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REVIEWS

Reversed-Phase HPLC on Monomeric Reversed Phases: Factors Determining Adsorbate Retention

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Abstract—In this brief review, we consider various characterizations of "monomeric" reversed phases for elucidating the interactions governing adsorbate retention in liquid chromatography. Conventional methods related to the assessment of retention capacity and hydrophobicity (specifically methylene selectivity) using single mobile phase compositions are discussed with a focus on dispersion interactions, along with their inherent strengths and limitations. An alternative approach involving separation maps through relative retention analysis is proposed. It is noted that, in real reversed-phase adsorbents, the density of the attached alkyl chains is typically one half of that of solid *n*-alkanes. In this case, adsorbate molecules to penetrate into the attached phase, and the process depends on the molecular shape. Consequently, conventional "monomeric" reversed phases exhibit specific selectivity towards substances with specific structures. The review also notes that current analytical methods often do not pay sufficient attention to the difference between the substance retention mechanisms, absorption and adsorption, because the predominant parameters of these mechanisms are quite different. Moreover, in the two most widely used very interesting and informative methods, linear solvation energy relationships (LSERs) and the hydrophobic-subtraction model, this characteristic has not received due attention. Taking into account that the method does not distinguish adsorbates retained by different mechanisms, absorptive versus adsorptive, to the obtained significant discrepancies between the calculated and experimental data do not seem extraordinary. The interpretation of the results of an LSER analysis is also complicated by uncertainties in the contributions of partial properties of adsorbates in both mobile and stationary phases to the total solvation energy, as only their difference is typically calculated. Nonetheless, a comparison of different columns in identical mobile phases can yield informative insights. A drawback of the second approach is the necessity of using multiple columns with substantial qualitative differences in the adsorbate retention among them. Furthermore, a possibility of the decomposition of all interactions into distinct types seems questionable, because the method does not involve any orthogonal (independent of the applied calculation method) properties.

Keywords: reversed-phase columns, structure of "monomeric" phases, column characterization, linear solvation energy relationships, hydrophobic-subtraction model

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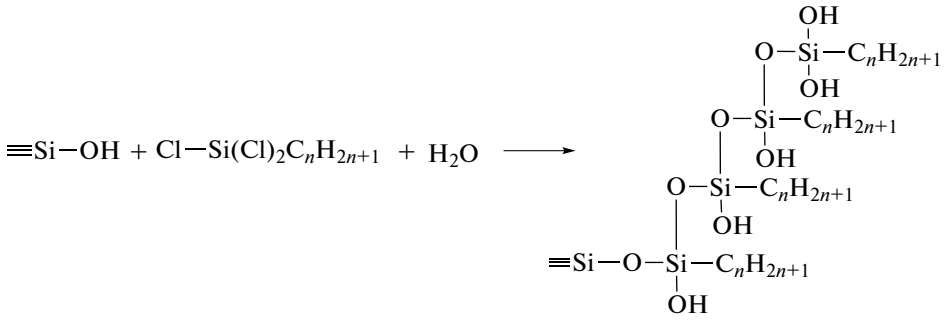
Reversed-phase adsorbents are originally normal-phase silicas with the surfaces derivatized by silylation by alkyl dimethylchlorosilane [1] through the covalent bonding via silanol groups, following through the reaction

$$\equiv Si-OH + Cl-Si(CH_3)_2C_nH_{2n+1} \xrightarrow[Py\text{-}HCl]{Py} \equiv Si-O-Si(CH_3)_2C_nH_{2n+1}$$

The mechanism described corresponds to the preparation of so-called "monomeric" reversed phases. In addition to monomeric phases, there are technologies for producing "polymeric" reversed phases, among which "polymeric monoliths" are the most well-known and widely used versions, obtained using silanes with three hydrolyzable groups. In polymeric monoliths, condensation occurs initially between the molecules of alkyl trichlorosilane, and then the resulting product is immobilized on the adsorbent,



Such phases have a fundamentally different structure compared to monomeric phases and exhibit a significantly different selectivity [2], but they are not considered in this study.

In analyzing the structure and properties of monomeric reversed-phase adsorbents, one should consider that the number of silanol groups on the surface of the fully hydroxylated silica is approximately 4.8 groups per nm^2 (Zhuravlev–Kiselev rule [3]). However, due to the presence of two methyl groups at the silicon atom of the silylating agent molecule, there are steric hindrances that allow for the derivatization of only about one half of the surface silanol groups [1]. Simple analysis [4] shows that, in such phases, the density of the attached alkyl groups is approximately one half of that of *n*-alkanes in a solid phase. Therefore, the space between the attached radicals can be filled with components of the mobile phase or utilized by adsorbates to penetrate into the depth of such a layer, ensuring a distribution mechanism of retention [5, 6].

On the other hand, highly hydrophilic compounds can be retained via an adsorption mechanism, remaining on the surface of the stationary phase if they are unable to displace the molecules of the organic modifier filling the gaps between the attached alkyl radicals. Initially, the adsorption mechanism of retention was proposed as the first retention mechanism in reversed-phase HPLC under the name of hydrophobic exclusion [7].

Therefore, retention in reversed-phase HPLC can occur by two fundamentally different mechanisms: absorption and adsorption. Additionally, a hybrid mechanism, termed "floatation," is possible, in which part of a molecule penetrates into the attached phase, while its hydrophilic part remains on the surface of the stationary phase [8] (Fig. 1).

Differentiation between these mechanisms based on the chromatographic behavior on a single stationary phase is not possible. However, we can use the retention analysis of sorbates on stationary phases with different lengths of the attached layers. The retention factor of sorbate i on stationary phase j, $k_j(i)$, is determined by its distribution constant between the stationary and mobile phases, $K_j(i)$, and column phase ratio, φ_j,

$$k_j(i) = K_j(i)\varphi_j. \quad (1)$$

If columns packed with stationary phases synthesized on the same silica are used, the phase ratios for the sorbates retained by adsorption mechanisms should be similar, and the retention of sorbates is minimally dependent on the length of the attached radical. Thus, in going from a C18 phase to a C8 phase, the adsorptive properties of the surfaces are quite similar, although the residual activity of the silanol groups in the C8 phase is higher than that in the C18 phase. In contrast, for an absorption mechanism, the volume of the attached layer decreases by more than half in the same transition. Therefore, the retention factor should decrease by more than half as well [9].

Dispersion interactions. The covalent immobilization of silanes with nonpolar alkyl groups on the silica surface transforms a polar adsorbent into a nonpolar one. The attached alkyl groups have nearly zero dipole moment; hence, the interaction between the adsorbate and the adsorbent primarily occurs through dispersion interactions, leading to increased retention with (a) increasing hydrophobicity of the adsorbate, (b) increasing hydrophobicity of the stationary phase, and (c) increasing polarity of the mobile phase.

From a thermodynamic standpoint, the retention of adsorbates is generally determined by the difference in their free energies between the stationary and mobile phases (within the linear region of the adsorption isotherm). However, in the literature, different variants are distinguished, in which the following dominating factors are considered: (a) hydrophobic interactions of the adsorbate with components of the aqueous-organic mobile phase [7] and (b) lipophilic interactions of the adsorbate with the stationary phase [10].

Monomeric reversed phases can vary in several aspects:

— Properties of silica used for derivatization: this includes specific surface area, pore size and volume, and surface cleanliness;

— Degree and quality of derivatization: this can vary with or without embedding and the denticity of the attached group;

— Activity of residual silanol groups: this is affected by additional treatments, such as endcapping, either nonpolar or polar.

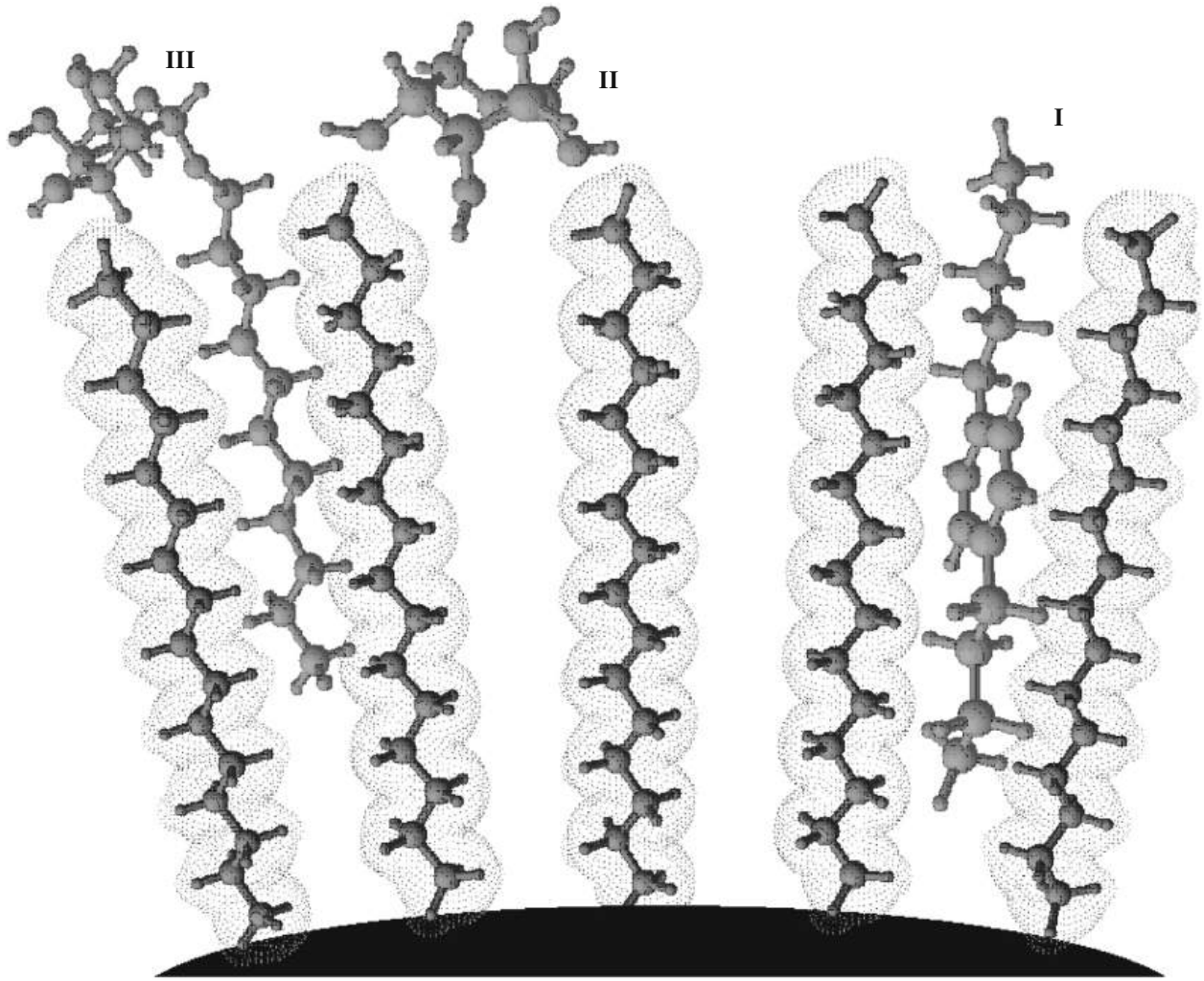


Fig. 1. Mechanisms of adsorbate retention on monomeric reversed phases: I, absorption; II, adsorption; III, floatation.

For these reasons, significant differences arise in the adsorption properties of adsorbents from different brands (and sometimes between the batches of the same brand). Parameters have been introduced to characterize the stationary phases' interaction with adsorbates in reversed-phase columns. For instance, the absolute retention of ethylbenzene gives an estimate of the column retentiveness, or column strength, which is proposed to be determined in an eluent containing 80% methanol and 20% water at 23°C according to the US standard [11]. According to Galushko's proposal [12], the hydrophobicity of columns can be evaluated using the semisum of the retention factors of benzene and toluene. Another approach suggests the use of the logarithm of the retention factor approximated at the zero organic modifier content [12] as a measure of column hydrophobicity. However, applying these three parameters to compare the properties of columns from different manufacturers introduces uncertainty due to potential (and significant) differences in the phase ratio in Eq. (1). Nevertheless, using the ratio of retention factors of two substances on the same column as a property parameter reduces phase ratio ambiguity,

$$\frac{k_j(a)}{k_j(b)} = \frac{K_j(a)}{K_j(b)}\frac{\varphi_j}{\varphi_j} = \frac{K_j(a)}{K_j(b)}. \quad (2)$$

Such a ratio (referred to as selectivity) is a more reliable parameter for determining the hydrophobicity of columns. The hydrophobicity parameters (or methylene selectivity) according to the Tanaka test are determined as the ratio of retention factors of amylbenzene to butylbenzene, the ratio of retention factors of ethylbenzene to toluene (Engelhardt test), and even of anthracene to benzene (Walters test), which no longer have the for the sense of methylene selectivity [12].

In this regard, an analysis of second-type separation maps [13] (Fig. 2) offers several advantages. Figure 2 shows the retention dependence of 4-caffeoylquinic acid (4CQA) on 5-caffeoylquinic acid (5CQA) in acetonitrile–0.3% orthophosphoric acid–water elu-

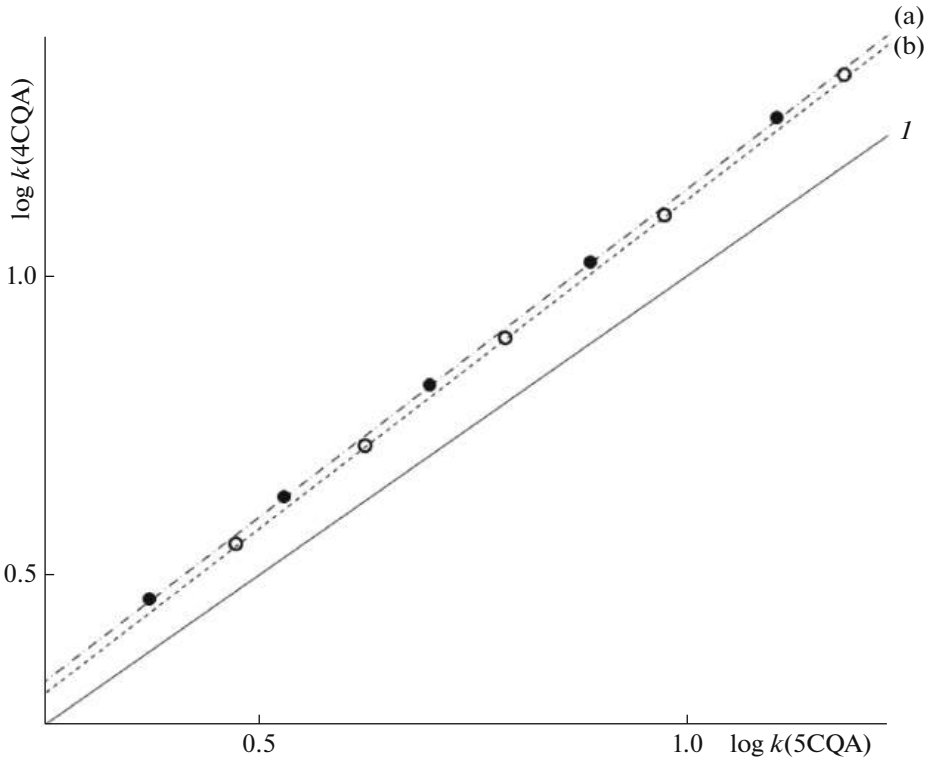


Fig. 2. Second-type separation map: retention of 4-caffeoylquinic acid (4CQA) relative to 5-caffeoylquinic acid (5CQA) in the acetonitrile–0.3% orthophosphoric acid–water eluents in two Symmetry C18 columns (a and b).

ents. The lines representing the relative retention of 4CQA have a steeper slope compared to the line for 5CQA. This indicates that the selectivity of separating these two isomers increases with their retention. Methylene selectivity also depends on the composition of the mobile phase. The proposed separation map allows a comparison of the properties of different stationary phases. This allows for detecting differences in the retention of these compounds in replacing one column with another of the same brand but subjected to long-term use under harsh conditions (in a mobile phase containing 10% formic acid with a pH around 1.5). As pH no lower than 2 is the upper limit of acceptable acidity for mobile phases with the guaranteed stability of conventional C18 phases [14], prolonged operation in more acidic mobile phases causes the hydrolysis of some attached alkyl groups. This results in a noticeable decrease in retention; the selectivity of the separation of these isomers also decreases, albeit to a lesser extent.

Another example was given in [15]. Here, the logarithm of the retention factor of toluene relative to the logarithm of the retention factor of benzene for 21 columns, analyzed according to the US standard [11] at the same mobile phase composition (as mentioned above), showed a linear relationship close to the straight line for the relative retention of these same compounds in the same column but with different mobile phase compositions. Expecting a perfectly strict matching of relative retention on different columns was not reasonable in principle, because the logarithms of the phase ratios involved varied for different stationary phases, as mentioned earlier.

Selectivity to the shape of adsorbates. This property of reversed-phase stationary phases is fundamentally important for separating complex mixtures. In focusing on selectivity regarding the shape of adsorbates, this property is more commonly attributed to polymeric reversed phases. The sensitivity of retention to the shape of adsorbates for polymeric and monomeric phases depends on various factors. In the case of monomeric reversed phases,

(a) Conditionally linear *n*-alkyl groups can easily penetrate into the attached layer. However, in the case of adsorbates with double C=C bonds in the *cis* configuration attempting to penetrate into the attached layer, a conformational rearrangement of the alkyl chains in the attached layer is required, necessitating an expenditure of free energy within this layer. This requirement is more pronounced for the bonds closer to the "head" methyl group in the alkenyl radical penetrating into the attached layer [4, 16] (Fig. 3). Such a model of the structure of a monomeric reversed phase is the sole justification for the applicability of the topological model, which explains a difference in the

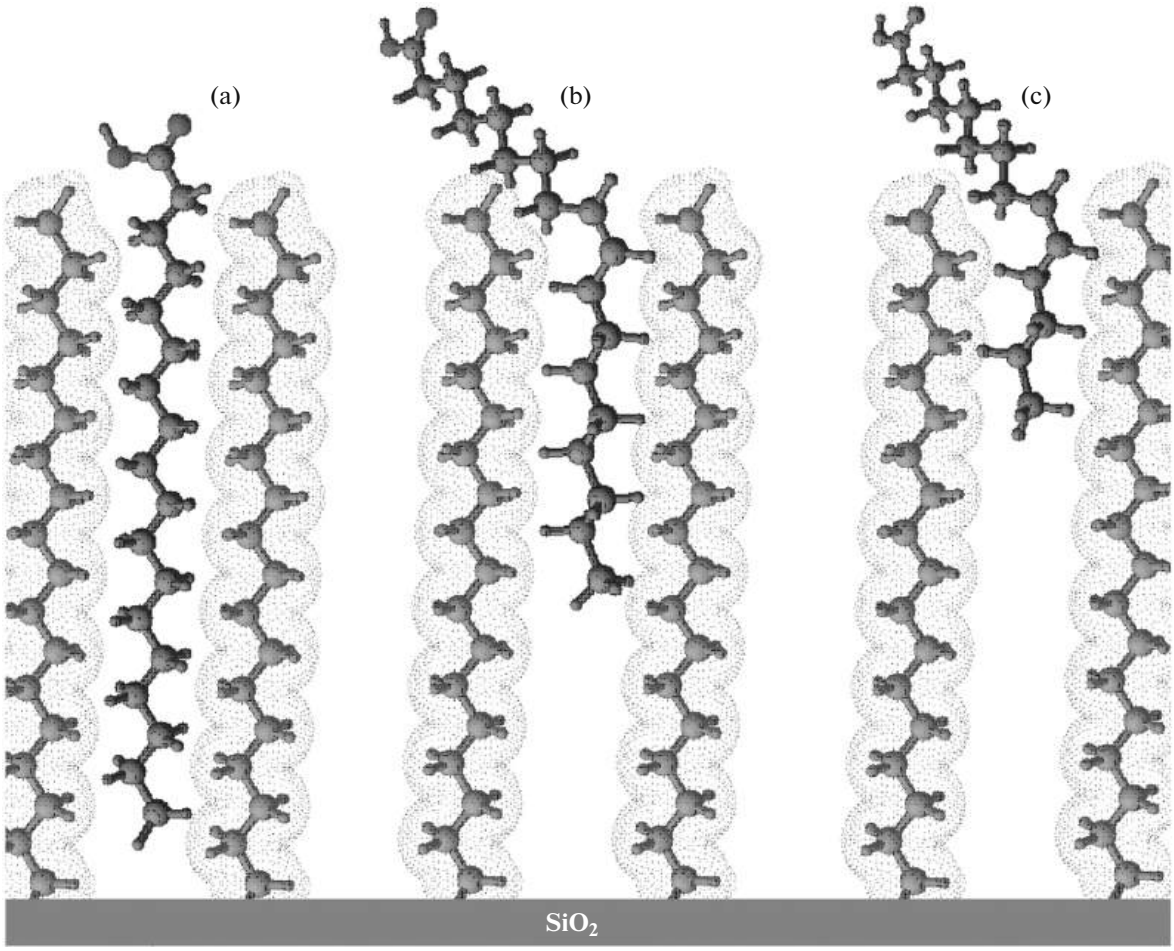


Fig. 3. On the ease and steric hindrances of fatty acid adsorption: (a) *n*-alkanoic acid, (b) ω-9-*n*-alkenoic acid, and (c) ω-5-*n*-alkenoic acid.

adsorbate retention with isomeric structures of alkyl radicals [17];

(b) In the distribution mechanism of carotenoid adsorption, the complete immersion of carotenoid molecules into the attached layer necessitates the presence of linear rows with nonderivatized silanol groups on the adsorbent surface. Conversely, the chaotic derivatization positions and the irregular structure of amorphous silica require additional conformational rearrangements of the alkyl chains in the attached layer during the carotenoid adsorption. This concept helps to explain, for instance, the experimentally observed elution order of fully *trans*-, 9-*cis*-, 13-*cis*-, and 15-*cis*-isomers of β-carotene [18–20];

(c) For flat adsorbates, penetration into the attached layer is easier compared to the nonflat adsorbates. This aspect of adsorption by specifically selected monomeric reversed-phase columns is well recognized. In the Tanaka test, for example, the retention factor ratio of flat triphenylene and nonflat *ortho*-terphenyl (due to the repulsion of hydrogen atoms from the first and third phenyl rings) is determined (Fig. 4) for monomeric reversed-phase columns, although the high efficiency of polymeric phases in differentiating adsorbates based on their spatial structure has made their use preferable in HPLC [21]. The flat structure of quercetin, in which the OH group at position 3 is partially shielded by aromatic ring B, explains its significantly higher retention compared to nonflat dihydroquercetin, despite the C=C bond being replaced with a more lipophilic CH_2–CH_2 group (Fig. 4) [22].

Activity of residual silanol groups. Polar interactions between the adsorbate and adsorbent the for monomeric reversed-phase adsorbents are determined by the availability of residual silanol groups, the amount of which is approximately one half of those present initially on the silica surface before derivatization. The activity of these residual silanol groups causes significant differences in the adsorption properties of reversed-phase columns from different manufacturers. Because of the randomness of the silanization of silanol groups and the irregular structure of amorphous silica at the initial stage, regions may appear on

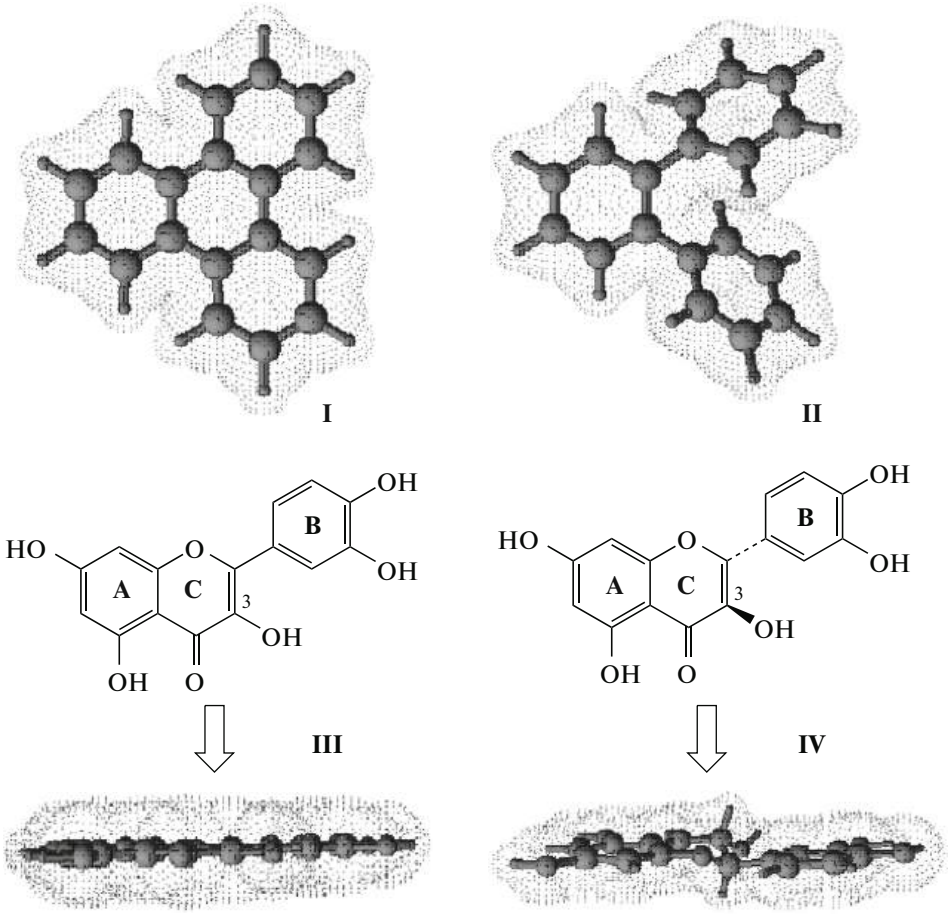


Fig. 4. Chemical structures of (I) triphenylene, (II) *o*-terphenyl, (III) quercetin, and (IV) dihydroquercetin.

its surface where two free silanol groups rather than one can group are formed between the attached groups. In such cases, the additional silanization of one of these groups with trimethylchlorosilane allows a researcher to reduce their total activity, thea process known as endcapping [23]. Another technology exists for reducing the activity of residual silanol groups—embedding [24]. However, stationary phases of this type are not as widespread as phases with endcapping, and therefore they are not discussed in this review.

Therefore, in conventional monomeric reversed-phase columns with endcapping, adsorbate molecules with polar groups capable of penetrating into the attached phase can form hydrogen bonds or other types of polar interactions with residual silanol groups. To suppress such interactions, additives competing with the adsorbates for the opportunity to form hydrogen bonds are introduced into the mobile phase. For instance, based on our laboratory's experience in determining higher fatty acids derived from fat hydrolysis, chromatograms often show broadened peaks with tailing. However, adding less than 1% triethylamine or acetic acid to the mobile phase significantly improves peak shapes.

Several different tests have been proposed to assess the activity of silanol groups. The work [25] listed test options:

(a) Engelhardt's test evaluates "silanophilic interactions" (a misleading term, because silanes and silanol groups are chemically quite distinct entities) by determining the retention factors of aniline and phenol in a mobile phase containing 55% methanol in water at 40°C;

(b) Walters' test measures the retention factor ratio of *N*,*N*-dimethyl-*m*-toluidine (DMT) and anthracene in 100% acetonitrile at 40°C;

(c) Galushko proposed a peculiar equation taking into attention the retention factors of aniline and phenol,

$$S_G = 1 + 3\left[\frac{k(\text{aniline})}{k(\text{phenol})} - 1\right]. \quad (3)$$

In [12], Engelhardt's test for silanol activity involves the assessment of the peak asymmetry of diethyleneamine at 5% of the peak height in the eluents 49 : 51 or 55 : 45 (v/v) methanol–water at 40°C. Tanaka's test evaluates the ability of forming hydrogen bonds using the ratio of the retention factors of caf-

feine, a significantly more stable compound upon storage compared to aniline, and phenol using uracil as the dead time marker in an eluent containing 30% methanol in water. Additionally, ion exchange capacity tests are conducted under different pH conditions by determining the ratio of the retention factors of benzylamine and phenol in the eluents 30 : 70 (v/v) methanol–water with a 0.02 M phosphate buffer solution at pH 7.6 and a 0.02 M aqueous phosphate buffer solution at pH 2.7.

In the US standard [11], amitriptyline and chrysazin are used as reference compounds. Chrysazin (1,4-dihydroxyanthraquinone) acts as a metal-chelating agent, making its chromatographic behavior dependent on the presence or absence of metals in the chromatographic system. When the adsorbent exhibits low activity towards chelating agents, symmetrical peaks are formed. Conversely, high activity results in asymmetrical peaks with tailing. Chrysazin is typically eluted after ethylbenzene and before amitriptyline. In columns with certain types of embedding, chrysazin may be eluted the last with a good peak symmetry. Amitriptyline, a tricyclic antidepressant ($pK_a = 9.4$), is a primary pharmaceutical compound widely used by column manufacturers for characterization. The elution of organic bases with tailing peaks is often attributed to high silanol activity, making peak asymmetry a suitable measure of the silanol activity.

As in the previously proposed approach to mitigate the shortcomings of point methods, an analysis of second-type separation maps [26] can be employed.

Thus, in the retention of adsorbates on monomeric reversed-phase adsorbents, two distinct adsorption mechanisms may take place (as well as an intermediate mechanism known as "floating" [8]). Each of these mechanisms possesses its own stereospecific properties; therefore, the evaluation of the relationship between the structure of the adsorbates and their retention must be performed after their separation by a particular mechanism.

Linear Solvation Energy Relationships (LSERs). The method has become widely adopted based on an approach developed by the renowned experts in physical organic chemistry Kamlet, Taft, and others [27]. This method aims at determining the contributions of different types of intermolecular interactions to the retention energy of substances in reversed-phase chromatography, assuming the additivity of these characteristics. The Gibbs free energy of the transfer of a sorbate from the mobile phase to the stationary phase is proportional to the logarithm of its retention factor. To eliminate the effect of phase composition, the logarithm of the retention factor of a reference substance is subtracted from this logarithm. According to the method proposed by Abraham et al. [28, 29], the retention of adsorbates in reversed-phase chromatography is determined by the equation:

$$\log k = \log k_0 + M(\psi_s - \psi_m)V_2 + S(\pi_s^* - \pi_m^*)\pi_2^* + A(\beta_s - \beta_m)\alpha_2 + B(\alpha_s - \alpha_m)\beta_2. \quad (4)$$

In Eq. (4), the subscripts s and m refer to the stationary and mobile phases, respectively. The subscript 2 denotes partial properties of the adsorbate, such as molar volume (V), polarity and polarizability (π), acidity (α) as a hydrogen bond donor activity, and basicity (β) as a hydrogen bond acceptor activity. The coefficients at these parameters represent the difference in complementary properties between the stationary and mobile phases. It is assumed that the coefficients denoted by uppercase letters, as well as $\log k_0$, are adjustable parameters independent of the adsorbate and the nature of the chromatographic phases.

The term $M(\psi_s - \psi_m)V_2$ is the most complex term in Eq. (4). It represents the term of the cavity, which is necessary to accommodate the adsorbate molecule in both phases, expressed as the cohesion energy defined by Hildebrand parameters: $M_1(\delta_{H,s}^2 - \delta_{H,m}^2)V_2$, stabilized by dispersion interactions between the adsorbate molecules and the dispersion media, $M_2(D_2 - D_m)V_2$.

Alkyl radicals in the attached phase would seemingly impart zero partial components related to polarity (and polarizability) and the ability of forming hydrogen bonds. However, upon the adsorption of water and organic modifiers inside the attached phase, they acquire certain values of these parameters (ranging from 0.7 to 1.1 for π^*).

For the fixed stationary and mobile phases, the equation simplifies to

$$\log k = \log k_0 + mV_2 + s\pi_2^* + a\alpha_2 + b\beta_2. \quad (5)$$

The main objective of the method is to assess the corresponding parameters of the adsorbate using any suitable approache. For V_2, the use of the McGowan parameter V_x [30] is convenient. Other parameters are determined through the study of various properties of adsorbates, such as their chromatographic behavior under gas chromatography conditions [31]. Once all descriptors are determined, the task is reduced to a multiple linear regression for determining the terms related to the chromatographic system—specifically, the column used and the composition of the mobile phase. This approach should identify the primary types of interactions governing sorption processes and quantify their contribution to the overall retention.

The equations for linear relationships between solvation energies have been slightly changed or supplemented in many publications. Thus, in [32], the following equation was used instead of Eq. (6):

$$\log k = \log k_0 + vV_2 + s\pi_2^* + a\sum\alpha_2^H + b\sum\beta_2^H + rR_2, \quad (6)$$

in which an additional term (last) appeared—the excess molar refraction of the adsorbate R_2,—and the complementary value of r, depending on the mobile and stationary phases.

An analysis of the coefficients associated with the stationary and mobile phases reveals the following trends for typical monomeric C18 phases:

• Coefficient v is positive and decreases with increasing concentration of acetonitrile in the mobile phase. This suggests a greater contribution of the dispersion interactions and solvation energy of the adsorbate in the stationary phase compared to the mobile phase. The decrease in this difference is associated with an increased lipophilicity of the mobile phase with increasing acetonitrile concentration;

• Coefficient s, related to the polarity or polarizability of the adsorbate, is negative and remains almost unchanged with increasing acetonitrile concentration in the mobile phase. This indicates that polar interactions in the mobile phase are stronger than in the stationary phase;

• Coefficients α and β are also negative, but their absolute values decrease with increasing acetonitrile concentration in the mobile phase. This suggests that water, a critical component responsible for hydrogen bonding, exerts a stronger effect in the mobile phase compared to the stationary phase.

In going to a C8 phase (in which the availability of residual silanol groups on the adsorbent increases), coefficient s remains positive but is approximately one and a half times lower than for C18 phases. This suggests the strengthening of polar interactions in the stationary phase due to an increased availability of the residual silanol groups on the adsorbent. As anticipated, the energies associated with hydrogen bonding (both acidity and basicity) decrease in absolute terms under identical mobile phase compositions.

Thus, the method allows for assessing the contributions of various interactions in both phases, but a quantitative separation of this contribution into the roles of the stationary and mobile phases is unlikely. An explicit limitation of the model is its applicability only to substances for which descriptors were determined beforehand.

According to Wilson et al. [33], using the equations presented above as a method for characterizing columns is limited. Firstly, the predictive accuracy of the method is no better than 10–20%, which is often unacceptable, especially for substances with R_s values ranging from 0.5 to 2.0. Secondly, the use of contributions to the overall retention of some (a limited number of) parameters requires justification; furthermore, the use of adsorbate descriptors determined by the methods other than HPLC does not guarantee their applicability to HPLC.

Additionally, this method indiscriminately accounts for the geometric parameters of adsorbates, and not all adsorbates used are classified based on their retention mechanisms. This lack of classification may be responsible for the typically high dispersion between the calculated retention factor values and the experimental data.

Hydrophobic-subtraction model. As mentioned above, the retention of adsorbates in reversed-phase chromatography depends not only on dispersion interactions between the adsorbate and the stationary phase but also on other types of interactions. Upon comparing the logarithms of retention factors for 90 different adsorbates and 10 different columns, it was found that, for the Intersil ODS-3 column relative to the StableBond C18 column, there exists a linear relationship with the slope close to unity (1.01), a high correlation coefficient ($R^2 = 0.995$), and a relatively small standard deviation (0.034) [24]. An analysis of these data enabled a conclusion that the contributions of the major interaction types are qualitatively similar for most adsorbates used (excluding strong bases and aliphatic amides), but significant differences occur for substances of the basic type. Therefore, a comprehensive model must take into account contributions from various types of interactions.

The hydrophobic-subtraction model [25] suggests the use of the selectivity of the separation of all adsorbates relative to nonpolar ethylbenzene according to the equation

$$\log\alpha = \log\left(\frac{k}{k_{eb}}\right) = \eta' H - \sigma' S + \beta' A + \alpha' B + \kappa' C, \quad (7)$$

where k is the retention factor of an adsorbate in question, k_{eb} is the retention factor of ethylbenzene, measured under identical conditions; the remaining symbols indicate empirical, dependent on the composition of the mobile phase and temperature, descriptors related to a given adsorbate (η', σ', β', α', and κ'), or related to the adsorbent, independent of the composition of the eluent and temperature, adsorbent descriptors (H, S, A, B, and C).

The approximate nature (actually determined only after the classification of the adsorbates used in the method) of the contributions considered in the equation includes:

• A parameter related to hydrophobicity (first product);

• A parameter associated with steric factors (second product);

• A, a descriptor assessing the propensity of nonionized residual silanol groups to forming hydrogen bonds as hydrogen bond donors;

• B, an identical parameter, but relating to hydrogen bond acceptors, interacting with water molecules adsorbed on the silica surface;

• C accounting for the cation exchange properties of the adsorbent.

Additive parameters (η', σ', β', α', and κ') indicate the corresponding complementary properties of the adsorbates. A detailed method for determining these parameters was described in [34]. Ultimately, the authors of the cited work found that the reliability of the correlations obtained is significantly higher than in the method of linear relationships between the solvation energies.

In the proposed method, the parameters obtained actually lack clear physical meaning, as they have not been correlated with any specific physical properties of the adsorbates. Initially, relative retentions of all 67 adsorbates (relative to the retention of ethylbenzene used as a reference substance) were compared, identifying those exhibiting linear dependences across all columns relative to the reference column (SB 100). Subsequently, substances lacking such correlations were grouped based on linearities in retention differences between pairs of substances across 10 different stationary phases used. Using multiple linear correlations across the entire set of adsorbates, descriptors for the adsorbates were determined. Finally, a multiple linear correlation for the relative retention of all adsorbates was utilized to refine the parameters of the columns (in reality, the chromatographic systems, which included both mobile and stationary phases).

In classifying interaction types, the authors do not model steric selectivity, assuming that it manifests itself automatically. Ultimately, their approach is applicable to analyzing retention across a broad range of adsorbates on columns with intentionally varied partial (interaction type) properties. Application of the method to columns with similar surface chemistry is not meaningful. Finally, as in the previous method, the initial data contain high negative values of retention factor logarithms, the numerical values of which heavily depend on the reliability of the dead time determination method used.

CONCLUSIONS

Thus, an analysis of literature data indicates that the challenge of determining the types of intermolecular interactions governing adsorbate retention on monomeric reversed-phase stationary phases is highly complex. This complexity exists not only at a quantitative but also at a qualitative level. Primarily, it stems from the difficulty in accounting for the sensitivity of the stationary phases to the volumetric factors of adsorbates in the adsorption mechanism, for which justified descriptors are currently unknown. Lastly, there is a need in differentiating the adsorbates retained through adsorption or absorption mechanisms, the retention patterns of which can significantly differ across all types of interactions.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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