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Effect of phosphate salts concentrations, supporting electrolytes, and calcium phosphate salt precipitation on the pH of phosphate buffer solutions

Yoonjee Park[a], Sook Heun Kim[a], Sadis Matalon[b], Nien-Hwa Linda Wang[a], Elias I. Franses[a,*]

[a] School of Chemical Engineering, Purdue University, 480 Stadium Mall Drive, West Lafayette, IN 47907-2100, USA
[b] Department of Anesthesiology, University of Alabama at Birmingham, BMR2 224, 901 19th St. South, Birmingham, AL 35205-3703, USA

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ABSTRACT

A monobasic and a dibasic phosphate salt are usually added to water to form a standard phosphate buffer solution. The solution pH depends primarily on the concentration ratio *R* of the two salts, and secondarily on their concentrations and on the concentrations of the supporting electrolytes such as NaCl or KCl. Various ideal and non-ideal solution thermodynamic models are presented. The ideal solution model predicts that the pH should depend only on *R*, and underestimates the pH at physiological osmolarities by 0.3–0.5 pH units at both 298 K and 310 K, depending on the phosphate, NaCl, and KCl concentrations. Model predictions using the non-ideal extended Debye–Hückel (D–H) equation agree with the data up to ca. ±0.1 pH units at 298 K and 310 K. The pH predictions for the standard isotonic phosphate buffer saline, containing 2 mM of monobasic and 8 M of dibasic sodium phosphate, 137 mM of NaCl, and 2.7 mM of KCl agree well with the data. When $CaCl_2$ is added, up to 3 mM, a phosphate salt often precipitates, affecting the free Ca^{2+} ion concentration, the phosphate ion concentrations, and the pH. The conditions for the formation at equilibrium of hydroxyapatite ($Ca_5(PO_4)_3(OH)$), or dicalcium phosphate dehydrate ($CaHPO_4{\cdot}2H_2O$), or tricalcium phosphate ($Ca_3(PO_4)_2$), are predicted. The results are important in determining and predicting electrolyte concentrations for achieving desirable pH values while avoiding precipitation for various biological or biomedical applications.

1. Introduction

For obtaining biologically or medically relevant results with aqueous systems of proteins, lipids, or cells, one needs to use physiological buffer conditions. A “physiological” buffer should be isotonic, with about 300 mOsm/kg H_2O. Usually, the pH should range from 7.0 to 7.4. There should be a substantial buffer capacity, i.e. the ability to maintain the pH in the specified range when some acid, base, protein, lipid, or other ion is introduced in the solution. A combination of sodium chloride, potassium chloride, sometimes calcium chloride, and other electrolytes may be used. In this report, we focus on using phosphate buffers, with sodium monobasic phosphate and sodium dibasic phosphate, either alone or with added salts, developing thermodynamic models for predicting their pH, and testing these models experimentally.

For the phosphate salts, there are standard published pH data for various ratios, $R \equiv C_2^0/C_3^0$, of the concentrations of the monobasic (C_2^0) to the dibasic (C_3^0) sodium phosphate salt, primarily for $C_T^0 = C_2^0 + C_3^0 = 66.6\,\mathrm{mM}$ [1]. No general model is available. These concentrations are mostly non-isotonic, and contain much higher phosphate salts concentrations than in the standard phosphate buffer saline, or “PBS,” which contains 137 mM of NaCl, 2.7 mM of KCl, and $C_2^0 = 2\,\mathrm{mM}$ and $C_3^0 = 8\,\mathrm{mM}$, for a pH of 7.4 at 298 K or at 310 K [2]. Few data and no model predictions are available for these and similar isotonic solutions. When some $CaCl_2$ is added to have a typical physiological Ca^{2+} ion concentration of about 2.5 mM, some calcium phosphate salt may precipitate. Then the pH may shift beyond the acceptable range. For this reason, when there is a possibility that Ca^{2+} ions may cause precipitation, some researchers may opt to use a different buffer, such as containing HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), an organic molecule [3].

The goal of this article is to develop and test a set of thermodynamic models which can predict accurately the pH and other ion concentrations, so that one can choose the phosphate salt concentrations for specified concentrations of C_{H^+}, $C_{Ca^{2+}}$, C_{Na^+}, C_{K^+}, and other ions.

To predict calcium phosphate precipitation at a given value of C_{CaCl_2}, one needs to predict the phosphate ions concentrations and have accurate and reliable values of the solubility products, K_{sp}, of the various phosphate salts which may precipitate. Tricalcium

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Abbreviations: DCPD, dicalcium phosphate dihydrate; D–H, Debye–Hückel; HAP, hydroxyapatite; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PBS, phosphate buffer saline; TCP, tricalcium phosphate.

[*] Corresponding author. Tel.: +1 765 494 4078; fax: +1 765 494 8050.
E-mail address: franses@ecn.purdue.edu (E.I. Franses).

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phosphate salt, $Ca_3(PO_4)_2$, or TCP, is sparingly soluble and can precipitate. Hydroxyapatite, $Ca_5(PO_4)_3(OH)$, or HAP and dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$, or DCPD, can also precipitate [4–7]. In this report, we present three models with the assumption that only one of three salts may precipitate, after considering the ionic product K_{ip} vs. the solubility products K_{sp} of one of each of the phosphate salts which may precipitate.

2. Experimental details: materials and methods

Sodium chloride (NaCl), potassium chloride (KCl), and sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) were purchased from Mallinckrodt Specialty Chemicals Co. (Paris, KY). Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) were purchased from Fluka Chemical Corp. (Milwaukee, WI). Water from a Millipore unit, with organic adsorption, ion exchange and ultrafiltration columns, was used. Concentrations were prepared by weight. Measurements of pH, ±0.1 or better (±0.02), were done using a EXA PH202 pH meter from Yokogawa at 298 and 310 K. At least $n = 5$ measurements were done at each set of conditions. The averages and standard deviations are reported.

For certain solutions containing Ca^{2+} and forming precipitates, the solutions were heated first to 55 °C, and were cooled to 298 or 310 K. This was done to induce some precipitation at 55 °C, and bring the systems closer to equilibrium. Measurements of pH for those conditions were time-dependent. Hence, some reported values, which were obtained at 24 h after heating, may not be strictly at equilibrium.

3. Thermodynamic models, sample calculations, and comparisons to data

3.1. Introduction to the models

Several models are developed to study the effect of the concentrations of two phosphate salts, NaH_2PO_4 and Na_2HPO_4, and certain supporting electrolytes, such as NaCl, KCl, and $CaCl_2$, on the pH at 298 and 310 K (Table 1). In order to find out this effect for non-ideal solution models, the activity coefficients are calculated with the simplest one-parameter Debye–Hückel equation (Models 2a, 3a, etc.), and with the two-parameter Debye–Hückel equation (Models 2b, 3b, etc.). At most concentrations four possible phosphate forms, H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, and PO_4^{3-}, with concentrations, in mol/L, and activity coefficients, C_1, C_2, C_3, and C_4, and γ_1, γ_2, γ_3, and γ_4, respectively, are predicted from the three dissociation equilibrium constants below.

3.2. Phosphate salts solutions with no added electrolyte

3.2.1. Some general equations for ideal and non-ideal solutions

The dissociation equilibria of the phosphate species with activities, $\alpha_i = \gamma_i C_i$ are as follows [8,9]:

$$H_3PO_4 \overset{K_1}{\rightleftharpoons} H^+ + H_2PO_4{}^-, \tag{1}$$

$$H_2PO_4{}^- \overset{K_2}{\rightleftharpoons} H^+ + HPO_4{}^{2-}, \tag{2}$$

$$HPO_4{}^{2-} \overset{K_3}{\rightleftharpoons} H^+ + PO_4{}^{3-}, \tag{3}$$

where

$$\frac{\alpha_{H^+}\alpha_2}{\alpha_1} = \frac{\gamma_{H^+}\gamma_2}{\gamma_1}\frac{C_{H^+}C_2}{C_1} = K_1, \tag{4}$$

$$\frac{\alpha_{H^+}\alpha_3}{\alpha_2} = \frac{\gamma_{H^+}\gamma_3}{\gamma_2}\frac{C_{H^+}C_3}{C_2} = K_2, \tag{5}$$

and

$$\frac{\alpha_{H^+}\alpha_4}{\alpha_3} = \frac{\gamma_{H^+}\gamma_4}{\gamma_3}\frac{C_{H^+}C_4}{C_3} = K_3. \tag{6}$$

The values of the equilibrium constants, $pK_i \equiv -\log_{10} K_i$, at 298 K are 2.12, 7.21, and 12.67, respectively. At 310 K, the values are 2.18 [12], 7.18 [10], and 12.36 [11], respectively. The total input concentration of phosphate is $C_T^0 = C_2^0 + C_3^0$. Phosphorus atom conservation implies that

$$C_1 + C_2 + C_3 + C_4 = C_T^0 = C_2^0 + C_3^0. \tag{7}$$

The ion concentrations should also satisfy the electro-neutrality principle.

$$C_{H_2PO_4{}^-} + 2C_{HPO_4{}^{2-}} + 3C_{PO_4{}^{3-}} + C_{OH^-} = C_{Na^+} + C_{H^+}. \tag{8}$$

The sodium ions come from the two phosphate salts. The hydrogen ion and hydroxide ion concentrations ($C_{OH^-} = 10^{-14}/C_{H^+}$) are usually neglected here, in all models except Models 5 and 6, since the pH of interest of the solution is between 6 and 8. When the pH reaches values outside this range, these ion concentrations may be considered for more accurate calculations. Then, Eq. (8) reduces to

$$C_2 + 2C_3 + 3C_4 = C_2^0 + 2C_3^0. \tag{9}$$

3.2.2. Ideal solution: Model 1

The simplest model, “Model 1,” is the ideal solution model in which the activity coefficients are equal to 1,

$$\gamma_1 = \gamma_2 = \gamma_3 = \gamma_4 = \gamma_{H^+} = 1. \tag{10}$$

The five unknown concentrations, C_1–C_4 and C_{H^+}, are found by solving the three non-linear equilibrium equations, Eqs. (4)–(6) (with $\gamma_i = 1$), along with the two balance Eqs. (7) and (9). This system of

Table 1
List of models studied.

Model	Solutions of	Osmolarity (mEq/kg)	γ-Model	Salt precipitation considered (Y, N)
1	Phosphates[a]	2–200	Ideal	N
2a	Phosphates[a]	2–200	Model a	N
2b	Phosphates[a]	2–200	Model b	N
3a	Standard PBS[b]	∼300	Model a	N
3b	Standard PBS[b]	∼300	Model b	N
4a	Standard PBS[b] + $CaCl_2$	∼300	Model a	N
4b	Standard PBS[b] + $CaCl_2$	∼300	Model b	N
5	Standard PBS[b] + $CaCl_2$	∼300	Ideal	Y
6a	Standard PBS[b] + $CaCl_2$	∼300	Model a	Y
6b	Standard PBS[b] + $CaCl_2$	∼300	Model b	Y

[a] Na_2HPO_4 and NaH_2PO_4.
[b] 137 mM of NaCl, 2.7 mM of KCl, 8 mM of Na_2HPO_4 and 2 mM of NaH_2PO_4.