

Retention of ionisable compounds on high-performance liquid chromatography

XV. Estimation of the pH variation of aqueous buffers with the change of the acetonitrile fraction of the mobile phase

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Abstract

The most commonly used mobile phases in reversed-phase high-performance liquid chromatography (RP-HPLC) are hydro-organic mixtures of an aqueous buffer and an organic modifier. The addition of this organic solvent to buffered aqueous solutions involves a variation of the buffer properties (pH and buffer capacity). In this paper, the pH variation is studied for acetic acid–acetate, phosphoric acid–dihydrogenphosphate–hydrogenphosphate, citric acid–dihydrogencitrate–citrate, and ammonium–ammonia buffers. The proposed equations allow pH estimation of acetonitrile–water buffered mobile phases up to 60% (v/v) of organic modifier and initial aqueous buffer concentrations between 0.001 and 0.1 mol L⁻¹, from the initial aqueous pH. The estimated pH variation of the mobile phase and the pK_a variation of the analytes allow us to predict the degree of ionisation of the analytes and from this and analyte hydrophobicities, to interpret the relative retention and separation of analyte mixtures.

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1. Introduction

Careful pH control and measurement of the mobile phase is essential for a reproducible and successful chromatographic analysis of ionisable analytes. There are three different pH scales commonly used in pH measurement of reversed-phase high-performance liquid chromatography (RP-HPLC) mobile phases. The IUPAC recommends to measure pH in the mobile phase, after mixing aqueous buffer and organic modifier. The pH electrode system can be calibrated with aqueous buffers and thus the pH readings provide directly the ^s_wpH values of the mobile phase, i.e. the pH value

in the mobile phase solvent (s) relative to water (w) as standard state solvent [1]. Alternatively, the pH electrode system can be calibrated with buffers prepared in the water organic solvent mixture used as mobile phase, and the pH readings provide ^s_spH values, i.e. the pH value in the mobile phase solvent (s) relative to the same solvent (s) as standard state solvent [1]. The two IUPAC pH scales can be easily related by means of the δ parameter [2–4]:

$$^s_w\text{pH} = ^s_s\text{pH} + \delta \quad (1)$$

The δ parameter includes the primary medium effect and the difference between the liquid-junction potentials of the electrode system in the mobile phase and water. The primary medium effect depends only on the solvent at which pH is measured (mobile phase solvent composition), but the liquid-

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