-allergic drug, *N*-[4-(1,4-benzodioxan-6-yl)-2-thiazolyl]oxamic acid, ethanolate salt (I-EA). The synthesis and pharmacology of this compound and of others in the same series has been previously reported (Hargrave et al., 1983). Unexpected and unreproducible solubility

* *Present address:* Glaxo Laboratories, Research Triangle Park, Chapel Hill, NC 27514, U.S.A.

Correspondence: N.K. Pandit Pharmaceutics Department R&D, Boehringer Ingelheim Pharmaceuticals Inc., 90 East Ridge, P.O. Box 368, Ridgefield, CT 06877, U.S.A.

and distribution coefficient values were obtained during routine preformulation experiments with **I**. The problem was traced to the presence of various types and concentrations of buffer salts present in the experimental solutions. The solubility results can be explained in terms of the solubility products of various salts. The increases in distribution coefficients observed in the presence of salts are examined in light of the two explanations mentioned above that have been proposed in the literature. The availability of accurate solubility and distribution values is essential in predicting the absorbability of a compound. In addition, the presence of ions may have an effect on the absorption of drugs (Davis et al., 1984).

Materials and Methods

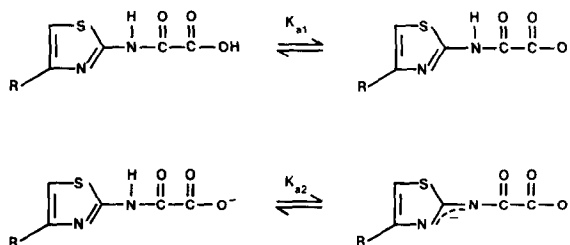
Materials

I-EA (1980 US Patent 4238496) was 99.9% pure and was used without further purification. Sodium chloride, potassium chloride, calcium chloride, and 1-octanol (all ACS grade, Fisher Scientific), and benzalkonium chloride (Mason Chemicals) were used as received. Water was deionized and distilled.

Methods

Determination of pK_a . The two ionization constants of **I** were determined spectrophotometrically according to the method of Albert and Serjeant (1984) at 22°C. Solutions of **I**-EA, at a concentration of 4.4×10^{-5} M, and ranging in pH from 0.6 to 12 were prepared in suitable buffers at a constant ionic strength of 0.2. Absorbances were recorded at 245 and 256 nm to obtain pK_{a1} and pK_{a2} , respectively; these are defined in Scheme 1.

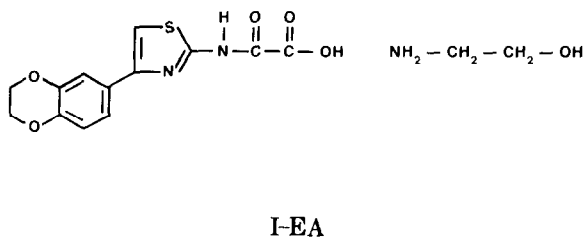
HPLC assay for **I.** A Waters chromatograph consisting of a model 590 pump, a WISP 701B autoinjector and a model 480 variable wavelength detector was used. The column was C18 reverse-phase, 5 μ m (μ Bondapak), and the mobile phase consisted of 20% acetonitrile, 24% methanol and 56% 0.05 M phosphate buffer at pH 3.5. Under these conditions **I** eluted at 4.6 min and was monitored at 269 nm.



Scheme 1.

Determination of pH-solubility profile. A saturated solution of **I**-EA in water was titrated with HCl. The solution was allowed to equilibrate after each addition, the pH of the solution was measured, and a filtered aliquot was analyzed by HPLC. This experiment was performed in duplicate at 22°C. These data were also used to calculate pK_{a1} (Albert and Serjeant 1984), to confirm the value obtained spectrophotometrically. These solutions were unbuffered and at uncontrolled ionic strengths because any added salts affect the solubility.

Determination of solubility products. The solubility products of the sodium, potassium and calcium salts of **I** were determined by adding known concentrations of NaCl, KCl or CaCl_2 to a saturated solution of **I**-EA in water. The solid phase that precipitated out was confirmed to be **I**-Na, **I**-K or **I**-Ca through elemental and/or infrared analysis. The concentration of **I** remaining in solution was determined by HPLC, the concentration of the added cation remaining was calculated stoichiometrically, and the K_{sp} calculated for each salt. The pH value of all solutions was 6.0. All experiments were carried out in triplicate at 22°C, with a CV not exceeding 4% for



Scheme 2.

the calculated K_{sp} . The K_{sp} of I-EA was estimated from the plateau of the pH-solubility profile.

In order to confirm that the ethanolamine in solution did not affect the K_{sp} of the other salts, the K_{sp} of I-Na was determined using the I-Na salt itself. The value obtained was identical to the one obtained by adding a NaCl solution to a saturated solution of I.

Determination of distribution coefficients. Water and 1-octanol were presaturated with each other prior to the experiment. 10 ml of each phase, with the aqueous phase containing appropriate concentrations of I-EA (and added salts, if required) were shaken together for 2 h at 22°C; this length of time was shown to be adequate to achieve equilibrium. The concentrations of I-EA had to be low enough such that the solubility products of various salts were not reached in the experimental solutions. The mixture was centrifuged, and the concentration of I in the aqueous phase determined by HPLC. The pH of the aqueous phase before and after equilibration was checked to make sure that it had not changed. Each experiment was carried out in triplicate, with typical CV values around 5% for the distribution coefficient.

Results and Discussion

Ionization constants and solubility products

The ionization for I is shown in Scheme 1; pK_{a1} was determined to be 1.32 from spectrophotometric measurements and 1.34 from solubility measurements. The value of pK_{a2} was only determined spectrophotometrically and found to be 10.31. Solubility and distribution experiments to examine effects of added cations were all carried out at pH 6, where the compound exists almost entirely in the monoanionic state. Fig. 1 shows the pH-solubility profile, where the solid line is calculated based on a pK_a of 1.33. K_{sp} values for some salts of I are indicated on Fig. 1 and are also listed in Table 1.

Effect of pH on distribution coefficients

The distribution coefficients of I-EA were determined as a function of pH in the absence of buffers. Only HCl was used to adjust pH, and the

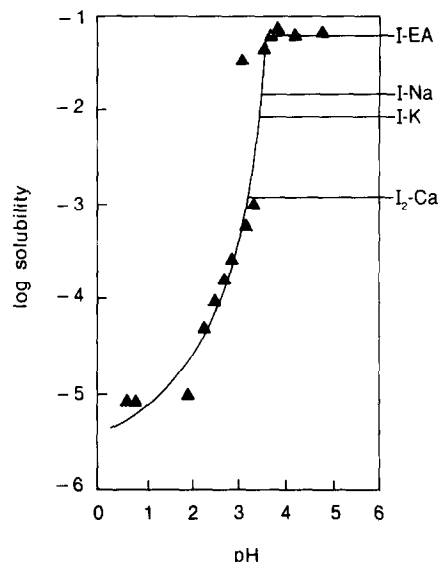


Fig. 1. Profile of molar solubility vs pH for I in unbuffered aqueous solutions at 22°C. Each point represents an average of two measurements, the curve is calculated, and the horizontal lines show the limiting solubility of the various salts.

pH was measured before and after equilibration with the organic phase. The pH did not change significantly during the experiment. At pH values above 3, it was found that the results were not sufficiently reproducible when the experiment was carried out in glass containers. Since the distribution coefficient is low at these pH values, it was felt that trace amounts of alkali ions leaching out from the glass may have affected the results. All experiments were therefore carried out in plastic labware after ensuring that I did not adsorb to plastic. The distribution coefficients obtained were very reproducible, and were lower than those obtained in glass containers, indicating that the

TABLE 1

Solubility product (K_{sp}) values for sodium, potassium and calcium salts of I at 22°C

Salt	K_{sp}	Stoichiometry (I: cation)
Sodium	$2.4 \times 10^{-4} \text{ M}^2$	1:1
Potassium	$9.2 \times 10^{-5} \text{ M}^2$	1:1
Calcium	$1.8 \times 10^{-9} \text{ M}^3$	2:1
Ethanolamine	$9.8 \times 10^{-4} \text{ M}^2$	1:1

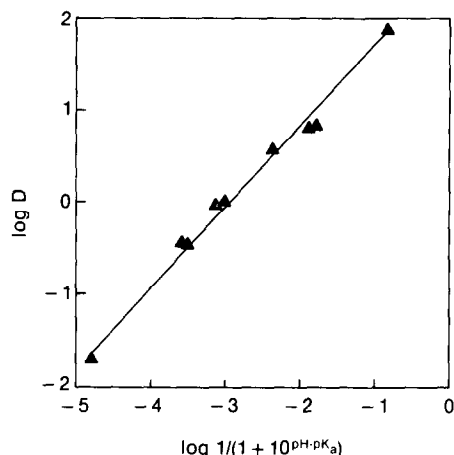


Fig. 2. Plot of distribution coefficients (D) of **I** as a function of pH of the unbuffered aqueous phase, according to Eqn. 1; $T = 22^\circ\text{C}$.

leaching of alkali ions was increasing the distribution coefficients. Assuming that only the unionized form of **I** (**IH**) can distribute into the organic phase, the pH versus distribution coefficient results are plotted in Fig. 2 according to the equation:

$$\log D = \log P + \log \left[\frac{1}{1 + 10^{\text{pH} - pK_{a1}}} \right] \quad (1)$$

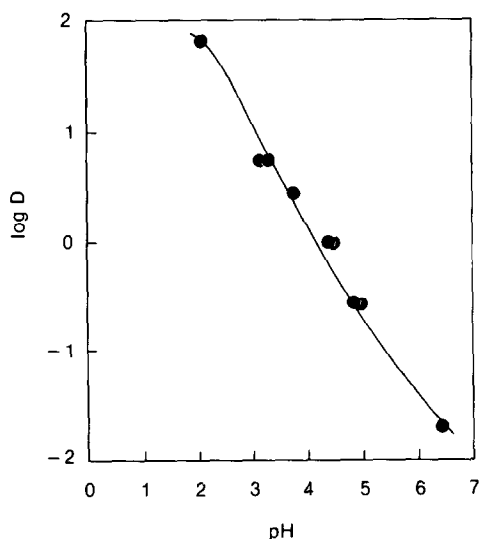


Fig. 3. Distribution coefficients (D) of **I** plotted as a function of the pH of the unbuffered aqueous phase; $T = 22^\circ\text{C}$.

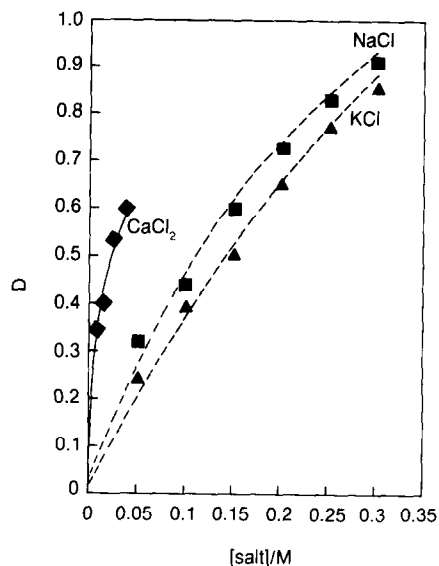


Fig. 4. Distribution coefficients of **I** at pH 6 as a function of added salt concentration; $T = 22^\circ\text{C}$.

where D is the observed distribution coefficient, P is the partition coefficient of the unionized form, and pK_{a1} is as defined in Scheme 1. The good linearity observed indicates that the distribution of **I** obeys the pH-partition hypothesis in the absence of added ions. The value of P is determined to be 514 from the intercept, and the slope of the line is 0.94, which is close to the unit slope expected. Fig. 3 shows the data plotted as $\log D$ versus pH, where the solid line is calculated based on Eqn. 1.

Effect of added ions on the distribution coefficient

The results for distribution coefficients as a function of sodium chloride, potassium chloride and calcium chloride concentration are plotted in Fig. 4. Distribution coefficients as a function of a large organic counterion, benzalkonium chloride, are shown in Fig. 5. It is clear that a significant increase in the distribution coefficient is obtained in the presence of relatively low concentrations of added salt, even in the case of the inorganic salts. In order to find out if this increase is entirely due to the change in activity coefficients in the presence of salts, we need to identify the effect of salts on all the species present in solution, and then look at its impact on the measurement of D . Since

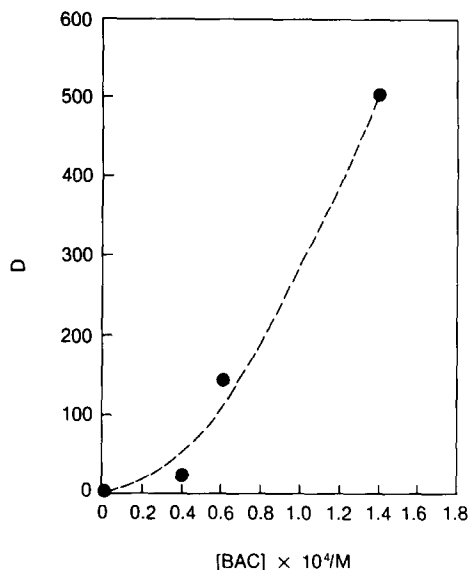


Fig. 5. Distribution coefficients of I at pH 6 as a function of benzalkonium chloride (BAC) concentration; $T = 22^\circ \text{C}$.

only pK_{a1} is of relevance in the pH range studied, we will use K_a to mean K_{a1} from here on.

In the absence of any added salts, the following equilibria exist in a distribution experiment:



where **IH** is the unionized form, I^- is the monoanion, the subscripts w and o denote the aqueous and octanol phases, respectively, and P and K_a are as previously defined. The observed distribution coefficient, D , is given by:

$$D = C_o/C_w = [\text{IH}]_o / \{[\text{IH}]_w + [\text{I}^-]_w\} \quad (4)$$

where C_o and C_w are the total concentrations of **I** in the octanol and water, respectively. If activities, activity coefficients and concentrations are represented by a , γ and C , respectively, the thermodynamic ionization constant, K'_a , is given by:

$$K'_a = a_{\text{H}_w^+} a_{\text{I}_w^-} / a_{\text{IH}_w} = K_a \gamma_{\text{I}_w^-} / \gamma_{\text{IH}_w} \quad (5)$$

where K_a is the "mixed" ionization constant measured. Similarly, the thermodynamic distribution coefficient, D' , is given by:

$$D' = a_{\text{IH}_o} / (a_{\text{IH}_w} + a_{\text{I}_w^-}) \quad (6)$$

The measured distribution coefficient, D , can be written as:

$$\begin{aligned} D &= C_{\text{IH}_o} / (C_{\text{IH}_w} + C_{\text{I}_w^-}) \\ &= C_{\text{IH}_o} / \{C_{\text{IH}_w} (1 + K_a / a_{\text{H}_w^+})\} \end{aligned} \quad (7)$$

$$D = \left[a_{\text{IH}_o} / \{a_{\text{IH}_w} (1 + K_a / a_{\text{H}_w^+})\} \right] (\gamma_{\text{IH}_w} / \gamma_{\text{IH}_o}) \quad (8)$$

Although K'_a and D' , being defined in terms of activities, will not change with salt concentration, the measured values K_a and D will. This is a result of changes in the activity coefficients γ_{IH} and γ_{I^-} . Since no individual ions are expected to partition into octanol we can assume that γ_{IH} will remain constant.

Kojima and Davis (1984) have shown that the distribution coefficient of a non-electrolyte such as phenol is affected by the addition of sodium chloride. The increase in distribution coefficient was ascribed to salting out of phenol in the aqueous phase, thus resulting in an increase in its activity coefficient. The authors fitted their data to an equation analogous to the Setschenow formula, which has been used to describe the effect of salts on the activity of non-electrolytes:

$$\log(D/D_0) = kC \quad (9)$$

where D is the distribution coefficient in the presence of salt, D_0 the distribution coefficient in the absence of salt, C the concentration of salt and k the Setschenow proportionality constant, which can be related to the salt–non-electrolyte interaction parameter.

In water, the absolute value of k ranges between 0.1 and 1 (Gordon, 1975), and it is generally expected that salts in concentrations less than 0.5

M will have negligible ($< 10\%$) effects on non-electrolyte activity coefficients. Kojima and Davis (1984) obtained a k value of 0.168 for the phenol-sodium chloride system at 25°C which is consistent with a salting-out process. Examination of their data confirms that significant increases in the distribution coefficient occur only at sodium chloride concentrations 0.5 M and above.

If we attempt to interpret our results on the basis of a salting-out effect, we obtain excessively high values for k . For example, k turns out to be 1.84 for the I/NaCl system. Examination of our results shows, furthermore, that significant increases in D , even for the inorganic salts, are obtained at salt concentrations less than 0.4 M. Thus, salting out effects due to changes in the activity coefficient of unionized I (T_{IH}) cannot account for the increases in distribution coefficient observed.

The greatest effect of increased ionic strength is expected to be on T_{I^-} . At the relatively low ionic strengths studied, T_{I^-} should decrease with increasing salt concentration, and, assuming that the effect on T_{IH} will be much smaller, K_a should increase. As a result, D should decrease. Thus, D is expected to decrease with increasing salt concentration if changes in ion activity coefficients are the only consequences. (Since the measurement of pH during the experiments in fact measures hydrogen ion activity, no activity coefficient corrections are required for H^+).

The increase in D of I observed at low salt concentrations must, therefore, be primarily due to some other phenomenon. Ion pairing with added ions has been proposed as a means of enhancing distribution coefficients and extraction efficiencies of drugs. Most of the reports deal with large organic counterions where substantial increases in D are found (Tomlinson and Davis, 1979), as in our experiments with benzalkonium chloride. There is generally no dispute that distribution of an ion pair of the drug and the counterion is responsible for the increase in D in these systems.

Some authors feel that inorganic ions can also participate in such ion pairing (Murthy and Zografis, 1970; Dearden and George, 1978; Cools

and Janssen, 1983; Davis et al., 1984), resulting in enhancements of D , albeit not quite as large as those with large organic ions. These ion pairs are probably not present in water, but form either in the organic phase or at the interface. We carried out a few experiments with sodium, potassium, and calcium ion-selective electrodes to attempt to demonstrate that a stoichiometric amount of the cation was transferred into the octanol phase along with I. Unfortunately, the low K_{sp} of the sodium, potassium, and calcium salts and the sensitivity of the electrodes were not appropriate to show this at the concentrations we were restricted to using. We attempted to fit our distribution data to the model proposed by Cools and Janssen (1983), where they fit their distribution data for 4-hydroxycoumarins in the presence of sodium ions to the following equation:

$$\log P_{\text{app}} = \log P_{\text{NaA}} - p\text{Na} + pK_{\text{NaA}} \quad (10)$$

where P_{app} is the observed distribution coefficient in the presence of Na ions, P_{NaA} is the partition coefficient of the sodium ion pair, and K_{NaA} is the dissociation constant of the sodium ion pair in water. A plot of $\log P_{\text{app}}$ against $p\text{Na}$ should give a straight line with a slope of -1 . When our data were plotted in this fashion, straight lines were obtained, but the slopes were -0.7 for the I-Na and I-K systems and -0.6 for the I_2 -Ca system. This deviation of the slope from -1 cannot be easily explained. Cools and Janssen also found anomalous slopes for 2 of the 6 compounds they studied, which could not be explained. This suggests that the model may not be applicable to all such systems.

Oxamic acids have been reported to form complexes with several inorganic ions even in aqueous media, because of their ability to coordinate to the cation through both oxygen atoms or one oxygen and one nitrogen (Kouinis et al., 1978; Perlepes et al., 1980; Tsangaris and Veltsistas, 1981). Since I is an oxamic acid derivative, it can be expected to behave similarly. Thus, an interaction with an added cation, especially in the presence of an organic phase into which the ion pair (or complex) can distribute, is quite possible.

Conclusions

The solubility and distribution behavior of **I** is dramatically affected by the presence of added cations. Based on all the available information, we feel that the enhancement of the distribution coefficient of **I** in the presence of both large organic ions as well as small inorganic ions is primarily due to ion pairing/complex formation. The effect of changing activity coefficients in the presence of salts is expected to be negligible, and may in fact tend to decrease the distribution coefficients overall. No matter what the reason, it has now been demonstrated in several reports that distribution coefficients of drugs can be significantly altered in the presence of simple electrolytes commonly used in distribution experiments. If these measurements are to have any meaning at all, care has to be taken to ensure that the results are not compromised by such effects.

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