

Retention of ionisable compounds on high-performance liquid chromatography XVII

Estimation of the pH variation of aqueous buffers with the change of the methanol fraction of the mobile phase

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Abstract

The use of methanol–aqueous buffer mobile phases in HPLC is a common election when performing chromatographic separations of ionisable analytes. The addition of methanol to the aqueous buffer to prepare such a mobile phase changes the buffer capacity and the pH of the solution. In the present work, the variation of these buffer properties is studied for acetic acid–acetate, phosphoric acid–dihydrogenphosphate–hydrogenphosphate, citric acid–dihydrogencitrate–hydrogencitrate–citrate, and ammonium–ammonia buffers. It is well established that the pH change of the buffers depends on the initial concentration and aqueous pH of the buffer, on the percentage of methanol added, and on the particular buffer used. The proposed equations allow the pH estimation of methanol–water buffered mobile phases up to 80% in volume of organic modifier from initial aqueous buffer pH and buffer concentration (before adding methanol) between 0.001 and 0.01 mol L⁻¹. From both the estimated pH values of the mobile phase and the estimated pK_a of the ionisable analytes, it is possible to predict the degree of ionisation of the analytes and therefore, the interpretation of acid–base analytes behaviour in a particular methanol–water buffered mobile phase.

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1. Introduction

Chromatographic retention of acid–base analytes in reversed phase high performance liquid chromatography (RP-HPLC) depends on the hydrophobicity of the analytes and on their ionisation degree, which in turn depends on the pH and analyte acid–base constant (pK_a) in the particular mobile phase used. The effect of both the pH and the pK_a on ionisation degree and therefore, on retention times in HPLC has been already extensively reported [1–22]. To achieve reproducible and successful chromatographic analysis, a careful control and accurate measurement of pH is essential. We recommend the measurement of pH in the hydroorganic mobile phase, rather than in the aqueous buffer, because the pH variation when adding an organic modifier depends on the particular buffering system,

on its concentration and on the fraction of organic solvent in the mixture [2,3,7,8,10,12–14]. When the measurement of pH in the mobile phase is not easy, e.g. in the case of highly automated HPLC experiments where independent reservoirs of buffer and organic solvent are pumped into and mixed within the apparatus, it may be very useful to estimate the pH variation for a particular buffer when the organic modifier is added. This pH modelling may also be useful to provide the chromatographers with a buffered mobile phase adequate to solve a particular separation problem. This is useful in case of mixtures of analytes with similar acid–base properties, because without performing any measurement it is possible to predict the particular composition of the mobile phase in which the differences on ionisation degree between the analytes are significant enough. This *a priori* optimization could avoid fruitless time and reagent consuming experiments. On the basis of previous works, on pH estimation in acetonitrile–aqueous buffer mobile phases [11,18], we present in this paper a model developed for the pH variation of the most commonly used buffers in methanol–water mobile phases. From

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