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Martin's rule revisited. Its molecular sense and limitations

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Abstract

The linear relation $\ln k' = Bn + \ln A$ between the retention factor k' in liquid adsorption chromatography (LAC) and the number of repeat units n within a homologous series of oligomers is called Martin's rule. This empirical relation was supported by the retention behavior of the homologous series of different classes of oligomers but had no theoretical justification. In this paper, it is demonstrated that Martin's rule is a consequence of the general theory of liquid chromatography and the molecular sense of coefficients B and A is clarified: B is the Gibbs energy of the repeat unit of the long polymer chain adsorbed at the wall surface, and A is a combination different parameters which characterize the column and the adsorption correlation length H.

The theory predicts the deviations from the linear dependence under conditions of weak adsorption between repeat units and stationary phase when H is close to radius of gyration R_g. Experimental data for retention volumes and selectivity of poly(ethylene glycol)s are given for normal and reversed-phase LAC on different columns in acetone–water and methanol–water as mobile phases. These data show excellent agreement between the theory and experiments. It is shown that Martin's rule holds under special conditions, which are theoretically defined by the relation $H > R_g/1.5$.

Keywords: Martin's rule; Adsorption chromatography; Distribution coefficient; Adsorption; Poly(ethylene glycol)

1. Introduction

It has been a persistent goal in chromatography to predict the dependence of the retention time (or volume) on molar mass, solute and adsorbent's characteristics. For this purpose, both theoretical and pragmatic approaches have been used to relate the retention volume to a variety of parameters, including the molecular structure of polymer chains and the thermodynamic properties of stationary and mobile phases.

In order to make different columns comparable, the dimensionless retention factor k' has been introduced, which is given by the retention volume V_R of the solute and the total void volume V_0 of the column: $k' = (V_R - V_0)/V_0$ is thus independent of column dimensions. An important problem is the correct determination of the void volume [6,12–14]. We follow the definition of the mobile phase volume as the total volume of liquid phase in the column.

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The retention factor k' is frequently used in studies of the retention mechanism in liquid chromatography instead of the elution volume or elution time. A simple relation between the retention factor k' and the number of repeat units n within a homologous series of oligomers was suggested by Martin [1] in 1949, who found empirically, that within homologous series k' increases exponentially with the number of repeat units n (e.g. in reversed-phase chromatography with the number of methylene groups).

$$k' = A \exp(Bn) \tag{1}$$

The logarithmic form of Eq. (1) is well known as Martin's rule.

$$\ln k' = Bn + \ln A \tag{2}$$

wherein A and B are empirical coefficients. This linear relationship between $\ln k'$ and the chain length has been observed experimentally for many homologous series of different classes of oligomers, such as alkyl benzenes [2,3], aliphatic ketones [4], alkyl aryl ketones [4], and alcohols [5].

It was shown that B strongly depends on the composition of the mobile phase. This dependence was expressed by empirical equations in polynomial form with two and three terms [6]. Some scientists also described a dependence of A on mobile phase composition and tried to establish a correlation between A and B [7].

The linear dependence (Eq. (2)) has been used for various purposes, such as the evaluation of solvent eluotropic strength [7], the optimization of separation, etc. [8,9]. An overview is given in several reviews [7,10–12].

The validity of Eq. (2) was so generally accepted that it was also used as a basis for estimating column dead volumes [6,12–14], which is a persistent problem in HPLC. There was, however, no rigorous thermodynamic reason to justify this linear behavior.

Most of the authors considered the dependence of $\ln k'$ on n as straight lines, although considerable deviations were observed in the lower molecular range. This was demonstrated experimentally for several homologous series, in which a wide range of solute chain length and different solvents were included [15]. The slope for long chain solutes was virtually constant but as the chain length decreased the slope of the curve increased slightly [6,11].

The non-linearity of retention data for homologous series becomes even more obvious, when the selectivity $S = k'_{n+1}/k'_n$ is plotted versus n. According to Martin's rule, the selectivity has to be constant but in reality the value of selectivity increases with decreasing n [7,11,16].

Most of these experimental data on retention behavior of oligomers were obtained in the case of strong adsorption for rather short homologous series (with typically no more than 10–15 repeat units). The deviations from linearity were typically observed in the region below 4–5 repeat units, depending on the series [11,17].

Some scientists explained these deviations in terms of a gradual loss of conformational entropy of the solute with increasing n [11,18]. It was also suggested that deviations should be expected when n becomes larger than a critical value, which depends on the length of the bonded moieties in the stationary phase [11,17]. There were other suggestions, too, such as a discontinuity of the gradual increase of London forces between the solute and support [7], but none of them could provide a really convincing explanation.

Recently, we have studied the retention behavior of PEG and PPG with up to 70 repeat units in isocratic regime using different mobile phase compositions [19]. Obviously, this can only be achieved under conditions of weak adsorption interaction between repeat units and stationary phase. It was shown that deviations from linearity occurred below a certain number of repeat units, which increased with decreasing strength of the interaction.

In this paper, we will discuss the nature of these deviations and show that Martin's rule is a result of the theory of liquid adsorption chromatography (LAC). We shall use the term LAC in the subsequent text as a synonym to interaction chromatography, regardless of the nature of the interaction (whether adsorption or partition) [20,21]. We will show the molecular sense of the coefficients A and B. Moreover, we will present the criteria, under which Martin's rule holds, and discuss the behavior of selectivity.

2. Experimental

These investigations were performed using the density detection system DDS70 (CHROMTECH, Graz,

Austria), which has been developed in our group. Data acquisition and processing was performed using the software package CHROMA, which has been developed for the DDS 70.

Columns and density cells were placed in a thermostatted box, in which a temperature of 25.0 °C was maintained for all measurements. In both systems, the columns were connected to two column selection valves (Rheodyne 7060, from Rheodyne, Cotati, CA, USA).

In reversed-phase LAC, the mobile phase was delivered by a Jasco 880 PU pump (Japan Spectrosopic Company, Tokyo, Japan) at a flow rate of 0.5 ml/min. A Bischoff 8110 RI detector (Bischoff, Leonberg, Germany) was connected to the DDS 70. The following columns were used in RP-LAC:

- Prodigy 5 μm ODS3 (from Phemonenex, Torrance, CA, USA), silica-based octadecyl phase, 250 mm × 4.6 mm, particle diameter: 5 μm, nominal pore size: 10 nm.
- Spherisorb ODS2 (from Phase Separations Ltd., Deeside, Clywd, UK), silica-based octadecyl phase, 250 mm × 4.6 mm, particle diameter: 5 μm, nominal pore size: 8 nm.
- Spherisorb S5× C18 (from Phase Separations Ltd., Deeside, Clywd, UK), silica-based octadecyl phase, 250 mm × 4.6 mm, particle diameter: 5 μm, nominal pore size: 30 nm.
- Spherisorb S5P (from Phase Separations Ltd., Deeside, Clywd, UK), silica-based phenyl phase, 250 mm × 4.6 mm, particle diameter: 5 μm, nominal pore size: 8 nm.
- Jordi Gel 500 RP (from Jordi, Bellingham, MA, USA): 100% divinylbenzene, 250 mm × 4.6 mm, particle diameter: 5 μm, nominal pore size: 50 nm.

In normal phase LAC, a flow rate of 0.5 ml/min was maintained with an ISCO 2350 HPLC pump (ISCO, Lincoln, NE, USA). A Spherisorb S3W column (from Phase Separations Ltd., Deeside, Clywd, UK), plain silica, 150 mm × 4.6 mm, particle diameter: 3 μm, nominal pore size: 8 nm was used for all NP-LAC measurements.

Samples were injected using a Spark 125 autosampler equipped with an 20 μl loop. A Sedex 45 ELSD apparatus (Sedere, Vitry sur Saine, France) was connected to the DDS 70. Nitrogen was used as the carrier gas, the pressure at the nebulizer was set to 2.0 bar and the temperature of the evaporator to 30 °C.

The solvents (acetone, methanol and water, all HPLC grade) were purchased from Roth, Karlsruhe, Germany. The mobile phase compositions are always given in wt.% (i.e. 85% methanol denotes methanol–water 85:15 (w/w)). Polyethylene glycols were purchased from Sigma–Aldrich, Vienna, Austria and Fluka, Buchs, Switzerland.

The radius of gyration of PEG was estimated using the relation $R_g = a\sqrt{n/6}$ according to literature data [22] corresponding to the unperturbed dimensions of polyoxyethylene.

3. Theoretical considerations

The general concept of LC considers the column as a combination of the interstitial (free) volume V_i—the volume of the mobile liquid phase, and the pore volume V_p—the volume of stagnant liquid phase. The surface of the pores can be solid (e.g. plain silica in normal phase LC) or modified with surface grafted alkyl chains (as is typically the case in reversed-phase LC). The surface outside the pores is not taken into account. The retention volume is then given by:

$$V_R = V_i + KV_p \tag{3}$$

wherein the distribution coefficient K reflects the partitioning of solutes between the free volume V_i and pore volume V_p. The distribution coefficient K is defined as the ratio of the average polymer concentration in the pore and the concentration in the interstitial volume.

From thermodynamically point of view the distribution coefficient $K = \exp(-G/RT)$ is related to the change of the Gibbs energy G of the polymer chain when it transfers from the free volume V_i into the pore volume V_p. We will use the state of the polymer chain in free volume as a referent state and use G as the change of the Gibbs energy instead ΔG. The Gibbs energy G can be determined experimentally using:

$$-\frac{G}{RT} = \ln K = \ln\frac{V_R - V_i}{V_p} \tag{4}$$

Relation (3) is typically applied in size-exclusion chromatography (SEC) where $0 < K < 1$. We will use this relation in LAC where $K > 1$ and in critical chromatography (LC under critical conditions, LCCC),

where K is close to unity. The reason of this extension is connected with the physical meaning of V_R.

The retention volume V_R is the partition function of a polymer chain in a chromatographic column, which corresponds to the sum of the partition function of a polymer chain in free space and inside a pore at any adsorption interaction between polymer chain and pore surface. All conformations of polymer chain in the column can be separated into mobile and stationary conformations. Mobile conformations are the conformations of the polymer chain in the interstitial volume where a polymer chain exists as a coil in free volume. Stationary conformations are the conformations of the polymer chain inside the pore volume. These stationary conformations can be separated into floating and adsorbed conformations (see Fig. 1). Floating conformations have no contact with the pore surface. Adsorbed conformations have at least one unit of the polymer chain in contact with the wall surface. The statistical weight of each adsorbed conformation depends on the adsorption interaction. The weights of floating conformations do not depend on the adsorption interaction and are close to unity as for the coil in mobile conformations (in free volume). The amount of the adsorbed conformations is much smaller than the amount of floating conformations but the weight of the typical adsorbed conformation is much higher than unity.

Consequently, the full distribution coefficient K (or the full partition function) is the sum of the distribution coefficients K_{fl} and K_{ads} of floating and adsorbed states:

$$K = K_{fl} + K_{ads} \tag{5}$$

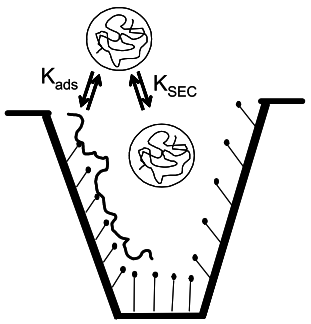

Fig. 1. Partitioning of polymer molecules in adsorption liquid chromatography (schematic representation).

Only the overall distribution coefficient K is important for retention, since a molecule moves through the column, when it is in the interstitial volume, and it is retained, as long as it is in the stagnant volume within the pores, regardless whether in floating or adsorbed states.

The general theory of LC considers the distribution coefficient as the partition function of the polymer chain in porous media. The overall distribution coefficient K results from two partitions, namely between the mobile phase in the interstitial volume and (a) floating state in the pores K_{fl} and (b) the adsorbed state K_{ads} as is shown schematically in Fig. 1.

The general theory of LC operates with two fundamental parameters: the radius of gyration R_g and the correlation length H. The radius of gyration R_g for the model of a polymer chain as a Gaussian coil is given by $R_g = a\sqrt{n/6}$, wherein n is the number of repeat units and a is the length of the segment.

We will assume that a (and thus R_g of a given oligomer) does not change dramatically when the eluent composition is changed. An accurate determination of R_g in mixed solvents is experimentally difficult. It is, however, not necessary in a study of retention in different regimes of interaction, if different columns with different selectivity are compared in the same mobile phase. As will be shown later on, the influence of mobile phase composition on R_g can be studied indirectly. The pore medium is considered as a slit-like pore with the width D. We will treat the case of wide pores ($D \gg R_g$), which is the most common situation in LAC.

For this model, the distribution coefficient K_{fl} which takes into account the conformations of the polymer chain inside the pore volume having no contacts with the pore surface was calculated by Casassa and Tagami [23,24] and formed the theory of size-exclusion chromatography:

$$K_{fl} = K_{SEC} \approx 1 - \frac{4}{\sqrt{\pi}}\frac{R_g}{D} \tag{6}$$

It must be mentioned that this equation is restricted to the linear part of the SEC calibration curve and assumes uniform pores.

The general theory of LC [25,26] takes into account the term K_{fl} and the term K_{ads}, which depends on adsorption interactions. The interaction between repeat units and the solid phase is characterized by the

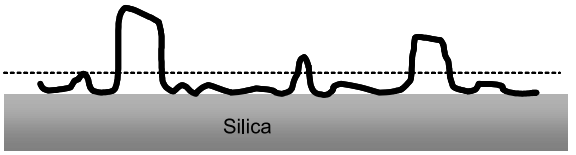

Fig. 2. Schematic representation of a polymer chain adsorbed on a solid surface, corresponding to the normal phase LAC. The average thickness of the adsorbing layer H is indicated by the dotted line.

correlation length H. The value of H is measured in nanometer and has a simple physical sense in LAC. Let us consider a long polymer chain grafted at the solid surface, as is shown schematically in Fig. 2. In the case of adsorption, this chain exists in more or less flat conformations with loops and trains and forms the adsorbing layer. The average thickness of this layer is H. Alternatively, the interaction parameter $c = 1/H$ can be used to characterize the interaction [27].

In reversed-phase LAC, the polymer is generally believed to sink into a brush of alkyl chains grafted on the surface of the stationary phase. It is possible to introduce an effective average thickness H, as is shown schematically in Fig. 3.

Depending on the structure of the bonded stationary phase, different models have been discussed [28]. It must be mentioned that reversed-phase LAC can as well be performed on non-polar solid surfaces, as is the case on the Jordi columns, which consist of 100% poly(divinyl benzene), and do not contain any brush-like structure. There is also experimental evidence that bonded alkyl chains are mainly in the

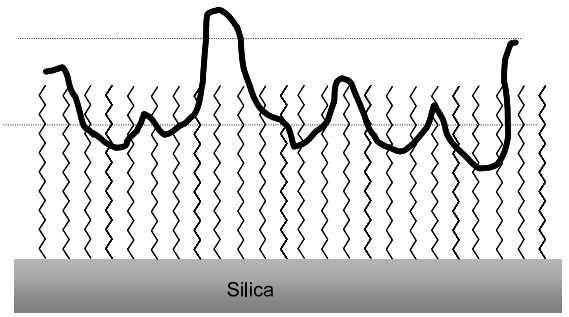

Fig. 3. Schematic representation of a polymer chain adsorbed on an alkyl bonded stationary phase, corresponding to the usual model of reversed-phase LAC. The average thickness H of the adsorbing layer is indicated by dotted lines.

self-associated (collapsed) state [29] and that there is an adsorbed layer of organic solvent on the hydrophobic layer of reversed-phase adsorbents [30].

Because the structure of the adsorbing layer on a solid surface is similar to the structure on top of the brush, we will apply this model to both normal and reversed phases. Therefore, the flat conformation of the adsorbing chain with $H \ll R_g$, which is typical for the situation of strong interaction can be presented schematically as in Fig. 2 for NP as well as for RP.

The value of H strongly depends on the mobile phase composition, but it does not depend on D and n, and thus not on R_g. According to the general theory of LC the distribution coefficient K_{ads} has a form:

$$K_{ads} = \frac{2H}{D}\left[Y\left(-\frac{R_g}{H}\right) - 1\right] \tag{7}$$

wherein $Y(-t) = \exp(t^2)\,[1 - \mathrm{erf}\,(-t)]$: erf$(-t)$ is the error function.As has been shown [25,26], the function $Y(-t)$ can be replaced in LAC by $2\exp(t^2)$. Consequently, one may write:

$$K_{ads} = \frac{4H}{D}\exp\left(\frac{R_g^2}{H^2}\right) \tag{8}$$

which is the main equation of LAC, which holds, however, only in the case of sufficiently strong interaction.

According to the theory [31,32], the Gibbs energy of adsorbing long chain grafted to a plain surface is proportional to $(R_g/H)^2$. The stronger the adsorption the smaller is H. Therefore, the term $\exp(R_g^2/H^2)$ is the partition function of the polymer chain grafted to the wall surface.

The term $4H/D$ connects with the probability to become some unit of the chain close to the wall surface at first attachment. It reflects the "memory" of the polymer chain about the time when it existed in flow conformations and chaotically wandered in wide pore D finding by her repeat units the adsorption region H close to the wall.

From the definitions of K and k', it is clear that these parameters are related by:

$$k' = (K - 1)\frac{V_p}{V_0} \tag{9}$$

Using Eqs. (6), (8) and (9), we have in LAC:

$$k' = \left[\frac{4}{\sqrt{\pi}}\frac{R_g}{D} + \frac{4H}{D}\exp\left(\frac{R_g^2}{H^2}\right)\right]\frac{V_p}{V_0} \tag{10}$$

At $H < R_g/1.5$ one may neglect the first term, and put:

$$k' \cong K_{ads}\frac{V_0}{V_p} = \frac{4V_p}{V_0}\frac{H}{D}\exp\left(\frac{R_g^2}{H^2}\right) \quad (11)$$

From Eq. (11) one obtains the relations:

$$\ln k' = \frac{1}{6}\left(\frac{a}{H}\right)^2 n + \ln\left(4\frac{V_p}{V_0}\frac{H}{D}\right) \quad (12)$$

which describes the retention of homologous series in LAC.

It must be mentioned that this relation holds only for non-functional molecules, i.e. chains, the end groups of which do not contribute to retention, regardless the nature of the interaction. For functional molecules, quite different situations occur [33–35].

Eq. (12) corresponds to Martin's rule: in such graphs, the slope $B = 1/6(a/H)^2$ corresponds to the Gibbs energy of the repeat unit of the long polymer chain grafted to the wall surface. It strongly depends (as H^{-2}) on the mobile phase. The intercept $\ln A = \ln(4(V_P H/V_0 D))$ depends on characteristics of the column; it shows only a weak dependence on mobile phase composition (as $\ln H$).

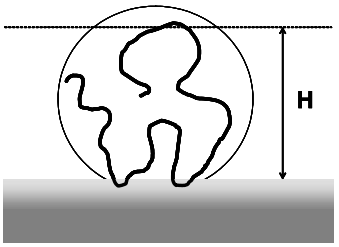

Fig. 4. Schematic representation of a polymer chain close to the critical point of adsorption, corresponding to LCCC.

Substitution of H yields:

$$A\sqrt{6B} = 4\frac{V_p}{V_0}\frac{a}{D} \quad (13)$$

If the adsorption interaction becomes weak, the average thickness of the adsorbed layer increases, while the loops and tails become larger.

Fig. 4 shows the conformation with H close to R_g which are typical for weak adsorption. The region of weak interaction with $H \sim R_g$ corresponds to LCCC. Eq. (12) breaks down when the layer thickness H is comparable with the coil size R_g. The analysis shows that beyond the region $H < R_g/1.5$ we are leaving

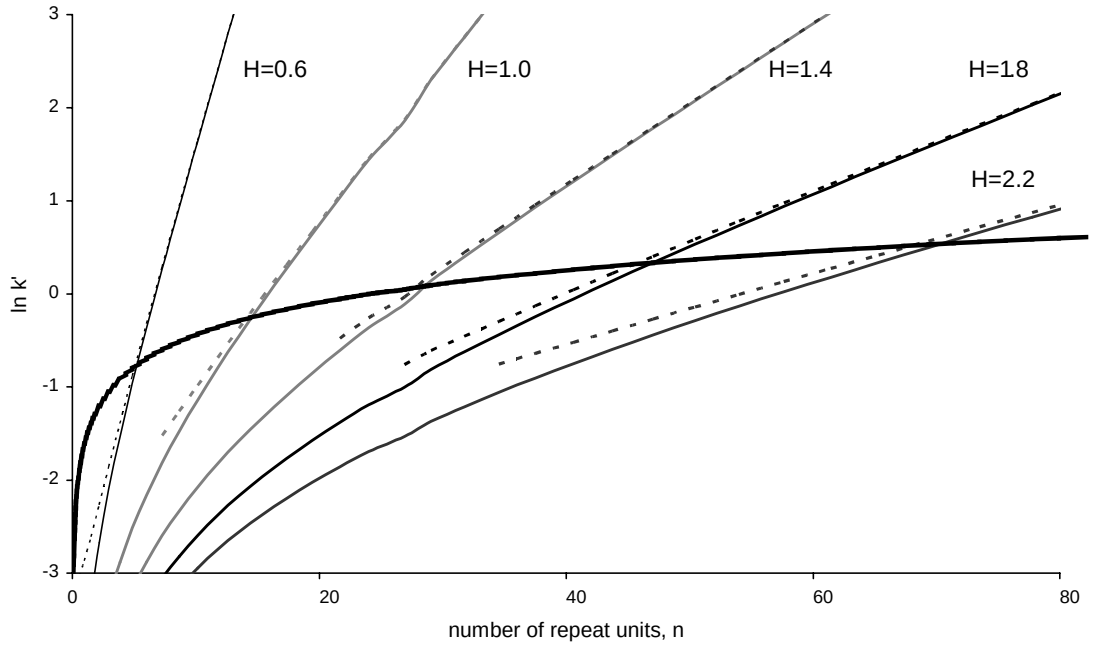

Fig. 5. Calculated values of $\ln k'$ as a function of n for different parameters $H = 0.6$, 1, 1.4 and 1.8. The shaded line indicates the demarcation line (Eq. (14)).

LAC and entering in LCCC. In this case, the main Eq. (9) of LAC starts to be incorrect, and the general Eqs. (5)–(7), and (9) have to be applied.

The border between LAC and LCCC is, however, diffuse. We will use the condition $R_g/H \approx 1.5$ as the demarcation line. It corresponds to:

$$k' \approx 25\frac{V_p}{V_0 D}R_g \quad (14)$$

The theoretical curves of $\ln k'$ versus n are shown in Fig. 5 for several values of H. The shaded line shows the demarcation line [14].

4. Results and discussion

Fig. 6 shows $\ln k'$ as a function of the number of repeat units of PEG on the reversed-phase column Jordi in methanol–water of different composition as mobile phase.

Fig. 7 shows a plot of B and $\ln A$ as a function of the mobile phase composition. As could be expected, the slope depends on mobile phase composition, while the intercept is fairly constant. According to Eq. (13) the combination $A\sqrt{6B}$ depends on a/D and V_p/V_0 only.

Fig. 8 shows that there is a weak dependence of $A\sqrt{6B}$ on the mobile phase composition, which can originate from variation of either R_g or the pore volume with the water content. As has been shown by other authors, the dimensions of a pore may change with the composition of the mobile phase [15,29,36]. (Others have also described a dependence on temperature [6,37].)

Fig. 9 shows the dependencies of $\ln k'$ on n (similar to Fig. 6) for PEG dimethyl ethers on the normal phase column S3W in acetone–water of different composition.

It must be mentioned that PEGs with terminal hydroxy groups can be considered as non-functionals in reversed-phase LAC, but as difunctionals in normal phase LAC, as the interaction of the polar hydroxy groups with the silica surface must be expected to be stronger than that of the EO unit. Consequently, PEG dimethyl ethers had to be used for this comparison in the normal phase LAC.

As can be seen from Figs. 6 and 9, the agreement between Martin's rule and the experimental curves is quite good for longer chains (with a higher number of repeat units), but there are considerable deviations from linearity at lower n.

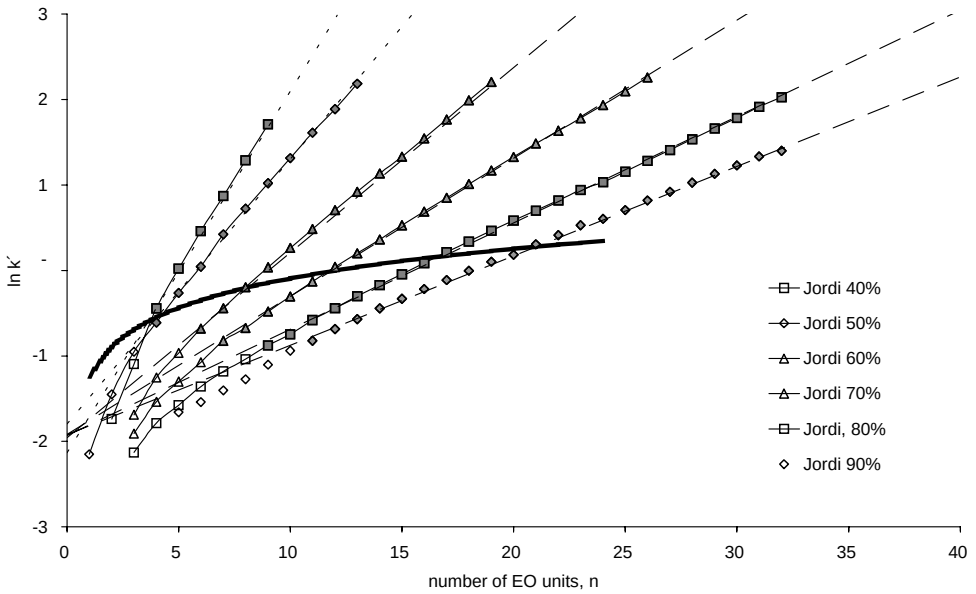

Fig. 6. $\ln k'$ as a function of the number of repeat units of PEG. Experimental data obtained on the Jordi column in methanol–water of different composition (dotted lines from linear regression of higher oligomers (filled symbols)). The shaded line represents the demarcation line (Eq. (14)).

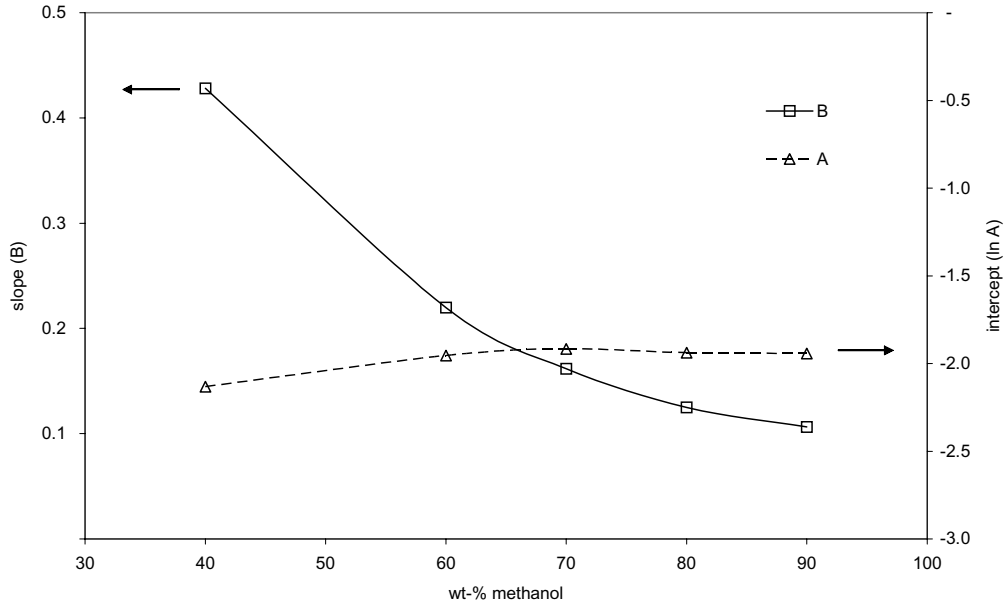

Fig. 7. Slope (B) and intercept ($\ln A$) in the Martin plot of PEG on the Jordi column in methanol–water of different composition.

The reason is physically clear: for long chains the partition function of the adsorbing conformations dominates in the full partition function. The stronger are the adsorption interactions (the smaller the thickness of the adsorption layer H) the shorter chains can be described by the adsorbing conformations only. If the same mobile phase is used on different columns, R_g will be constant, anyway.

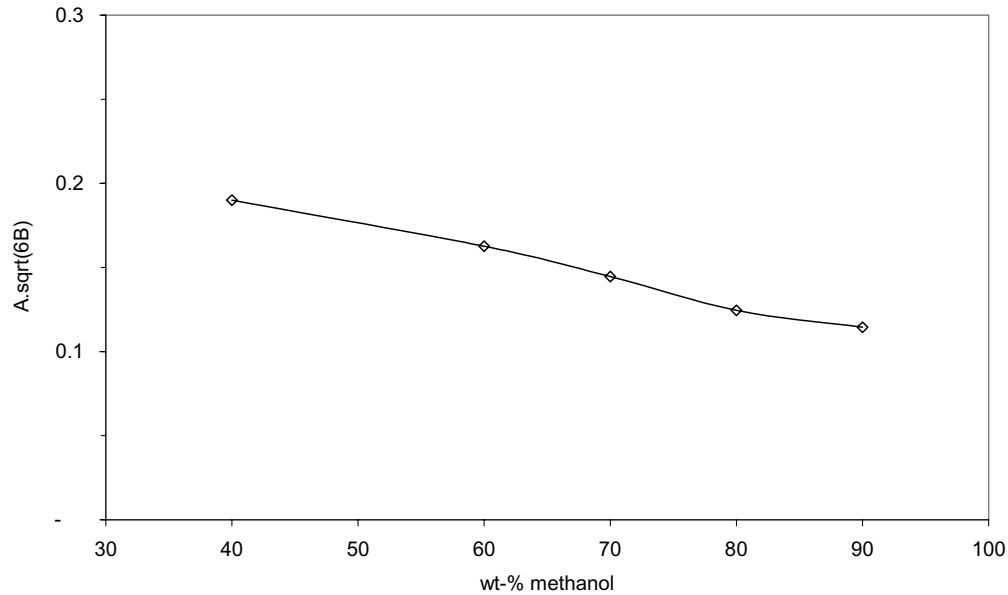

Fig. 8. Plot of $A\sqrt{6B}$ as a function of mobile phase composition (PEG on Jordi in methanol—water mobile phases).

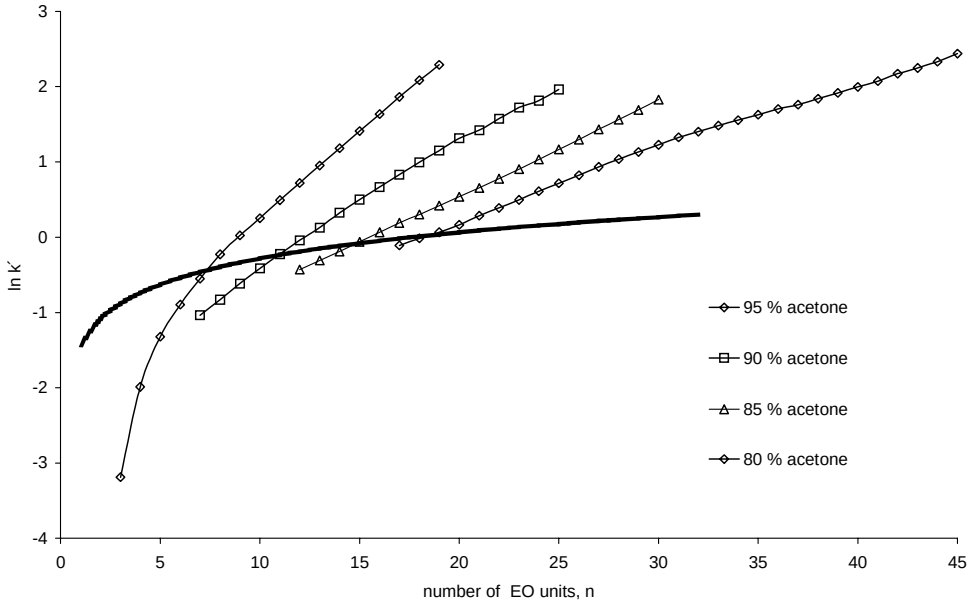

Fig. 9. $\ln k'$ as a function of the number of repeat units of PEG dimethyl ethers, as obtained on the S3W column in acetone–water of different composition. The shaded line represents the demarcation line (Eq. (14)).

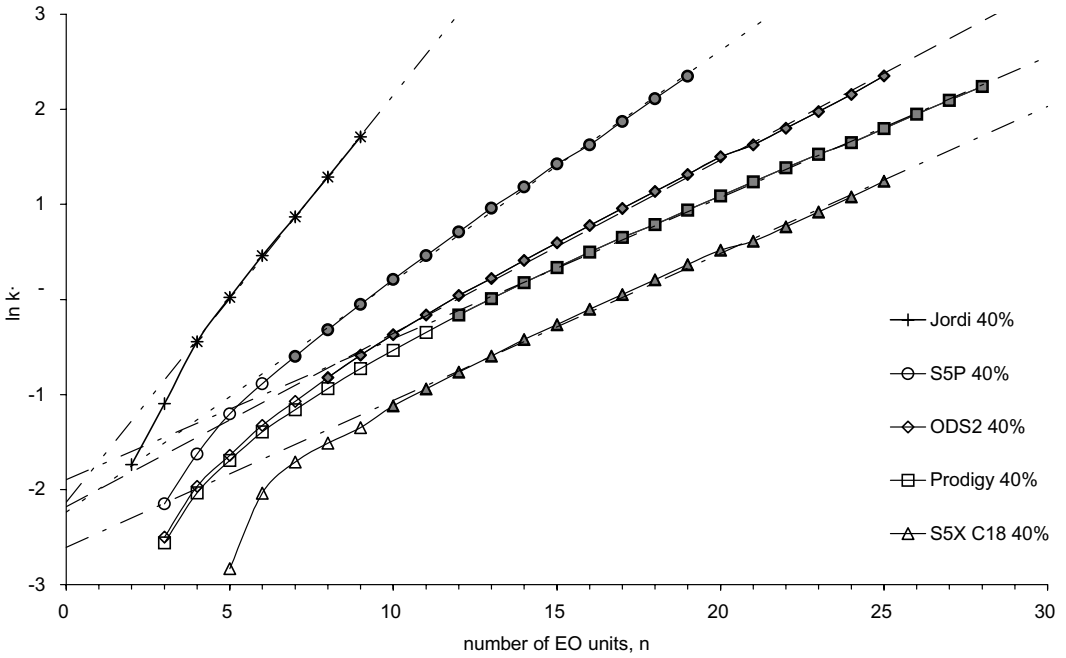

Fig. 10. $\ln k'$ as a function of repeat units of PEG on different columns in methanol–water 40:60 (w/w) (dotted lines from linear regression of higher oligomers (filled symbols)).

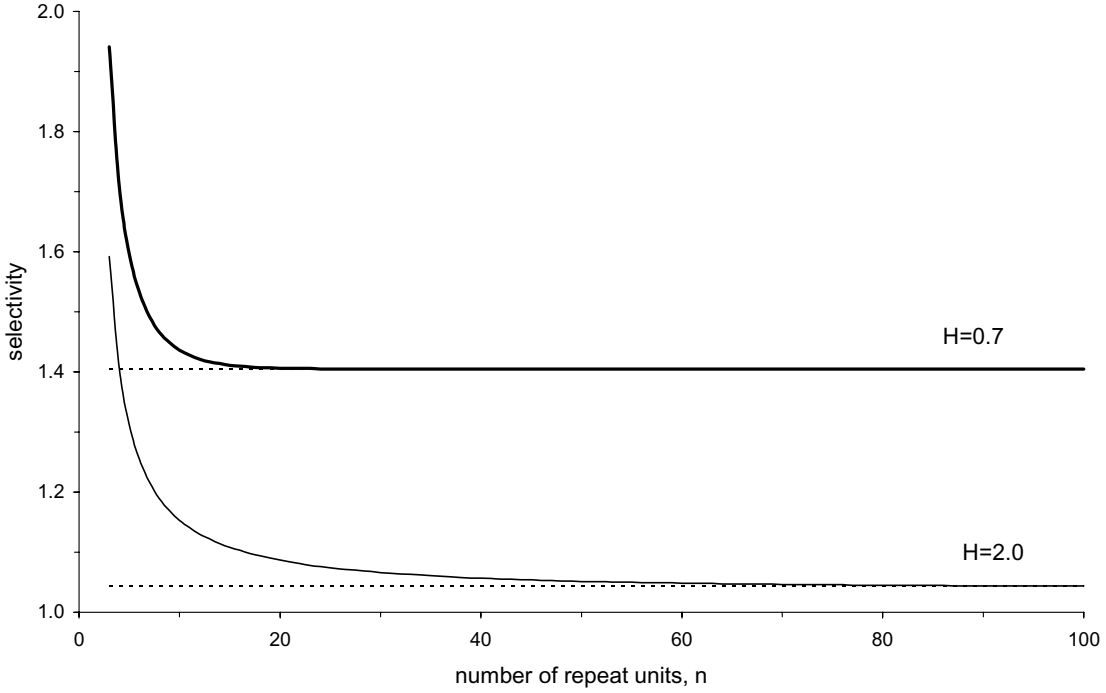

Fig. 11. Calculated selectivity as a function of the number of repeat units for two values of $H = 0.7$ and 2.0.

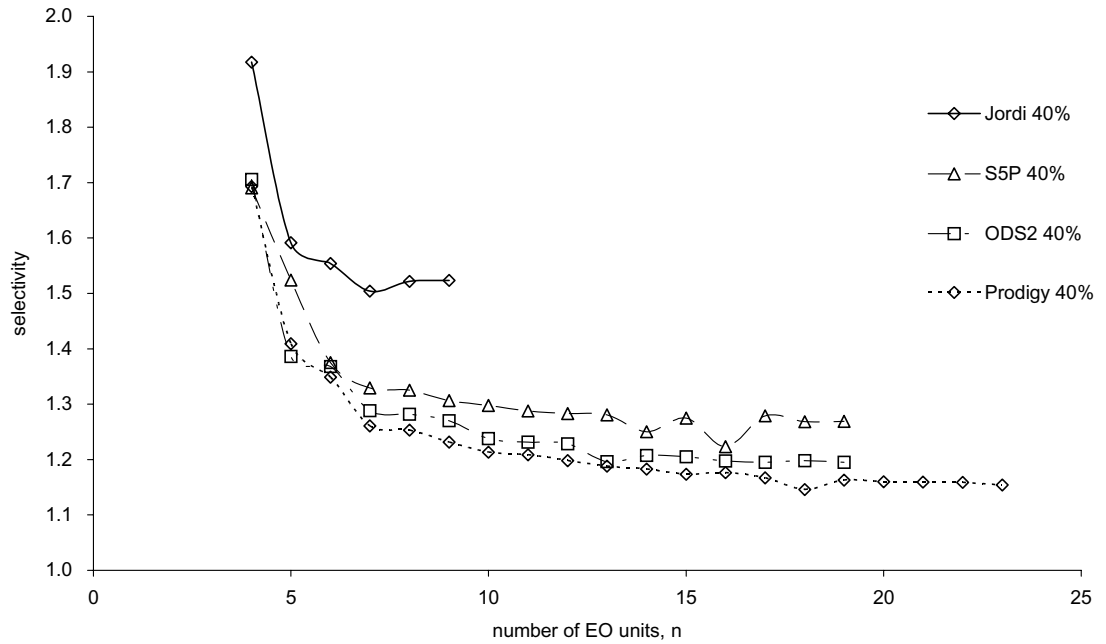

Fig. 12. Selectivity (for the EO unit) on different stationary phases in methanol–water 40:60 (w/w) as mobile phase.

In Fig. 10, the experimental data (open symbols) are shown together with the regression lines obtained for the higher oligomers (filled symbols), which correspond to what would be expected from Martin's equation. The slopes of the straight lines are quite similar for the C18 columns (S5×; Prodigy, and ODS2), but different for the phenyl phase and for the Jordi column. The intercept of the wide pore C18 column is different from those of the other columns.

The deviation from linearity becomes even more obvious when one looks at the selectivity, which is defined by $S = k'(n+1)/k'(n)$. The selectivity should be constant in the range where Martin's rule holds. As it follows from Eq. (12), the selectivity in LAC is equal to $(a/H)^2/6$ and increases with increase of adsorption interaction as H^{-2} and does not depend on molar mass. The general theory of chromatography (Eq. (8)) predicts, however, that selectivity should depend on molar mass.

Fig. 11 shows the selectivity in dependence on n, as calculated by Eqs. (8) and (12). The shaded lines show the limiting values $(a/H)^2/6$. As can be seen, at strong adsorption the selectivity is practically constant for long chains and starts to grow when $n < 10$ only.

In a mobile phase producing the condition of weak adsorption selectivity depends on n in a wide region of n (up to several dozen). The analysis shows that selectivity is roughly proportional to $1/n$. The corresponding experimental data are shown in Fig. 12. As can be seen, there is at least a reasonable qualitative agreement.

5. Conclusions

The general idea of this paper was to apply the theory of LAC for describing the dependencies of k' from molar mass (or R_g) for different mobile phases and different sorbents with normal and reverse phases. We have demonstrated that the retention factor and selectivity as functions of n have a similar behavior on normal and reverse phases. In fact, this similar behavior is a result of the similar adsorption conformations of a polymer chain on normal or reversed phases.

On the basis of the general theory of liquid chromatography, we have studied the molecular sense of the coefficients B and A in Martin's rule (Eq. (2): B is related to the Gibbs energy, and the intercept A is a combination different parameters), which characterize the column and the correlation length H. The value of B has been usually identified with full Gibbs energy, which is related to the distribution coefficient: $G/RT = -\ln K$ and therefore depends on pore size.

As we have shown, B is the Gibbs energy of the repeat unit of the long polymer chain grafted to the wall surface. It does not depend on column volume or pore size and depends only on the relation a/H of the polymer rigidity a and correlation length H.

In the literature, the relation $k' = K_L\phi$ is generally applied, wherein $\phi = V_{st}/V_{mob}$. The value of K_L is often defined as the thermodynamic equilibrium constant between the volumes of mobile and stationary phase [38]. Especially in new publications [18,30,39], K_L is identified as the distribution coefficient K, which is related to the change of the full Gibbs energy G/RT.

The volume of the mobile phase V_{mob} is considered as the total volume of the liquid phase in the column, and the volume of the stationary phase V_{st} is considered as the volume of the silica-based alkyl brushes. The value V_{st} should strongly depend on the structure of the derivatized silica material (on the length of the alkyl group, bonding type, bonding density).

Experimental data [16,40] show, however, no dramatic changes of $\ln k'$ with the chain length of the grafted alkyl chains (from C_1 to C_{18}) in the same mobile phase. It is obvious that this definition cannot be applied to normal and reversed phases without alkyl groups on the surface.

As we have shown, the relation $k' = K_{ads}V_p/V_0 = (K-1)V_p/V_0$ between retention factor k' and the distribution coefficient K_{ads} of adsorbed state should be used. This fundamental relation is just a result of the existence of floating and adsorbed conformations of polymer chain in the pore volume, and the definition of retention factor. The retention factor k' is defined by the retention volume of a peak and the void volume or mobile phase volume: $k' = (V_R - V_{mob})/V_{mob}$. We follow the determination of the mobile phase volume (or hold-up volume) as "the total volume of the liquid phase in the column" [15]. This definition is equivalent to the "nothing is adsorbed" convention [41].

To obtain the void volume, "an unretained substance" (which could be methanol, carbon tetrachloride, acetonitrile or chloroform) can be injected [6]. Consequently, $V_{mob} = V_0 = V_i + V_p$.

On the other hand, there is the definition of the mobile phase as the *minimal* retention volume on a given column [42]. It can be determined by injecting an unretained substance with minimal retention volume, i.e. a polymer with a molar mass above the exclusion limit. In this case, there are no stationary (adsorbed or floating) conformations inside the pores and mobile conformations exist only outside the pores. As a result, V_{mob} will be equal to exclusion limit V_i, and the relation $k' = (V_R - V_i)/V_i$ could be applied. Combination with the main relation of liquid chromatography $V_R = V_i + KV_p$ yields $k' = KV_p/V_i$ which is in accordance with the definition $k' = K\phi$, if we put $V_{mob} = V_i$ and $V_{st} = V_p$.

Therefore, the solution of all problems we have discussed before is the correct definition of the mobile phase volume V_{mob}. In the case of the convention "nothing is adsorbed", $V_{mob} = V_i + V_p$, and $k' = (K - 1)V_p/(V_i + V_p)$. If the convention "minimal retention volume" is applied, $V_{mob} = V_i$, and $k' = KV_p/V_i$. In all cases, the pore volume V_p is used as the volume of the stagnant phase.

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References

[1] A.J.P. Martin, Biochem. Soc. Symp. 3 (1949) 4.
[2] E. Grushka, H. Colin, G. Guiochon, J. Chromatogr. 248 (1982) 325.
[3] P. Jandera, J. Chromatogr. 352 (1986) 91.
[4] R.M. Smith, J. Chromatogr. 236 (1982) 313.
[5] B. da Silva Junkes, R. Dias de Mello Castanho Amboni, R. Augusto Yunes, V.E.F. Heinzen, Anal. Chim. Acta 477 (2003) 29.
[6] C.A. Rimmer, C.R. Simmons, J.G. Dorsey, J. Chromatogr. A 965 (2002) 219.
[7] H. Colin, A.M. Krustulovic, M.F. Gonnord, G. Guiochon, Z. Yun, P. Jandera, Chromatographia 17 (1983) 9.
[8] C.H. Lochmuller, D.R. Wilder, J. Chromatogr. Sci. 17 (1979) 574.
[9] C.H. Lochmuller, C. Reese, A.J. Aschman, S.J. Breiner, J. Chromatogr. A 656 (1993) 3.
[10] A. Tchapla, S. Heron, E. Lesellier, H. Colin, J. Chromatogr. A 656 (1993) 81.
[11] A. Tchapla, H. Colin, G. Guiochon, Anal. Chem. 56 (1984) 621.
[12] A.M. Krstulovic, H. Colin, G. Guiochon, Anal. Chem. 54 (1982) 2438.
[13] H.J. Möckel, T. Freyholdt, Chromatographia 17 (1983) 215.
[14] J.H. Knox, R. Kaliszan, J. Chromatogr. 349 (1985) 211.
[15] Y.V. Kazakevich, H.M. McNair, J. Chromatogr. A 872 (2000) 49.
[16] H. Engelhardt, G. Ahr, Chromatographia 14 (1981) 227.
[17] G.E. Berendsen, L. De Galan, J. Chromatogr. 196 (1980) 21.
[18] R. Tijssen, P.J. Schoenmakers, M.R. Böhmer, L.K. Koopal, H.A.H. Billiet, J. Chromatogr. A 656 (1993) 135.
[19] A. Gorbunov, A. Skvortsov, B. Trathnigg, M. Kollroser, M. Parth, J. Chromatogr. A 798 (1998) 187.
[20] R.P.J. Ranatunga, P.W. Carr, Anal. Chem. 72 (2000) 5679.
[21] P. Nikitas, A. Pappa-Louisi, P. Agrafiotou, J. Chromatogr. A 946 (2002) 9.
[22] M. Kurata, Y. Tsunashima, M. Iwama, K. Kamada, in: J. Brandrup, E.H. Immergut (Eds.), Polymer Handbook, second ed., Wiley, New York, NY, 1975, p. IV.
[23] E.F. Casassa, Y. Tagami, Macromolecules 2 (1969) 14.
[24] E.F. Casassa, J. Polym. Sci. B 5 (1967) 773.
[25] A.M. Skvortsov, A.A. Gorbunov, J. Chromatogr. 358 (1986) 77.
[26] A.A. Gorbunov, A.M. Skvortsov, Adv. Colloid Interface Sci. 62 (1995) 31.
[27] A.M. Skvortsov, G.J. Fleer, Macromolecules 35 (2002) 8609.
[28] A. Vailaya, C. Horvath, J. Chromatogr. A 829 (1998) 1.
[29] I. Rustamov, T. Farcas, F. Ahmed, F. Chan, R. LoBrutto, H.M. McNair, Y.V. Kazakevich, J. Chromatogr. A 913 (2001) 49.
[30] Y.V. Kazakevich, R. LoBrutto, F. Chan, T. Patel, J. Chromatogr. A 913 (2001) 75.
[31] P.G. De Gennes, Scaling Concepts in Polymer Physics, Cornell University Press, Ithaca, NY, 1979.
[32] P.G. de Gennes, Rep. Prog. Phys. 32 (1969) 187.
[33] B. Trathnigg, A. Gorbunov, J. Chromatogr. A 910 (2001) 207.
[34] A. Gorbunov, B. Trathnigg, J. Chromatogr. A 955 (2002) 9.
[35] C. Rappel, B. Trathnigg, A. Gorbunov, J. Chromatogr. A 984 (2003) 29.
[36] Y.V. Kazakevich, H.M. McNair, J. Chromatogr. Sci. 31 (1993) 317.
[37] H.J. Mockel, J. Chromatogr. A 675 (1994) 13.
[38] S.M. Petrovic, S.M. Lomic, Chromatographia 27 (1989) 378.
[39] H.J.A. Philipsen, H.A. Claessens, H. Lind, B. Klumperman, A.L. German, J. Chromatogr. A 790 (1997) 101.
[40] K. Karch, I. Sebestien, I. Halasz, J. Chromatogr. 122 (1976) 3.
[41] F. Riedo, E.S. Kovats, J. Chromatogr. 239 (1982) 1.
[42] D.A. Skoog, F.J. Holler, T.A. Nieman, Principles of Instrumental Analysis, Brooks/Cole Publishing Co., Pacific Grove, CA, USA, 1997.