Estimation of Drug Precipitation upon Dilution of pH–Cosolvent Solubilized Formulations

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This manuscript is a study of precipitation of insoluble drug upon dilution from the pH–cosolvent solubilized formulation with simulated blood fluid. An equation is developed to estimate drug precipitation upon dilution of combined pH–cosolvent solubilized formulations. This is an extension of a previous equation used for the estimation of drug precipitation from simple pH controlled formulations. The proposed equation considers the effect of the cosolvent and its concentration on the pK_a of the drug as well as all buffering species. According to the proposed equation and our experimental data, the addition of cosolvent on the pH solubilized formulation could increase total drug solubility in the formulation. However, the solubility after dilution became lower than the 0% ethanol formulation because of the change in both drug and buffer pK_a values. Since this equation is based on the equilibrium condition, it is the worst case scenario for precipitation. This equation provides useful information regarding the feasibility of the successful use of pH–cosolvent combinations in drug formulation.

Key words precipitation; solubility; simulation; formulation; injectables; log P

A variety of techniques have been reported to enhance the solubility of drugs. These have found application in the development of liquid formulations for oral as well as parenteral administration. The use of pH control and cosolvents are two important examples of such techniques. An exponential enhancement in the solubility has been achieved for a number of drugs with the use of these approaches. In fact, the combination of pH and cosolvent solubilization is used in several commercial products.[1] However, caution must be practiced while using these techniques, as the drug runs a risk of precipitation upon dilution with the body fluids following administration. Drug precipitation may significantly affect its bioavailability and pharmacokinetics. Furthermore, the cases of thromobophlebitis resulting from drug precipitation have been reported for several commercial products.[2]

Recently, we reported a useful equation to estimate drug precipitation upon dilution of pH controlled formulations based on the Henderson–Hasselbalch theory.[3] The solubility of the ionized form of a drug ($S_{i,w}$) at a particular pH is given by:

$$S_{i,w}=S_{u,w}\times 10^{pH-pKa} \quad (1)$$

where, $S_{u,w}$ is the solubility of unionized drug (intrinsic solubility).

In the present study, we have expanded this equation to cover the combined use of pH control and cosolvency for drug solubilization. Cosolvents such as ethanol and propylene glycol have been commonly used to enhance the solubility of poorly soluble drugs.[4,5] Many commercial products utilize either a single cosolvent or mixture of cosolvents.[6] According to the log-linear model,[7,8] the drug solubility increases exponentially with cosolvent concentration:

$$\log S_{mix}=\log S_w+\sigma_{0.5}f_c \quad (2)$$

where, S_{mix} and S_w are the solubilities in the cosolvent solution and water, respectively; and f_c is the volume fraction of cosolvent. The term $\sigma_{0.5}$ is the semi-log slope between 0—50% of cosolvent. It is specific for each drug–cosolvent combination.

As expected from Eq. 1, the drug solubility is exponentially related to the cosolvent concentration. And the total solubility is also exponentially related to the difference between pH of the diluted media and the pK_a of drug.[3] Thus, dilution of a pH–cosolvent solubilized formulation with body fluids might result in greater and more complicated change in drug's solubility as compared to using either of these approaches individually.

In this study, a new equation has been developed to estimate drug precipitation upon dilution of pH–cosolvent formulations. The accuracy of this equation is tested experimentally using various phenytoin formulations. Phenytoin is chosen as a model compound, since it is generally known to pose precipitation concerns upon dilution.[3,9]

Experimental

Materials Phenytoin (free acid form) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were analytical grade.

Estimation of Solubility in Cosolvent–Water System To estimate the solubilities of the unionized and ionized forms of the drug in a cosolvent–water system, the following equations can be prepared based on Eq. 1:

$$\log S_{u,mix}=\log S_{u,w}+\sigma_{0.5,u}f_c \quad (3)$$

$$\log S_{i,mix}=\log S_{i,w}+\sigma_{0.5,i}f_c \quad (4)$$

where, $S_{u,mix}$ and $S_{i,mix}$ are solubilities of the unionized and the ionized species in cosolvent–water mixture, and $\sigma_{0.5,u}$ and $\sigma_{0.5,i}$ are solubilization powers of the cosolvent for the unionized and ionized forms, respectively.

The total drug solubility in a pH–cosolvent solubilized formulation can be calculated by combining Eqs. 1 through 4.

$$S_{total}=S_{i,mix}+S_{u,mix}=S_u(10^{\sigma_{0.5,u}f_c}+10^{pH-pK_a+\sigma_{0.5,i}f_c}) \quad (5)$$

Estimation of Solubility Based on pH-Control In water, an acidic drug (DH) has ionization equilibria as described by:

$$DH \overset{K_D}{\rightleftharpoons} D^- + H^+ \quad (6)$$

When a triprotic acid buffer is used in the formulation (H_3A) and as a diluents (H_3B), the ionic equilibria is given as:

$$H_{3A} \overset{K_{A1}}{\rightleftharpoons} H_2A^- + H^+ \quad (7)$$

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$$H_2A \overset{K_{A2}}{\rightleftarrows} HA^{2-}+H^+ \quad (8)$$

$$HA^{2-} \overset{K_{A3}}{\rightleftarrows} A^{3-}+H^+ \quad (9)$$

$$H_3B \overset{K_{B1}}{\rightleftarrows} H_2B^-+H^+ \quad (10)$$

$$H_2B^- \overset{K_{B2}}{\rightleftarrows} HB^{2-}+H^+ \quad (11)$$

$$HB^{2-} \overset{K_{B3}}{\rightleftarrows} B^{3-}+H^+ \quad (12)$$

If α_i, β_i and χ_i denote the fraction of each species such that:

$$\alpha_i = \frac{[H_{3-i}A^{i-}]}{C_A} \quad (i=0,1,2,3) \quad (13)$$

$$\beta_i = \frac{[H_{3-i}B^{i-}]}{C_B} \quad (i=0,1,2,3) \quad (14)$$

$$\chi_i = \frac{[H_{1-i}D^{i-}]}{C_D} \quad (i=0,1) \quad (15)$$

where, C_A, C_B and, C_D are sum of different charged forms of triprotic acid buffer in the formulation, buffer, and drug, respectively. As previously reported, we can simplify the relation. Surakitbanharn *et al.* showed that the fraction of formulation (*ff*) in the diluted mixture is given by,[9]

$$ff = \frac{[OH^-]-[H^+]-C_B[Y]}{C_A[X]-C_B[Y]+C_D[Z]} \quad (16)$$

where

$$[X] = \frac{a[H^+]^3+bK_{A1}[H^+]^2+cK_{A1}K_{A2}[H^+]-dK_{A1}K_{A2}K_{A3}}{[H^+]^3+K_{A1}[H^+]^2+K_{A1}K_{A2}[H^+]+K_{A1}K_{A2}K_{A3}} \quad (17)$$

$$[Y] = \frac{k[H^+]^3+lK_{B1}[H^+]^2+mK_{B1}K_{B2}[H^+]-nK_{B1}K_{B2}K_{B3}}{[H^+]^3+K_{B1}[H^+]^2+K_{B1}K_{B2}[H^+]+K_{B1}K_{B2}K_{B3}} \quad (18)$$

$$[Z] = \frac{\chi_1[H^+]-\chi_0K_D}{[H^+]+K_D} \quad (19)$$

where, a, b, c and d are $\alpha_1+2\alpha_2+3\alpha_3$, $\alpha_2+2\alpha_3-\alpha_0$, $\alpha_3-2\alpha_0-\alpha_1$ and $3\alpha_0+2\alpha_1+\alpha_2$, respectively. Similarly, k, l, m and n are $\beta_1+2\beta_2+3\beta_3$, $\beta_2+2\beta_3-\beta_0$, $\beta_3-2\beta_0-\beta_1$ and $3\beta_0+2\beta_1+\beta_2$, respectively. Note that *ff* varies from unity for the pure formulation (*ff*=1) to infinite dilution (*ff*≈0).

All the parameters in Eqs. 16 through 19 are constant for each mixing ratio except $[H^+]$ and $[OH^-]$. Using the above equation, the *ff* can be calculated at any pH in the mixture.

Since all the pK_a values are affected by the ionic strength of the solution, they were corrected with the use the Davies' modification of the Debye–Huckel equation.[9]

$$\log\gamma_i = z_i^2\left[0.15I - \frac{0.51\sqrt{I}}{1+\sqrt{I}}\right] \quad (20)$$

where, γ_i is the activity coefficient of ion i, having a charge z_i in each dilution of ionic strength I. Although, the Davies' equation gives the geometric mean ionic activity coefficient, it can be applied to the individual ionic activity coefficients at low ionic strengths.[3,10]

Another factor that must considered is the effect of cosolvent concentration on the pK_a of the drug and all buffer species. The experimental pK_a at a given cosolvent concentration must be used if available. However, these values are generally not available and have to be estimated. Rubino reported the shift in the pK_a values of various drugs and buffer species in ethanol–water mixtures.[11] From this report it can be estimated that the pK_a of acidic drugs increases about 1 unit in 50% ethanol solution. Based on the assumption that the pK_a shift is linearly related to the cosolvent concentration, it can be calculated for any ethanol–water mixture.

Following this information, more precise *ff* values can be estimated at any pH. We first calculated *ff* values at 10 points between the initial pH of drug formulation and that of the diluted solutions. After calculating the *ff* values, further correction of the dissociation constants based on the change in the ionic strength and ethanol concentration was performed. These calculations were iterated until all the values converged.

The total drug concentration was expressed as the product of $ff \times C_D$. If $ff \times C_D$ is smaller than the S_{total} defined by Eq. 5, no precipitation is expected. On the other hand, if $ff \times C_D > S_{total}$, there is a supersaturated condition and the possibility for precipitation. Therefore, we could estimate the *ff* values and also the amount of drug precipitating at any pH.

Table 1. Physicochemical Parameters Used for This Study

Constant	Drug substance Phenytoin	Formulation buffer Carbonate	Diluent buffer Phosphate
pK_{a1}	8.33[a]	6.35[a]	2.35[a]
pK_{a2}	—	10.33[a]	7.20[a]
pK_{a3}	—	—	12.38[a]
S_{int}	0.0152 mg/ml[a]	—	—
C log P	1.243[b]	—	—
C log P	2.085[c]	—	—

a) Narazaki *et al.*[3] *b*) Calculated value using Clog P® for the ionized form. *c*) Calculated value using Clog P® for the unionized form.

Formulation (Drug Solution) 1 mg/ml phenytoin solutions in 50 mM carbonate buffer at pH 10.5 containing 0, 5, 10, or 15% ethanol were used as model formulations. To the of Na_2CO_3 and $NaHCO_3$ buffer described in a previous report.[3] 0—15% ethanol was added. The drug substance was dissolved completely and the pH was controlled using Na_2CO_3 and $NaHCO_3$ solution. Final drug concentration was controlled to 1 mg/ml.

Dilution As a surrogate blood model, Sorensen's phosphate buffer (SPB; 67 mol, pH 7.4) was used for dilution. After dilution, pH was measured and samples were kept at 25 °C for 2 weeks to attain equilibrium. The samples were then filtered through Acrodisc® LC 13 mm Syringe Filter 0.45 μm (Pall Corporation, Ann Arbor, MI, U.S.A.). These filtrates were diluted with methanol and used for HPLC analysis.

HPLC Analysis Phenytoin was analyzed using a Pinnacle ODS 5 μm column (150×4.6 mm; RESTEK, Bellefonte, PA, U.S.A.). The mobile phase consisted of 50% acetic acid solution (0.01%) and 50% methanol at a flow rate of 1 ml/min. The injection volume was 50 μl and the absorbance was measured at 258 nm.

Physicochemical Parameters Table 1 contains the physicochemical parameters of phenytoin and the buffers used in this study. The carbonate buffer has two pK_a values. Since the proposed estimation program is based on three pK_a values, we inputted a value of 20, which is significantly higher than 14, as the third pK_a (pK_{A3}) to assume no further dissociation. These pK_a values listed in Table 1 are intrinsic pK_a values at I (ionic strength)=0 M. As explained before, pK_a values were varied by ionic strength and cosolvent concentration.

To estimate the solubility in cosolvent–water system, the solubilization powers ($\sigma_{0.5,u}$ and $\sigma_{0.5,i}$) are required. According to Li and Yalkowsky, for ethanol–water systems the $\sigma_{0.5}$ of a solute is related to its calculated octanol-water partition coefficients (Clog P® software)[8] as:

$$\sigma_{0.5}^{EtOH} = 0.791 \times C\log P + 1.274 \quad (21)$$

Using this relationship, the $\sigma_{0.5,i}$ and $\sigma_{0.5,u}$ for phenytoin were calculated as 2.26 and 2.92, respectively.

Result and Discussion

Figures 1 and 2 show the effect of ethanol concentration on both pH and drug solubility upon dilution.

The accuracy of both the pH and solubility predictions is evident in Fig. 3 ($0<ff<0.4$). The solubility in the formulation containing 15% ethanol at $ff=0.25$ is almost half of that in the formulation in the absence of ethanol. It is interesting that the drug solubilized using both pH and cosolvent results in more precipitation than the mere pH controlled formulation. This is because the increase in the solubility by the cosolvent is offset by the decrease in the solubility induced by the shift in drug and buffer pK_a values.

It is generally believed that the presence of cosolvent increases the solubility of poorly soluble drugs significantly. Actually, as shown in Eqs. 3 and 4, the solubility in cosolvent increases exponentially with the cosolvent concentration. However, in case of an ionizable drug, the presence of cosol-

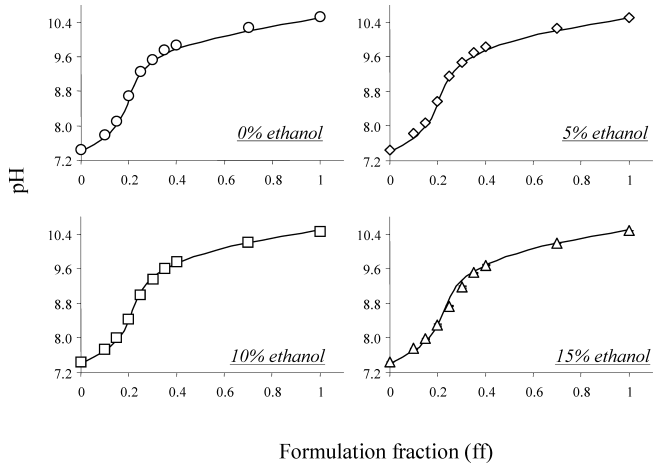

Fig. 1. Effect of the Ethanol Concentration in the Formulation on the pH of the Diluted Solutions

Formulations; 1 mg/ml phenytoin formulation (50 mM carbonate buffer at pH 10.5, containing 0—15% ethanol). Diluents: 67 mM phosphate buffer at pH 7.4. The solid curves represent the predicted values.

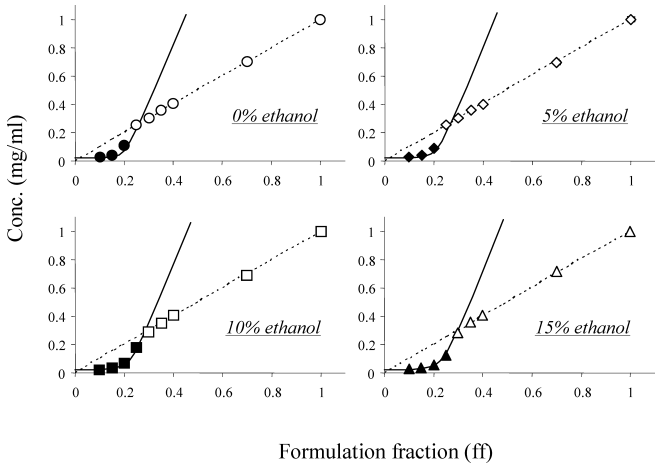

Fig. 2. Effect of the Ethanol Concentration in the Formulation on the Phenytoin Concentration in the Diluted Solutions

Formulations; 1 mg/ml phenytoin formulation (50 mM carbonate buffer at pH 10.5, containing 0—15% ethanol). Diluents: 67 mM phosphate buffer at pH 7.4. The closed symbols represent that the precipitation was observed. The solid curves represent the predicted values and the broken lines represent the dilution lines.

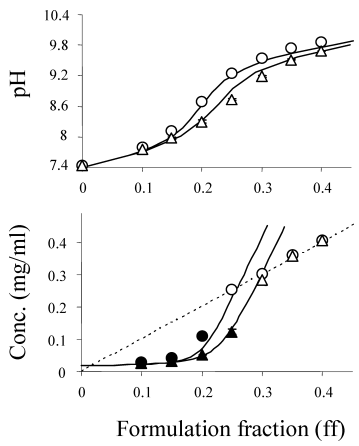

Fig. 3. Effect of the Ethanol Concentration on the Properties of the Diluted Solutions (Enlargement of Figs. 1 and 2)

Ethanol concentration: 0% (○), 15% (△). The closed symbols represent that the precipitation was observed. The solid curves represent the predicted values and the broken lines represent the dilution lines.

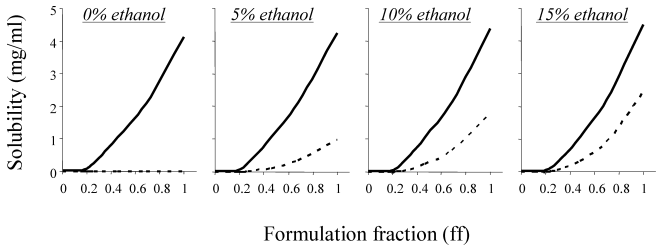

Fig. 4. Estimated Solubility upon Dilution

Formulations; 1 mg/ml phenytoin formulation (50 mM carbonate buffer at pH 10.5, containing 0—15% ethanol). The solid curves represent the total solubilities and the broken curves represent the solubilities in ethanol fraction.

vent produces a shift of the pK_a values of the drug and all buffer species. Due to the shift of pK_a values of all species, the pH is also affected. In the case of the 15% ethanol containing formulation, the pH of the diluted formulations is slightly shifted to neutral compared to the one without any ethanol. Moreover, the pK_a value of phenytoin increases in the presence of ethanol. Thus, the difference between the pH of the solution and the drug's pK_a reduces more as compared to the dilution of a simply buffered formulation. This alters the equilibrium between the ionized and unionized forms of the drug. As can be seen from figures, the experimental data supports the predicted curves.

Figure 4 shows the calculated solubility under the same conditions. As shown in Fig. 4, the total solubilities of all formulation are over 4 mg/ml and ethanol increases the total phenytoin solubility only slightly. While the ethanol dependent solubility is increasing, the fraction of ionized specie is decreased. As the result, just a small increase of the total solubility is observed.

It might be misunderstood that the pK_a shift induced by cosolvent is very slight and could be neglected in calculating the total solubility. However, these slight pK_a's shifts affect not only the drug but also all buffer species in the mixture. And, in the case of a pH controlled formulation, this slight shift could affect the solubility, significantly.

Interestingly, when *ff* is small, the 15% ethanol formulation has less solubility after dilution than the 0% ethanol formulation. However, when *ff* is large, the 15% ethanol formulation has higher solubility than the 0% ethanol formulation. Our equation estimates that this inversion occurs when *ff* is about 0.60.

The possibility of precipitation can be predicted with this equation by using just a few physicochemical parameters of the drug such as the $S_{u,w}$, pK_a, $\sigma_{0.5,u}$, and $\sigma_{0.5,i}$. These values may be either measured experimentally or estimated from other measured properties. The intrinsic solubility, $S_{u,w}$ can be estimated from the melting point and $C\log P$ value.[5] As mentioned previously, the $\sigma_{0.5,u}$ and $\sigma_{0.5,i}$ can also be estimated from $C\log P$ values (Eq. 21). This equation is established only for ethanol–water cosolvent system. You can use the different equation for other cosolvent system.[5] Also, some softwares are available for the estimation of the pK_a (ACD/LC®, pkalc®, SPARC v.3.1, *etc.*). In addition, as we mentioned previously, we also assume that the pK_a shift of acidic compound has a linear relationship with the ethanol concentration, and 50% ethanol induce the pK_a shift about 1 unit. These results suggest that our assumptions are acceptable for drugs without information about the shift of pK_a

against cosolvent. Thus, this equation will be useful not only for drugs with known their physicochemical properties but also for new chemical entities for which limited information are available.

Conclusion

An equation to estimate drug precipitation upon dilution of a pH–cosolvent solubilized formulation has been developed. According to the equation, the effect of a cosolvent on the pK_a of the drug and the buffering species plays an important role in determining the drug solubility upon dilution. In the case of phenytoin, more precipitation occurs upon diluting a pH–cosolvent formulation as compared to a mere pH controlled formulation. This equation provides a useful and an accurate tool for screening of the formulation for potential precipitation following dilution.

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