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Investigations on the chromatographic behaviour of zwitterionic stationary phases used in hydrophilic interaction chromatography

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ABSTRACT

Two commercial stationary phases possessing a sulfobetaine zwitterionic bonded ligand (ZIC-HILIC and Nucleodur HILIC) were compared under hydrophilic interaction chromatographic (HILIC) conditions. First of all, the separation of 12 model compounds chosen among neurotransmitters and presenting a diversity of ionization states (anionic, cationic and zwitterionic) was studied under varied operating conditions. The effects of the percentage of acetonitrile, ammonium acetate concentration and temperature of the mobile phase were compared on the two columns. Secondly, a generally applicable retention model was established, based on chromatographic retention data (log k) acquired for 76 model compounds. The chosen compounds are small molecules presenting a wide diversity of molecular structures and are relevant to biomedical and pharmaceutical studies. To account for their retention behaviour, a modified version of the solvation parameter model was designed: two additional molecular descriptors were introduced, to account for ionic interactions with anionic and cationic species. The retention equations obtained allow a rationalization of the interactions contributing to retention and separation in the HILIC systems considered.

1. Introduction

The mechanism of retention in hydrophilic interaction chromatography (HILIC) appears to be complex. The primary retention mechanism in HILIC is supposed to be partition of the analyte between the organic-rich mobile phase and the immobilized aqueous layer at the surface of the stationary phase, as per the speculation by Alpert [1]. Any polar stationary phase which can retain water could be used in the HILIC mode. Interaction between the solute and the functional groups of the stationary phase (dipole–dipole, hydrogen bonding and electrostatic interactions) also occurs, as evidenced by the different separations observed when the stationary phase is varied [2–5]. Both partition and adsorption mechanisms are thus believed to contribute to the overall retention of analytes in HILIC conditions.

Among the existing HILIC stationary phases, phases with zwitterionic ligands bonded on silica (ZIC-HILIC) or polymer substrates (pZIC-HILIC) were first introduced by SeQuant/Merck. Macherey-Nagel has recently introduced an identical sulfobetaine-bonded silica phase (Nucleodur HILIC). Nesterenko et al. [6] recently reviewed zwitterionic stationary phases used in ion separations (ion chromatography and HILIC). True zwitterionic stationary phases contain equal amounts of groups bearing opposite permanent charges in their ligands. Indeed, these functional groups are not sensitive to pH, as is the case with the quaternary ammonium and sulfonate groups present in the sulfobetaine stationary phases. In the latter stationary phase, the positive charge is closest to the silica surface, while the sulfonate groups are the terminal end of the bonded ligand and are thus more accessible for interaction with the analytes. As a result, cations are generally more retained than anions on this stationary phase. However, oppositely charged functional groups may also self-associate, yielding a weak ion-exchanger. Sulfobetaine phases have proven to be useful for the HILIC separation of a variety of polar compounds [4,7–17].

A major objective of this study was to investigate the retention characteristics of sulfobetaine stationary phases and hopefully gain some insights into chromatographic retention mechanisms in the HILIC mode. The zwitterionic columns were chosen for their popularity, as a starting point to develop a stationary phase characterization method.

First of all, the retention of 12 model analytes was systematically investigated on the above two commercial sulfobetaine phases, by varying the chromatographic conditions. Plots of k as a function

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of chosen operating parameters were expected to provide some starting information on retention mechanisms.

In addition, a better understanding of the mechanisms that govern the retention of solutes in a particular chromatographic system can be achieved through the use of quantitative structure–retention relationships (QSRRs). A QSRR is a mathematical model relating the retention of a given analyte to physicochemical and structural parameters. Thus QSRRs can help us to gain some insights into the separation mechanisms that occur at the molecular level.

HILIC has only rarely been the topic of QSRR studies. The development of retention prediction models on unmodified silica, diol-, polyvinyl alcohol- and polyamine-bonded stationary phases under the HILIC mode has been described [18–22]. Models were derived using multiple linear regression analyses and artificial neural networks. Jandera et al. [23] used linear solvation energy relationships (LSERs) to describe retention of phenolic acids and flavone compounds on a variety of columns, including the ZIC-HILIC stationary phase. They compared the models obtained for each column used either in the RPLC mode, or in the HILIC mode. Michel et al. used QSRRs to compare different stationary phases, comprising the ZIC-HILIC phase, for the separation of peptides [24]. However, as the latter study was carried out in the RPLC mode, it is not very informative regarding the HILIC retention mechanism. In another study, Michel [25] performed QSRR studies to describe the retention of pesticides, this time using the ZIC-HILIC phase in the HILIC mode and comparing it to other polar stationary phases used in RPLC mode. But in this work, eight-descriptor models were used to describe the retention of only five solutes, which does not seem a reasonable basis for drawing generally applicable conclusions.

The second purpose of this work was thus to develop a reasonable and chemically sound QSRR for the description of retention in HILIC and to ascertain the possibility of using this model for the conjoint modelling of the retention of neutral compounds and ionized compounds with different charge state (anions, cations and zwitterions).

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from J.T. Baker (Noisy-le-Sec, France) and perchloric acid from VWR Prolabo (Darmstadt, Germany). Ammonium acetate and acetic acid were purchased from Fluka (St.-Quentin-Fallavier, France).

The 76 solutes used in this study are presented in Table 1, along with their structures and molecular descriptors. The chemicals were obtained from several different manufacturers.

Deionized (18 MΩ) water, purified using an Elgastat UHQ II system (Elga, Antony, France) was used for preparation of analyte and mobile phase solution.

2.2. Standards

Stock standard solutions of each analyte prepared at a concentration of 1000 μg mL^{-1} were obtained by dissolving a weighed amount of each compound with the appropriate solvent. For compounds 8, 9, 27–32, 37, 39, 50–52 and 57–59 MeOH was used; for catecholamine, indolamine, and metabolite (compounds 65–76) 0.2 M perchloric acid was used; for all the other compounds in Table 1 the standard solutions were prepared in deionized water.

The use of perchloric acid, for catecholamine dissolution, is dictated by the fact that the neurotransmitter analysis was inscribed in a larger study aiming at analyzing these molecules in brain extracts, which are prepared in perchloric acid. We wished to maintain identical conditions throughout the whole method development process. All catecholamine stock solutions were stored at −80 °C.

The solutions used were obtained by diluting the corresponding stock standard solutions in buffer/organic modifier mixture in order to have an injection solvent as close as possible to the mobile phase and a final analyte concentration of 10 μg mL^{-1} for the catecholamines and 50 μg mL^{-1} for all the other compounds. In the HILIC mode the injection solvent composition is very important. Retention time shift can be caused by the slightest difference in the organic phase/aqueous phase ratio between the injection solvent and the mobile phase, particularly for solutes experiencing little retention. Moreover, it is imperative to have similar salt concentration and nature in the injection solvent and the mobile phase in order to obtain good peak symmetry [26].

The mobile phase was a mixture of acetonitrile and ammonium acetate buffer ${}_{w}^{w}$pH 4. PhoEBus, an application program aid for buffer studies (Analis, Namur, Belgium) was used for the preparation of aqueous salt solutions. It was prepared by specifying the salt concentration and ${}_{w}^{w}$pH. The ${}_{w}^{w}$pH value of the ammonium acetate solution was adjusted with 1 M acetic acid aqueous solution. The buffer ${}_{w}^{w}$pH was then adjusted before the addition of organic solvent, then the buffer–acetonitrile ${}_{w}^{S}$pH was measured, with the pH-meter calibrated in aqueous buffers. When mixing the ${}_{w}^{w}$pH 4 buffer to 80% acetonitrile, the resulting ${}_{w}^{S}$pH was 6.2.

2.3. Instrumentation

The chromatographic systems consisted of a Merck-Hitachi quaternary pump model Lachrom L-7100 (Darmstadt, Germany), a Rheodyne (Cotati, CA, USA) model 7725 injection valve fitted with a 20 μL loop, column oven Jet Stream 2 Plus, a 785A UV–visible HPLC Detector (Applied Biosystems, Courtaboeuf, France) and an evaporative light scattering detector ELSD model Sedex 55 (SEDERE, Alfortville, France). The UV detection was carried out at 280 nm for catecholamine analysis and 254 nm for the rest of the analysed compounds. The usual ELSD settings were as follows: photomultiplier, 7; evaporative temperature, 50 °C; air pressure, 2.3 bar. The chromatographic data handling was accomplished using EZChrom Server software (Merck, Darmstadt, Germany).

The two columns studied were: ZIC-HILIC (SeQuant/Merck) 150 × 4.6 mm, 5 μm and Nucleodur HILIC (Macherey-Nagel) 125 × 3 mm, 3 μm. The mobile phase flow rate was 0.5 mL min^{-1} for the ZIC-HILIC column (in accordance with recommendations from the manufacturer) and 0.3 mL min^{-1} for the Nucleodur HILIC column.

2.4. Methods

The neurotransmitters (solutes 65–76 in Table 1) were injected one-at-a-time, and no experimental design was considered. The effect of acetonitrile percentage was studied with a ${}_{w}^{w}$pH 4 40 mM ammonium acetate buffer; temperature was set at 20 °C. Temperature effect was investigated with a mobile phase comprised of ACN and 25 mM aqueous solution of ammonium acetate ${}_{w}^{w}$pH 4 80:20 (v/v). Salt concentration effect was investigated with a mobile phase comprised of ACN and aqueous solution of ammonium acetate ${}_{w}^{w}$pH 4 80:20 (v/v) at 20 °C. Retention factors (k) were recorded under all conditions tested.

For the linear solvation energy relationship (LSER) characterization, all solutes in Table 1 were analyzed under the following mobile phase conditions: ACN/100 mM ammonium acetate aqueous solution ${}_{w}^{w}$pH 4, 80:20 (v/v) at 20 °C. As our aim was to investigate the differences in stationary phase properties, it was important to choose some operating conditions that would be suitable to both columns under investigation. The operating conditions also needed to be consistent with common practice of HILIC today,