Note

---

pK* Values of phosphate buffer components in methanol–water reversed-phase eluents

A. LEITOLD and Gy. VIGH*

Institute for Analytical Chemistry, University of Chemical Engineering, Veszprém (Hungary)

(Received November 1st, 1982)

Phosphate buffers are the workhorse of reversed-phase liquid chromatography (RPLC) and ion-pair RPLC. Aqueous methanol eluents are used in the majority of these cases. The separations often depend on the balance of solute pK and eluent pH, *e.g.*, in ionization-controlled RPLC[1–3].

pH is generally reported either as the pH of the aqueous buffer component before it is mixed with methanol, or as the operational pH of the hydroorganic eluent as measured by a glass electrode which is standardized with aqueous buffer solutions, *e.g.*, ref. 2. Generally, the latter approach is followed when the eluent pH has to be finely adjusted. Eluent preparation could be greatly simplified if the thermodynamically meaningful protonation constants, pK, of the buffer components in various hydroorganic eluents were known. At present, such pK values are very scarce. Paabe *et al.*[4] reported the pK values of HPO_4^{2-} for a number of methanol–water mixtures. However, the corresponding values of $H_2PO_4^-$ under chromatographically relevant conditions are not available. The purpose of this communication is to present the titrimetrically determined pK of $H_2PO_4^-$ as a function of the methanol concentration in various methanol–water binary mixtures.

Bates and co-workers[4–7] have comprehensively discussed the meaning and determination of pH in mixed solvents, and Karger[8] has reviewed the main principles. For details the reader is referred to these publications and references therein. Essentially, a thermodynamically meaningful pH value in mixed media, pH_x^*, is defined as

$$pH_x^* = pH_{st} + \frac{E_x^* - E_{st}}{0.059} + \frac{E_{j,x}^* - E_{j,st}}{0.059}$$

where pH_x^* is the hydrogen ion-activity-based pH value referenced to the mixed solvent, pH_{st} is the assigned pH of an aqueous standard buffer solution, E_x^* and E_{st} are the e.m.f. values measured in the mixed solvent and the aqueous standard buffer, respectively, and $E_{j,x}^*$ and $E_{j,st}$ are the liquid junction potentials in the mixed solvent and the aqueous standard buffer, respectively. The last term is called the correction factor, δ, which is available for methanol–water systems[7,8]. The sum of the first two terms is what we obtain with a pH meter and a glass electrode calibrated with aqueous buffer solutions. It is often called pH_x^{app}, the apparent pH of the mixed medium.

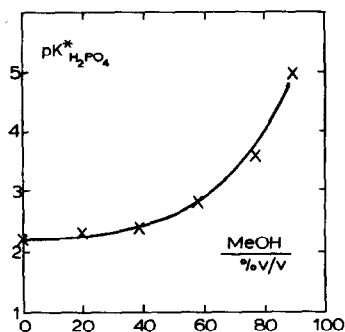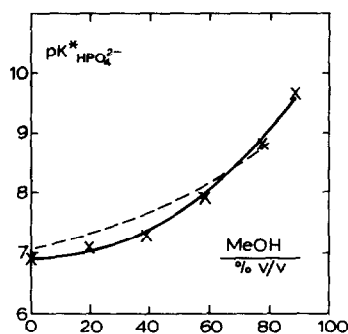Fig. 1. pK^* of $H_2PO_4^-$ as a function of the % (v/v) methanol (MeOH) concentration in methanol–water mixtures.

Fig. 2. pK^* of HPO_4^{2-} as a function of the %(v/v) methanol (McOH) concentration in methanol–water mixtures. The broken line represents data from ref. 4.

Albert and Serjeant[9] discussed the various methods used for the determination of pK values and gave detailed instructions to insure reliability of the determined values. Their method and equipment as given in Chs. 2–4 of ref. 9 were used.

Potentiometric titrations were carried out in a thermostatted vessel at 25.0°C purged with carbon dioxide-free nitrogen. Carbonate-free potassium hydroxide solutions (0.05 M) were prepared with the various methanol–water mixtures tested. Calculated amounts of 1 M KOH solutions were weighed into a 100-ml volumetric flask. Weighed amounts of methanol were added to the flask, and the volume was then brought almost to the mark with distilled water. The solution was equilibrated at 25.0°C and the final drops of water were added. Then the flask was reweighed. Thus % (w/w), mole per cent, % (v/v) and % (w/v) concentrations could be calculated.

The solutions tested are listed in Table I.

0.005 M H_3PO_4 solutions were similarly prepared.

A calibrated 12-ml Schellbach burette and an OP-208/1 precision digital pH meter (Radelkis, Budapest, Hungary) equipped with a combined glass electrode was used. Three parallel titrations were carried out, but the precision advocated by Albert

TABLE I

COMPOSITION OF THE METHANOL–WATER MIXTURES USED IN THE TITRATION EXPERIMENTS, AND THE pK^* VALUES

Methanol concentration	No. of solution					
	1	2	3	4	5	6
% (w/w)	0	15.44	31.77	49.37	69.25	83.68
mole per cent	0	9.32	20.80	35.58	56.17	74.17
% (v/v)	0	19.01	38.02	57.03	76.04	88.72
% (w/v)	0	15	30	45	60	70
pK^* of $H_2PO_4^-$	2.2	2.3	2.4	2.8	3.6	4.9
pK^* of HPO_4^{2-}	6.9	7.1	7.3	7.9	8.8	9.6

and Serjeant[9] could not be achieved in each case, so we do not claim the accuracy of the pK^* values to be better than ± 0.2 pH units.

Calculations were carried out with an iterative program[9] using a PTK-1096 programmable calculator (Hiradástechnika KTSZ, Budapest, Hungary). The pH values used in the calculations were corrected pH_x^* values.

The calculated pK^* values are listed in Table I. As an example, the pK^* values of $H_2PO_4^-$ and HPO_4^{2-} are plotted in Figs. 1 and 2 against the % (v/v) concentration of methanol. In Fig. 2 the pK^* values of HPO_4^{2-} as determined by Paabe et al.[4] are also shown for comparison. The pK^* of acetate and succinate ions as a function of the methanol concentration of binary methanol–water mixtures changed in a similar manner[4].

The pK^* values thus obtained allow the direct calculation of the buffer compositions in methanol–water eluents with thermodynamically meaningful pH_x^* values.

REFERENCES

1 Cs. Horváth, W. Melander and J. Molnár, *Anal. Chem.*, 49 (1977) 142.
2 J. L. M. Van de Venne, J. L. H. M. Hendrikx and R. S. Deelder, *J. Chromatogr.*, 167 (1978) 1.
3 Gy. Vigh, Z. Varga-Puchony, Á. Bartha and S. Balogh, *J. Chromatogr.*, 241 (1982) 169.
4 M. Paabe, R. A. Robinson and R. G. Bates, *J. Amer. Chem. Soc.*, 87 (1965) 415.
5 R. G. Bates, *Determination of pH. Theory and Practice*, Wiley-Interscience, New York, 1973.
6 R. G. Bates, *CRC Crit. Rev. Anal. Chem.*, 10 (1981) 247.
7 R. G. Bates, M. Paabe and R. A. Robinson, *J. Phys. Chem.*, 67 (1963) 1833.
8 B. L. Karger, in Cs. Horváth (Editor), *High Performance Liquid Chromatography*, Vol. 1, Academic Press. New York, 1980, p. 113.
9 A. Albert and E. P. Serjeant, *The Determination of Ionization Constants*, Chapman and Hall, London, 1972.