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Retention of ionisable compounds on high-performance liquid chromatography XVIII: pH variation in mobile phases containing formic acid, piperazine, tris, boric acid or carbonate as buffering systems and acetonitrile as organic modifier

Xavier Subirats, Elisabeth Bosch, Martí Rosés*

Departament de Química Analítica, Universitat de Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain

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ABSTRACT

In the present work dissociation constants of commonly used buffering species, formic acid, piperazine, tris(hydroxymethyl)–aminomethane, boric acid and carbonate, have been determined for several acetonitrile–water mixtures. From these pK_a values a previous model has been successfully evaluated to estimate pH values in acetonitrile–aqueous buffer mobile phases from the aqueous pH and concentration of the above mentioned buffers up to 60% of acetonitrile, and aqueous buffer concentrations between 0.005 (0.001 mol L^{-1} for formic acid–formate) and 0.1 mol L^{-1}. The relationships derived for the presently studied buffers, together with those established for previously considered buffering systems, allow a general prediction of the pH variation of the most commonly used HPLC buffers when the composition of the acetonitrile–water mobile phase changes during the chromatographic process, such as in gradient elution. Thus, they are an interesting tool that can be easily implemented in general retention models to predict retention of acid–base analytes and optimize chromatographic separations.

1. Introduction

pH in mobile phases plays an outstanding role in the chromatographic retention of analytes with acid/base properties, because it affects the ionisation degree of the ionisable analytes. In fact, slight variations in the mobile phase pH when it is close to the pK_a of the analytes, may cause notable changes in retention times [1–5]. In previous works a model has been developed to estimate the pH variation in acetonitrile– [6,7] and methanol–water [8] buffered mobile phases from the aqueous pH value and concentration of the buffer, and from the organic solvent content of the mixture. The buffering systems studied in these works were acetic, phosphoric and citric acids, and ammonia. In the present work this model has been extended to acetonitrile–water mobile phases containing formic acid, piperazine, tris(hydroxymethyl)–aminomethane, boric acid or carbonate as buffering systems.

Our aim is set up relationships between pH, on one hand, and buffer concentration and solvent composition, on the other hand, for the most common HPLC buffers. The ultimate purpose is to implement these relationships in general models predicting retention of acid–base analytes from mobile phase composition and pH [9,10] which can be useful to optimize chromatographic separations. We think that our equations can be especially useful when mobile phase composition, and thus buffer pH changes during the elution process, such as in gradient elution.

2. Experimental

2.1. Apparatus

Potentiometric measurements were taken with a Crison (Barcelona, Spain) 5014 combination electrode (glass electrode and a reference electrode with a 3.0 mol L^{-1} KCl solution in water as salt bridge) in a Crison GLP22 pH meter with a precision of ±0.1 mV (±0.002 pH unit). All solutions were thermostated externally at 25 ± 0.1 °C. Titrations were carried out using an autoburette Metrohm (Herisau, Switzerland) 665 Dosimat.

2.2. Chemicals

Acetonitrile was RP HPLC gradient grade from Merck (Darmstadt, Germany) and water purified by the Milli-Q plus system (to 18 MΩ) from Millipore (Bedford, MA, USA). The studied buffers were prepared from tris(hydroxymethyl)aminomethane (Aldrich, Steinheim, Germany; 99.9+%), piperazine anhydrous (Fluka, The Netherlands; ≥99%), sodium carbonate (Merck, for analysis), formic

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[*] Corresponding author. Tel.: +34 93 403 92 75; fax: +34 93 402 12 33.
E-mail address: marti.roses@ub.edu (M. Rosés).

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acid (Baker, Deventer, Holland; 98–100%) and boric acid (Fluka, Buchs, Switzerland; ≥99%). Sodium carbonate was dried in a muffle at 270 °C for 2 h before use. Titrating solutions were prepared from hydrochloric acid 1 mol L^{-1} (Merck, Darmstadt, Germany, Titrisol®) and potassium hydroxide 1 mol L^{-1} (Merck, Darmstadt, Germany, Titrisol®), and they were prepared just before use. Titration solutions were standardized using potassium hydrogenphthalate (Probus, Barcelona, Spain; ≥99%) and tris(hydroxymethyl)aminomethane. These substances were dried in the oven for 1 h at 110 °C just before use. Hydrochloric acid (Merck, 25%, for analysis) and potassium hydroxide (Panreac, for analysis) were used to adjust the pH to the wanted value when it was necessary.

2.3. *Procedure*

2.3.1. *pK_a determination of the buffering species*

Carbonate, tris and piperazine solutions of concentration 5×10^{-3} mol L^{-1} were prepared for the studied acetonitrile–water compositions (0, 20, 40 and 60% in volume) and titrated with hydrochloric acid solution of concentration 0.1 mol L^{-1}. The same procedure was used for formic and boric acids, which were titrated with potassium hydroxide solution. The solution containing the buffer was under argon atmosphere to avoid CO_2 uptake from laboratory air. Room temperature was controlled and fixed to 20 °C. Molarity concentration scale was employed and solvent volume contraction when acetonitrile was added to the aqueous solutions was taken into consideration [11]. The electrode system was calibrated using the usual aqueous standard reference buffers of potassium hydrogenphthalate (pH 4.01 at 25 °C) and potassium dihydrogenphosphate–disodium hydrogenphosphate (${}_{w}^{w}\mathrm{pH}$ 7.00 at 25 °C). All buffer solutions were thermostated externally at 25 ± 0.1 °C and titrated with potassium hydroxide or hydrochloric acid solutions of the same solvent composition of the buffer solutions. All pH readings were done in the hydroorganic mixture and thus were obtained in the ${}_{w}^{s}\mathrm{pH}$ scale [12]. Titrations were performed automatically by the Valora program [13], which controls the burette and records the readings of the potentiometer. A pH reading was considered to be stable when pH variation within a minute was lower than 0.005 units. Electrode system was calibrated after each titration, and potential values corresponding to standard buffers were controlled to guarantee the glass electrode good condition.

Once obtained the titration curve, the equivalence point was calculated through the first derivative and confirmed through a home-made program called Ona implemented in Matlab [14].

2.3.2. *Experimental testing of the model*

The required aqueous acid and base concentrations for the selected pH was calculated to provide a total buffer aqueous concentrations of 0.001, 0.005, 0.01, 0.05 and 0.1 mol L^{-1}. If necessary, the pH was finally adjusted by addition of small amounts of concentrated solutions of potassium hydroxide or hydrochloric acid. Acetonitrile–water buffers were prepared by addition of the organic solvent to the aqueous buffers using an autoburette (10, 20, 30, 40, 50 and 60% in volume). In all instances, the electrode system was calibrated using the usual aqueous standard reference buffers of pH 4 and pH 7. All pH readings were done in the ${}_{w}^{s}\mathrm{pH}$ scale, i.e. after mixing aqueous buffer with acetonitrile. All buffer solutions were thermostated externally at 25 ± 0.1 °C.

Table 1

Properties of relevant interest for pH measurements in acetonitrile–water mixtures at 25 °C [6].

%MeCN (v/v)	x_{MeCN}	A	a_0B	${}_{s}^{s}\mathrm{p}K_{ap}$	δ
0	0.000	0.528	1.52	14.00	0.00
10	0.040	0.566	1.55	14.24	–0.01
20	0.079	0.604	1.59	14.47	–0.03
30	0.130	0.655	1.63	14.74	–0.04
40	0.186	0.712	1.68	15.08	–0.14
50	0.260	0.791	1.74	15.48	–0.22
60	0.339	0.877	1.80	15.90	–0.46

x_{MeCN}: molar fraction of acetonitrile in the mixture; A and a_0B: Debye–Hückel equation parameters; ${}_{w}^{s}\mathrm{p}K_{ap}$: autoprotolysis constant of the solvent mixture; δ: interconversion parameter between ${}_{s}^{s}\mathrm{pH}$ and ${}_{w}^{s}\mathrm{pH}$ scales.

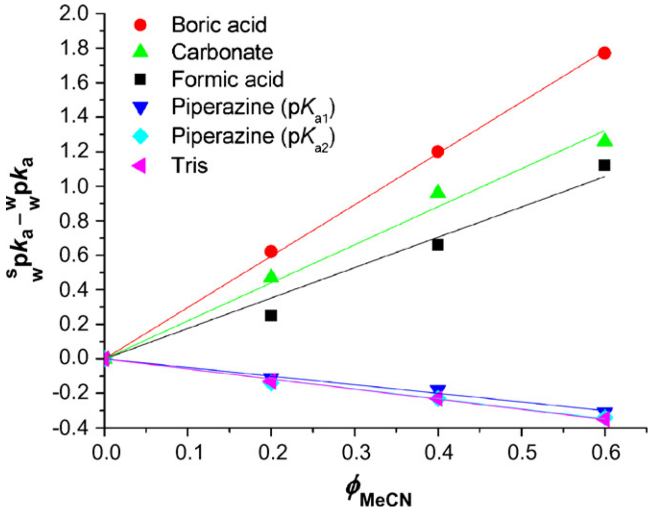

Fig. 1. Variation of the pK_a values (${}_{w}^{s}\mathrm{p}K_a - {}_{w}^{w}\mathrm{p}K_a$) of buffer components with the addition of acetonitrile: (●) boric acid–borate ($m_{pK} = 2.98$), (▲) hydrogencarbonate–carbonate (2.20), (■) formic acid–formate (1.76), (▼) piperazine pK_{a1} (–0.50), (◆) piperazine pK_{a2} (–0.58), and (◀) tris(hydroxymethyl)aminomethane (–0.59).

3. Results and discussion

3.1. *pK_a variation of the buffering species in acetonitrile–water mixtures*

The dissociation constants of formic acid, piperazine, tris–(hydroxymethyl)aminomethane, boric acid and carbonate at 0, 20, 40 and 60% in volume of acetonitrile were determined

Table 2

Determined pK_a values for the studied buffers in acetonitrile–water mixtures with their associate standard error (S.E.).

Buffering system	0%		20%			40%			60%		
	${}_{w}^{s}\mathrm{p}K_a = {}_{s}^{s}\mathrm{p}K_a$	S.E.	${}_{w}^{s}\mathrm{p}K_a$	${}_{s}^{s}\mathrm{p}K_a$	S.E.	${}_{w}^{s}\mathrm{p}K_a$	${}_{s}^{s}\mathrm{p}K_a$	S.E.	${}_{w}^{s}\mathrm{p}K_a$	${}_{s}^{s}\mathrm{p}K_a$	S.E.
Formic acid–formate	3.72	0.02	3.96	3.99	0.03	4.40	4.54	0.02	4.87	5.33	0.01
Piperazine (pK_{a1})	5.37	0.01	5.26	5.29	0.01	5.19	5.33	0.05	5.06	5.52	0.04
Piperazine (pK_{a2})	9.76	0.00	9.62	9.65	0.02	9.53	9.67	0.03	9.42	9.88	0.04
Tris(hydroxymethyl)aminomethane	8.08	0.01	7.94	7.97	0.04	7.85	7.99	0.03	7.72	8.18	0.05
Boric acid–borate	9.23	0.01	9.85	9.88	0.02	10.43	10.57	0.04	11.00	11.46	0.03
Hydrogencarbonate–carbonate	10.35	0.02	10.82	10.85	0.03	11.31	11.45	0.02	11.62	12.08	0.04

Acetonitrile content in volume, zero ionic strength, 25 °C.