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Is hydrophilic interaction chromatography with silica columns a viable alternative to reversed-phase liquid chromatography for the analysis of ionisable compounds?

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Abstract

The separation of acidic, neutral and particularly basic solutes was investigated using a bare silica column, mostly under hydrophilic interaction chromatography (HILIC) conditions with water concentrations >2.5% and with >70% acetonitrile (ACN). Profound changes in selectivity could be obtained by judicious selection of the buffer and its pH. Acidic solutes had low retention or showed exclusion in ammonium formate buffers, but were strongly retained when using trifluoroacetic acid (TFA) buffers, possibly due to suppression of repulsion of the solute anions from ionised silanol groups at the low $^{s}_{s}$pH of TFA solutions of aqueous ACN. At high buffer pH, the ionisation of weak bases was suppressed, reducing ionic (and possibly hydrophilic retention) leading to further opportunities for manipulation of selectivity. Peak shapes of basic solutes were excellent in ammonium formate buffers, and overloading effects, which are a major problem for charged bases in RPLC, were relatively insignificant in analytical separations using this buffer. HILIC separations were ideal for fast analysis of ionised bases, due to the low viscosity of mobile phases with high ACN content, and the favourable Van Deemter curves which resulted from higher solute diffusivities.

Keywords: HPLC; HILIC; Silica column; Aqueous normal phase

1. Introduction

Hydrophilic interaction chromatography (HILIC) is a method where a polar stationary phase (for example naked silica, or a polar bonded phase) is used in conjunction with a mobile phase containing a mixture of an appreciable amount of water (typically at least 2.5 vol.%) and a less polar solvent (typically >70% acetonitrile). It has been used for many years for carbohydrate separations [1,2]. The term HILIC was first employed by Alpert [3] who used hydrophilic polymeric stationary phases bonded to silica in order to separate (amongst other polar compounds), amino acids, which were eluted in opposite order to that found in RPLC. He suggested the mechanism involved (mostly) partitioning between the hydrophobic mobile phase and a layer of mobile phase enriched with water and partially immobilised on the stationary phase. This "HILIC retention" seemed to be a major factor contributing to retention with >70% ACN in the mobile phase, although ionic retention was also suggested. Alpert considered that dipole–dipole interactions (hydrogen bonds) might contribute to partitioning into the stationary phase layer. He noted that charged basic groups in a solute lead to pronounced hydrophilicity and retention. Yoshida similarly considered that HILIC retention encompassed both hydrogen bonding (which depends on Lewis acidity/basicity) and dipole–dipole interactions (dependent on dipole moments and polarisability of molecules). He showed that the elution pattern was similar to that in (non-aqueous) normal phase chromatography, and thus proposed therefore that the mechanism must be similar [4]. Hydrogen bonding, especially when using mobile phases of low water content, has also been proposed as a retention mechanism by others [5]. Increasing the concentration of water in the eluent decreases retention when, as is usual in HILIC, high acetonitrile concentrations are used [6].

Other groups have experimented with bare silica using mobile phases of high (but <70%) concentrations of organic solvent. Bidlingmeyer et al. [7] separated organic amines with good peak shape using 60% ACN containing 4 mM ammonium phosphate pH 7.8. At this lower concentration of ACN, *decreasing*

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retention for lipophilic amines was observed as the ACN concentration increased from 30 to 70% (the opposite from under HILIC conditions), attributed to a RP type of retention on hydrophobic siloxane groups (ACN concentrations outside this range were not studied). RP retention was considered to be combined with an ionic attraction between ionised silanols and charged bases. Later, Euerby and co-workers [8] achieved acceptable separations on bare silica (albeit with rather low efficiency and somewhat tailing peaks) of basic drugs using buffered mobile phases mostly containing ~30% methanol, similarly attributing the mechanism as mostly ion exchange but with a significant contribution of hydrophobic retention.

Clearly, the mechanism of HILIC separations (>70% organic solvent) is complex and partition, ion exchange and hydrophobic retention contribute to various degrees, dependent on the particular conditions employed [9]. The possible separation mechanisms, and other aspects of HILIC have been recently reviewed by Hemström and Irgum [2]. In particular, they debated whether HILIC is principally an adsorption or a partition mechanism.

Despite the complexity of the mechanism, the technique is simple in practice and its general advantages can be summarised as follows:

(1) Reasonable peak shapes seem to be obtained for bases.
(2) Mass spectrometer sensitivity is enhanced due to the high organic content in the mobile phase and the high efficiency of spraying and desolvation techniques.
(3) Direct injection can often be made of extracts eluted from C18 solid phase extraction columns with solvents of high organic content [10].
(4) The order of elution of solutes is generally the opposite of that found in RP separations, giving useful alternative selectivity.
(5) Good retention of polar compounds is obtained in HILIC, whereas very poor retention is often obtained in RP-HPLC [11,12].
(6) Higher flow rates are possible due to the high organic content of typical mobile phases.

Our present study was directed mostly (but not exclusively) to an investigation of basic compounds, in which we have long had an interest, due to the majority of pharmaceuticals and biomedically relevant compounds which contain basic groups [13]. Their analysis can still cause considerable problems in RP chromatography, due to detrimental interactions with ionised silanol groups and to overloading effects [13,14], where peak shape for charged bases (and charged acids) deteriorates rapidly with increasing sample mass. We have examined the behaviour of bare silica columns, which continue to be the most popular stationary phase for HILIC separations [2]. The aims of our study were principally:

(1) To investigate how the selectivity of HILIC separations can be manipulated by change of column, nature of the buffer and pH.
(2) To compare the peak shapes obtained in HILIC with those typically obtained in RP-LC for basic compounds.
(3) To compare the overloading of charged compounds in HILIC in various buffer systems with overloading under typical conditions in RPLC.
(4) To examine how the low viscosity of the mobile phase in HILIC might be exploited for fast or high efficiency analysis, e.g. by comparing Van Deemter curves under typical HILIC and RP conditions.

2. Experimental

An 1100 binary high-pressure mixing HPLC system (Agilent, Waldbronn, Germany) with Chemstation, UV detector (1 μl flow cell), and Rheodyne 7725 valve (5 μl injections) was used in all experiments. Connections were made with minimum lengths of 0.01 cm i.d. tubing to minimise extra-column volume. Temperature was maintained at 30 °C by immersing the column and injector in a thermostatted water bath. A 3 m × 0.5 mm i.d. stainless steel tubing connected between the pump and injector and also immersed in the bath was used to regulate the temperature of the incoming mobile phase; flow was 1.0 $cm^3\ min^{-1}$ except where stated. The columns (25 cm × 0.46 cm i.d., 5 μm particle size) used were: Atlantis silica, pore diameter 93 Å; XTerra RP18, pore diameter 137 Å, C18 coverage 2.4 μmol/m^2 (Waters, Milford, MA, USA); Luna Silica (2), pore diameter 100 Å (Phenomenex, CA, USA); Ascentis silica, pore diameter 93 Å; Discovery C18, pore diameter 180 Å, C18 coverage 3.0 μmol/m^2 (Supelco, Bellefonte, PA, USA).

Ammonium formate buffers were prepared by adjusting the solution in water with formic acid to the required pH. Mobile phase pH was measured both before ($^{w}_{w}$pH) and after the addition of organic solvent ($^{s}_{w}$pH), with the meter calibrated in aqueous buffers in both cases. The true thermodynamic $^{s}_{s}$pH can be calculated from $^{s}_{w}$pH according to the relationship:

$$^{s}_{s}\mathrm{pH} = {}^{s}_{w}\mathrm{pH} - \delta$$

The δ term incorporates both the Gibbs energy for transference of 1 mol of protons from the standard state in water to the standard state in the hydroorganic solvent at a given temperature, and the residual liquid junction potential (the difference between the liquid junction potential established during calibration in aqueous solutions, and that established in the hydroorganic mixture). Recently, δ values have been published in ACN–water solutions up to 90% acetonitrile [15]. The effects of such high concentrations of ACN on the performance of some types of glass combination pH electrodes has been studied [15], but nevertheless the $^{s}_{w}$pH values quoted in the current study should be treated with some caution, and are always accompanied by the equivalent $^{w}_{w}$pH measurement. For TFA, the acid was added to both aqueous and ACN channels, allowing a constant concentration of TFA (0.1%, w/v) to be established at any concentration of ACN.

The column efficiency (N) was determined from peak widths at half height or for the statistical moments method, from the square of the first moment divided by the second moment. The asymmetry factor (A_s) was calculated at 10% of the peak height