Journal of Chromatography A, 1395 (2015) 57–64

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Contributions to reversed-phase column selectivity III. Column hydrogen-bond basicity

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ARTICLE INFO

Article history:
Received 16 December 2014
Received in revised form 17 March 2015
Accepted 18 March 2015
Available online 24 March 2015

Keywords:
HPLC
Reversed-phase
Column selectivity
Hydrophobic–subtraction model
Column hydrogen-bond basicity
Retention mechanism

ABSTRACT

Column selectivity in reversed-phase chromatography (RPC) can be described in terms of the hydrophobic–subtraction model, which recognizes five solute–column interactions that together determine solute retention and column selectivity: hydrophobic, steric, hydrogen bonding of an acceptor solute (i.e., a hydrogen-bond base) by a stationary-phase donor group (i.e., a silanol), hydrogen bonding of a donor solute (e.g., a carboxylic acid) by a stationary-phase acceptor group, and ionic. Of these five interactions, hydrogen bonding between donor solutes (acids) and stationary-phase acceptor groups is the least well understood; the present study aims at resolving this uncertainty, so far as possible. Previous work suggests that there are three distinct stationary-phase sites for hydrogen-bond interaction with carboxylic acids, which we will refer to as column basicity I, II, and III.

All RPC columns exhibit a selective retention of carboxylic acids (column basicity I) in varying degree. This now appears to involve an interaction of the solute with a pair of vicinal silanols in the stationary phase. For some type-A columns, an additional basic site (column basicity II) is similar to that for column basicity I in primarily affecting the retention of carboxylic acids. The latter site appears to be associated with metal contamination of the silica. Finally, for embedded-polar-group (EPG) columns, the polar group can serve as a proton acceptor (column basicity III) for acids, phenols, and other donor solutes.

1. Introduction

The experimentally derived hydrophobic-subtraction model was introduced in 2002 [1] and has since been significantly extended, as summarized a decade later [2]. Solute retention (the retention factor k) in reversed-phase chromatography (RPC) can be described as a function of solute and column properties:

$$\log k = \log k_{EB} + \underset{(i)}{\eta' H} - \underset{(ii)}{\sigma' S^*} + \underset{(iii)}{\beta' A} + \underset{(iv)}{\alpha' B} + \underset{(v)}{\kappa' C} \quad (1)$$

The quantity k_{EB} refers to the retention factor for a reference compound (ethyl benzene) that corrects for differences in column surface area, while remaining terms *i*–*v* represent contributions to k from hydrophobic attraction (*i*), steric repulsion (*ii*), hydrogen bonding between an acceptor solute and a donor column site (a silanol) (*iii*), hydrogen bonding between a donor solute and an acceptor or "basic" stationary-phase group (*iv*), and ionic attraction between oppositely charged groups in the solute and stationary phase (*v*). In each case, Greek symbols (η', σ', etc.) refer to interaction properties of the solute, and capital letters (H, S^*, etc.) refer to complementary interaction properties of the column.

The various column-selectivity parameters (H, S^*, etc.) are measured for a given column by means of the retention of 16 test solutes, using a specified mobile phase and temperature. The five column parameters H, S^*, etc. thus define column selectivity, are known for >600 RPC columns, and can be used for various purposes [2] by means of the United States Pharmacopeia website [3].

Previous papers [2] have examined terms *i*–*iii* and *v* of Eq. (1) in detail. For all but term *iv* of Eq. (1), values of the solute and column parameters are generally consistent with solute molecular structure, the properties of the column (ligand length and concentration, pore diameter, end-capping), and the theoretical basis of the associated solute-column interactions. There is thus a reasonable understanding of how column properties affect these four interactions and RPC column selectivity. For term *iv* of Eq. (1), α' and B have been attributed, respectively, to the hydrogen-bond (H–B)

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http://dx.doi.org/10.1016/j.chroma.2015.03.044
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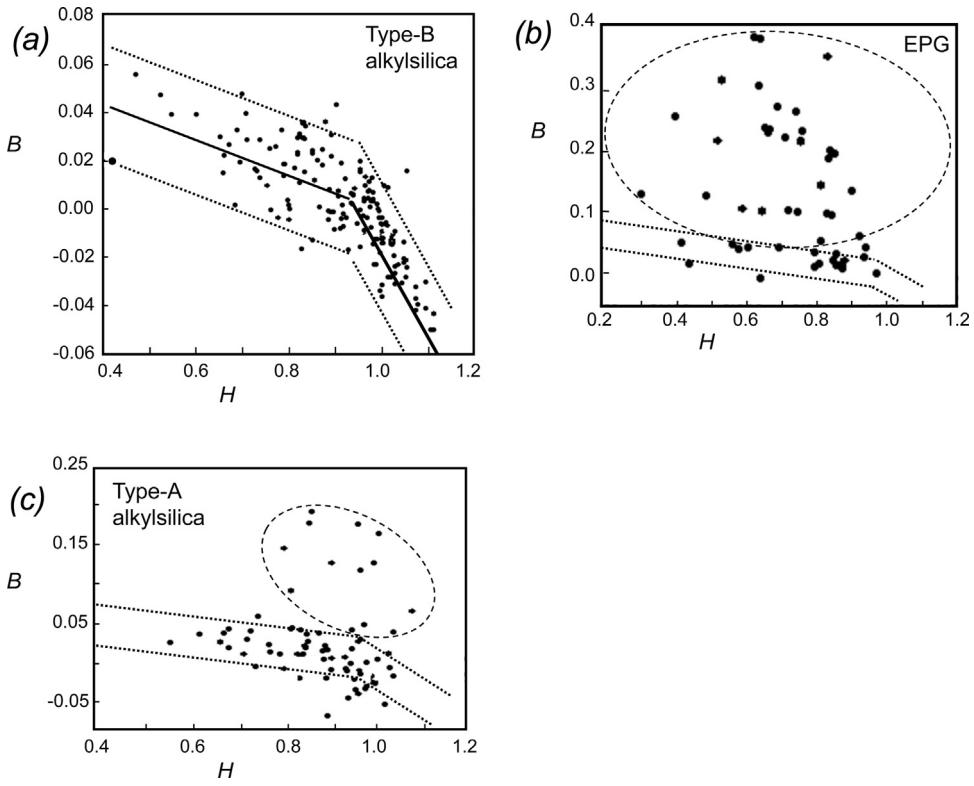

Fig. 1. Column H–B basicity *B* vs. hydrophobicity *H* for different column types. (a) Type-B alkylsilica columns (C_1–C_{30}); (b) type-B embedded-polar-group columns; (c) type-A alkylsilica columns (C_4–C_{18}). Lines (. . .) represent ±2SD from average values (—); circles (_ _ _) enclose values with "excess column basicity" *B* (values of B exceeding 2SD from the average values of (a) for a given value of *H*). Adapted from [2], data from [3]; see text for details.

donor acidity of the solute and the H–B acceptor basicity of the column. Attempts to reconcile values of α' and *B* with experiment, in similar fashion as for terms *i*, *ii*, *iii*, and *v* of Eq. (1), have until now proved less successful. The present study addresses term *iv* in more detail and completes a recent series of papers [4,5] aimed at a detailed understanding of the nature of the five interactions summarized by Eq. (1).

2. Experimental

The present report is based on previously reported data for a mobile phase of 50% acetonitrile/buffer, a buffer of 30 mM potassium phosphate (pH 2.8), and a temperature of 35 °C. Values of the parameter α' for 87 solutes are from [1,6], and column parameters (*H*, *S**, etc.) are reported for 167 type-B alkylsilica columns described in [4]. Values of *H*, *S**, etc. for other columns discussed here are taken from [3].

3. Discussion

3.1. Evidence for three different basic sites

Values of column H–B basicity *B* are determined mainly by the retention of two benzoic acids (4-*n*-butylbenzoic acid and mefenamic acid) that form part of the 16-compound column test mixture used to characterize column selectivity by means of Eq. (1). As seen in Fig. 1a for type-B alkylsilica columns (i.e., C_1–C_{30}), *B* correlates inversely with column hydrophobicity *H*. The (bent) solid line in this figure defines average values of *H* vs. *B*, while the dotted lines bound the data within ±2 standard deviations (SD); see [5] for details. We do not ascribe any chemical significance to the apparent break in the average-value line of Fig. 1a. In the following text we will use the term "basicity" to refer to "hydrogen-bond acceptor strength."

Consider next a comparison of this relationship for type-B alkylsilica columns with similar plots (Fig. 1b and c) for two other column types; in each case, the dotted lines are identical to those in Fig. 1a. For embedded-polar-group (EPG) columns (Fig. 1b), a number of data points cluster within the two dotted lines (i.e., column basicity I), but the preponderance of these 48 columns have values of *B* that are much larger than expected (data within the dashed ellipse, falling outside the ±2SD limits from Fig. 1a). A similar plot is shown in Fig. 1c for 73 type-A alkylsilica columns. Here a smaller fraction of all type-A columns exhibit excess column basicity; i.e., larger than-expected values of *B*. As shown previously [2], it appears that the more basic stationary-phase sites present in EPG and type-A alkylsilica columns are not the same; thus type-A alkylsilica columns interact strongly with benzoic acids by hydrogen bonding, but only weakly with phenols (similar to type-B columns), while both phenols and benzoic acids interact strongly with EPG columns [2]. The latter behavior for EPG columns is consistent with simple hydrogen bonding between donor solutes and H–B basic polar groups within the stationary phase (amide, carbamate, urea, etc.). We will refer to these three contributions to *B* as column basicity I (Fig. 1a), II (outliers in Fig. 1b for EPG columns), and III (outliers in Fig. 1c for type-A columns).

The origin or nature of column basicity for type-B alkylsilica columns (Fig. 1a) has so far remained elusive. Plots of *B* vs. *H* for certain other columns prepared from type-B silica (phenyl, cyano, polar-end-capped) resemble that for type-B alkylsilica columns; i.e., few outliers > 2SD from plots as in Fig. 1a [2]. These various results suggest that the less-basic acceptor sites present in type-B alkylsilica columns (column basicity I) are common to all silica-based reversed-phase columns, and are therefore likely associated with the silica surface (rather than the bonded phase).