Solubilization of Ionized and Un-ionized Flavopiridol by Ethanol and Polysorbate 20

PING LI,[†] S. ESMAIL TABIBI,[‡] AND SAMUEL H. YALKOWSKY*,[†]

Contribution from *Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, Arizona 85721, and Pharmaceutical Resources Branch, National Cancer Institute, NIH, Bethesda, Maryland 20892.*

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Abstract □ Because the ionized species is more polar than its un-ionized counterpart, it is often assumed that the ionized species of the drug does not make a meaningful contribution to solubilization by either cosolvents or surfactants. This report extends previous studies on solubilization of the ionic species by a combination of pH control and complexation to pH control and micellization and to pH control and cosolvency. The total aqueous solubility is expressed as the addition of the concentration of all contributing species: free un-ionized drug [D_u], free ionized drug [D_i], un-ionized drug micelle [D_uM], and ionized drug micelle [D_iM] for surfactant, and free un-ionized drug [D^c_u] and free ionized drug [D^c_i] for cosolvent. The equations indicate that under certain conditions the ionized species can be more important in determining the drug total solubility than the un-ionized species. Flavopiridol, a weak base, is used to test these newly generated equations. As expected, the micellar partition coefficient and solubilization power for ionized flavopiridol are both less than those of the un-ionized species. However, at acidic pH, the solubilities of the ionized drug in surfactant micelles [D_iM] and in cosolvent–water [D^c_i] are both much greater than that of the un-ionized drug. This difference is because the solubilization of the ionized drug is proportional to its aqueous solubility, and its solubility [D_i] can be as much as 24-fold greater than that of the free un-ionized species [D_u].

Introduction

The control of pH with either a cosolvent, a surfactant, or a complexant is often used to improve the aqueous solubility of drugs.[1] It is commonly believed that the un-ionized species makes the major contribution to the improvement of the total aqueous drug solubility.[2] In a recent study we found that a change of pH favoring ionization of the drug significantly increased not only the solubility of the drug in water but also the amount of the drug solubilized by the complexant.[3] In fact, the ionized species were shown to be solubilized to a greater extent than the un-ionized species.[3−6]

In this report, we extend our studies on the role of the ionized species in improving aqueous solubility to pH control combined with either cosolvency or micellization. The newly developed equations are verified using flavopiridol [5,7-dihydroxy-8-(4-*N*-methyl-2-hydroxy-pyridyl)-6′-choloroflavone hydrochloride], a derivative of rohitukine that has been developed for the treatment of breast cancer, as a model drug. The drug is weakly basic with an apparent pK_a of 5.68 and a low intrinsic solubility of 0.025 mg/mL for its zwitterionic form.[7]

* To whom correspondence should be addressed.
[†] Department of Pharmaceutical Sciences.
[‡] Pharmaceutical Resources Branch.

Background

Solubilization by Surfactant—A surfactant or surface-active agent has two distinct regions: one polar and one nonpolar. When it is added to aqueous media, the surfactant molecules orient themselves to form micellar aggregates, provided their concentration exceeds the critical micellar concentration (cmc).[1] Because the interiors of these micelles are much less polar than water, they can more effectively dissolve the nonpolar drug molecules. As a result, the apparent aqueous solubility is increased.

For a given surfactant solution there is an equilibrium among four species: un-ionized drug [D_u], ionized drug [D_i], un-ionized drug in the micelle [D_uM], and ionized drug in the micelle [D_iM]. The total concentration of the drug [D^{tot}] is

$$[D^{tot}] = [D_u] + [D_i] + [D_uM] + [D_iM] \quad (1)$$

For a basic drug, the concentrations of the ionized drug and the un-ionized drug are related by the Henderson−Hasselbalch equation

$$[D_i] = [D_u]10^{(pK_a - pH)} \quad (2)$$

The value of [D_uM] is related to the micellar partition coefficient for the un-ionized species κ_u and the micellar concentration [C_m] by[1]

$$[D_uM] = \kappa_u[D_u][C_m] \quad (3)$$

Note that [C_m] is equal to the total surfactant concentration when the cmc is small enough to be ignored. Similarly, the value of [D_iM] is related to the micellar partition coefficient for the ionized species κ_i by

$$[D_iM] = \kappa_i[D_i][C_m] \quad (4)$$

Therefore eq 1 can be expanded to

$$[D^{tot}] = [D_u] + [D_u]10^{(pK_a - pH)} + \kappa_u[D_u][C_m] + \kappa_i[D_u]10^{(pK_a - pH)}[C_m] \quad (5)$$

Equation 5 indicates that the total drug aqueous solubility is a function of [D_u], pK_a, κ_u, κ_i, [C_m], and solution pH. The ionized species will be solubilized to a greater extent than the un-ionized species (i.e., [D_iM] > [D_uM]) if $\kappa_i[D_i] > \kappa_u[D_u]$. This condition will be met if $\kappa_i 10^{(pK_a - pH)} > \kappa_u$ (i.e., if pK_a − pH > log κ_u − log κ_i). This equation is similar to a previously developed equation that describes solubilization by pH control and complexation.[3]

Solubilization by Cosolvent—Unlike surfactants, cosolvents form homogeneous solutions with water. These solutions act as new solvents that have polarities between

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that of water and the pure cosolvents. In any cosolvent–water mixture, the concentrations of the un-ionized species $[D^c_u]$ and the ionized species $[D^c_i]$ are in equilibrium as described by eq 2. The total solubility in the mixed solvent $[D^{tot}]$ is

$$[D^{tot}] = [D^c_u] + [D^c_i] \quad (6)$$

The value of $[D^c_u]$ is related to the volume fraction of cosolvent f in the mixture and the solubilizing power for the un-ionized drug σ_u by[1,2]

$$[D^c_u] = [D_u]10^{\sigma_u f} \quad (7)$$

Similarly, the value of $[D^c_i]$ is related to the solubilizing power for the ionized drug σ_i by

$$[D^c_i] = [D_i]10^{\sigma_i f} \quad (8)$$

Equation 8 can be expressed as

$$[D^c_i] = [D_u]10^{(pK_a - pH)}10^{\sigma_i f} \quad (9)$$

Substituting eqs 7 and 9 into eq 6 gives

$$[D^{tot}] = [D_u]10^{\sigma_u f} + [D_u]10^{(pK_a - pH)}10^{\sigma_i f} \quad (10)$$

The total drug solubility in a mixed solvent can therefore be described in terms of $[D_u]$, σ_u, σ_i, pK_a, f, and solution pH. In general, the solubilizing power of the cosolvent for the ionized species (σ_i) is smaller than that of the un-ionized species (σ_u). As a result, the un-ionized species is often assumed to be primarily responsible for the improvement of the total drug solubility. However, eq 10 indicates that the solubilization of the ionized species can be more important than that of the un-ionized species in determining the total drug solubility. In fact, the concentration of the ionized species will exceed that of the un-ionized species if the difference between the pK_a of the drug and the solution pH is greater than the difference between the solubilization powers of the cosolvent for the un-ionized species and that of the cosolvent for the ionized species (i.e., $pK_a - pH > \sigma_u - \sigma_i$).

In this report, the effect of the ionized species on the total drug solubility by either micellization or cosolvency (as predicted by eqs 5 and 10, respectively) is confirmed using a weakly basic drug, flavopiridol.

Materials and Methods

Materials—Flavopiridol was provided by the National Cancer Institute and used as received. All other chemicals were reagent grade and purchased from Sigma (St. Louis, MO) or Aldrich (St. Louis, MO) and used without further purification. Citrate-phosphate buffers were prepared according to Scientific Tables.[8] Samples for the solubilization studies were prepared in a series of either polysorbate 20 or ethanol solutions at concentrations of 0, 1.25, 2.5, 5, 10, and 20% in citrate-phosphate buffers at pH 4.3, 5.0, and 8.4.

Methods—*Solubility Determination*—An excess amount of flavopiridol was added to vials containing 0.5 mL of an aqueous solution of either polysorbate 20 or ethanol. The sample vials were then rotated at 20 rpm using an end-over-end mechanical rotator (Glas-Col Laboratory Rotator, Terre Haute, IN) at 25 °C for 6 days. Samples with drug crystals present were considered to have reached their equilibrium solubility and were removed from the rotator. The samples were filtered through a 0.45-μm filter and the pH at equilibrium was measured before performing the HPLC analysis.

HPLC Analysis—The HPLC assay used an EPS C18 column (100 cm × 4.6 mm, Alltech, Deerfield, IL) with a mobile phase composed of acetonitrile and 50 mM phosphate buffer at pH 3.0 (ratio, 35:65). The flow rate was controlled at 1 mL/min (125 Solvent Module, Beckman), and the effluent was detected at 263 nm (168 detector, Beckman). All experimental data are the average of duplicate values, with a relative standard deviation of <3%.

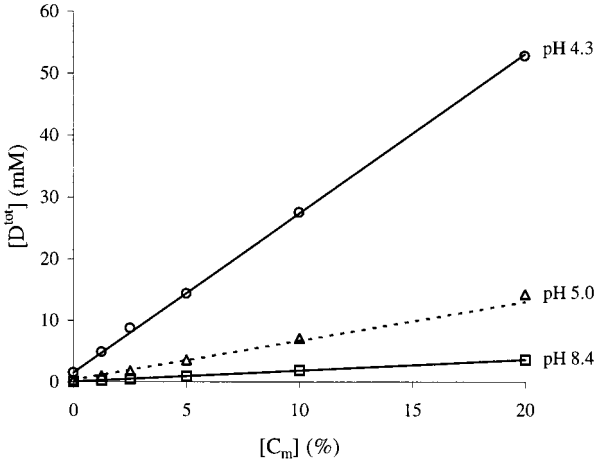

Figure 1—Experimentally determined total aqueous flavopiridol solubilities $[D^{tot}]$ (symbols) in polysorbate 20 solutions $[C_m]$ at different pHs.

Table 1—Results Determined with Equations 2, 3, and 4

pH	total $[D^{tot}]$	free un-ionized $[D_u]$	micelled un-ionized $[D_uM]$	free ionized $[D_i]$	micelled ionized $[D_iM]$
8.4	1.85	0.06	1.79	0.00	0.00
5.0	6.92	0.06	1.79	0.29	4.78
4.3	27.3	0.06	1.79	1.44	24.0

Results and Discussions

Solubilization by Surfactant—In Figure 1, the experimental total aqueous solubility is plotted against the concentration of polysorbate 20 at pH 4.3 (open circles) and pH 8.4 (open squares). Although linearity between the drug aqueous solubility and the surfactant concentration is observed at both pHs, the solubilization slopes are quite different. The solubilization slope at pH 4.3 is ≈15-fold higher than the slope at pH 8.4.

These solubilization slopes can be used to calculate polysorbate 20 micellar partition coefficients for un-ionized and ionized flavopiridol via eq 5. The calculated values are $\kappa_u = 333.3\ M^{-1}$ and $\kappa_i = 185.4\ M^{-1}$, respectively. The lower micellar partition coefficient for the ionized drug is obviously due to its greater affinity for water.

With $[D_u]$, pK_a, κ_u, and κ_i, we can calculate $[D_i]$, $[D_uM]$, and $[D_iM]$ at any given combination of surfactant concentration and solution pH by using eqs 2, 3, and 4, respectively. Table 1 lists these values and the experimental total drug solubility for a 10% surfactant concentration at pH 8.4, 5.0, and 4.3. The highest total drug solubility is achieved at pH 4.3 where most of the drug is ionized. At this pH $[D_i]$ accounts for 5% of total solubility and $[D_iM]$ accounts for ≈88%. The high concentration of the $[D_iM]$ is primarily responsible for the higher total solubility in the acidic solution than in the neutral solution. Note that $[D_uM]$ remains constant at all pH conditions because it is only determined by $[D_u]$ and κ_u, both of which are pH independent.

Solubilization by Cosolvent—Figure 2a shows the experimental aqueous solubility of flavopiridol versus the concentration of ethanol at pH 4.3 (open circles) and pH 8.4 (open squares). Unlike the linear surfactant solubilization curves, the drug solubility increases exponentially

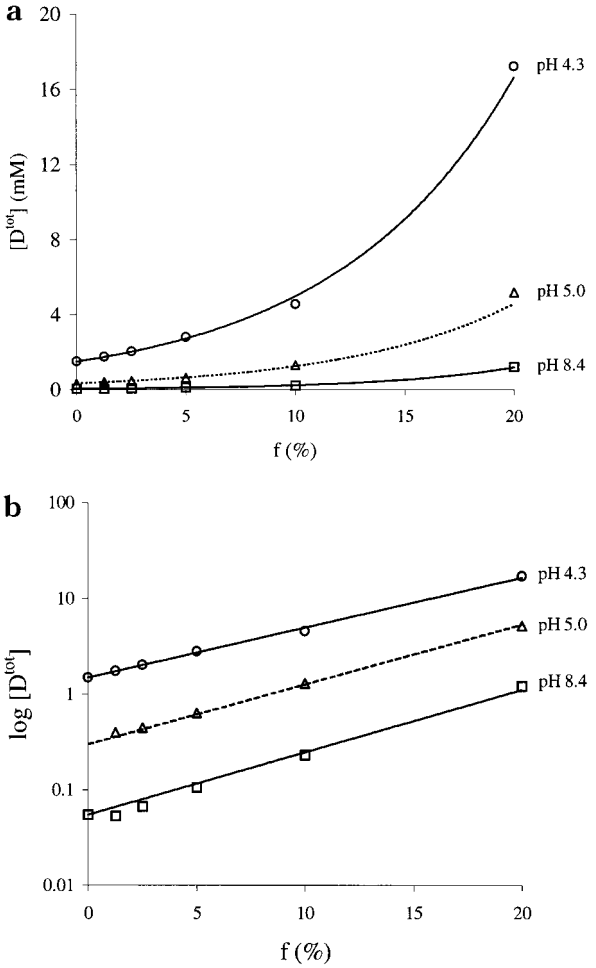

Figure 2—(a) Experimentally determined total aqueous flavopiridol solubilities [D^{tot}] (symbols) in ethanol solutions [*f*] at different pHs. (b) Schematic plot of the semilogarithmical total aqueous solubility of flavopiridol log[D^{tot}] against ethanol volume fraction *f*.

Table 2—Results Determined with Equations 7 and 9

pH	total [D^{tot}]	un-ionized [D^c_u]	ionized [D^c_i]
8.4	0.25	0.25	0.00
5.0	1.19	0.25	0.94
4.2	4.98	0.25	4.73

as the cosolvent concentration increases. Again, the solubilization curves are different at different solution pHs. The drug solubilities in both water and 10% ethanol solution at pH 4.3 are ≈20-fold greater than that at pH 8.4.

Figure 2a can be re-plotted semilogarithmically, as is seen in Figure 2b, where nearly parallel linear relationships between log[D^{tot}] and cosolvent volume fraction are evident at both pHs. This linearity persists up to 20% cosolvent. The slopes at pH 4.3 and 8.4 show that σ_u and σ_i are quite similar, with values of 0.06 and 0.05% $^{-1}$, respectively.

Table 2 lists the experimental total drug solubility [D^{tot}] in 10% cosolvent and the values of [D^c_u] and [D^c_i] calculated with eqs 7 and 9. As in surfactant solubilization, the total drug solubility in cosolvent is much higher at pH 4.3 than that at pH 8.4. Although the solubility of flavopiridol increases by nearly the same factor at both pH values, the amount of drug solubilized is much greater at the lower pH. This difference occurs because the concentration of ionized drug [D^c_i] far exceeds the concentration of the un-ionized drug [D^c_u] in the solution at pH 4.3. It is also noted that the value of [D^c_u] is pH independent.

Validation of Equations—The solubilities (dotted lines), calculated with eq 5 for polysorbate 20 solution and with eq 10 for ethanol solution, are compared with the experimental solubility data (open triangles) at pH 5.0 in Figures 1 and 2, respectively. The strong agreement between the predicted and the observed solubility data supports the validity of both eq 5 and eq 10.

Conclusion

A pH change that favors the ionization of the drug not only increases the solubility of the ionized species in water, but also increases the solubility of the ionized species in both micelles and cosolvent. This result is independent of the values of the micellar partition coefficient and the solubilization power. Furthermore, the solubilities of the ionized species in the micelles and cosolvent can exceed those of the un-ionized species.

For micellization:

$$[D_iM] > [D_uM], \text{ if } (pK_a - pH) > (\log \kappa_u - \log \kappa_i)$$

For cosolvency:

$$[D^c_i] > [D^c_u], \text{ if } (pK_a - pH) > (\sigma_u - \sigma_i)$$

Note that these relationships are similar to the following relationship, which was previously derived for complexation

$$[D_iL] > [D_uL], \text{ if } (pK_a - pH) > (\log K_u - \log K_i)$$

where K_u and K_i are complexation constants for the un-ionized and ionized solutes, respectively, and [D_uL] and [D_iL] are the concentrations of the corresponding complexes.

References and Notes

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