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Talanta 65 (2005) 495–500

Talanta

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Density measurements of potassium phosphate buffer from 4 to 45°C

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Received 7 June 2004; received in revised form 23 June 2004; accepted 30 June 2004
Available online 4 August 2004

Abstract

Potassium phosphate buffer is often used in methods such as equilibrium dialysis, high performance liquid chromatography (HPLC), and affinity capillary electrophoresis (ACE) for characterizing the binding of drugs and hormones with proteins or other ligands within the body. In these experiments, the buffer density is often approximated to be that of water and the concentrations of all reagents are assumed to be constant with temperature. However, some difference in density between phosphate buffer and water would be expected, and variations in this density could lead to significant changes in the concentrations of dissolved solutes with temperature. This, in turn, could affect the binding observed for a solute–ligand system in such a buffer. In this study, the densities of potassium phosphate buffers with concentrations up to 0.10 M were measured at or near physiological pH for temperatures ranging from 4–45 °C. The general change in density versus temperature followed a quadratic equation, while the changes in density with concentration and pH followed a linear response. The results were used to formulate a general equation that could be used to calculate the density of potassium phosphate buffer at any pH, temperature, and concentration within the tested range. This equation and more specialized relationships developed in the temperature, concentration, and pH studies were found to give much greater accuracy in describing the density of these buffers versus a previous relationship developed for solutions containing only potassium dihydrogen phosphate.

Keywords: Phosphate buffer; Density; Binding studies; High performance liquid chromatography; Affinity capillary electrophoresis

1. Introduction

Potassium phosphate buffer is commonly used in the preparation of biological samples and the study of biological interactions. This is especially true for pH 7.4, 0.067 M potassium phosphate buffer (KPB), which is often used in methods like equilibrium dialysis, high performance liquid chromatography (HPLC), and affinity capillary electrophoresis (ACE) for characterizing the binding of drugs and hormones with carrier proteins or other ligands within the body [1–7].

In these studies, the phosphate buffer is usually prepared at room temperature, combined with the desired additives or solutes, and adjusted to the final temperature for study (e.g., 37 °C for the characterization of drug binding to human plasma proteins). A change in temperature between preparation and use would be expected to give rise to a change in the density for the buffer, which would also affect the concentrations of any added solutes. However, this effect is commonly ignored and no information is currently available on its impact when using phosphate buffers. This is mainly due to a lack of data on the density of phosphate buffer at or near physiological conditions.

There have been a few previous studies examining the density of tribasic (K_3PO_4), dibasic (K_2HPO_4), and monobasic (KH_2PO_4) potassium phosphate solutions [8–10]. However, the temperatures and buffer concentrations examined in these reports did not include those most often used in biological binding studies (e.g., 4–45 °C and 0.067–0.10 M). In addition, information is not available on the temperature dependence of the densities for mixtures of the dibasic and monobasic forms of potassium phosphate, as would be used in preparing a buffer at or near physiological pH. In this current study,

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0039-9140/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.talanta.2004.06.029

such information was obtained by measuring the density of potassium phosphate buffers over the range of temperatures, buffer concentrations, and pHs that are commonly used when measuring solute–protein binding. The results can then be used to estimate the effects of density and volume changes during the use of such buffers and to correct for these effects during the preparation of solutions for biointeraction studies.

2. Experimental

Each potassium phosphate buffer was prepared at 25 °C by combining monobasic potassium phosphate (99.8% pure) and dibasic potassium phosphate (99.1% pure) in deionized water obtained from a Nanopure water system (Barnstead, Dubuque, IA). These phosphate salts were obtained from Mallinckrodt (Phillipsburg, NJ) and were measured on an analytical balance with a mass precision of ±0.1 mg. The pH of all buffers was measured using a Corning model 350 pH meter (Acton, MA).

Preparation of the 0.067 and 0.10 M potassium phosphate buffers consisted of first adding the monobasic and dibasic salts to a volumetric flask along with a volume of water near that of the final desired volume (1–2 L). The quantities of each salt necessary to achieve the desired concentration and pH were calculated using the Henderson–Hasselbalch equation with an activity coefficient-corrected $\mathrm{p}K_a$ value for phosphate of 6.860 at 25 °C. For example, 2 L of pH 7.4, 0.067 M potassium phosphate buffer was prepared by adding 4.0820 g of the monobasic salt and 18.114 g of the dibasic salt to 1.9 L of water. The pH of this solution was then adjusted to 7.400 by adding small amounts of 1.0 M sodium hydroxide or 2.5 M hydrochloric acid (note: the original pH of each solution was within 0.02 units of the desired value, so adjustments with NaOH or HCl contributed less than 0.2% to the total mass of solutes added to the solution, making their effects negligible on the final density). Next, the solution was diluted to the mark on the volumetric flask with water, mixed, and filtered under vacuum through a 0.45 μm nylon membrane filter to remove any solid contaminants. The pH 7.4, 0.025, and 0.037 M buffers were prepared by dilution of this 0.067 M buffer. All buffer solutions were stored at 4 °C.

A 25 mL Gay-Lussac pycnometer was used for all density measurements. The volume of this pycnometer was calibrated between 4 and 45 °C using deionized water. In these measurements, a previously weighed pycnometer, in a beaker of water and a container of deionized water were placed in a water bath containing a 30:70 (v/v) mixture of ethylene glycol in water. After these items had been equilibrated at the desired temperature (±0.1 °C), the pycnometer was removed from the water bath, filled with deionized water, capped, and wiped dry. The pycnometer was then reweighed. The temperature and humidity of the air at the time of this mass measurement were also noted for later use in correcting for buoyancy effects. All masses were determined in replicate ($n = 5$) at each temperature used in this study.

During calibration of the pycnometer, all measured masses were corrected for buoyancy effects by using the following formula [11]

$$m_{\mathrm{H_2O}} = w_{\mathrm{H_2O}} \frac{(1 - \rho_{\mathrm{air}}/\rho_{\mathrm{ref}})}{(1 - \rho_{\mathrm{air}}/\rho_{\mathrm{H_2O}})} \qquad (1)$$

where $m\mathrm{H_2O}$ is the true mass of deionized water in the pycnometer, and $w\mathrm{H_2O}$ is the recorded weight of water in the pycnometer (i.e., as determined by taking the difference in the measured weights of the empty and filled pycnometer). The terms ρ_{air}, $\rho_{\mathrm{H_2O}}$, and ρ_{ref} represent the densities of the surrounding air, deionized water, and reference weight used to calibrate the analytical balance. By using Eq. (1) to find the true mass of deionized water in the pycnometer at a given temperature, this value could then be used along with the known density of pure water at the same temperature to obtain the internal volume of the pycnometer at the given temperature.

In this calibration process, the value of ρ_{ref} was found from the manufacturer of the balance to be 8.0 g cm^{-3}**,** and $\rho_{\mathrm{H_2O}}$ was obtained from literature values for the density of pure water [12]. The density of the surrounding air (ρ_{air}) was calculated by using the temperature and humidity of the air at the time of measurement. This was accomplished with the following equation [13],

$$\rho_{\mathrm{air}} = 0.0012929 \frac{273.13}{T} \frac{(P - 0.3783h)}{760} \qquad (2)$$

where P is the measured barometric pressure (in mm Hg), h is the vapor pressure (in mm Hg), and T is the temperature of the air (in Kelvin). Prior to using Eq. (2), the vapor pressure was calculated from the saturation vapor pressure ($e_s(T)$), as given in Eq. (3), where $e_s(T)$ is in units of kPa [14,15].

$$e_s(T) = 0.61078 e^{[17.27(T-273.16)/(T-35.86)]} \qquad (3)$$

The result of Eq. (3) was then combined with the percent relative humidity (RH, in %) through the use of Eq. (4) to give the vapor pressure (h) in units of mm Hg [16].

$$h = \frac{760}{101.325} e_s(T) \frac{\mathrm{RH}}{100} \qquad (4)$$

The resulting value of h was then placed into Eq. (2) to determine the density of air at the time of the mass measurement.

The density of each phosphate buffer was measured in replicate ($n = 5$) at each calibrated temperature using the same weighing procedure and correction for buoyancy effects as described for calibration of the pycnometer. One change required in this experiment was the need to substitute the actual and measured masses for the phosphate buffer (m_{KPB} and w_{KPB}) for the actual and measured masses of water ($m\mathrm{H_2O}$ and $w\mathrm{H_2O}$) in Eq. (1). Another change required was the use of ρ_{KPB} in place of $\rho_{\mathrm{H_2O}}$ in Eq. (1), which was then further replaced with $m_{\mathrm{KPB}}/V_{\mathrm{pyc}}$, where V_{pyc} is the calibrated volume of the pycnometer at the temperature of the experiment. When these substitutions were made in Eq. (1), the following expression for determining the true mass of phosphate buffer contained in the pycnometer was obtained.

$$m_{\mathrm{KPB}} = w_{\mathrm{KPB}}\left[1 - \frac{\rho_{\mathrm{air}}}{\rho_{\mathrm{ref}}}\right] + \rho_{\mathrm{air}} V_{\mathrm{pyc}} \qquad (5)$$

This true mass was then used along with the calibrated volume of the pycnometer to give the density of the phosphate buffer at the desired temperature, $m_{\mathrm{KPB}}/V_{\mathrm{pyc}}$.

3. Results and discussion

3.1. Changes in phosphate buffer density with temperature

Table 1 shows the average densities measured for pH 7.4, 0.067 M potassium phosphate buffer at temperatures ranging 4–45 °C. Also provided in this table is the 95% confidence interval for each density value. As shown by these data, all of the densities were measured to at least five significant figures, with relative precisions of ±0.0019–0.022%. The results indicate that the density of pH 7.4, 0.067 M potassium phosphate buffer differs by over 1% as the temperature is varied from 4 to 45 °C. Thus, depending on the temperatures of preparation and use for the buffer, variations in these temperatures can have a measurable effect on the buffer's density.

A plot of the density of pH 7.4, 0.067 M phosphate buffer versus temperature is shown in Fig. 1. A similar plot for pure water is included for reference, as prepared according to literature values [12]. These two plots are similar in shape, with potassium phosphate buffer having a higher density than water at any given temperature. The size of this difference was 0.0063–0.0096 g cm^{-3} (or 0.6–1.0%) at 4–45 °C. The plots in Fig. 1 for both potassium phosphate buffer and water gave a good fit to a quadratic equation, with correlation coefficients of 0.9985 (n = 9) and 0.9997 (n = 40), respectively. The best-fit parameters for these plots are given in the legend of Fig. 1. The errors between the experimental densities and those predicted by these best-fit equations were 0.0002–0.07%, with an average relative error of only 0.019%.

Table 1
Experimental densities and calculated concentrations of pH 7.4, 0.067 M potassium phosphate buffer prepared at 25 °C

Temperature (°C)	Density of buffer (g cm^{-3})[a]	Buffer conc. (M) vs. 0.067 M at 25°C[b]
4	1.00959 (± 0.00020)	0.0672257
10	1.00918 (± 0.00015)	0.0671984
15	1.00854 (± 0.00007)	0.0671558
20	1.00739 (± 0.00007)	0.0670792
25	1.00620 (± 0.00002)	0.0670000
30	1.00459 (± 0.00003)	0.0668927
35	1.00317 (± 0.00010)	0.0667982
40	1.00150 (± 0.00011)	0.0666870
45	0.99855 (± 0.00026)	0.0664908

[a] The value in parentheses for each density is the 95% confidence interval for five replicate measurements.

[b] These values show how a 0.067 M buffer prepared at 25 °C would change in its concentration if the buffer were later used at another temperature.

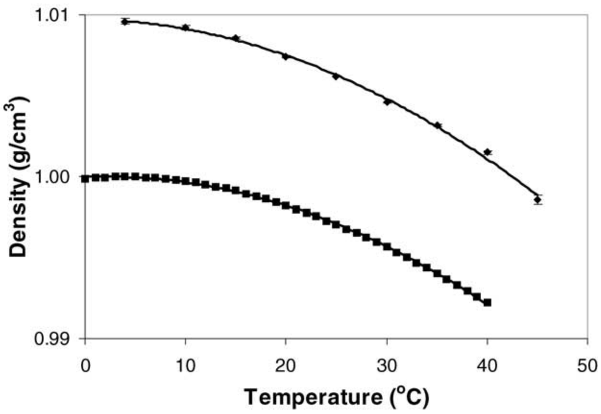

Fig. 1. Density of pH 7.4, 0.067 M potassium phosphate buffer vs. temperature (♦). The best-fit quadratic line to these results was $y = -5.2037 \times 10^{-6}x^2 - 8.1463 \times 10^{-6}x + 1.0097$, with a correlation coefficient of 0.9985. The error bars represent the 95% confidence interval for n = 5. Also shown is a plot of density vs. temperature for pure water (■) based on values obtained from Ref. [12]. This second graph was described by the quadratic equation $y = -5.4180 \times 10^{-6}x^2 + 2.0174 \times 10^{-5}x + 1.0000$, with a correlation coefficient of 0.9997.

It is often assumed in calculations involving dilute phosphate buffers that these solutions have densities similar to that of water. However, Fig. 1 indicates that this assumption gives rise to a systematic error of 1% for 0.067 M potassium phosphate buffer (i.e., a buffer often used in biointeraction studies). In addition, Fig. 1 shows that the change in density versus temperature for phosphate buffer is similar to, but not strictly parallel to, the change in density seen with water. This makes the use of water's density in place of the true density of phosphate buffer even less valid when such a buffer is to be used at a variety of temperatures.

If the density of phosphate buffer is known at a given temperature, the fact that volume (V) is inversely proportional to density (ρ) can be used to determine the corresponding change in the buffer's concentration. For instance, using the fact that $V_1/V_2 = \rho_2/\rho_1$ for the same buffer at two temperatures, it is possible to relate the concentrations M_1 and M_2 for this buffer at temperatures T_1 and T_2 by using Eq. (6).

$$M_1 \frac{\rho_2}{\rho_1} = M_2 \qquad (6)$$

Thus, if the concentration and density of the buffer is known at the first temperature and its density is known at the second, the buffer's concentration at the second temperature can also be obtained. The same relative change in concentration occurs for any solutes dissolved in this buffer.

To illustrate this last effect, Table 1 shows the expected change in concentration with temperature for a pH 7.4, 0.067 M potassium phosphate buffer that is originally prepared at 25 °C. As these results show, a change in temperature from 25 to 4 °C alters the buffer concentration by 0.3%, while a change in temperature from 25 to 45 °C alters the concentration by 0.8%. Thus, this is another possible source

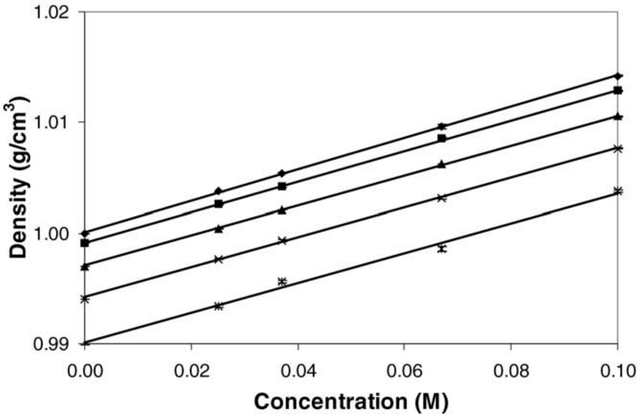

Fig. 2. Density of pH 7.4 potassium phosphate buffer vs. buffer concentration at 4 °C (♦), 15 °C (■), 25 °C (▲), 35 °C (×), and 45 °C (✳). Each of these densities was determined with a precision of at least ±0.0002 g cm^{-3}.

of systematic errors if such a buffer is prepared and used at different temperatures. Although this effect can be ignored in low precision measurements of binding constants or concentrations, it is necessary to consider this if high precision values with accuracies of 1% or better are desired.

3.2. Changes in phosphate buffer density with concentration

Fig. 2 shows how the density of pH 7.4 potassium phosphate buffer changes with its concentration at several temperatures. These densities were again determined with a precision of at least ±0.0002 g cm^{-3}. For all temperatures considered, a linear relationship was seen between the density and concentration of phosphate buffer from 0.0 to 0.10 M. All plots in Fig. 2 had correlation coefficients of 0.9970–0.9999 over five data points. The best-fit parameters for these equations are provided in Table 2. The standard error for each slope ranged from ±0.0007 to 0.0060 g cm^{-3} M^{-1} and the standard error for each intercept ranged from ±4.25 × 10^{-5} to 0.0003 g cm^{-3}.

The slopes for all the lines in Fig. 2 were statistically identical. The standard deviation for the mean of these slopes was ±0.001 g cm^{-3} M^{-1} and the relative standard deviation for the mean of these slopes was ±1%. This indicates that the change in density with concentration followed the same general pattern at all of the temperatures examined in this study. Furthermore, it is possible to use the best-fit equations in Table 2 to predict the density of potassium phosphate buffer at various concentrations over this temperature range. When the values predicted by these equations were compared to the experimental densities in Fig. 2, the relative errors ranged 0.0003–0.06%, with an average relative error of only 0.012%.

Table 2
Best-fit equations for the linear plots of density vs. buffer concentration[a]

Temperature (°C)	Best-fit line	Correlation coefficient, *r*
4	$y = 0.142x + 1.0001$	0.9998
15	$y = 0.138x + 0.9991$	0.9995
25	$y = 0.135x + 0.9971$	0.9999
35	$y = 0.135x + 0.9942$	0.9996
45	$y = 0.135x + 0.9901$	0.9970

[a] These best-fit lines were obtained for the data shown in Fig. 2.

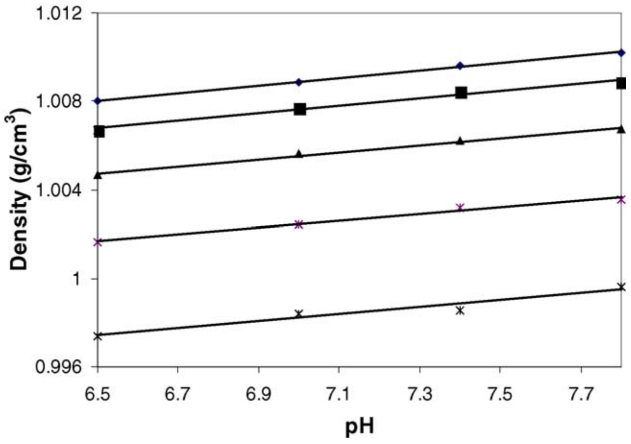

Fig. 3. Density of 0.067 M potassium phosphate buffer vs. pH at 4 °C (♦), 15 °C (■), 25 °C (▲), 35 °C (×) and 45 °C (✳). Each of these densities was determined with a precision of at least ±0.0002 g cm^{-3}.

3.3. Changes in phosphate buffer density with pH

Fig. 3 illustrates how the density of 0.067 M potassium buffer changed with pH at 4 to 45 °C. The precision in all of these measured densities was ±0.0002 g cm^{-3} or better. A linear relationship was again observed at each temperature for buffers in the pH range of 6.5–7.8. These lines gave correlation coefficients of 0.9711 to 0.9993 when using the best-fit parameters shown in Table 3. The standard error for each slope ranged ±4.0 × 10^{-5}–0.0002 g cm^{-3} M^{-1} and the standard error for each intercept ranged ±0.0003–0.002 g cm^{-3}. Over this pH range, the predicted values for density (i.e., as determined from the best-fit lines) had relative errors of 0.001–0.03%, with an average relative error of 0.010%.

The slopes for the lines in Fig. 3 were again quite similar, with a standard deviation for the mean of these five slopes of only ±3.8 × 10^{-5}. The relative standard deviation for the mean of these slopes was ±2.3%. From these results, it was found that the slope of these lines, and therefore the change in phosphate buffer density versus pH, was approximately the same at all of the sampled temperatures.

Table 3
Best-fit equations for the linear plots of density vs. buffer pH[a]

Temperature (°C)	Best-fit line	Correlation coefficient, *r*
4	$y = 0.0017x + 0.997$	0.9993
15	$y = 0.0017x + 0.996$	0.9903
25	$y = 0.0016x + 0.994$	0.9951
35	$y = 0.0015x + 0.992$	0.9941
45	$y = 0.0016x + 0.987$	0.9711

[a] These best-fit lines were obtained for the data shown in Fig. 3.

3.4. *Global predictions of phosphate buffer density*

Since the density of potassium phosphate buffer was found to be linear with respect to concentration and pH and the slopes of these lines were constant over the range of conditions examined in this study, it was possible to develop a master equation to estimate the density of such a buffer under these conditions. This was performed for buffer concentrations of 0–0.10 M, temperatures of 4–45 °C, and pH values of 6.5–7.8. This was accomplished by using the fact that both the plots for water and pH 7.4, 0.067 M potassium phosphate buffer in Fig. 1 gave a response that could be described by a quadratic equation. If the three coefficients for these plots are referred to as A, B, and C in the equation for phosphate buffer and A′, B′, and C′ for water, the difference between the density of the phosphate buffer and that for water is given by Eq. (7)

$$\mathrm{d}\rho = (A - A')T^2 + (B - B')T + (C - C') \qquad (7)$$

where T is the temperature (in °C) and $\mathrm{d}\rho$ is the difference in density between potassium phosphate buffer and water.

As shown in Tables 2 and 3, the density of potassium phosphate buffer gives a linear change with pH and concentration. It is also known that the slopes obtained for buffer density versus concentration or pH is essentially constant over the temperature range examined in this study. This allows a more general version of Eq. (7) to be developed in which the difference in density between water and a phosphate buffer with a pH other than 7.4 and a concentration other than 0.067 M can be determined. The result is shown in Eq. (8)

$$\mathrm{d}\rho = [(A - A')T^2 + (B - B')T + (C - C')]\frac{[\text{phosphate}]}{0.067}\,\frac{\text{pH}}{7.4} \qquad (8)$$

If the response for the density of pure water is added to Eq. (8) and the best-fit values from Fig. 1 are substituted in for A, B, C, A′, B′, and C′, a general expression describing the density of potassium phosphate buffer over a broad range of conditions is obtained, as shown in Eq. (9).

$$\rho_{\text{KPB}} = (-5.0 \times 10^{-6}T^2 + 2.0 \times 10^{-5}T + 1) + [-2.8 \times 10^{-5}T + 0.0097]\frac{[\text{phosphate}]}{0.067}\,\frac{\text{pH}}{7.4} \qquad (9)$$

To test the use of Eq. (9), the experimental densities measured in this study for potassium phosphate buffer were compared to those predicted by this general relationship. This yielded calculated densities with relative errors of only 0.0002–0.08% versus the actual values, with an average error of 0.025%. Thus, Eq. (9) was found to be a useful tool (with an accuracy of 0.08% or better) in estimating the density of potassium phosphate buffer throughout the range of temperatures, pH values, and buffer concentrations considered in this work.

A previous equation has been reported by Sohnel and Novotny to describe the effect of temperature and concentration on the density of potassium dihydrogen phosphate in aqueous solutions [10]. However, their study extended down to only a 2% solution, which is higher than the concentrations examined in this study (i.e., 1.7% or less). In addition, their equation did not cover solutions that contained a combination of the dibasic and monobasic salts of potassium phosphate [10].

The results predicted by this previous equation were compared directly to the experimental data obtained in this current study. This alternative equation always gave low estimates for the buffer densities, with relative errors in the range of 0.2–0.5% for the data shown in Fig. 1 and 0.1–0.5% for the data in Fig. 2. This is a significant difference when it is considered that the density of these buffers differs from water by only 1%. This level of error is much greater than that obtained when using Eq. (9) or the best-fit expressions in the legend of Fig. 1 or in Tables 2 and 3. Thus, it was determined that the equations developed in this current report gave a much more accurate description of the densities for common phosphate buffers and in determining the effects of temperature and concentration on these densities.

4. Conclusions

The densities of potassium phosphate buffers at or near a physiological pH were measured at temperatures ranging 4–45 °C with a precision of at least $\pm 0.0002\ \mathrm{g\,cm^{-3}}$. The general change in density versus temperature for these buffers followed a quadratic equation, while the changes in density with concentration and pH followed a linear response.

The results of this study have shown that temperature variations can have measurable effects on the density and concentration of a potassium phosphate buffer. These changes, in turn, may affect the binding of solutes to ligands in such buffers, leading to small errors in such experiments. For instance, the assumption that 0.067 M potassium phosphate buffer has the same density as water was found to lead to a 1% error. In addition, temperature effects on buffer density and concentration gave an error as high as 0.8% when going from 25 to 45 °C. This change in buffer density will not only affect its concentration, but will also alter the ionic strength of the solution and the concentrations of its dissolved solutes.

The data obtained in this work made it possible to develop a general equation for calculating the density of potassium phosphate buffer under a wide range of conditions seen in binding studies. This expression was found to have much greater accuracy than a previous equation developed for potassium dihydrogen phosphate [10] when describing the density of such buffers. It was also shown how knowledge of these density changes could be used to determine the resulting concentration changes in the buffer and its contents. Such a correction is necessary when the error in buffer and solute concentration must be less than 1%. This, in turn, should lead to greater accuracy in the use of such methods as equilibrium dialysis, HPLC and ACE in examining the binding of biological compounds in phosphate buffers.

Acknowledgements

This work was supported by the National Institutes of Health under grant RO1 GM44931. J.E. Schiel was supported by the University of Nebraska UCARE program. The authors thank C. Ohnmacht and R. Albro for their advice and help during this project.

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