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Precipitation of pH solubilized phenytoin

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Abstract

Precipitation of phenytoin often occurs as it is diluted by blood after intravenous injection. The presence and the amount of precipitate depend upon the initial pH and buffer capacity of the formulation vehicle. The prediction of phenytoin precipitation can be carried out in vitro using isotonic Sorensen's phosphate buffer (SPB) to simulate blood. An equation is developed to calculate the change in solubility resulting from the change in pH due to dilution. This equation is very difficult to solve analytically because it involves a high order polynomial. However, it can be solved numerically using a spreadsheet program. The relationship between pH or solubility and dilution can then be presented graphically. Therefore, the precipitation of any pH solubilized drug due to dilution under various conditions can be easily predicted. This is illustrated for several aqueous phenytoin solutions.

Key words: Formulation; Precipitation; Solubility; Buffer; Phenytoin; pH equation

1. Introduction

It is customary to increase the solubility of weakly acidic and weakly basic drugs by adjusting the solution pH by the addition of a buffer (Flynn, 1980). The increase in solubility is achieved by maximizing the percentage of ionized drug. It is not uncommon for buffer solubilized drugs to precipitate when they are mixed with i.v. admixtures or blood, which are also buffered, however, to a different pH. When precipitation occurs in the bloodstream, the result can be reduced bioavailability and/or thrombophlebitis (Yalkowsky, 1977). In order to ensure that precipitation will not occur, it is necessary to have a quantitative understanding of the alteration of pH and solubility that will be produced by a dilution.

In this report, we will attempt to quantitate the effect of dilution upon pH in terms of the buffer concentration and the pH of both a model of pure buffered formulation and dilution medium. This information allows us to determine the change in solubility as well as the potential of drug precipitation upon dilution.

Because of its low water solubility and high pK_a value, phenytoin was chosen as a model

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solute. Since this report considers only aqueous buffered solutions, no cosolvents were used.

2. Materials and methods

2.1. Reagents

Phosphate buffer was prepared from dibasic potassium phosphate (K_2HPO_4, Mallinckrodt Inc.). Monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$, Mallinckrodt Inc.) and K_2HPO_4 were used to prepare Sorensen's phosphate buffer (SPB). Phenytoin (Aldrich Chemical Co.) which has a molecular weight of 252.26, pK_a of 8.31 and intrinsic solubility (S_i) of 0.02 mg/ml (Philip, 1984) was used as received.

2.2. Apparatus

All pH measurements were carried out using a pH meter (Corning model 140) which was calibrated daily using pH 7.00 and 10.00 standard buffers (VWR Scientific). A HPLC system equipped with a C_{18} column (Econosphere C-18 5U, Alltech) was used. The mobile phase consisted of a mixture of equal volumes of acetonitrile and acetate buffer (0.10 M at pH 4.00).

2.3. Formulation

Phosphate buffer in concentrations of 0.01, 0.05, 0.10 and 0.20 M was used as a model formulation vehicle. Each of these were buffered to an initial pH of 9.00, 10.00 and 11.00. Phenytoin in concentrations of 0.10, 0.50 and 1.00 mg/ml was dissolved in the 0.10 M phosphate buffer at pH 11.00.

2.4. Dilution medium

SPB (1/15 M at pH 7.40) was used as a dilution medium. SPB is believed to be a better blood model than plasma because its buffer capacity of 0.036 is close to the values for fresh whole blood. The buffer capacity of whole blood is reported to be 0.0318 (Salenius, 1957) and 0.039 (Ellison et al., 1958). The buffer capacity for plasma is only 0.008 (Martin, 1993).

Furthermore, SPB has been certified by the National Bureau of Standards (NBS) as a pH standard for use in the biological range of interest between 7.3 and 7.5 (Bower, 1961).

2.5. Mixture of formulation and dilution medium

Various volumes of phosphate-buffered formulations (V_F) were mixed with a various volumes of SPB dilution medium (V_{DM}) to produce different dilution fractions (f) where:

$$f = V_F/(V_F + V_{DM}) \quad (1)$$

The value of f ranges from 1.0 for the pure formulation to 0.0 at infinite dilution. The dilution factor (F) is determined as:

$$F = 1 - f \quad (2)$$

The pH was measured for each dilution.

The model formulations containing phenytoin were also diluted as described above. For each dilution the presence or absence of precipitation was noted and the pH was measured. The precipitation was visually observed as white crystal or cloudiness. The mixture of buffered formulation and SPB were equilibrated for 2–3 days using a test tube rotator. If precipitation was observed, the mixture was centrifuged at 2800 rpm (Beckman Model TJ-6 Centrifuge) for 30 min and the concentration of drug in supernatant was determined using HPLC.

All of the experiments were carried out at room temperature (25°C) in duplicate.

3. Calculations

3.1. Dilution

When an intravenous formulation is injected, it is diluted with a dilution medium such as blood. The concentrations of all formulation components change from their initial values to zero at infinite dilution. At the same time the concentrations of the blood components in contact with the formulation change from zero to the

values in pure blood. The problem of diluting a buffered formulation of drug with blood or any buffered solution is mathematically equivalent to that of titrating one weak electrolyte solution with another.

3.2. Ionic equilibria

The dilution of a buffered formulation containing drug with blood can be modeled by dilution of a triprotic acid buffer (e.g., citric, phosphoric) with SPB which is used as a surrogate for blood. The ionization equilibria applicable to the dilution of a triprotic acid buffer containing a weakly acidic drug with SPB are given below:

$$H_3A \overset{K_{A1}}{\rightleftharpoons} H_2A^- + H^+ \quad (3)$$

$$H_2A^- \overset{K_{A2}}{\rightleftharpoons} HA^{2-} + H^+ \quad (4)$$

$$HA^{2-} \overset{K_{A3}}{\rightleftharpoons} HA^{3-} + H^+ \quad (5)$$

$$H_3B \overset{K_{B1}}{\rightleftharpoons} H_2B^- + H^+ \quad (6)$$

$$H_2B^- \overset{K_{B2}}{\rightleftharpoons} HB^{2-} + H^+ \quad (7)$$

$$HB^{2-} \overset{K_{B3}}{\rightleftharpoons} B^{3-} + H^+ \quad (8)$$

$$HD \overset{K_D}{\rightleftharpoons} D^- + H^+ \quad (9)$$

where H_3A is a triprotic acid and K_{A1}, K_{A2} and K_{A3} denote its successive dissociation constants; H_3B is a phosphoric acid and K_{B1}, K_{B2} and K_{B3} are its successive dissociation constants; HD is a monobasic acid drug and K_D is its dissociation constant.

Note that in this study, SPB is used as the dilution medium. If the formulation is phosphate buffer, the calculation does not simplify because their initial pH values and concentrations are different. For formulations buffered with mono- or diprotic acids the appropriate terms are simply omitted.

The charge balance equation of the pure buffered formulation is:

$$[Na^+] + [H^+] = [OH^-] + (\alpha_0 + \alpha_1 + \alpha_2 + \alpha_3)[H_2A^-] + 2[HA^{2-}] + 3[A^{3-}] \quad (10)$$

and the mass balance equation of the pure buffered formulation is:

$$[Na^+] = (\alpha_1 + 2\alpha_2 + 3\alpha_3)C_A \quad (11)$$

$$C_A = [H_3A] + [H_2A^-] + [HA^{2-}] + [A^{3-}] \quad (12)$$

where C_A is the initial concentration of buffered formulation and α_i denotes the concentration fraction of each species in pure buffered formulation given by:

$$\alpha_i = [H_{3-i}A^{i-}]/C_A \quad (13)$$

The proton balance equation of the pure buffered formulation can be obtained by substituting Eq. 11 and 12 into Eq. 10:

$$[OH^-] - [H^+] = a[H_3A] + b[H_2A^-] + c[HA^{2-}] - d[A^{3-}] \quad (14)$$

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.

Likewise, the proton balance equation for the blood model can be expressed by the following:

$$[OH^-] - [H^+] = k[H_3B] + l[H_2B^-] + m[HB^{2-}] + n[B^{3-}] \quad (15)$$

where 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, and β_i is the concentration fraction of each species in the blood model given by:

$$\beta_i = [H_{3-i}B^{i-}]/C_B \quad (16)$$

The initial concentration of blood model, C_B, is:

$$C_B = [H_3B] + [H_2B^-] + [HB^{2-}] + [B^{3-}] \quad (17)$$

The proton balance equation for the mono acidic drug is:

$$\gamma_1[HD] + [H^+] = [OH^-] + \gamma_0[D^-] \quad (18)$$

where γ_0 and γ_1 are the concentration fractions of each species in the drug. The initial concentration of drug, C_D, is:

$$C_D = [HD] + [D^-] \quad (19)$$

The proton balance equation of the mixture can be obtained from the summation of Eq. 14,

15 and 18 in the following:

$$[OH^-] - [H^+] = a[H_3A] + b[H_2A^-] + c[HA^{2-}] - d[A^{3-}] + k[H_3B] + l[H_2B^-] + m[HB^{2-}] - n[B^{3-}] + \gamma_1[HD] - \gamma_0[D^-] \quad (20)$$

3.3. pH and dilution

The titration or dilution process can be thought of as mixing a volume fraction f of formulation with volume fraction F of dilution medium. Therefore, f decreases from unity to zero and F increases from zero to unity. The total concentration of each component in the mixture is:

$$C_{mix} = (C_A + C_D)f + C_B(1-f) \quad (21)$$

Combining these equations gives the following equation which relates hydrogen ion concentration to the fractional dilution of the formulation with blood:

$$f = \frac{[OH^-] - [H^+] - C_B[Y]}{C_A[X] - C_B[Y] + C_D[Z]} \quad (22)$$

where

$$[X] = \{a[H^+]^3 + bK_{a1}[H^+]^2 + cK_{A1}K_{A2}[H^+] - dK_{A1}K_{A2}K_{A3}\} \times \{[H^+]^3 + K_{A1}[H^+]^2 + K_{A1}K_{A2}[H^+] + K_{A1}K_{A2}K_{A3}\}^{-1} \quad (23)$$

$$[Y] = \{k[H^+]^3 + lK_{B1}[H^+]^2 + mK_{B1}K_{B2}[H^+] - nK_{B1}K_{B2}K_{B3}\} \times \{[H^+]^3 + K_{B1}[H^+]^2 + K_{B1}K_{B2}[H^+] + K_{B1}K_{B2}K_{B3}\}^{-1} \quad (24)$$

$$[Z] = \frac{\gamma_1[H^+] - \gamma_0 K_D}{[H^+] + K_D} \quad (25)$$

and $[H^+]$ is the proton concentration after mixing the buffered formulation containing drug and SPB.

Note that all of the factor values of proton concentration in Eq. 23–25 are constant for any initial concentrations of buffers and drug. Therefore, they are constant for any formulation. Also, the initial proton concentrations in Eq. 23 and 25 are identical since they describe the same solution, i.e., the same undiluted formulation.

Although it is difficult to obtain and to present the analytical solution for pH in terms of the fractional dilution, it is relatively easy to calculate the fractional dilution for any given pH. A spreadsheet program (QPRO version 5.0) is used throughout this study for solving Eq. 22 which expresses f as a polynomial of hydrogen ion concentration. The dilution fractions at each buffer concentration were calculated by putting pH values ranging from the initial pH values of 9.00, 10.00 and 11.00 to the infinite dilution value of 7.40 with 0.02 pH unit increments into Eq. 22. These data can be plotted as pH vs f as shown in section 4.

3.4. Activity coefficient correction

The thermodynamic pK_a values of phosphate buffer and SPB (2.15, 7.20 and 12.31) and drug (8.31) were corrected in each dilution using Davies' modification of the Debye-Huckel equation (Freiser, 1992), i.e.,

$$\log \gamma_i = z_i^2 \left[0.15I - \frac{0.51\sqrt{I}}{1+\sqrt{I}} \right] \quad (26)$$

where γ_i is the activity coefficient of an ion i, having a charge z_i in each dilution of ionic strength I.

This equation can be used to correct the pK_a values where the concentration of buffer solution is less than 0.2 mol per l. If higher concentrations are used, Eq. 26 must be modified (Freiser, 1992).

3.5. Solubility and pH

The equation relating the total solubility (S_T) to the intrinsic solubility (S_i) and the pH of a weakly acidic drug is:

$$S_T = S_i[1 + 10^{(pH - pK_a)}] \quad (27)$$

3.6. Solubility and dilution

The dilution of a buffered formulation containing drug with SPB results in a change of pH which alters the solubility. A combination of Eq. 22 and 27 is used to calculate the change of solubility due to the dilution.

3.7. Concentration and dilution

The concentration of the drug decreases linearly as the buffer solution is diluted. The equation that relates the concentration of drug in the formulation in each dilution, C_f, to the initial concentration, C_i, is

$$C_f = fC_i \tag{28}$$

3.8. Data input

The data input required to perform the calculation include each component's pK_a values, initial pH, concentration and intrinsic solubility and molecular weight of drug as illustrated in Table 1 for the system studied in this report.

This program is also able to determine the tonicity of the buffered formulation and the blood model. If desired, the amount of NaCl required to adjust the tonicity will be calculated (Shargel, 1994) as shown in Table 1.

Table 1
An example of the data input in spreadsheet for the calculations of dilution fraction (f), dilution and solubility as a function of pH

Formulation of drug in buffer solution: weakly acidic drug Press Alt A to start the program			
	Drug (phenytoin)	Formulation (phosphate buffer)	Blood model (SPB)
pK_{a1} [b]	8.31	2.15	2.15
pK_{a2} [b]	N/A	7.2	7.2
pK_{a3} [b]	N/A	12.4	12.4
pH	[a]	11.00	7.40
Concentration (mol/l)	0.00396	0.05	0.06667
S_i (mol/l)	$7.9E-05$		
Molecular weight	256.26		
Isotonic (Y/N)	[a]		
NaCl added (g/l)	[a]	4.33	3.91

[a] Same as formulation.
[b] Type 'N/A' where pK_a is not applicable.

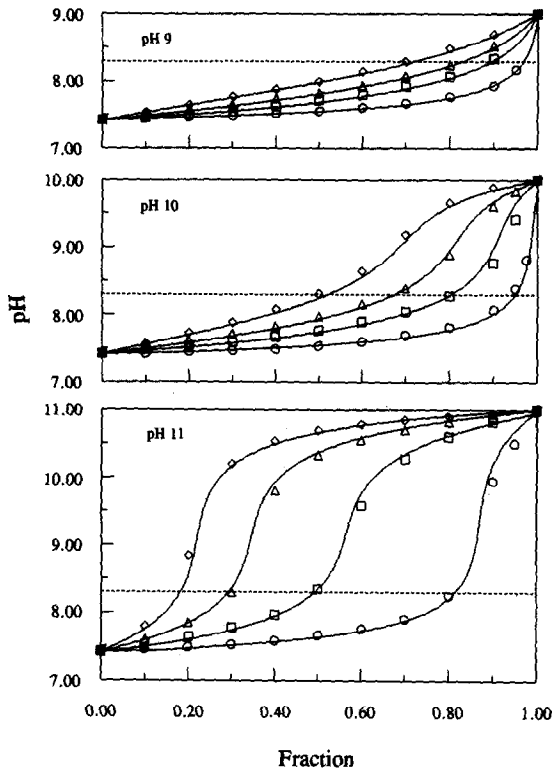

Fig. 1. Comparison of pH change from theoretical and experimental data (solid lines and symbols, respectively) due to the dilution of phosphate buffer with SPB at its different initial pH values and various concentrations: (○) 0.01 M, (□) 0.05 M, (△) 0.10 M and (◇) 0.20 M. The dashed lines represent the pK_a of phenytoin.

4. Results and discussion

4.1. pH of diluted formulation

The plots of observed pH vs dilution factor at various buffer concentrations and initial pH values are given in Fig. 1 along with the theoretical curves. It is clear from Fig. 1 that the theoretical curves obtained from Eq. 22 and 26 for the buffer system are confirmed very well by the experimental data.

The solubility of weakly acidic drugs like phenytoin will increase with increasing pH. This is the result of increasing the concentration of the ionized form of the drug. The concentrations of ionized and neutral form of drug will be identical where the pH is equal to its pK_a. Fig. 1 shows the importance of the initial pH of the formulation and its buffer capacity for keeping the pH higher than the pK_a of phenytoin (8.30) which is represented by the dashed line in Fig. 1. For any given buffer concentration the formulation with the higher initial pH will maintain its pH above the pK_a of phenytoin at a higher dilution (lower f). For instance, the 0.20 M buffers of pH 9.00, 10.00 and 11.00 reach pH 8.30 at F of 0.28, 0.48 and 0.82 dilution ($f = 0.72$, 0.52 and 0.18), respectively. Fig. 1 also shows that for any initial pH the formulation with the greater buffer capacity remains above the pK_a of phenytoin for a higher dilution. The pH 11.00 buffered formulation in Fig. 1 is shown as an example where the pH of the 0.01 M buffer concentration drops to the pK_a of phenytoin only after 0.18 dilution ($f = 0.82$), whereas it requires an F of 0.82 ($f = 0.18$) for the 0.20 M buffer concentration to reach this value.

4.2. Solubility of drug

The calculation of phenytoin solubility in phosphate buffer solutions under various conditions was carried out by applying Eq. 22, 26 and 27 and is illustrated in Fig. 2. The solubility curves shown in Fig. 2 were calculated based on the pH-f profiles of Fig. 1. The symbols in both figures have the same meaning. As expected, the higher pH values as well as the greater buffer concentrations in Fig. 1 correspond to the higher solubilities in Fig. 2. The dashed line in Fig. 2 corresponds to the solubility of phenytoin at a pH equal to its pK_a of 8.31 (i.e., twice its intrinsic solubility). Also, the intersections of the dashed and solid lines in Fig. 2 correspond to those in Fig. 1.

4.3. Precipitation of drug

The precipitation of phenytoin due to dilution of a formulation, which has an initial pH of 11.00 with SPB, is predicted by the superimposition of the solution curves of 0.1 M extracted from Fig. 2 with the dilution lines described by Eq. 27. These are illustrated in Fig. 3. Those dilution concentrations which are below the solubility lines represent stable solutions. They will not precipitate. Those dilution concentrations which are above the solubility lines represent unstable solutions. Although they have the potential to precipitate, they may or may not actually precipitate in a given period of time. The filled symbols represent solutions that did not precipitate. The open symbols correspond to the concentration of drug in the supernatant of solutions which did precipitate.

Note that in all cases the concentration line drops below the solubility line as infinite dilution

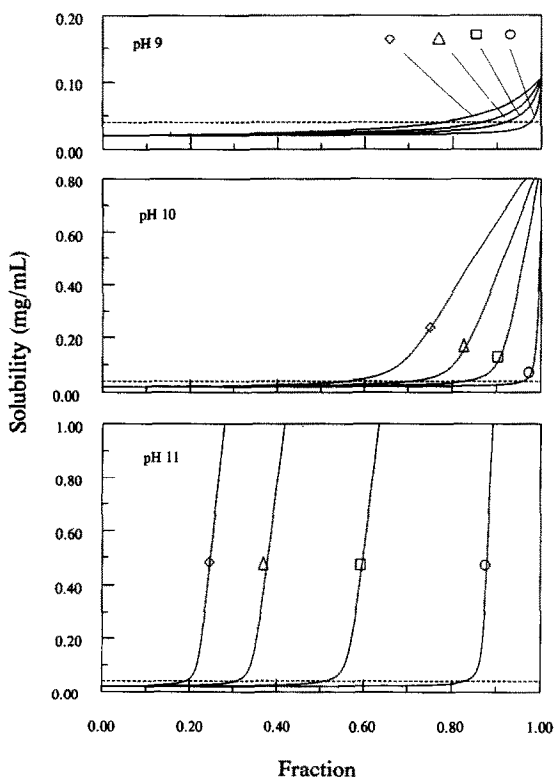

Fig. 2. Theoretical plots of solubility of phenytoin as a function of dilution fraction at various pH values and concentrations: (○) 0.01 M, (□) 0.05 M, (△) 0.10 M and (◇) 0.20 M. The dashed lines represent the solubility of phenytoin at its pK_a.

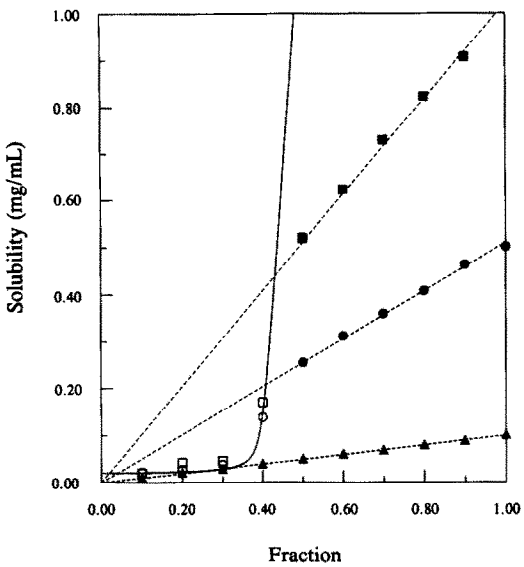

Fig. 3. Comparison of the precipitation of phenytoin from theoretical and experimental data in 0.10 M phosphate buffer (pH 11.00) at various initial concentrations of: (a) (■,□) 1.02 mg/ml, (b) (●,○) 0.51 mg/ml and (c) (▲) 0.10 mg/ml. The solid line represents the solubility limitation calculated from Eq. 16.

is approached. Under these conditions, solutions will not precipitate and precipitate that has formed in previous dilutions may redissolve. It is clear that the observations are in agreement with the prediction based upon the intersections of the solubility curve with the dilution curve.

5. Conclusions

When a formulation is diluted with pH 7.4 buffer, the pH of the mixture is changed towards that value, i.e., the pH of the phenytoin formulation is lowered and approaches pH 7.4 at infinite dilution. A graphical solution to the complex relationship between fractional dilution and pH has been developed. The pH change produces a decrease in the solubility of the drug. If the solubility is decreased below the concentration of diluted drug there is the possibility of precipitation. As the formulation is diluted further, the drug concentration will become less than the solubility (i.e., the solubility of drug in SPB), and the solution will not precipitate.

If SPB is a realistic model for human blood, the above represents a means of determining whether or not a formulation has the potential to precipitate when it is injected into the bloodstream. This approach provides an accurate, convenient method for predicting the physical stability of mixtures of parenteral drugs and i.v. admixtures.

Although this report is concerned with the effects of dilution upon the pH and drug solubility in purely aqueous solutions, the conclusions are applicable to one of the reasons for precipitation in more complex solutions such as the commercial phenytoin injection formulation.

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