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Estimation of Drug Precipitation upon Dilution of pH-Controlled Formulations

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Abstract: An equation is developed for estimating the precipitation that may occur upon diluting or injecting a (pH-)solubilized drug formulation. Since it is based on equilibrium, it is the worst case scenario for precipitation. This equation can be programmed in any commercially available spread sheet program such as Excel. According to the proposed equation, the type and the strength of the buffer species are the most significant factors that affect the pH and solubility of a drug in its microenvironment during dilution. To demonstrate the utility and robustness of the proposed equation, experimental measurements were performed using phenytoin as the model drug. The result suggests that the proposed equation can be used to indicate the possibility and the degree of precipitation that would occur upon injection. This provides a useful tool for the design of a successful pH-controlled solution formulation.

Keywords: Estimation; precipitation; pH; buffer; phenytoin

Introduction

Various techniques have been used for improving the solubility of poorly soluble drugs.[1−6] These techniques include the use of pH, cosolvents, surfactants, and complexing agents. To develop the formulation, we should consider not only the solubility of the drug in the formulation but also the destiny of drug during and after administration. It is generally expected that the drug will remain dissolved even after the formulation gets diluted with biological fluids such as blood and gastrointestinal contents. While this is most often the case, drug precipitation may occur if the drug concentration is more than its solubility in the biological fluid. Drug precipitation in the gastrointestinal tract or in blood vessels following administration may significantly affect its bioavailability and pharmacokinetic profile. In addition, precipitation in veins has been reported to cause thrombophlebitis for several commercial products.[7]

Precipitation is most commonly seen with pH-adjusted or cosolvent containing formulations. Common to both the techniques is the fact that dilution results in a linear decrease in the concentration and an exponential decrease in the solubility. Mixing of a buffered formulation with naturally buffered biological fluids generally results in a change in pH. This may cause the solubility of the drug to drop below its concentration, creating a supersaturated system and the potential for precipitation. Also, since the pK_a of the drug is

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sensitive to the ionic strength of the medium, dilution may cause it to shift.

Reasonably accurate means for estimating the solubility of drugs in aqueous media have been developed.[8−10] Unfortunately, similar means to predict the precipitation of drugs upon dilution are not widely reported. Schroeder and DeLuca[11] and Yalkowsky and Valvani[12] devised simple in vitro experiments to study drug precipitation. Yalkowsky el al[1] also reported a dynamic in vitro model to semiquantitatively measure the extent of precipitation.

Powis and Kovach[13] performed an in vivo study to demonstrate the precipitation of bisantrene in the rabbit ear vein following intravenous injection. Myrdal et al.[14] reported the effect of the formulation on the precipitation of levemopamil through in vivo and in vitro studies. Although these studies are very useful, they are difficult and time-consuming. Thus, a means to predict the likelihood and the extent of precipitation based on the properties of the drug and the formulation becomes important. Previously, Surakitbanharn et al.[15] have attempted to develop such a prediction technique.

In this study, the effects of the formulation pH, the drug concentration, and the strength and the type of buffering species are investigated. A new equation is developed for estimating the precipitation of a solubilized drug from a pH-adjusted formulation. The possible effect of dilution on the pK_a of the drug is also considered. The accuracy of the proposed equation has been tested experimentally using the buffered phenytoin formulation. Phenytoin is chosen as a model compound, since it is generally known to pose precipitation concerns upon dilution.[7,15]

Materials and Methods

Materials. Phenytoin was purchased from Sigma Chemical Co. (St Louis, MO). All other materials used were of analytical grade.

Estimation of Precipitation. In water, an acidic drug (DH) has ionization equilibrium as described by

$$DH \overset{K_D}{\rightleftharpoons} D^- + H^+ \tag{1}$$

where [DH] and [D^-] are concentrations of the un-ionized and the ionized forms, respectively.

The dissociation constant of the drug (K_D) is given by

$$pK_D = pH + \log \frac{[DH]}{[D^-]} \tag{2}$$

The total concentration of the drug is the sum of [DH] and [D^-]:

$$C_D = [DH] + [D^-] \tag{3}$$

Generally, buffer systems have multiple ionization sites and each of these warrants consideration. The ionic equilibrium of a triprotic acid buffer used in the formulation is given as

$$H_3A \overset{K_{A1}}{\rightleftharpoons} H_2A^- + H^+ \tag{4}$$

$$H_2A^- \overset{K_{A2}}{\rightleftharpoons} HA^{2-} + H^+ \tag{5}$$

$$HA^{2-} \overset{K_{A3}}{\rightleftharpoons} A^{3-} + H^+ \tag{6}$$

Similarly, the ionic equilibrium of the buffering agent (H_3B) in the dilution medium is given as

$$H_3B \overset{K_{B1}}{\rightleftharpoons} H_2B^- + H^+ \tag{7}$$

$$H_2B^- \overset{K_{B2}}{\rightleftharpoons} HB^{2-} + H^+ \tag{8}$$

$$HB^{2-} \overset{K_{B3}}{\rightleftharpoons} B^{3-} + H^+ \tag{9}$$

Similar to eq 3, the total concentrations of H_3A and H_3B are equal to

$$C_A = [H_3A] + [H_2A^-] + [HA^{2-}] + [A^{3-}] \tag{10}$$

$$C_B = [H_3B] + [H_2B^-] + [HB^{2-}] + [B^{3-}] \tag{11}$$

respectively.

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

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$$\alpha_i = \frac{[H_{3-i}A^{i-}]}{C_A} \qquad (i = 0, 1, 2, 3) \qquad (12)$$

$$\beta_i = \frac{[H_{3-i}B^{i-}]}{C_B} \qquad (i = 0, 1, 2, 3) \qquad (13)$$

$$\chi_i = \frac{[H_{1-i}D^{i-}]}{C_D} \qquad (i = 0, 1) \qquad (14)$$

The fraction of formulation, ff, is defined as formulation volume divided by the total volume of formulation and blood surrogate. Thus if one part formulation is mixed with 4 parts diluent, ff = 0.2. Surakitbanharn et al.[15] showed that this ff is given by

$$ff = \frac{[OH^-] - [H^+] - C_B[Y]}{C_A[X] - C_B[Y] + C_D[Z]} \qquad (15)$$

where

$$[X] = \frac{a[H^+]^3 + bK_{A1}[H^+]^2 + cK_{A1}K_{A2}[H^+] - dK_{A1}K_{A2}K_{A3}}{[H^+]^3 + K_{A1}[H^+]^2 + K_{A1}K_{A2}[H^+] + K_{A1}K_{A2}K_{A3}} \qquad (16)$$

$$[Y] = \frac{k[H^+]^3 + lK_{B1}[H^+]^2 + mK_{B1}K_{B2}[H^+] - nK_{B1}K_{B2}K_{B3}}{[H^+]^3 + K_{B1}[H^+]^2 + K_{B1}K_{B2}[H^+] + K_{B1}K_{B2}K_{B3}} \qquad (17)$$

$$[Z] = \frac{\chi_1[H^+] - \chi_0 K_D}{[H^+] + K_D} \qquad (18)$$

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. All the parameters in eqs 15−18 are constant for each mixing ratio except $[H^+]$ and $[OH^-]$. These two can be calculated from the final pH value after mixing. Thus, if we assume the final pH after mixing, we can calculate the fraction of formulation, ff, in the mixture.

We must also consider the effect of a change in ionic strength upon dilution on all the dissociation constant values. The Davies modification of the Debye−Hückel equation[15] can be used for this correction,

$$\log \gamma_i = z_i^2\left[0.15I - \frac{0.51\sqrt{I}}{1 + \sqrt{I}}\right] \qquad (19)$$

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 geometric mean ionic activity coefficient, it can be applied to the individual ionic activity coefficients at low ionic strengths.[16]Using this equation, we can calculate the actual pK_a values in each solution component.

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Using eq 15, the formulation fraction can be estimated at any pH. We first calculate ff values at 10 pH points between the pH of the drug and that of the diluent. After calculating the ff values, the ionic strengths in mixture were calculated. Using these calculated ionic strengths, we recalculated pK_a values and then recalculated ff values. These calculations were repeated until all the values converged.

The total solubility (S_{total}) at any pH is described as

$$S_{total} = S_{int}(1 + 10^{pH - pK_D}) \qquad (20)$$

On the other hand, the total concentration of drug after mixing is equal to ff·C_D. If $S_{total} >$ (ff·C_D), no precipitation will occur; whereas if $S_{total} <$ (ff·C_D), there is a supersaturated condition and the possibility for precipitation exists. Therefore, we can estimate the amount of drug precipitation at any ff.

Experimental Methods

Calculations. It is difficult to solve eq 15 for H^+ or OH^-. However, it is relatively easy to solve for ff numerically by inserting a series of values for pH in a spread sheet program. The values of H^+ and OH^- are readily calculated from pH and inserted into eq 15. Since all of the other terms in the equation are constant, ff is easily calculated and graphed.

The solubility of the drug at any pH is calculated by the Henderson−Hasselbalch equation and graphed using the same x-axis.

All calculations are based upon the assumption that the Henderson−Hasselbalch equation is applicable and that the ionic strength effect is adequately described by eq 19. It is also assumed that the dissociation constants of the drug, the vehicle, and the diluent are unchanged by the presence of each other.

Formulation (Drug Solution). Carbonate and phosphate buffers in concentrations of 10, 50, and 100 mM at pH 10−11 were prepared. Phenytoin was dissolved in these buffers at concentrations of 1 mg/mL and 0.5 mg/mL. Sodium chloride was added to the each sample to normalize the ionic strength.

Dilution. Each formulation was diluted with isotonic Sorensen's phosphate buffer (67 mM, pH 7.4; SPB). We diluted each formulation at 8−10 different ff values to maintain a total volume of 3 mL. After dilution, the pH was measured and the samples were kept at 25 °C for 2 weeks to attain equilibrium. The samples were then filtered through a 0.45 μm filter (Acrodisc LC13 mm Syringe Filter, Pall Corporation, Ann Harbor, MI). 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). The mobile phase consisted of 50% acetic acid solution (0.01%) and 50% methanol at a flow rate of 1 mL/min. The 50 μL injections were made and the absorbance was monitored at 258 nm.

Physicochemical Parameters. As mentioned above, pK_a values are variable against the ionic strength and temperature

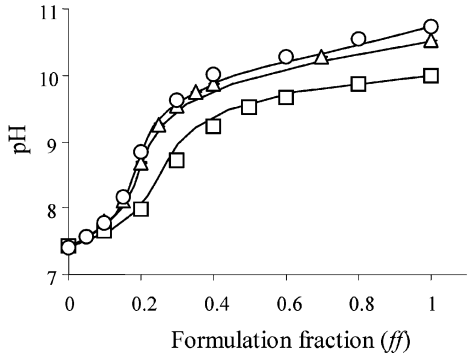

Figure 1. Effect of the initial pH of the formulation on the pH of the diluted solutions. Formulation: 1 mg/mL phenytoin formulation (50 mM carbonate buffer). Initial pH: pH 10 (□), pH 10.5 (Δ), and pH 10.74 (○). The solid curves represent the predicted values.

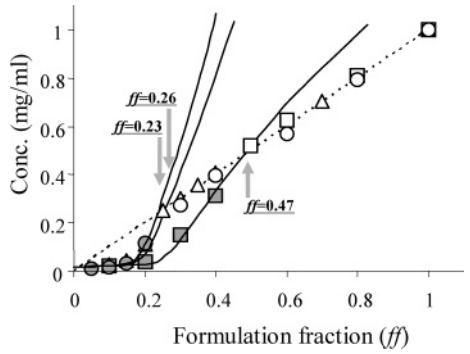

Figure 2. Effect of the initial pH of the formulation on the concentration of phenytoin upon dilution. Formulation: 1 mg/mL phenytoin formulation (50 mM carbonate buffer). Initial pH: pH 10 (□), pH 10.5 (Δ), and pH 10.74 (○). The shaded symbols represent that precipitation was observed. The solid curves represent the predicted solubility, and the broken line represents the concentration. When the solid curves are lower than the broken line, the possibility of precipitation is predicted.

etc. The intrinsic pK_a of phenytoin at ionic strength = 0 M and 25 °C was obtained from ACD/pK_a DB as 8.33. The intrinsic solubility of phenytoin is 0.0152 mg/mL (from Merck Index).

Results and Discussion

The Effect of Initial pH of the Formulation. Formulations of 1 mg/mL phenytoin in 50 mM carbonate buffer at pH 10.0, 10.5, and 10.74 were diluted with SPB. Figure 1 shows the effect of the initial pH of the formulation on the pH after dilution, and Figure 2 shows the resultant solubility of phenytoin. It can be seen that, for a formulation at pH 10, precipitation occurs at a formulation fraction ff < 0.5, while for an initial pH greater than 10.5, precipitation occurs only when ff is less than 0.2. The later formulations maintain a higher pH upon dilution, which inhibits precipitation.

The Effect of Strength of the Buffer Used in the Formulation. Figure 3 shows the effect of the buffer strength on the pH after dilution of formulations of 1 mg/mL phenytoin at pH 10 in 10, 50, and 100 mM carbonate buffers.

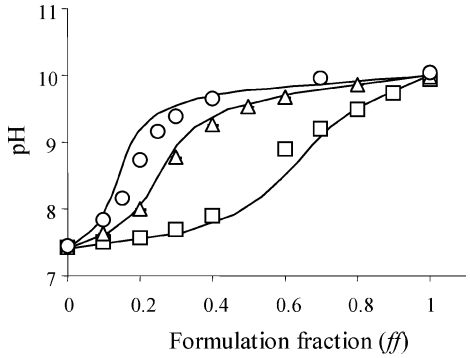

Figure 3. Effect of formulation buffer strength on the pH of the diluted solutions. Formulation: 1 mg/mL phenytoin formulation (carbonate buffer at pH 10.0). Buffer concentration: 10 mM (□), 50 mM (Δ), and 100 mM (○). The solid curves represent the predicted values.

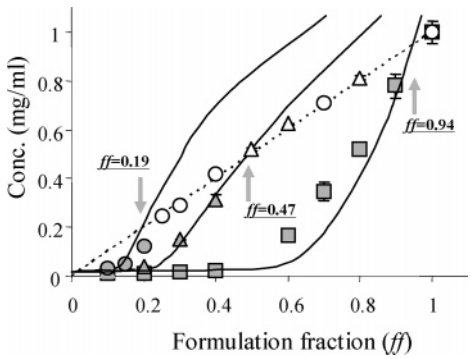

Figure 4. Effect of formulation buffer strength on the concentration of phenytoin upon dilution. Formulation: 1 mg/mL phenytoin formulation (carbonate buffer at pH 10.0). Buffer concentration: 10 mM (□), 50 mM (Δ), and 100 mM (○). The shaded symbols represent that the precipitation was observed. The solid curves represent the predicted solubility, and the broken line represents the concentration. When the solid curves are lower than the broken line, the possibility of precipitation is predicted.

It is clear that the buffer strength strongly affects the pH after mixing. This effect is more significant than the effect of the initial pH of the formulation.

The effect of buffer strength on drug precipitation is presented in Figure 4. For a 10 mM buffer, even a slight dilution results in precipitation. However, for a 100 mM buffer precipitation does not occur till ff < 0.2. Again, the buffer strength appears to be a more dominant factor than the initial pH.

The Effect of the Buffer Species Used in the Formulation. Formulations of 1 mg/mL phenytoin in 50 mM carbonate and phosphate buffers at pH 10.5 were diluted with SPB to illustrate the role of the pK_a of the buffer. It is evident from Figures 5 and 6 that there is a significant difference between phosphate and carbonate buffer. Carbonate buffer has pK_a values at 6.35 and 10.33. Thus, the carbonate formulation has a high buffer capacity at around pH 10. Since the pK_a values of phosphate buffer are 2.15, 7.20, and 12.38, it lacks strong buffer capacity in the region of between pH

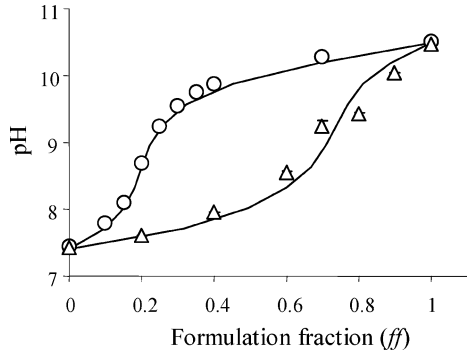

Figure 5. Effect of formulation buffer type on the pH of the diluted solutions. Formulation: 1 mg/mL phenytoin formulation (50 mM buffers at pH 10.5). Carbonate buffer (○), phosphate buffer (Δ). The solid curves represent the predicted values.

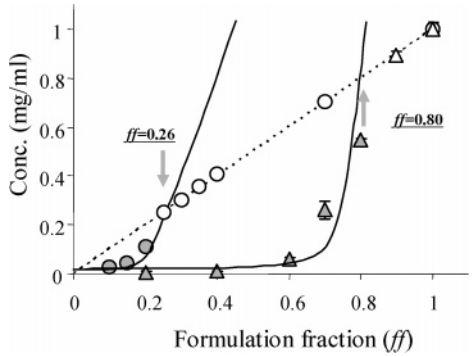

Figure 6. Effect of formulation buffer type on the concentration of phenytoin upon dilution. Formulation: 1 mg/mL phenytoin formulation (50 mM buffers at pH 10.5). Carbonate buffer (○), phosphate buffer (Δ). The shaded symbols represent that the precipitation was observed. The solid curves represent the predicted solubility, and the broken line represents the concentration. When the solid curves are located lower than the broken line, the possibility of precipitation is predicted.

7.4 and 10.5. Therefore, the pH of the diluted phosphate solution and the resultant drug solubility decrease significantly. No precipitation was noticed for formulation buffered with carbonate at ff > 0.25. But, for the formulation containing phosphate buffer, precipitation occurred over a wide range of dilutions.

The Effect of Initial Drug Concentration. The formulations of 0.5 and 1 mg/mL phenytoin in 50 mM carbonate buffer at pH 10 were diluted with SPB. As shown in Figure 7, the initial drug concentration had a negligible effect on the pH of the mixture. However, drug concentration is a key factor in formulation development. Figure 8 shows that the critical point for precipitation changed from ff = 0.47 for 1 mg/mL formulation to ff = 0.32 for 0.5 mg/mL.

The ionic strength of the medium is a function of the buffering species, their concentrations, and the amount of added salt. Many relationships have been put forth for the estimation of individual ion activity coefficients. In this proposed model, we have used the Debye−Hückel equation

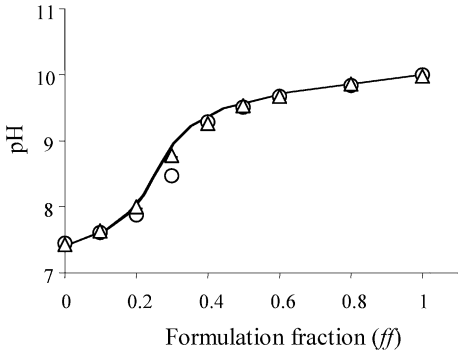

Figure 7. Effect of phenytoin concentration in the formulation on the pH of the diluted solutions. Formulation: phenytoin formulation (50 mM carbonate buffer at pH 10.0). Initial phenytoin concentration: 0.5 mg/mL (○), 1 mg/mL (Δ). The solid curves represent the predicted values. Each curve is almost overlapped.

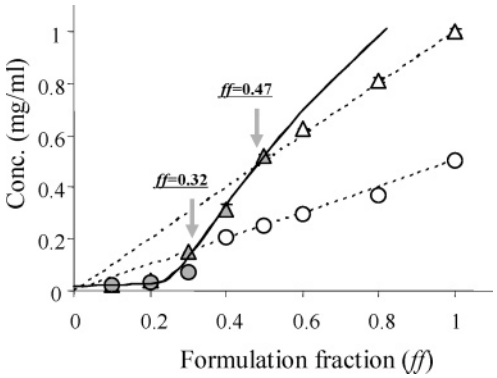

Figure 8. Effect of phenytoin concentration in the formulation on the concentration upon dilution. Formulation: Phenytoin formulation (50 mM carbonate buffer at pH 10.0). Initial phenytoin concentration: 0.5 mg/mL (○), 1 mg/mL (Δ). The shaded symbols represent that the precipitation was observed. The solid curves represent the predicted solubility, and the broken lines represent the concentration. When the solid curves are located lower than the broken line, the possibility of precipitation is predicted.

modified by Davies (eq 19). When the ionic strength (I) of the buffer is 0.1 M, the pK_a shift (ΔpK_a) of a monoacidic drug is about −0.23. When ionic strength is increased to 0.2 M, ΔpK_a becomes about −0.30. This small change can be significant because the solubility of an acidic drug is exponentially related to the (pH − pK_a) value. Thus, a shift of −0.30 in the pK_a may increase the drug solubility almost 2 times. The pK_a shift affects the un-ionized−ionized equilibrium for all species, which in turn affects the ionic strength after mixing. Therefore, it is necessary to consider the effect of ionic strength on all of the pK_a values.

Conclusions

An equation has been developed for estimating the precipitation of drugs that may occur upon diluting the formulation. To demonstrate the robustness of this model, the effects of pH, the buffer species and strength, and drug

concentration were evaluated. In this study, pH affected the solubility of the drug in formulation, but upon dilution, drug solubility was more affected by the buffer species and strength than by the initial pH or the initial drug concentration. The drug concentration affected the precipitation, but did not affect the pH of diluted formulation.

Since our model is based upon equilibrium values, it describes a worst case scenario. In other words, if precipitation is predicted, it may be observed. But, if precipitation is not predicted, it will not occur.

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