



5th Conference by Nordic Separation Science Society (NoSSS2009)

Simulating chromatographic separation of topologically different polymers

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Abstract

Chromatography which is sensitive to the sizes of macromolecules and to their adsorption serves as an appropriate method to separate complex polymers. Unfortunately, the molar mass also influences the chromatographic retention, thus making quite difficult the problem of separation of polydisperse polymers by their topology.

By using a theory of chromatographic behavior of macromolecules, we simulate chromatograms of polydisperse polymers that differ solely in their topology, and discuss possibilities to separate complex polymers (such as eight-, tadpole-, theta-, manacle-shaped polymers, etc.) from their linear, branched, or macrocyclic precursors or topo-isomeric products.

As follows from the simulations, two approaches towards the separation of polydisperse polymers by topology are especially promising. The first one is the chromatography at optimized (critical or near-critical) interaction conditions, where molar-mass effects are minimized; The second one consists in combing different chromatographic modes, which allows obtaining a separation by both molar mass and topology in a 2D chromatogram.

Some of the simulated chromatographic separations are qualitatively very similar to the real ones, the others are the theoretical prediction.

Keywords: complex polymers; separation by topology; two-dimensional chromatography; theory; simulation

1. Introduction

Both synthetic and natural macromolecules may differ by their topology. Generally macromolecules may have branch-points and contain linear and cyclic fragments (Figure 1 shows several examples of topologically different structures). Complex polymers have many interesting properties that are different from the properties of simple linear macromolecules [1]. A considerable progress is achieved in the synthesis of complex polymers [2-4], and many interesting topological structures of polymers have been realized [5-9].

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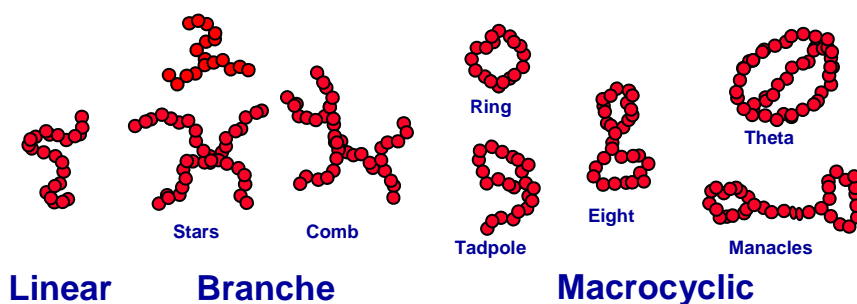


Fig.1. Examples of topologically different polymeric structures.

Liquid chromatography is widely used for the analysis and characterization of cyclic polymers, as well as for the separation of cyclic products from linear precursors [9-17]. Many reports describing successful chromatographic analysis of branched, and star polymers have appeared as well [18-23]. Mostly the systems were studied with strong correlations between topology and other molecular characteristics of polymers. Quite often a correlation exists between topology and molar mass. A typical experimental problem is for example to separate stars with different number of equal arms from each others and from a linear precursor of an arm. In this situation topologically different polymers – stars with different number of arms – have quite different molar mass (and different molecular radii), and therefore can be separated by means of either size-exclusion chromatography (SEC) or adsorption chromatography (AC). Such successful separations have been really achieved and reported [18-21]. Another correlation is used when specific groups differing in their adsorption properties from regular chain units are present in branched polymers (such groups can be situated on the chain ends or/and in the branch-points). There are several successful attempts to use the liquid chromatography for the characterization of such star-shaped and branched polymers [21-23]. In fact, in such cases chromatography separates polymers not by topology itself, but by the property which is correlated with chain topology (by molar mass or by the number of specific groups). So far, the problem of chromatographic separation by topology in its pure sense has not been given sufficient attention. We shall study this problem theoretically by considering a situation where there is no difference in molar mass and in the adsorption interaction of topologically different polymers.

The problem becomes especially difficult when topologically different polymers are heterogeneous in molar mass. It may happen that reasonable topological separation cannot be achieved by any mode of one-dimensional chromatography. However, even in such cases the separation may become possible, in principle, if one will combine different adsorption interaction conditions in a two-dimensional chromatographic process.

Two-dimensional chromatography is a very powerful tool for the analysis of complex polymers, which are heterogeneous in more than one dimension [24-30]. A fully automated two-dimensional chromatographic system was developed and successfully applied for the analysis of heterogeneous polymers by Kilz et al. [30-32]. A general benefit of two-dimensional chromatography is that it provides more space for a separation: although sample components co-elute in any single dimension, the component zone can be well resolved in two dimensions.

In order to achieve good results in topological separation of polymers, the experimental conditions (i.e. the combination of stationary and mobile phases as well as the operating temperature) must be selected very carefully to provide the optimal chromatographic mode. Experimental work on finding good separation conditions could be facilitated applying theoretical approaches. Development of a molecular theory and simulation of the chromatographic behavior of macromolecules seems to be the most straightforward way to the goals of optimization.

Molecular-statistical theory of size-exclusion chromatography (where adsorption interactions are absent) has been developed by Casassa [33]; this theory used a model of ideal polymer chains in slit-like pores. Gorbunov and Skvortsov [34] took into account the adsorption interactions and have generalized the Casassa theory in order to describe other modes of chromatography of linear polymers. Similar theory has been developed [34] for ring-shaped polymers. The theories for linear and ring polymers have proven to describe very reasonably many features of chromatography of real linear and ring polymers. These theories have been used to predict new effective separation

techniques. In particular, the possibility of effective separation of linear and ring polymers at the point of critical interaction has been predicted [34].

Analogous theories describing chromatography of topologically more complex polymers have been presented as well. Casassa and Tagami [35] have developed a theory of SEC for star-shaped polymers, while the theories of interactive chromatography of stars were developed by Kosmas et. al. [36], and by Gorbunov and Vakhrushev [37,38]. The theories are also available now for several types of complex macrocyclic structures, such as eight-shaped and daisy-like macromolecules [39], and theta-shaped polymers [40].

Our work on the creation of the theory for other types of complex polymers is in progress. Some of our recent theoretical results and examples of simulated separation of topologically different polydisperse polymers in various modes of one- and two-dimensional chromatography are presented here. By using the theory and simulations we conclude about the optimal conditions for topological separation.

2. Theory

2.1. A basic theory describing chromatography of linear polymers

A theory of chromatography of linear polymers [34] is based on the thermodynamic approach, according to which the chromatographic behavior of macromolecules is determined by the equilibrium partitioning of the chains between the mobile and the stationary phases, and therefore can be described by the equilibrium partition coefficient, K [33]. The chromatographic elution volume $V=V_i+V_pK$ is determined by the partition coefficient K (the interstitial volume V_i and the pore volume V_p are the parameters of the chromatographic system). In order to calculate the partition coefficient K , a model of an ideal chain and a slit-like pore (Fig. 2) was used by the theory [34]. The model parameters are the average radius of gyration of an unconfined polymer molecule, R , the width of a slit, $2d$ (which serves in this model as the pore size), and the adsorption interaction parameter, c .

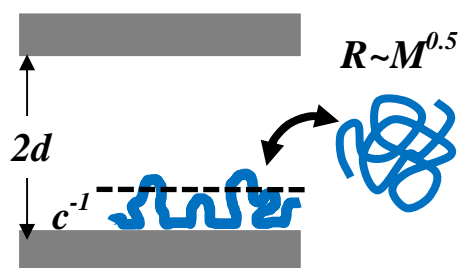


Fig. 2. A model describing the partitioning of a flexible polymeric chain between mobile and stationary chromatographic phases. The model parameters: R is the radius of gyration of an unconfined macromolecule, $2d$ is the width of a slit-like pore, and c is the adsorption interaction parameter.

The conformations of an ideal chain are described by the diffusion equation, while the boundary condition proposed by de Gennes [41] is applied on the pore walls to account for the adsorption interaction. A parameter c entering into the boundary condition, serves in the theory as the adsorption interaction parameter. Negative values of c correspond to effective repulsive forces between the chain units and pore walls ($c \rightarrow -\infty$ corresponds to the conditions of SEC). The point $c = 0$ is usually referred to as a critical point of adsorption [34]. Positive values of c correspond to adsorption. At $c > 0$ a sufficiently long macromolecule forms an adsorption layer on the surface; the average thickness of this layer is equal to c^{-1} .

In chromatography adsorption interaction is controlled by selection of adsorbent and solvent; by variation of temperature and composition of a mixed mobile phase.

According to the theory [34], the partition coefficient of a linear polymer is the function of three parameters:

$$K = \varphi(R, d, c) \quad (1)$$

The main results of the theory [34] are visualized in Figure 3. At $c \rightarrow \infty$ the results of the Casassa theory of SEC are recovered: K decreases with increasing R (that is with the increase of molar mass M). At positive c values, that correspond to adsorption chromatography, K exponentially increases with M . At the critical point $c=0$ the partition coefficient is independent of molar mass: $K=1$. This special point corresponds to liquid chromatography at the critical condition (LCCC) which is especially useful in the analysis of chemically heterogeneous polymers [24].

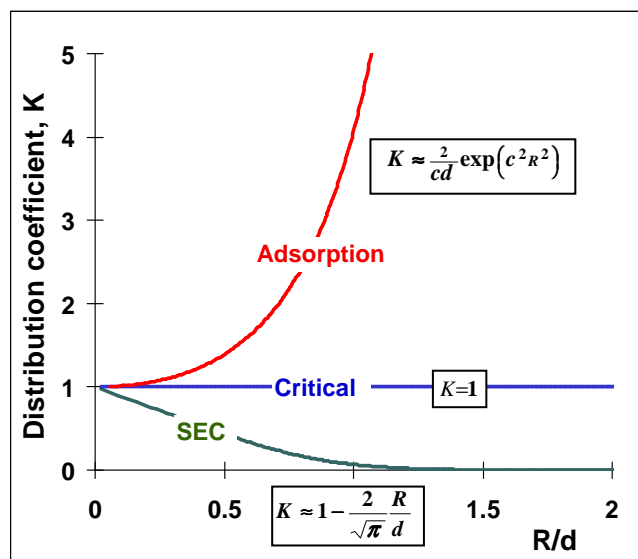


Fig.3. Theoretical dependences of the partition coefficient K of a linear polymer on the ratio R/d in the SEC, critical, and adsorption modes of chromatography.

2.2. Theories for topologically complex polymers

According to the theories [37-40], the partition coefficient of a topologically-complex polymer composed of m sub-chains has the following general form:

$$K = \Phi(R_1, R_2, \dots, R_m, d, c) \quad (2)$$

where R_i is the radius of gyration of i -th chain fragment ($i=1, 2, \dots, m$), and the mathematical form of the function Φ depends on the topological structure of a polymer.

The particular exact equations have been reported for ring-shaped polymers [34], for stars [37], and for several types of complex macrocyclic polymers [38-40]. Similar equations are also developed by us for tadpole-shaped and manacle-shaped polymers, and we are presenting these theoretical results altogether.

Figure 4a shows the theoretical dependences of the SEC partition coefficient K on the ratio R/d for polymers of different topology, Figure 4b visualizes the theoretical results at the critical interaction point $c=0$, while Figure 4c shows the results at the conditions of adsorption chromatography. For complex polymers R denotes here the radius of gyration of a referent linear chain, which has the same molar mass as the given complex polymer. This means that the separation of topologically different polymers of the same molar mass can be analyzed by comparing K values at the same R , while each dependence $K(R)$ characterizes the separation of the corresponding polymer by molar mass.

As it follows from Figures 4a-c, a topological separation of polymers with the same average molar mass and with the narrow molar-mass distributions in principle can be achieved by using different modes of chromatography. The problem is however in the fact that polymers are usually polydisperse, and the molar mass heterogeneity may cause difficulties for separation by topology. To study this problem and to formulate conditions for good separation,

basing on the theory we simulate chromatograms for model mixtures of topologically different polydisperse polymers.

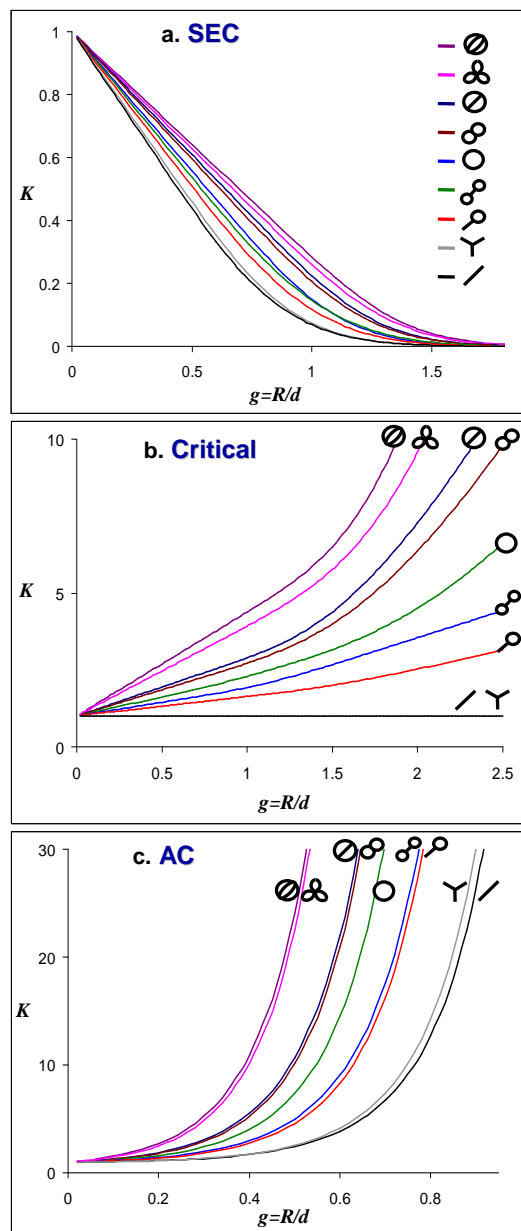


Fig. 4. Theoretical dependences of K on R/d for different types of polymers in (a) SEC, (b) critical, and (c) adsorption modes of chromatography.

2.3. A virtual chromatograph

In order to investigate the problem of chromatographic separation of polydisperse polymers by topology and to visualize expected one- and two-dimensional separations, we use the virtual chromatography technique [42]. The virtual chromatography has been already used in studies on chromatographic separation of polydisperse linear

homopolymers and block-copolymers; simulation results have proven to be very similar to real chromatograms obtained for polyethylene glycols and fatty alcohol ethoxylates [42-45].

The virtual chromatograph is specially developed software tool, where the simulation procedures are based on the theoretical equations for a variety of topologically different polymers. A polydisperse linear polymer represents an ensemble of individual macromolecules of different chain length (molar mass). A polydisperse complex polymer represents a more complex ensemble in which molar masses of all sub-chains can be different. The chromatographic elution volume $V=V_i+V_pK$ is determined by the partition coefficient K (the interstitial volume V_i and the pore volume V_p are the parameters of the chromatographic system which can be assumed as constants). Therefore, by calculating partition coefficients for the members of a given ensemble of macromolecules one can construct a theoretical chromatogram for a polydisperse polymer [37-40,42].

A similar procedure, modified in a straightforward way, is used to simulate two-dimensional chromatograms [38]. This procedure includes calculation of pairs of values K_1 and K_2 for the macromolecules of an ensemble. In order to account for different chromatographic conditions in two dimensions, K_1 and K_2 are calculated at different values of pore size d and interaction parameter c , which correspond to the first and the second dimensions.

3. Simulating chromatographic separation of polymers by topology

By using this technique we simulated expected chromatographic separations for model samples composed of topologically different polymers. The separation patterns and the conditions for good separation were of course dependent of the type of the polymers in the model mixtures. Basing on the simulations, we have concluded, however, that generally two approaches towards the separation of polydisperse polymers by topology are especially promising. The first one is the chromatography at optimized (critical or near-critical) interaction conditions, where molar-mass effects are minimized. The second one consists in combing different chromatographic modes, which allows obtaining a separation by both molar mass and topology in a 2D chromatogram. These two approaches are discussed in the following sections.

3.1. Topological separation of complex polymers at the critical adsorption point

Figures 5a-c show simulated chromatograms obtained for the same model sample at different interaction conditions. A sample was a mixture of two poly(THF) polymers (having star- and theta-shaped topology); these two polymers were assumed to have the same average molar mass $M_w=9600$ and to have the same heterogeneity index $M_w/M_n=1.7$ for the arms in a star-polymer and sub-chains in a theta-polymer.

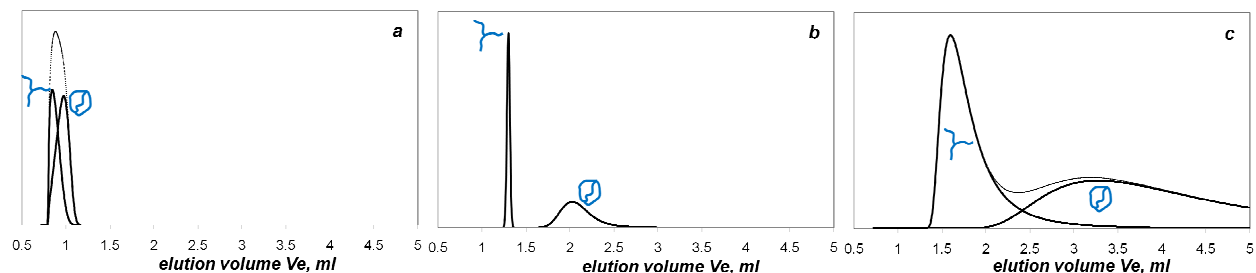


Fig. 5. Theoretical chromatograms for star- and theta-shaped polyTHFs under the condition of (a) SEC; (b) critical interaction, and (c) more strong adsorption. Simulation parameters: $M_w=9600$; arms in a star-polymer and sub-chains in a theta-polymer are of equal $M_w/M_n=1.7$; values of the interaction parameter, c : (a) -5000, (b) 0 and (c) 0.17; pore width $2d=8$ nm, pore volume $V_p=0.8$ ml, interstitial volume $V_i=0.5$ ml. Dotted lines represent the sum of the chromatograms of the components.

Figure 5a corresponds to the conditions of SEC. One can see a superposition of two peaks; although the peak positions are somewhat different, peaks are broad and overlap considerably. So, one can expect rather poor topological separation by SEC if polymers are heterogeneous in molar mass.

In the AC mode (Figure 5c) peak maxima are separated considerably, but to the other hand there is a huge peak broadening due to the molar-mass heterogeneity; this results in rather poor separation of these two polymers.

As can be seen, the simulated separation pattern becomes perfect at the interaction corresponding to the critical point (Figure 5b). At the critical condition a star polymer (even of a considerable polydispersity) elutes in a very narrow zone and is well-separated from the theta-shaped analogue.

If the goal is to separate two or several heterogeneous macrocyclic polymers, the exact critical interaction point is not an optimal condition, because (as it can be seen in Figure 4b) at the critical point the molar-mass dependences of the retention disappear for linear and branched structures, but still exist for macrocyclic polymers. However, as it was shown theoretically [34, 39], at some pre-critical condition (which is slightly shifted from the critical point to the exclusion side), the molar mass dependences for macrocyclic polymers become weaker, which will result in better separation of such polymers. Figure 6 shows an example of a quite good separation pattern obtained by simulation under such near-critical condition for a model mixture of polydisperse linear, ring-, eight-, and trefoil-shaped polymers.

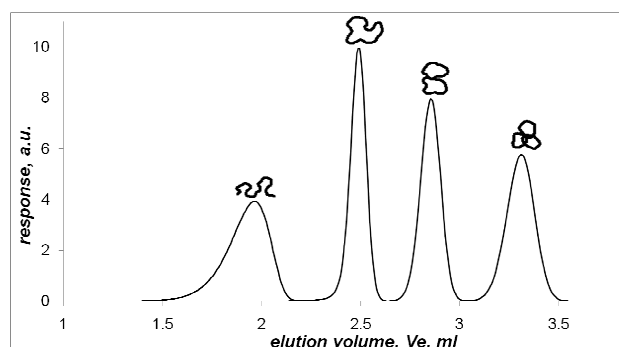


Fig. 6. A chromatogram simulated for a mixture of polystyrenes of different topology under the pre-critical interaction condition. Simulation parameters: $M_w=15000$ and $M_w/M_n=1.16$ are the same for each type of polymer in the mixture; the interaction parameter $c = -0.1$; pore width $2d=10$ nm, pore volume $V_p=1.2$ ml, interstitial volume $V_i=1$ ml.

3.2. Topological separation by using two-dimensional chromatography

When polymers of different topology have broad molar-mass distributions, it may happen that at any interaction conditions reasonable separation cannot be achieved. In such cases it will make sense to combine different interaction conditions in a two-dimensional chromatographic process.

Figure 7 shows a virtual 2D-chromatogram for a model mixture of polydisperse manacle- and theta-shaped polymers of the same average molar mass. In the first dimension we simulate LCCC, while the second dimension corresponds to SEC. As it can be seen in Fig. 7, by combining LCCC and SEC it is possible to improve the topological separation (which is not perfect in the first dimension, and is very poor in the second one).

The 2D combination of AC and SEC also seems appropriate for the purposes of topological separation of polymers. Fig. 8 shows a simulated 2D chromatogram (which is given in a form of the top-view contour map) for a mixture of polydisperse ring- and eight-shaped polymers; the AC - SEC combination is modeled here. Although in both 1D AC and SEC the separation of these two polymers is very poor, the simulated 2D separation pattern of Fig. 8 is perfect.

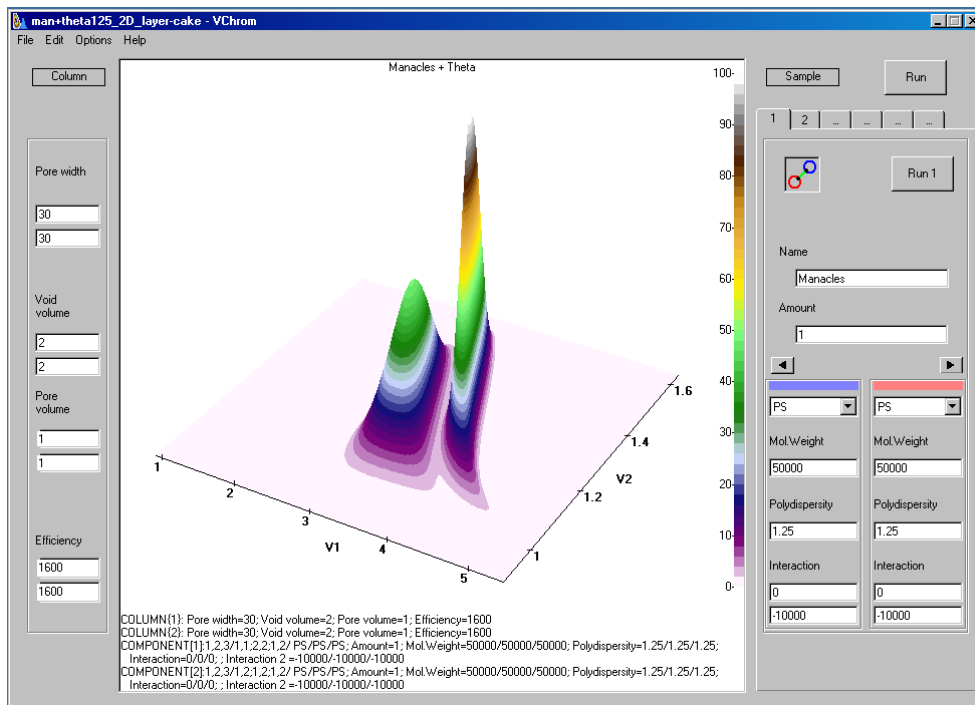


Fig. 7. A virtual separation of symmetric manacle- and theta-shaped polystyrenes with sub-chains of $M_w=50000$ and $M_w/M_n=1.25$ by two-dimensional chromatography. The first dimension corresponds to the critical interaction condition ($c=0$); the second dimension is SEC ($c = -10000$). Column relevant parameters: pore width $2d=30$ nm; $V_i=V_p=1$ ml.

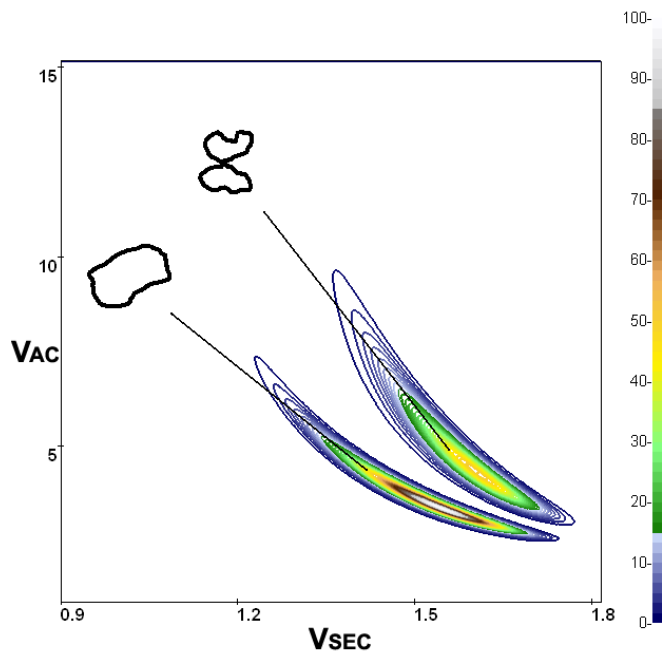


Fig. 8. A simulated two-dimensional (AC-SEC) chromatogram of a model mixture of polydisperse ring- and eight-shaped polystyrenes. Simulation parameters: $M_w=30000$, $M_w/M_n=1.25$; $d=10$ nm; $V_i=V_p=1$ ml; $c = 0.1$ nm⁻¹ (AC dimension), $c = -10000$ nm⁻¹ (SEC dimension).

One important particular problem is the separation of polydisperse linear and different star-shaped polymers [38]. According to the theory, the chromatographic behavior of linear polymers and stars at the critical point is identical (Fig. 4b). There are some differences in the retention of linear polymers and stars at the conditions of SEC and AC, but these differences are rather small, while the molar-mass effects are quite significant in both SEC and AC (Figs 4a and 4c). If linear and star polymers are polydisperse, both molar mass and topology influences the retention. As the result, polydisperse linear and star polymers will not be separated by SEC or by AC. In such situations the two-dimensional (AC – SEC) is the only chromatographic method where the separation can be expected.

Figures 9a and 9b show one-dimensional chromatograms simulated at SEC and AC conditions for 3-, 4-, and 5-arm star-shaped polystyrenes (of equal molar mass, and of the same molar mass heterogeneity of arms) in the mixture with corresponding linear polystyrene. As can be seen, in both cases peaks corresponding to species of different topology are somewhat shifted, but not separated because of the considerable peak broadening, which results from the molar mass distribution. Figure 9c, where a corresponding two-dimensional AC-SEC chromatogram is presented, shows a substantial separation of all topologically different components. The more detailed analysis showed that reasonable separation can be expected at $M_w/M_n < 1.1$ [38].

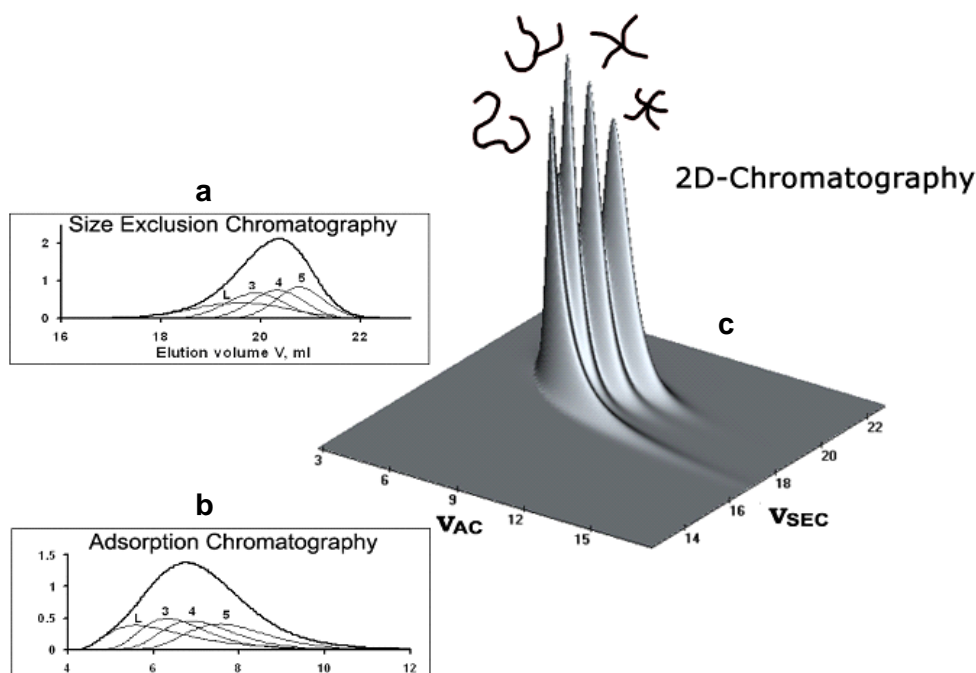


Fig. 9. One-dimensional (a) SEC, (b) AC chromatograms; and (c) two-dimensional SEC-AC chromatogram, simulated for the mixture of linear (L) polystyrenes and PS stars with three (3), four (4) and five (5) arms. Simulation parameters: $M_w=30000$; $(M_w/M_n)_{arm}=1.03$; $2d = 15$ nm; (a): $V_i= 14$ ml, $V_p=18$ ml; (b): $c=0.32$ nm⁻¹, $V_i= 3.1$ ml, $V_p=0.4$ ml; (c): parameters for SEC and AC dimensions are those of (a) and (b) respectively. Dashed lines show chromatograms for the components of the mixture; solid lines represent resulting chromatograms.

Additional advantage of 2D chromatography is that chromatograms like those of Figs. 7-9 represent both topological and molar-mass heterogeneity of the sample, while such information cannot be obtained in principle by using any one-dimensional mode of chromatography.

4. Theory and experiment

Predicted by the theory [34] chromatographic separation of linear and cyclic polymers at the critical interaction point, was realized by many groups, and now the method of liquid chromatography at the critical conditions has become a recognized technique in separation and characterization of macrocycles [12-15].

Recently Tezuka and co-workers have studied experimentally the chromatographic behavior of theta-shaped polymers in comparison with that of its star-shaped analogues [17,40]. Figure 10, which is plotted by using the data of Ref. [40], shows the overlaid chromatograms of star and theta poly(THF)s of the same $M=9600$ at various mobile phase composition. These chromatograms can be qualitatively compared with the theoretical ones of Fig. 5. The chromatograms of Fig. 10a, obtained in pure THF, are very similar to those of Figure 5a, simulated at the SEC condition: both figures show very poor separation by topology. The interaction conditions of Fig. 10b are close to the critical interaction point, therefore Fig 10b can be compared with Fig. 5b. Although not as perfect as in the theory, quite good separation is really seen in Fig. 10b. Figure 10c corresponds to stronger adsorption interaction, and thus can be compared with Fig. 5c. The separation in Fig. 10c is obviously poorer than in Fig. 10b; this fact also well agrees with the theory.

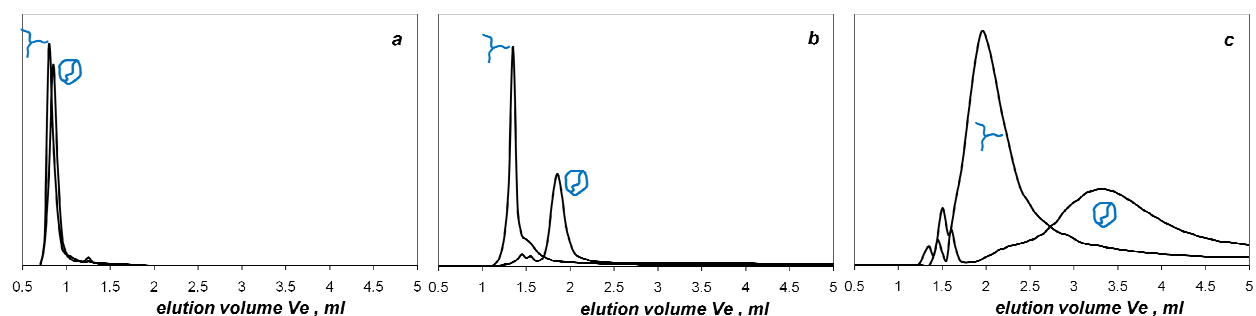


Fig. 10. Chromatograms of star and theta polymers of $M_w=9600$ on TSK ODS-80TS column at eluent compositions of THF/acetonitrile of 100/0 (a), 52/48 (b), and 48/52 (c) (Data - from Ref. [40]).

The relevant experimental data on the 2D chromatography (with the gradient AC in the first dimension, and SEC in the second one) of linear and star-shaped polystyrenes have been reported by Gerber and Radke [46]. In the experiments [46] the star sample was a mixture of stars with different number of equal arms; stars in this mixture were of different molar mass. The experiments [46] resulted in clear 2D-separations of the linear and star-shaped structures, and it was shown that separation between the linear and star polymers of the same molar mass improves with increasing arm number. These experimental findings are in a good qualitative agreement with the theory and simulation [38].

The other simulated separations have not been realized in the experiments; these simulations are still the theoretical prediction.

5. Conclusion

A theory of chromatography of complex macromolecules is used to simulate separations of polydisperse topologically different polymers. As follows from these simulations, two approaches are especially promising: the first one is the chromatography at optimized (critical or near-critical) interaction conditions; the second one consists in combing different chromatographic modes, which allows obtaining a separation by both molar mass and topology in a 2D chromatogram. Some of the simulated chromatographic separations are qualitatively very similar to the real ones.

Acknowledgements

This work was supported by grant 08-03-00891 from the Russian Foundation for Basic Research.

We thank Prof. Yasuyuki Tezuka (Tokyo Institute of Technology) for many fruitful discussions on topologically complex polymers.

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