



Short communication

Retention of ionisable compounds on high-performance liquid chromatography XIX. pH variation in mobile phases containing formic acid, piperazine and tris as buffering systems and methanol as organic modifier

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ARTICLE INFO

Article history:

Received 12 March 2009

Received in revised form 6 May 2009

Accepted 14 May 2009

Available online 21 May 2009

Keywords:

Mobile phase composition

Methanol–water mixtures

pH

Buffers

Chromatographic retention

ABSTRACT

In previous works a model to estimate the pH of methanol–aqueous buffer mobile phases from the aqueous pH and concentration of the buffer and the fraction of organic modifier was developed. This model was successfully applied and validated for buffers prepared from ammonia, acetic, phosphoric and citric acids. In the present communication this model has been extended to formic acid, piperazine and tris(hydroxymethyl)aminomethane buffers. Prior to the modelling work, the pK_a values of the studied buffers at several methanol–water compositions were determined.

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1. Introduction

It is well known that the pH of the mobile phase, is a determining factor in the retention of ionisable analytes, and it plays a well defined role in general models that estimate retention as a tool to optimize chromatographic separations [1,2]. With the aim of providing a useful tool to estimate the pH in methanolic mobile phases throughout the elution process, a model for buffers prepared from ammonia, acetic, phosphoric and citric acids was developed [3]. In this communication this model has been successfully extended and evaluated for three new buffers: formic acid, piperazine and tris(hydroxymethyl)aminomethane (tris).

With the present work we conclude a systematic study of the pH variation of chromatographic mobile phases for commonly used buffers with the addition of methanol or acetonitrile as organic modifiers. The objective of this extensive work has been to provide reliable pH values to retention models dealing with ionisable analytes, which can be very useful when working in gradient elution mode, because of the change in the mobile phase composition during the analysis.

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 pHmeter with a precision of $\pm 0.1 \text{ mV}$ ($\pm 0.002 \text{ pH unit}$). All solutions were thermostated externally at $25 \pm 0.1 \text{ }^\circ\text{C}$. Titrations were carried out using an autoburette Metrohm (Herisau, Switzerland) 665 Dosimat.

2.2. Chemicals

Methanol was RP HPLC gradient grade from Merck (Darmstadt, Germany) and water purified by the Milli-Q plus system (to $18 \text{ M}\Omega$) from Millipore (Bedford, MA, USA). The studied buffers were prepared from tris(hydroxymethyl)aminomethane (Aldrich, Steinheim, Germany, 99.9+%), piperazine anhydrous (Fluka, The Netherlands, $\geq 99\%$) and formic acid (Baker, Deventer, Holland, 98–100%). 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, $\geq 99\%$) and tris(hydroxymethyl)aminomethane. These sub-

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