Journal of Chromatography A, 1218 (2011) 7110–7129

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Contributions to reversed-phase column selectivity. II. Cation exchange

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ARTICLE INFO

Article history:
Received 8 April 2011
Received in revised form 18 June 2011
Accepted 26 July 2011
Available online 3 August 2011

Keywords:
HPLC
Reversed-phase
Column selectivity
Hydrophobic-subtraction model
Cation-exchange
Retention mechanism

ABSTRACT

The contribution of cation exchange to solute retention for type-B alkylsilica columns (made from high-purity silica) has been examined in terms of the hydrophobic-subtraction (H-S) model of reversed-phase column selectivity. The relative importance of cation exchange in the separation of ionized bases by reversed-phase chromatography (RPC) varies with (a) column acidity (values of the column cation-exchange capacity C), (b) mobile-phase pH and buffer concentration, and (c) the nature of the buffer cation. The effects of each of these separation variables on cation retention were examined. The contribution of cation exchange (and other ionic interactions) to solute retention is represented in the H-S model by properties of the solute (κ') and column (C), respectively. Values of κ' for 87 solutes have been examined as a function of solute molecular structure, and values of C for 167 type-B alkylsilica columns have been related to various column properties: ligand length (e.g., C_8 vs. C_{18}) and concentration (μmol/m^2), pore diameter (nm), and end-capping. These results contribute to a more detailed picture of the retention of cationic solutes in RPC as a function of separation conditions. While previous work suggests that the ionization of type-B alkylsilica columns is generally negligible with mobile-phase pH < 7 (as a result of which cation exchange then becomes insignificant), the present study provides evidence for cation exchange (and presumably silanol ionization) at a pH as low as 3 for most columns.

1. Introduction

Retention in RPC as a function of column selectivity can be described by the so-called *hydrophobic-subtraction* (H-S) model [1,2], which recognizes five separate solute–column interactions (Section 2.1 below). A previous report [3] has examined two of these contributions to retention in greater detail: *steric interaction* and *hydrophobic interaction*. The present paper describes a comparable study of a third solute–column interaction: *cation exchange* or ion–ion interaction. Additional goals of the present investigation are a better understanding of (a) the interaction of charged solutes with type-B alkylsilica columns and (b) the H-S model as a description of RPC retention. The present study also illustrates the use of the H-S model as a tool for the further interpretation of experimental retention data.

Basic molecules are frequent components of both natural and man-made mixtures. The retention of a basic compound *X:* in RPC can be affected by either of two solute–column interactions, each involving stationary-phase silanols. For neutral bases, hydrogen bonding of the solute to a silanol can occur:

$$\mathrm{X:}_{(m)} + {-}\mathrm{SiOH}_{(s)} \leftrightharpoons {-}\mathrm{SiOH}{-}{-}{-}\mathrm{:X}_{(s)} \quad (1)$$

where (m) and (s) refer to species in the mobile or stationary phase, respectively. For retention of a protonated base XH^+, ion exchange can take place with a buffer-cation (e.g., K^+) held by an ionized silanol ($-SiO^-$) in the stationary phase:

$$\mathrm{XH^+} + {-}\mathrm{SiO^-\,K^+} \leftrightharpoons {-}\mathrm{SiO^-\,XH^+} + \mathrm{K^+} \quad (2)$$

A considerable literature exists concerning the nature of these silanols and their chromatographic consequences (e.g., several hundred papers cited in [4–6]). Some studies have found that alkylsilica columns made from pure (type-B) silica do not exhibit significant cation-exchange retention at low pH and presumably are not ionized [7,8]. Another investigation [9] with a mobile-phase pH of 4.7–5.2 reported that cation-exchange predominates for a type-A column (Novapak C_{18}), vs. ion-pair behavior for a type-B column (Symmetry C_{18}). Still other work suggest that at pH 2.8 different type-B columns are ionized to various extents [2]. One goal of the present study was to clarify the extent and nature of stationary-phase ionization for type-B columns at low pH.

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doi:10.1016/j.chroma.2011.07.085

Hydrogen bonding as in Eq. (1) will be discussed here only for comparison with cation exchange (i.e., because both processes involve silanols). A more complete examination of solute–column interactions based on hydrogen bonding – including both donor and acceptor stationary phases – will be reported elsewhere.

2. Background and theory

Three topics which relate to the retention of cationic solutes in RPC will first be reviewed: (a) the H-S model (Section 2.1), (b) ionization of the stationary phase as a function of the column and mobile phase (Section 2.2), and (c) the retention of ionized solutes as a function of mobile-phase buffer concentration (Section 2.3). Here and elsewhere in this report, we will assume the use of type-B alkylsilica columns (which are further described in Section 2.2).

2.1. The hydrophobic-subtraction model of column selectivity

The development [1–3] and some representative applications [10–14] of the hydrophobic-subtraction (H-S) model have been described; solute retention factors k are given by this model as

$$\log k = \log k_{EB} + \underset{(i)}{\eta' H} - \underset{(ii)}{\sigma' S*} + \underset{(iii)}{\beta' A} + \underset{(iv)}{\alpha' B} + \underset{(v)}{\kappa' C} \qquad (3)$$

Terms i–v of Eq. (3) describe five different solute–column interactions: (i) hydrophobic; (ii) steric, (iii) hydrogen bonding of a basic solute to an acidic stationary-phase group (presumed to be a silanol–SiOH), (iv) hydrogen bonding of an acidic solute to a basic stationary-phase group, and (v) ion-exchange (or other ionic interaction) between an ionized solute and a charge-bearing column. Each of interactions i–v of Eq. (3) is the product of a column parameter (H, S^*, A, B, or C) and a solute parameter (η', σ', β', α', or κ'). In the case of phenyl or cyano columns [15], two additional solute–column interactions (π–π, dipole–dipole) can contribute to retention, although each of these interactions is relatively unimportant for most samples.

The column parameters include hydrophobicity (H), steric resistance to penetration of the solute into the stationary phase (S^*), hydrogen-bond (H-B) acidity (A), H-B basicity (B), and cation-exchange capacity (C). While we have referred to the column parameter C as "cation-exchange capacity," it should be noted that this usage differs from that of the ion-exchange community (total ionizable groups in the stationary phase). Our usage, as will be clear from the following discussion, refers (approximately) to the net charge of all *ionized* groups in the stationary phase. The parameters η', σ', β', α', and κ' correspond to complementary properties of the solute; for example, β' is solute H-B basicity, and κ' is related to the charge on an ionized solute molecule. Values of $\kappa' \approx +1$ for a completely ionized, singly protonated base, $\kappa' = 0$ for a non-ionized molecule, and values of κ' will be negative for negatively charged solutes (e.g., ionized acids). The quantity k_{EB} is the value of k for the reference solute ethylbenzene; k_{EB} corrects for differences in column surface area, but does not directly affect column selectivity. For type-B alkylsilica columns and the separation conditions of Section 3.1, Eq. (3) describes values of k with an accuracy of about ±1% [2], but is less accurate for other column types (cyano, phenyl, type-A alkylsilica, etc.).

The original recognition of the cation-exchange term $\kappa' C$ of Eq. (3) [1,2,16] merits a brief review, because of its relevance to the following discussion, as well as providing evidence for ionic solute–column interactions at pH 2.8 that significantly affect the retention of cationic solutes. Values of k were initially measured for 87 solutes of widely varied structure and nine different type-B C_{18} columns (same temperature and mobile phase). Plots of log k for one column vs. another were found to be linear, as predicted by Eq. (3) if terms ii–v are small relative to term i and can therefore be ignored. This is illustrated in Fig. 1 for a plot of values of $\log k$ for an Inertsil ODS-3 column vs. *average* values of $\log k$ for all nine columns. Deviations $\delta \log k$ from such plots (as for the five protonated bases shown as solid circles (●) in Fig. 1) then measure the relative contribution from terms ii–v of Eq. (3) for each solute and column (see the example of Fig. 2a, which is an expansion of the enclosed area of Fig. 1).

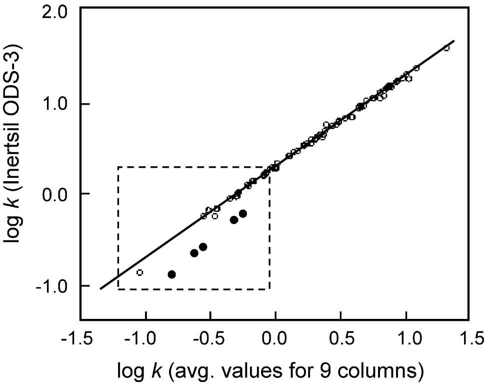

Fig. 1. Plot of $\log k$ for Inertsil ODS-3 column vs. average values of $\log k$ for nine different type-B C_{18} columns (87 solutes; data of [1,16]). Conditions: 50% acetonitrile/pH 2.8 buffer, 25 °C. Five fully protonated strong bases shown as (●).

In the similar (expanded) examples of Fig. 2a–d for four different columns, data for five fully protonated strong bases are again highlighted as (●). Note that in Fig. 2a the latter data-points lie well *below* the best-fit curve through remaining data, and well *above* the curve in Fig. 2d; the relative retention of these basic solutes (vs. other solutes) increases by a factor of ~3 for the StableBond column (d) vs. the Inertsil column (a). The only unique characteristic of these five strong bases is that each is fully protonated with a molecular charge of +1, while separation conditions other than the column do not change for the examples of Fig. 2. This implies that the charge on each of the stationary phases of Fig. 2 is different, becoming more negative (or less positive) in the sequence

Inertsil (a) < Symmetry (b) < Eclipse (c) < StableBond (d)

so as to result in increased binding of cationic solutes. By "stationary-phase charge" we refer to the net charge on ionized groups attached to the stationary phase, apart from their counterions.

For the latter five strong bases, the deviations ($\delta \log k$) for any two solutes (and all nine columns) are highly inter-correlated ($r^2 > 0.99$), implying that only term v of Eq. (3) contributes significantly to the non-hydrophobic retention of these solutes (see the further discussion of [3]). The average value of $\delta \log k$ for these five solutes and each column is then approximately equal to C at pH 2.8 (referred to as "C-2.8"), which defines the net attraction of that column for cationic solutes; values of C-2.8 for each column are noted in Fig. 2. Thus, a value of C-2.8 appears to measure the relative ionic charge on the stationary phase of a given column (with larger values of C-2.8 indicating a more negative charge).

2.2. Stationary-phase ionization as a function of silica type and mobile-phase pH

The extent of silanol ionization in RPC columns ($-\mathrm{SiOH} \leftrightharpoons -\mathrm{SiO}^- + \mathrm{H}^+$) varies with both mobile-phase pH and silica type, and has been approximated by the cation-exchange retention of an inorganic cation such as Li^+ [7,8]. This is illustrated in Fig. 3b for three different C_{18} columns, as well as for the corresponding unbonded-silica columns in Fig. 3a (60% methanol/buffer