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Comparison of peak shape in hydrophilic interaction chromatography using acidic salt buffers and simple acid solutions

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ABSTRACT

The retention and peak shape of neutral, basic and acidic solutes was studied on hydrophilic interaction chromatography (HILIC) stationary phases that showed both strong and weak ionic retention characteristics, using aqueous–acetonitrile mobile phases containing either formic acid (FA), ammonium formate (AF) or phosphoric acid (PA). The effect of organic solvent concentration on the results was also studied. Peak shape was good for neutrals under most mobile phase conditions. However, peak shapes for ionised solutes, particularly for basic compounds, were considerably worse in FA than AF. Even neutral compounds showed deterioration in performance with FA when the mobile phase water concentration was reduced. The poor performance in FA cannot be entirely attributed to the negative impact of ionic retention on ionised silanols on the underlying silica base materials, as results using PA at lower pH (where their ionisation is suppressed) were inferior to those in AF. Besides the moderating influence of the salt cation on ionic retention, it is likely that salt buffers improve peak shape due to the increased ionic strength of the mobile phase and its impact on the formation of the water layer on the column surface.

1. Introduction

Hydrophilic interaction chromatography (HILIC) is rapidly establishing itself as a complementary technique to reversed-phase separations (RP), particularly for polar and/or ionised compounds that are poorly retained using the latter method. It is a technique well-suited to the analysis of pharmaceuticals and compounds of biomedical significance [1–3]. The stationary phase in HILIC is typically bare silica, or polar groups bonded to a silica or an organic polymer matrix [4–6]. The hydro-organic mobile phase is similar to that used in RP, except typically employs much higher concentrations of acetonitrile (>70%). There is appreciable overlap in the applicability of these two techniques to compounds of moderate hydrophilicity, particularly for basic compounds. These can be retained by ionic interactions which occur on all silica-based phases as well as by hydrophilic interactions [4,7]. Hydrophilic interactions are likely to result from a combination of solute partition between a water layer held on the surface of the column and the bulk mobile phase, and by adsorption onto polar groups that may be partially deactivated by the presence of the water layer [6].

HILIC separations are usually performed in ACN-water mobile phases containing additives or buffer components, particularly when the analysed solutes are ionogenic. The buffer serves to control the ionisation of the stationary phase surface groups and silanols in silica-based phases, as well as the ionisation of the solute. The choice of salt buffers for HILIC is limited to those that have sufficient solubility in high concentrations of ACN. Typically, ammonium acetate or ammonium formate (AF) is used; these salts have the additional advantage that they are volatile and thus compatible with nebuliser-based detectors e.g. electrospray ionisation mass spectrometry. However, use of salt buffers can cause depression of the electrospray signal that increases with concentration over the typical range (5–50 mM) employed [8–10]. Even at the 5 mM level, it was shown that AF can cause greater signal suppression for acidic and basic pharmaceuticals compared with the use of simple acidic solutions of 0.1% formic acid (FA), which are commonly used. An added advantage of these acid solutions is that they are easier to prepare than mobile phases containing salt buffers. Nevertheless, it has been shown that ACN-water mixtures containing formic acid alone can give rise to poor peak shape in HILIC for

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Table 1
pH, ionic strength and buffer capacity of aqueous buffer solutions; $_{w}^{s}$pH measured in 85% ACN.

Buffer	$_{w}^{w}$pH	$_{w}^{s}$pH	Ionic strength (mmol/L)	Buffer capacity (mmol/L pH)
0.1% Formic acid (v/v)	2.7	2.9	2.2	9.7
5 mM Ammonium formate pH 3.0	3.0	5.2	6.1	14.7
0.1% Phosphoric acid (v/v)*	2.1	2.0	7.9	26.1

* This was used as 0.1% of an 85% solution (14.6 mM/L).

acidic and basic solutes, whereas good peak shapes were obtained with AF buffers [11]. However, these studies were performed solely on a bare silica column. It is possible that the strong ionic interactions with ionised silanols on this type of phase are contributory to this poor peak shape with FA, and that salt buffers are unnecessary with other types of HILIC columns [7]. For example, bonded phase (e.g. with amide ligands) materials prepared on inorganic–organic hybrid silicas show much reduced ionic interactions. Furthermore, silica hydride materials (Type C silica) are available for HILIC-type separations. It is claimed that this new type of stationary phase has significant differences in terms of chemical structure to traditional silicas, which are mainly populated with polar silanol groups. In contrast, Type C silica apparently has surface silicon-hydride groups [12,13]. The term "aqueous normal phase" (ANP) has been suggested to describe separations on this type of silica phase to distinguish them from "classical" HILIC separations. Nevertheless, ANP is also a term more generally used as an alternative to HILIC for classical separations, reflecting the possibility that adsorption is at least a contributory mechanism along with partition to the overall retention mechanism. It could be supposed that these Type C stationary phases would contribute considerably less ionic interactions, so the use of salt buffers might be unnecessary with such phases, if ionised silanol groups were the cause of peak shape problems. Indeed, separations on these phases are often reported with ACN-water mixtures containing only 0.1% formic or acetic acids [12–14] although no comment has been made in these reports concerning the lack of use of salt buffers, or whether their absence gave rise to any detrimental (or even beneficial) effects.

The aims of this paper were to compare the use of salt buffers with acid solutions for acidic, basic and neutral solutes separated on a variety of stationary phases, including bare silica, amide bonded onto hybrid silica, zwitterionic and silica hydride phases. These materials are considerably different in their retention characteristics towards ionised solutes, and therefore might produce different results in the various mobile phases. In this way we hoped to gain information to assist appropriate mobile phase selection for use in HILIC and HILIC with mass spectrometric detection.

2. Experimental

All experiments were performed with a 1290 binary high pressure mixing UHPLC instrument (Agilent, Waldbronn, Germany) with Chemstation, photodiode array UV detector (0.6 μL flow cell) and 5 μL injections. The columns used (all 25 × 0.46 cm ID, except where stated) were Cogent Silica C (4 μm particle size, pore size 100 Å, surface area 350 m^2/g) from Microsolv (Eatontown, USA), Atlantis silica (5 μm particle size, pore Size 100 Å, surface area 360 m^2/g) from Waters (Milford, USA), ZIC-HILIC (5 μm particle size, pore size 200 Å, surface area 140 m^2/g) from Merck-Sequant (Umeå, Sweden) and XBridge BEH Amide (15 cm × 0.46 cm, 3.5 μm particle size, pore size 140 Å, surface area 190 m^2/g) from Waters. By replacing the column with a zero dead volume fitting, the extra-column bandspreading of the instrument was estimated to reduce column efficiency by less than 5% even for a non-retained peak on the most efficient column. Temperature was maintained at 30 °C using the Agilent column compartment. Acetonitrile (far UV grade), ammonium formate and orthophosphoric acid were obtained from Fisher (Loughborough U.K.). AF buffers were prepared by adjusting aqueous solutions to pH 3.0 with formic acid such that the overall concentration of AF in the mobile phase after organic solvent addition was 5 mM. Standards were prepared at a concentration of 50 mg/L and made up in the exact mobile phase. The pH values of the mobile phase quoted are those either in the aqueous portion of the buffer ($_{w}^{w}$pH) or alternatively as measured in the organic-aqueous combination with the electrode calibrated in aqueous buffers ($_{w}^{s}$pH). All test solutes, and rubidium nitrate were obtained from Sigma-Aldrich (Poole, U.K.). Log *D* and log *P* values were calculated as the average from three different programs: ACD version 12.0 (ACD labs, Toronto, Canada), Marvin (ChemAxon, Budapest, Hungary) and MedChem Designer (Simulations Plus, Lancaster, USA). pK_a and solute charge was calculated from the average estimate given by the first two calculators. Column efficiency was measured from the first and second statistical moments according to the relationship

$$N = \frac{M_1^2}{M_2}$$

Asymmetry factor was measured at 10% of peak height by dividing the width of the trailing edge of the peak by that of the leading edge. The columns were operated in the region of their optimum flow (1.0 mL/min for silica and hydride silica, 0.5 mL/min for zwitterionic and amide).

3. Results and discussion

3.1. Buffer and solute properties

Table 1 indicates the pH, ionic strength and buffer capacity of the three mobile phases used, 5 mM ammonium formate (AF) adjusted to pH 3.0 with formic acid, 0.1% (v/v) formic acid (FA), and 0.1% (v/v) orthophosphoric acid (PA), if prepared in aqueous solution. Ammonium formate and formic acid are soluble in high concentrations of ACN; they are also volatile additives and thus extremely suitable for use in HILIC with mass spectrometry detection [2]. PA is an alternative acid additive used by several column manufacturers e.g. [15]. It was used successfully by Mant and Hodges for the HILIC separation of peptides using a 0.2% concentration in 85% ACN, using UV detection [16]. These authors sought a more hydrophilic acid additive than trifluoroacetic acid (TFA). We showed by experiment in the present study that 0.1% PA was completely soluble even in 100% ACN, with no evidence of precipitation. PA is not volatile and is thus unsuitable for use with mass spectrometry detection. However, PA was studied due to the lower $_{w}^{w}$pH and $_{w}^{s}$pH given by this relatively strong acid, and thus its better ability to suppress the ionisation of residual silanol groups. PA is also not expected to give substantial ion pair effects (see the discussion of these effects in Section 3.2). Ion pairing could lead to lower retention of ionised bases due to reduction in ionic interactions with the stationary phase and the reduced hydrophilicity of the paired species. In contrast, trifluoroacetic acid, which is a stronger acid and is more hydrophobic than PA can give quite pronounced ion pair effects [11], which we believed might have confounded the interpretation of the results by affecting retention times.