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Adsorption of water from aqueous acetonitrile on silica-based stationary phases in aqueous normal-phase liquid chromatography☆

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ABSTRACT

Excess adsorption of water from aqueous acetonitrile mobile phases was investigated on 16 stationary phases using the frontal analysis method and coulometric Karl–Fischer titration. The stationary phases include silica gel and silica-bonded phases with different polarities, octadecyl and cholesterol, phenyl, nitrile, pentafluorophenylpropyl, diol and zwitterionic sulfobetaine and phosphorylcholine ligands bonded on silica, hybrid organic-silica and hydrosilated matrices. Both fully porous and core-shell column types were included. Preferential uptake of water by the columns can be described by Langmuir isotherms. Even though a diffuse rather than a compact adsorbed discrete layer of water on the adsorbent surface can be formed because of the unlimited miscibility of water with acetonitrile, for convenience, the preferentially adsorbed water was expressed in terms of a hypothetical monomolecular water layer equivalent in the inner pores. The uptake of water strongly depends on the polarity and type of the column. Less than one monomolecular water layer equivalent was adsorbed on moderate polar silica hydride-based stationary phases, Ascentis Express F5 and Ascentis Express CN column at the saturation capacity, while on more polar stationary phases, several water layer equivalents were up-taken from the mobile phase. The strongest affinity to water was observed on the ZIC cHILIC stationary phases, where more than nine water layer equivalents were adsorbed onto its surface at its saturation capacity. Columns with bonded hydroxyl and diol ligands show stronger water adsorption in comparison to bare silica. Columns based on hydrosilated silica generally show significantly decreased water uptake in comparison to stationary phases bonded on ordinary silica. Significant correlations were found between the water uptake and the separation selectivity for compounds with strong polarity differences.

1. Introduction

In the past 10 years, aqueous normal-phase liquid chromatography (ANP LC), more often called hydrophilic interaction liquid chromatography (HILIC), has become increasingly popular as the primary method for separation of polar pharmaceuticals, biopolymers or metabolites, which are too strongly retained in non-aqueous normal-phase mode (NP), but—on the other hand—are insufficiently retained in reversed-phase (RP) systems [1–12]. Typical ANP LC techniques employ a polar stationary phase, mobile phases usually containing acetonitrile and less than 5–40% (v/v) of water, often with buffer additives adjusting the pH and ionic strength to reach stable retention [13–15].

In non-aqueous normal-phase adsorption chromatography (NPC), the retention is primarily based on the competition between the molecules of the solute and of the polar solvent for the localized adsorption sites on the surface of a polar adsorbent, usually bare silica gel. Polar solvents, especially water, are strongly adsorbed on polar adsorbents from mixed mobile phases. This effect should be taken into account in gradient elution NPC, where the composition of the mobile phase changes during the separation, so that the concentration of the polar solvent in the mobile phase is lower with respect to the pre-set gradient program; then the breakthrough of the polar solvent is observed when the column capacity is saturated. This effect, known as “solvent demixing” may cause decreased reproducibility of the retention data and long column re-equilibration times after the end of the gradient, which has been the reason of prejudice against using gradient elution in NPC. Water is more polar, and hence, more strongly retained

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than any organic solvent. Traces of water in the mobile phase can detrimentally decrease the adsorbent activity and retention. These inconveniences can be alleviated if dried mobile phases are used [16–18].

The ANP technique is believed to be less affected by the solvent demixing effect than the non-aqueous NP LC. As postulated by Alpert in 1990, water is preferentially adsorbed from mixed aqueous–organic mobile phases and forms a diffuse water-rich layer at the surface of the adsorbent [13]. This HPLC mode is also known as hydrophilic interaction liquid chromatography (HILIC), in which the retention is believed to be largely controlled by partition between the bulk mobile phase and the adsorbed water-rich layer. However, because of the miscibility of polar organic solvents and water, no fixed boundary between the two liquid phases can be fixed in the column. The retention may also be contributed by the adsorption of the solute onto the surface of a polar stationary phase. Aqueous mobile phases were employed for separation of carbohydrates and other compounds on polar stationary phases many years ago [19–21] and have been further elaborated by many later works [10,22–25]. These studies have also shown that the functional groups on the polar stationary phases contribute to the selectivity in ANP via hydrogen bonding, dipole–dipole interactions and electrostatic interactions between polar stationary phases and charged solutes. Further, residual silanols may contribute to cation exchange and repulsion of anions, interactions that would be superimposed on partitioning and adsorption as retention mechanism.

The type of the stationary phase plays the most important role in the ANP retention and separation selectivity, as it affects the nature of the retention mechanism. The ANP mode can be performed on a variety of silica-based or polymer chromatographic materials. In addition to the original poly(2-sulphoethyl aspartamide), strong cation-exchange ANP material introduced by Alpert [13], silica gel with decreased surface concentration of silanol groups, such as hydrosilated silica [26,27], was found suitable for separation of polar compounds. Due to its more hydrophobic surface, hydrosilated silica is believed to form less-dense adsorbed water surface layer than other, more polar stationary phases employed for HILIC separations. Further, various chemically bonded amino- [28,29], amido- [30], cyano- [31], carbamate [32] and diol- [33] phases can be used for ANP separations. Some columns may show dual retention mechanism, HILIC (ANP) in organic-rich mobile phases and reversed-phase (RP) with highly aqueous mixed mobile phases.

Zwitterionic chromatographic materials represent important group of HILIC stationary phases [34], containing both positive and negative charges on the material surface [35,36]. Because the functional moieties contain two oppositely charged groups in close proximity at a stoichiometric ratio, the electrostatic interaction between the charged groups of the stationary phase and oppositely charged analytes is weaker compared to normal ion exchangers. Ion-exchange interactions with charged functional groups may contribute to the retention of ionic or partly ionized samples, so that the resulting separation mechanism may be quite complex.

Properties and applications of various types of polar columns suitable for HILIC (aqueous-normal phase) chromatography have been reviewed recently [15,37,38].

It is generally assumed that a water-rich layer formed at the surface of the polar stationary phases may participate in the sample distribution in the ANP mode. However, some authors assume that the water uptake is less important with the less polar silica-based stationary phases such as stationary phases based on hydrosilated silica, chemically modified with non-polar groups, in comparison to the ordinary silica containing high concentrations of silanols [39–41]. In the present work, we investigated the excess adsorption of water in the ANP mode on various types of polar and non-polar stationary phases by frontal analysis, to compare the role of different functionalities and the role it may play in the ANP retention mechanism. The present study extends our recent work on hydrosilated silica-based columns [42].

2. Materials and methods

2.1. Samples and chemicals

Thiourea, uracil, toluene, phenol and polystyrene standard (1,800,000 g/mol) were purchased from Sigma–Aldrich in the best available purity. Acetonitrile, tetrahydrofuran (both LiChrosolv grade), ammonium acetate and formic acid (both reagent grade) were obtained from Merck, Darmstadt, Germany. Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Coulomat® AG, a reagent for coulometric Karl Fischer titration, was purchased from Sigma Aldrich.

2.2. Equipment

For the measurement of water adsorption, an ECOM pump (ECOM, Prague, Czech Republic) was used, connected to a chromatographic column and an automated fraction collector (CF-1 Fraction Collector, Spectrum Chromatography, Houston, TX, USA). Columns were placed in a thermostatted compartment set at 40 °C. The concentration of water in the collected fractions was determined using an 831 KF Coulometer-coulometric Karl–Fischer titrator equipped with a 728 Ti Stand-magnetic stirrer (Metrohm, Herisau, Switzerland).

All the chromatographic experiments were measured using an HPLC setup including a high-pressure pump (ECOM, Prague, Czech Republic) connected with a variable UV detector from the same manufacturer. The columns were heated in a thermostatted column compartment and the detection wavelength was set to 210 nm.

2.3. Columns and their characteristics

The dimensions and characteristics of 16 columns tested are listed in Table 1. The columns tested include two zwitterionic columns (sulfobetaine and phosphorylcholine), diol silica-based stationary phases (YMC Triart Diol, LiChrospher 100 DIOL, Luna HILIC), ethylene-bridged hybrid silica (XBridge HILIC), silica (Atlantis HILIC and fused core-shell silica Ascentis Express HILIC), chemically bonded fused core-shell silica stationary phases (pentafluorophenylyhexyl silica, silica with highly polar ligand possessing 5 hydroxyl groups, silica modified with diisopropylcyanopropylsilane) and modified and unmodified hydrosilated silica.

2.4. Methods—determination of excess water isotherms

The data necessary for the determination of excess concentration of water, q_{ex}, adsorbed onto the surface of the columns tested were measured using the frontal analysis method for 12 feed solutions of water (0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 15.0 vol.% of water, v/v), in LiChrosolv acetonitrile.

The sample retention is strongly affected even by traces of water contained in the organic solvent. Using Karl–Fischer titration, we found that the LiChrosolv-grade acetonitrile used in this work contained approximately 0.4% water. To eliminate the effect of the water adsorption from pure acetonitrile, the organic solvent was dried by molecular sieves, using a traditional approach applied in non-aqueous normal-phase chromatography. Before each measurement, the column was rinsed with 50-column hold-up volumes of acetonitrile. The column was then disconnected, the pump and the connecting capillaries were filled with the feed solution of water in acetonitrile, the column was again connected to the system and the fraction collector and the isocratic pump delivering the