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Relevance of π–π and dipole–dipole interactions for retention on cyano and phenyl columns in reversed-phase liquid chromatography

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Abstract

Previous work suggests that π–π interactions between certain solutes and both phenyl and cyano columns can contribute to sample retention and the selectivity of these two column types versus alkylsilica columns. Recent studies also suggest that dipole–dipole interactions are generally unimportant for retention on cyano columns. The present study presents data for 44 solutes, three columns and two different mobile phases that were selected to further test these conclusions. We find that π–π interactions can contribute to retention on both cyano and phenyl columns, while dipole–dipole interactions are likely to be significant for the retention of polar aliphatic solutes on cyano columns. When acetonitrile/water mobile phases are used, both π–π and dipole–dipole interactions are suppressed, compared to the use of methanol/water.

Keywords: π–π Interactions; Dipole–dipole interaction

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1. Introduction

Differences in relative retention or selectivity for one column versus another are determined by the various interactions between different solutes and the stationary phase. Five such interactions have been characterized and measured for more than 300 reversed-phase (RP-LC) columns [1], based on the following equation:

$$\log k = \log k_{\mathrm{EB}} + \eta' H - \sigma' S^* + \beta' A + \alpha' B + \kappa' C \qquad (1)$$

The retention factor k for a solute can be related to various properties of the column: H, hydrophobicity; S^*, steric resistance to penetration of the solute into the stationary phase; A, hydrogen-bond acidity; B, hydrogen-bond basicity; C, cation-exchange capacity of the column. The parameters η', σ', etc. represent complementary properties of the solute (see Nomenclature, Section 6); k_{EB} refers to the retention factor k for ethylbenzene (reference compound). Values of the column parameters H, S^*, etc. can be used to characterize column selectivity; e.g., for the selection of (a) columns of equivalent selectivity that are interchangeable in a routine RP-LC method [2], or (b) columns of quite different selectivity for the development of an orthogonal separation [3].

An additional possible contribution to solute retention for phenyl columns is π–π interaction [4]; phenyl groups in the stationary phase can interact strongly with nitro-substituted aromatics and polycyclic aromatic hydrocarbons (PAH), and less strongly with other aromatics. Several prior reports have proposed that π–π interactions between solute and column are also significant for retention on cyanopropyl ("cyano") columns [5–7]. The application of Eq. (1) to several commercial cyano columns has been reported [8], but no test of the relative importance of π–π interactions was carried out in that study. Dipole–dipole interactions between solute and column also seem possible for cyano columns, because of the large dipole moment of the cyano group. However, limited studies with acetonitrile/buffer mobile phases and aromatic solutes appeared to rule out significant contributions of this kind [1].

The identification of various solute–column interactions and an assessment of their relative importance in affecting retention on different columns can be important to the practicing chromatographer, inasmuch as these interactions affect column selectivity and the ability of various columns to achieve the separation of different samples. A further study of retention

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on a C_8, phenyl and cyano column was therefore carried out, in order to further clarify the relative importance of π–π and dipole–dipole interactions for both cyano and phenyl columns (these interactions can be assumed absent for retention on alkylsilica columns).

2. Background and theory

2.1. Cyano columns

The prior conclusion that π–π interactions contribute to the retention of aromatic and other unsaturated solutes on cyano columns [5–7] is based on (a) the preferential retention of aromatic versus aliphatic solutes on cyano columns versus C_{18} columns, and (b) a decrease in this preferential retention when using mobile phases that contain increasing amounts of acetonitrile (ACN), compared to mobile phase containing methanol (MeOH). The suppressed retention of aromatics with ACN as B-solvent can be attributed to competitive π–π interactions between ACN and the solute molecule in the mobile phase (a similar interaction of ACN with stationary phase cyano groups would also reduce π–π interaction between solute and column). On the basis of previously reported experimental data, we find this interpretation for retention on cyano columns to be suggestive, but not compelling. Also, these (and other) past studies of π–π interaction in reversed-phase liquid chromatography (RP-LC) have usually involved mobile phases which are rich in the organic solvent (e.g., 70–100% B). Since most RP-LC separations carried out today employ mobile phases with lower concentrations of the B-solvent, conclusions based on higher %B may not be generally applicable.

2.2. Phenyl columns

Evidence for the importance of π–π interactions between phenyl columns and aromatic solutes has been summarized in [4], based on comparisons of retention for different solutes on phenyl versus C_8 columns. It was found that retention on phenyl columns (relative to that on alkylsilica columns) tends to increase in the order aliphatics < substituted benzenes < PAHs ≈ nitro-substituted aromatics, which is the order of increasing π-activity of the solute. The preferential retention of PAH's and nitro-substituted aromatics on phenyl columns is also significantly greater when MeOH is used as B-solvent, compared to the use of ACN. It is plausible to attribute the reduced retention of aromatics with ACN as B-solvent to π–π interactions of ACN with either aromatic solutes in the mobile phase or phenyl groups in the stationary phase (just as for retention on cyano columns; see above).

3. Experimental

3.1. Equipment, materials and procedures

These were as described previously [4,8]: model LC-10 (Shimadzu); 40% (v/v) acetonitrile/water or 60% methanol/water as mobile phase (except where noted otherwise); 35 °C; 2.0 mL/min; 500-ng injection of each solute; UV detection at 205 nm.

3.2. Columns

The columns used in this study were described previously [4,8]: (a) Restek Ultra C8 ("C_8"); (b) Kromasil KR-60-5CN ("cyano"); (c) Jones Genesis cyano ("cyano-2"); (d) Phenomenex Prodigy phenyl-3 ("phenyl"). Each column had dimensions of 15 cm × 0.46 cm; columns *a*, *b* and *d* were packed with 5-μm-diameter particles, while column *c* contains 4-μm-diameter particles. The Kromasil cyano column was used for all of the following comparisons of retention versus column type, except for the experiments summarized in Fig. 3.

3.3. Samples

The 44 solutes used in the present study are listed in Table 1. These can be classified as "substituted benzenes" (#1–18), "π-active" solutes (#19–32), and "aliphatics (#33–44).

3.4. Calculations

Values of the retention factor k were determined as $k = (t_R - t_0)/t_0$, where t_0 equals the retention time for thiourea. The extra-column volume of the HPLC system was not corrected for, as the latter quantity cancels out when values of k are compared for different columns (as in the present study).

4. Results and discussion

The approach followed here for the interpretation of the data of Table 1 is similar to that described in [1,4]. Values of log k for a given mobile phase and two different columns are plotted versus each other, with deviations from a best-fit line through the data being interpreted as the result of differences in solute–column interaction. The larger the deviations or more scattered the plot, the less similar are the two columns in terms of solute retention. Fig. 1 illustrates this for comparisons of retention on (a) the cyano versus the C_8 column, and (b) the cyano versus the phenyl column, with 60% MeOH as mobile phase in each case. In each plot of Fig. 1, a roughly linear correlation of retention times is observed, which is primarily the result of hydrophobic interactions between solute and column (the usually-dominant $\eta' H$ term of Eq. (1)). The scatter of data around this correlation line can be attributed to other solute interactions: π–π, dipole–dipole, etc.

Table 2 summarizes the results of similar plots for all three columns (C_8, cyano, phenyl) and both mobile phases (40% ACN, 60% MeOH). For each mobile phase, it is seen that there is a better correlation for the cyano versus phenyl column ($r^2 = 0.99$, 0.87), than for either of these columns versus the C_8 column ($r^2 = 0.93$–0.95, 0.71–0.82). These results suggest that the phenyl and cyano columns are more similar in their solute–column interactions than are the cyano and C_8 or phenyl