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General mechanism of the chromatography of macromolecules: Experimental test of the universal nature of adsorption effects

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Chromatographic data have been obtained for polyethylene glycols, polypropylene glycols, and polyvinylpyrrolidones, based on the use of four different adsorbents at different temperatures and pH values of aqueous buffer eluents. The experimental results are interpreted from the standpoint of a general theory of chromatography of polymers, accounting for adsorption interactions. Comparison between experiment and theory has made it possible to determine the adsorption interaction parameter, c, and the mean thickness of long adsorbing macromolecules, H, at different pH values and temperatures for various polymer–adsorbent systems.

According to the theory, the parameter $U = (K - K_{GPC})/(1 - K_{GPC})$ is a universal function of the product of gyration radius R and adsorption parameter c. In fact, the entire set of experimental data fit the same U *versus* cR relationship. Predicted from theory, the universal character of manifestation of adsorption effects in the chromatography of polymers has been tested experimentally for most diverse systems, viz. polymers of various chemical nature and molecular weights, adsorbents having various structures and pore sizes, aqueous and organic eluents, as well as for different methods to realize and change adsorption interactions. © 1997 Elsevier Science Ltd.

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INTRODUCTION

It is well known that under GPC conditions the distribution coefficient of macromolecules, K_{GPC}, depends on one dimensionless parameter: the ratio of molecule size R to pore size d[1]. It is the universal K_{GPC} *versus* R/d relationship that determines the size exclusion effect in the chromatography of polymers under GPC conditions.

At the same time, changes in the chromatographic conditions (such as adsorbent or solvent changes, temperature or pH variations, etc.) leading to the emergence of adsorption forces will entail changes in the mechanism of chromatography[2–5]. Up to a certain critical value of the adsorption interaction parameter, the molecular weight sequence of elution of macromolecules corresponds to the elution sequence in GPC, but the universal law characteristic for GPC no longer holds under these changed conditions, and the chromatographic behaviour of macromolecules becomes dependent upon the adsorption interactions[6,7]. After the critical level of adsorption interactions is reached, the molecular weight sequence of elution of macromolecules becomes even opposite to that of under GPC conditions, this fact being typical for adsorption chromatography of polymers.

A general theory of chromatography of macromolecules, accounting for the effects of adsorption of polymeric chain units over pore walls, has been developed[8,9]. According to this theory, the adsorption interactions in the chromatography of polymers may be described with the use of the only one universal parameter. This means that the adsorption effects manifest themselves in the chromatography of polymers in an universal manner, independent of the method, in which the adsorption interactions are realized.

In the literature[10,11] the general theory of chromatography of macromolecules was compared with the experimental data[3,12] derived from chromatography of polystyrenes on macroporous glasses at varied temperatures and mixed organic solvent compositions. Various modes of chromatographic behaviour have been observed also for water-soluble polymers[13–18]. Although the data presented[13–18] have not been quantitatively compared with the theory, these data seem to be in a qualitative agreement with the general theory[8].

The present paper is devoted to the more detailed examination of mechanisms involved in the chromatography of water-soluble polymers based on the use of different adsorbents and varied temperatures and aqueous buffer eluent pH values. We demonstrate here that the very different chromatographic systems can be characterized unambiguously by the same adsorption interaction parameter, and that the adsorption effects in these systems actually do manifest themselves in the same universal manner.

EXPERIMENTAL

The following compounds were studied for their chromatographic behaviour: polyethylene glycols (PEG) from Fluka (Buchs, Switzerland), Loba (Wien, Austria), and Ferak (Berlin, Germany), with molecular weights up to 4×10^4;

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low molecular weight polypropylene glycols (PPG) from Aldrich Chemical Company Inc. (Milwaukee, WI, USA); polyvinylpyrrolidones (PVP) with molecular weights from 10×10^3 to 24×10^3 from Fluka.

Four different adsorbents were used. The first was Butyl-Toyopearl 650 M (with particle size 45–90 μm) from TOYA SODA (Tokyo, Japan). Three other adsorbents used were of Russian make. These were cross-linked copolymers of methacrylic acid and butyl methacrylate, differing one from another in the proportions of the constituent monomeric components[19]. Their generic name is SOLOZA, and a specific adsorbent is suffixed to denote the molar percentage of the butyl methacrylate present. We used three adsorbents from this series: SOLOZA K-0, SOLOZA K-33 (particle size about 200 μm), and SOLOZA KG-8 (particle size 60–80 μm). SOLOZA adsorbents are slightly swellable in water.

The pore sizes of all adsorbents were determined by the dextran-based GPC porosimetry method[20]. This chromatographic method permits adsorbent investigations in the presence of liquid mobile phase, under conditions identical with, or close to, conditions of practical usage, which is of particular importance for swellable polymeric adsorbents. The pore diameters determined in this way were within 20–28 nm. GPC porosimetric measurements did not reveal appreciable variation of pore structure parameters in the studied range of temperature and pH of mobile phase.

The experiments were carried out on a chromatographic installation comprising a Model 12000 Varioperpex peristaltic pump (LKB, Bromma, Sweden) and a Model R 401 differential refractometer (Waters, Milford, MA, USA) coupled to a Model 2066 two-channel potentiometric recorder from LKB. The columns used were 120 mm long, 4 mm i.d., and 50 mm long, 10 mm i.d. The flow rate was 0.2 ml min^{-1}, sample size 10–30 μl, polymer concentration 0.3 wt%. The eluent was phosphate buffer (0.05 M in experiments with Butyl-Toyopearl, and 0.1 M in all other cases), with pH and temperature varied.

The retention volumes, V, of the polymers studied were determined from the maxima of the chromatographic peaks, and the distribution coefficients K were calculated from the formula $K = (V - V_0)/(V_t - V_0)$, where V_0 and V_t are the retention volumes for high molecular weight dextran ($M = 40 \times 10^6$) and glucose, respectively. The typical values for these reference volumes were: $V_0 \simeq 1.8$ ml, $V_t \simeq 3.4$ ml for the Butyl-Toyopearl 650 M adsorbent, and $V_0 \simeq 0.9$ ml, $V_t \simeq 1.5$ ml for the SOLOZA adsorbents. Each polymer fraction was chromatographed three to five times; the statistical error for K values was estimated as about 2–5%.

RESULTS AND DISCUSSION

Figure 1 shows molecular weight dependencies of the distribution coefficients at various pH values for PEG macromolecules chromatographed using Butyl-Toyopearl 650 M adsorbent at 43°C (*Figure 1a*) and SOLOZA K-33 adsorbent at 22°C (*Figure 1b*). *Figure 2* gives chromatographic results for PEG and three adsorbents: Butyl-Toyopearl (*Figure 2a*), SOLOZA K-33 (*Figure 2b*), and SOLOZA K-0 (*Figure 2c*). These results were obtained at fixed pH, but at different temperatures. Similar relationships are represented in *Figure 3* for PVP at several pH values and a temperature of 22°C, and in *Figure 4*, for a PPG–SOLOZA KG-8 system at pH 5 and several different temperatures.

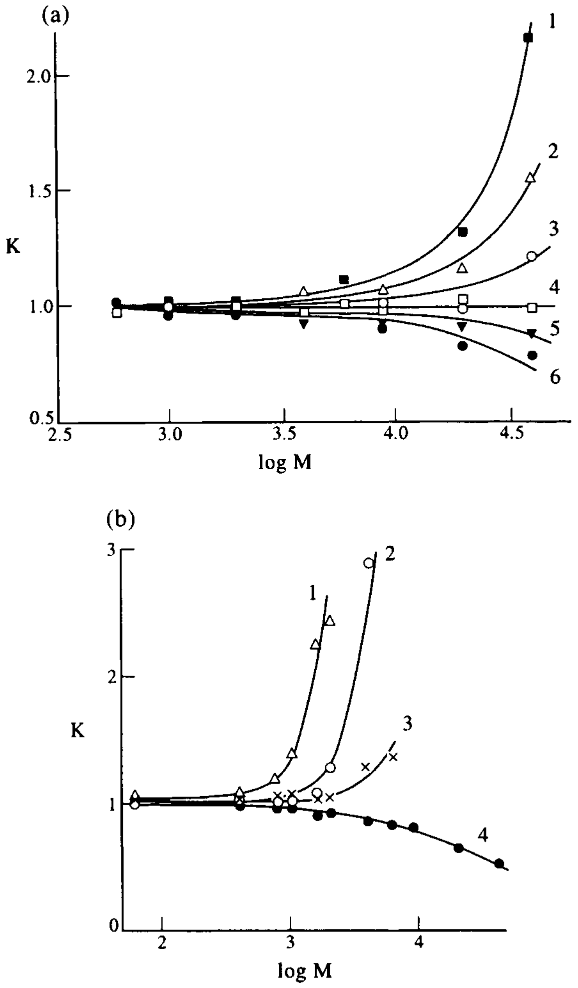

Figure 1 Distribution coefficient *versus* molecular weight for PEG chromatographed using Butyl-Toyopearl 650 M adsorbent (a), and SOLOZA K-33 adsorbent (b). Experimental conditions: (a) temperature 43°C and pH values of 3.9 (1), 4.6 (2), 5.2 (3), 5.8 (4), 6.5 (5), and 8.8 (6); parameter c values: 0.1 (1), 0.066 (2), 0.032 (3), 0.0013 (4), −0.031 (5), and −0.084 nm^{-1} (6); (b) temperature 22°C and pH values of 5 (1), 5.5 (2), 6 (3), 6.5 (4); c = 0.95 (1), 0.5 (2), 0.28 (3), and − 0.37 nm^{-1} (4). Solid lines show the theoretical relationships (equations (1)–(3))

As may be seen from *Figures 1–4*, pH and temperature variations, as well as polymer and adsorbent changes, lead to changed adsorption interactions, resulting in changes in the aspect and nature of the *K versus M* relationship.

In our experiments we observed all known chromatographic modes for polymers, viz. the GPC mode realizable in the absence of adsorption interactions; GPC-like modes complicated by adsorption effects, with weak adsorption interactions (characteristic of these modes, just as of GPC, is decreasing K with increasing M); chromatography under critical conditions, when $K = 1$; and, finally, adsorption chromatography characterized by increasing K with increasing M.

Theoretical interpretation and calculation of the adsorption interaction parameter

A general theory of chromatography of polymers, accounting for adsorption interactions, has been developed[8]. According to this theory, distribution coefficient K is a function of two dimensionless parameters: $g = R/d$ and $\Gamma = -cR$, where R is the radius of gyration of a macromolecule, d is the pore radius of an adsorbent, and c is the measure of the adsorption interaction in the system.

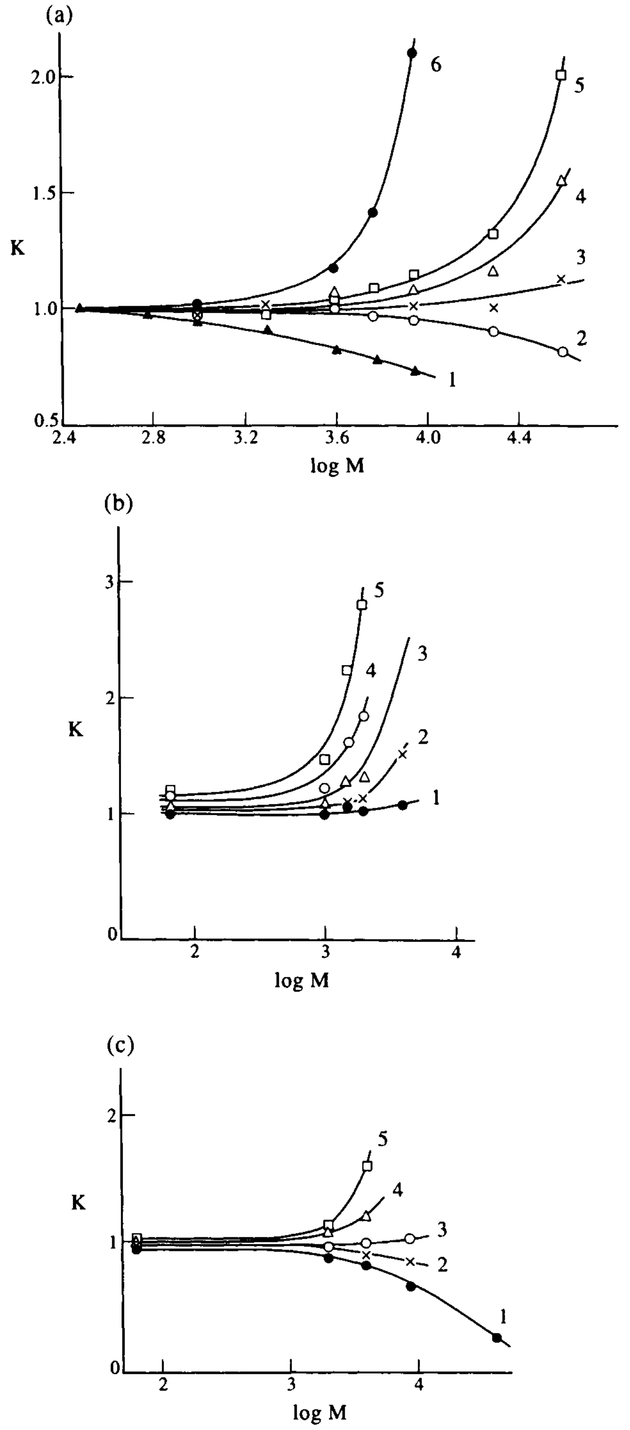

Figure 2 Molecular weight dependencies of distribution coefficient for PEG chromatographed using Butyl-Toyopearl 650 M (a), SOLOZA K-33 (b), and SOLOZA K-0 (c) adsorbents. (a) pH 4.6 and temperatures of 18°C (1), 37°C (2), 40°C (3), 43°C (4), 45°C (5), 50°C (6); parameter c values: −2.6 (1), − 0.047 (2), 0.019 (3), 0.066 (4), 0.093 (5) and 0.34 nm^{-1} (6); (b) pH 5 and temperatures of 1.5°C (1), 6°C (2), 9°C (3), 18°C (4) and 27°C (5); c = 0.1 (1), 0.36 (2), 0.53 (3), 0.77 (4) and 0.94 nm^{-1} (5); (c) pH 4.5 and temperatures of 8°C (1), 15°C (2), 18.5°C (3), 26°C (4) and 30°C (5); c = −0.77 (1), −0.12 (2), 0.036 (3), 0.24 (4) and 0.36 nm^{-1} (5). Solid lines: equations (1)–(3)

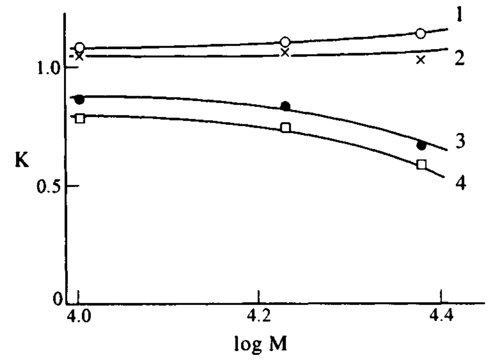

Figure 3 Distribution coefficient *versus* molecular weight for the PVP–SOLOZA K-33 system at 22°C and pH 5 (1), 6 (2), 7.1 (3) and 8 (4). Solid lines: equations (1)–(3) at c = 0.077 (1), 0.038 (2), −0.34 (3) and −1.25 nm^{-1} (4)

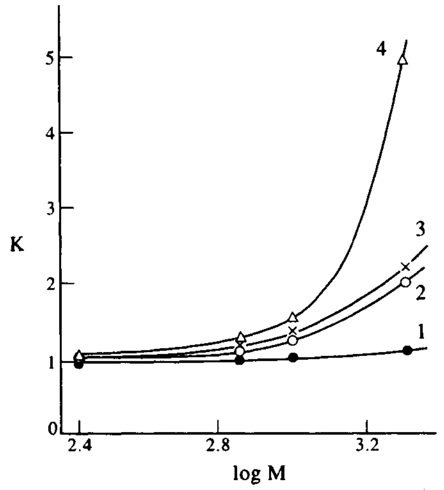

Figure 4 Molecular weight dependencies of distribution coefficient in the PPG–SOLOZA KG-8 system at pH 5 and various temperatures: 5°C (1), 13.5°C (2), 15°C (3) and 19.5°C (4). Parameter c values: 0.83 (1), 2.18 (2), 2.27 (3) and 2.7 nm^{-1} (4). Solid lines: equations (1)–(3)

The c parameter characterizes the deviation of a polymer–solvent–adsorbent system from the critical conditions. In GPC mode and in GPC-like chromatography, where adsorption effects are negligible or small, the adsorption interaction parameter c is negative; positive values of c correspond to adsorption chromatography, where adsorption effects are dominating; $c = 0$ corresponds to chromatography under critical conditions, characteristic of which is a compensation of entropy and enthalpy effects arising in the course of a macromolecule transition from the eluent into the adsorbent pores. More detailed discussion on the physical meaning of the adsorption interaction parameter will be presented in the last section of this paper.

At low g values, i.e. for small macromolecules and wide-pore adsorbents (which is exactly the case realized in our experiments), the theory presented in Ref. [8] provides the following expression for the distribution coefficient K of flexible-chain macromolecules:

$$K \simeq K_{\mathrm{GPC}} + \frac{g}{\Gamma}[1 - Y(\Gamma)] \qquad (1)$$

where

$$K_{\mathrm{GPC}} \simeq 1 - \frac{2}{\sqrt{\pi}}g \qquad (2)$$

and

$$Y(\Gamma) = \exp(\Gamma^2)[1 - \mathrm{erf}(\Gamma)] \qquad (3)$$

($\mathrm{erf}(\Gamma)$ being the well known probability integral, for which detailed tables are available). The theoretical equations (1)–(3) include two scaling parameters, g and Γ, these being expressible in terms of three parameters, d, R and c: $g = R/d$, $\Gamma = -cR$.

In order to compare theory and experiments, we determined the pore radii d of the adsorbents from the GPC-porosimetric measurements, and calculated the mean polymer radii R for macromolecules of each type and molecular weight M, using the formula $R = BM^{\alpha}$. As a rough approximation, we used for each polymer the following values of B and α at all temperatures and pH values (PEG: $B = 0.016$, $\alpha = 0.59$; PPG: $B = 0.018$, $\alpha = 0.5$; PVP: $B = 0.029$, $\alpha = 0.5$).

Knowing d and R values, it is possible to compare theory and experiment, using c as an adjustable parameter, accounting for adsorption interactions (note that according to the theory, each specified polymer–solvent–adsorbent system at fixed pH and temperature must be describable by the single value of the c parameter, which is the same for macromolecules of all molecular weights). To compare the theory with the experimental data and to find the unknown parameter c, the following procedure was adopted. A computer program based on the least squares technique was developed and used to determine a certain value of c (the same for all molecular weights) for each temperature and pH value. The c value which provided the best fit of the experimental points to the theoretical K *versus* M relationship (calculated using equations (1)–(3)) was selected.

The values of the c parameter so determined are indicated in the captions of *Figures 1–4*, while the corresponding theoretical curves are shown in these figures by solid lines. *Figures 1–4* show that with the adsorption interaction parameter c suitably selected, the entire set of the experimental points can be adequately described by equations (1)–(3).

Figures 5 and 6 show pH and temperature dependencies of c obtained for PEG macromolecules and three different adsorbents. These dependencies were used to determine the critical conditions (points of intersection between the curves and the abscissa axis).

De Gennes[21] suggested a linear relationship between the c parameter and a temperature. *Figure 6* shows that for the given systems this relationship holds but approximately, within a narrow range of temperatures near the critical point. Also found approximately linear was the relationship between c and pH value of the solution (*Figure 5*).

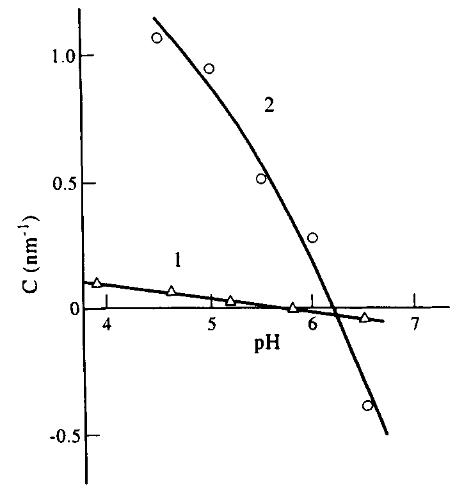

Figure 5 Adsorption interaction parameter, c, *versus* eluent pH in the PEG–Butyl-Toyopearl 650 M system at 43°C (1), and in the PEG–SOLOZA K-33 system at 22°C (2)

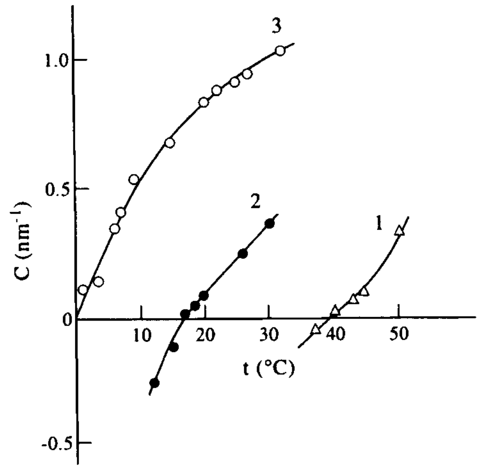

Figure 6 Temperature dependence of parameter of adsorption interactions of PEG macromolecules for the Butyl-Toyopearl 650 M adsorbent at pH 4.6 (1), the SOLOZA K-0 adsorbent at pH 4.5 (2), and the SOLOZA K-33 adsorbent at pH 5 (3)

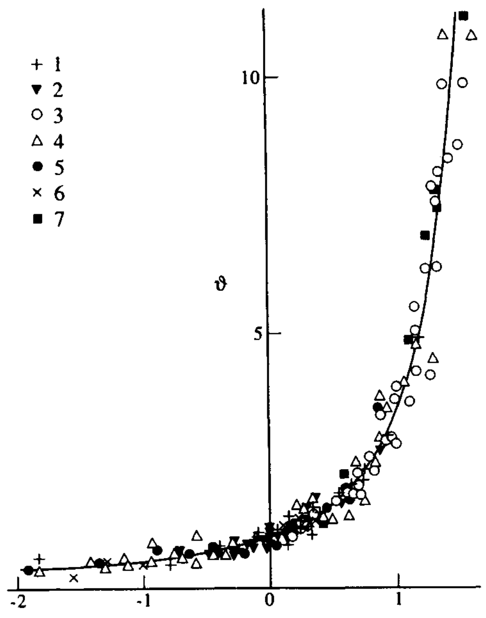

Figure 7 $U = (K - K_{GPC})/(1 - K_{GPC})$ *versus* adsorption interaction parameter $\Gamma = -cR$ for the PEG–Butyl-Toyopearl 650 M (1, 2), PEG–SOLOZA K-33 (3, 4), PEG–SOLOZA K-0 (5), PVP–SOLOZA K-33 (6), and PPG–SOLOZA KG-8 (7) systems. Solid line: equation (4). (1) pH 4.6, temperature varying from 18 to 50°C; (2) temperature 43°C, pH varying from 3.9 to 8.8; (3) pH 5, temperature varying from 1.5 to 32°C; (4) temperature 22°C, pH varying from 4.5 to 8; (5) pH 4.5, temperature varying from 8 to 30°C; (6) temperature 22°C, pH varying from 5 to 8; (7) pH 5, temperature varying from 5 to 19.5°C

Universal nature of adsorption effects in the chromatography of polymers

Theory[8–10] predicts the universal nature of adsorption effects in the chromatography of macromolecules. By excluding the exclusion effects from equation (1), the following equation was obtained in Ref. [10]:

$$\frac{K - K_{GPC}}{1 - K_{GPC}} = \frac{\sqrt{\pi}}{2}\Gamma^{-1}[1 - Y(\Gamma)] \qquad (4)$$

According to equation (4), the experimentally measured quantity $U = (K - K_{GPC})/(1 - K_{GPC})$ must be a function of one dimensionless parameter $\Gamma = -cR$.

Figure 7 gives the experimental data of *Figures 1–4* in the *U versus* Γ coordinates. The solid line in *Figure 7* corresponds to the theoretical relationship (equation (4)). It can be seen that the chromatographic data for all PEG, PPG, and PVP samples for four adsorbents at various temperatures and pH values are described by a common relationship of the form given in equation (4). Previously[10,11], it was shown that the same theoretical relationship is fitted by the entire set of experimental chromatographic data[3,12] for the system polystyrene–porous glass at various temperatures and mixed chloroform–carbon tetrachloride eluent compositions. Thus, the universal nature of the manifestation of adsorption effects in chromatography has been demonstrated experimentally for polymers of various chemical nature and molecular weight, for adsorbents of various structure and with various pore sizes, and for both organic and aqueous eluents, with the adsorption interactions varied in diverse ways.

Correlation length of adsorption and the mean thickness of adsorbed macromolecules

The adsorption interaction parameter c has the dimension of a reciprocal length, reciprocal parameter, $H = c^{-1}$, called the correlation length of adsorption. The correlation length of adsorption is a large scale characteristic which is independent either of the structural details of the polymer chain or of the microstructure of the adsorbent or of the nature and precise form of an adsorption interaction potential.

Under the conditions corresponding to the adsorption chromatography mode (at positive c values) interactions between a macromolecule and a surface of porous adsorbent are thermodynamically more beneficial than those between a macromolecule and solvent molecules, and this results in the formation of high monomer density regions near the pore walls. The high density layer with similar properties is also known to be formed in the course of polymer adsorption on the surface of non-porous particles at low polymer concentrations.

According to the theories of adsorption of long ideal macromolecules in pores and on a plane surface at low polymer concentration (see Ref.[22] for a review of these theories), the density of polymer chain units, μ, is the decreasing exponential function of distance from the plane (or from the adsorbing wall), z:

$$\mu(z) \approx \exp(-2cz) \qquad (5)$$

equation (5) is valid for $cR > 1$, i.e. for sufficiently long macromolecules. It follows from equation (5) that the correlation length, $H = c^{-1}$, has a meaning of the average thickness of a long adsorbed macromolecule, H being independent of molecular weight.

So, the chromatographic method (essentially equilibrium and thermodynamic) permits the determination of the structural parameter, H, i.e. the average thickness of a long adsorbed macromolecule.

As an example, the values of H, calculated from chromatographic measurements for the PEG–SOLOZA K-33 system, are plotted *versus* temperature in *Figure 8*. It follows from *Figure 8*, that at high temperatures (15–30°C) H values of PEG molecules adsorbed in the pores of SOLOZA K-33 are about 1 nm. It can be concluded that under these conditions the main motive of adsorbed PEG macromolecules is the alternation of flat adsorbed trains and loops. We suppose that at these high values of temperature the conformations of adsorbed PEG macromolecules with a mean thickness of about 1 nm, really do exist in the pores of SOLOZA adsorbent in the course of chromatographic experiments.

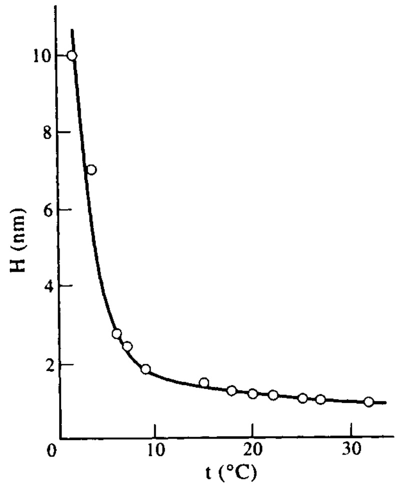

Figure 8 Temperature dependence of the mean thickness of long adsorbing macromolecules, *H*, in the PEG–SOLOZA K-33 system at pH 5

Decreasing the temperature in the system under investigation leads to a weakening of adsorption interactions, and consequently, to an increase in the mean thickness of adsorbed macromolecules. The effect of increasing H with decreasing temperature can be seen in *Figure 8*.

As follows from *Figure 8*, in this system H reaches a value of about 10 nm at 1.5°C. Even larger values of H—about 20 nm—were obtained for other systems (PEG–Butyl-Toyopearl, PEG–SOLOZA K-0 and PVP–SOLOZA K-33). These values of correlation length H exceed the mean sizes of PEG macromolecules in the solution (for example, the radius of inertia, R, of PEG molecules of $M = 4 \times 10^4$ is about 8 nm), and have the same order of magnitude as the pore diameters. It is evident that in the chromatographic experiments with rather short polymer molecules these large values of H can not be identified with any of the sizes of adsorbed molecules. But, as follows from the theory[9,22], these large values of correlation length H obtained in the chromatographic experiments with short PEG molecules may be considered as a prediction of the mean thickness of long adsorbed PEG macromolecules in the same experimental system. Even in the case where H values exceed pore sizes we may speculate upon the structure of adsorbed macromolecules: H values could be ascribed to the mean thickness of long macromolecules adsorbing on the surface of non-porous adsorbent of the same chemical structure.

It would be of interest to measure thicknesses of adsorbed polymer molecules, using other polymer thickness measuring methods (Ref. [23] presents a review of these methods), and to compare these data with the results, obtainable with the use of the proposed chromatographic method.

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REFERENCES

1. Casassa, E. F., *Journal of Polymer Science, Part B*, 1967, **5**, 773.
2. Belenkii, B. G. and Vilenchik, L. Z., *Modern Liquid Chromatography of Macromolecules*. Elsevier, Amsterdam, 1983.
3. Nefyodov, P. P. and Lavrenko, P. N., *Transportnyye Metody w Analiticheskoj Khimii Polimerow* (Transport Methods in the Analytical Chemistry of Polymers). Khimija, Moscow, 1978.
4. Entelis, S. G., Evreinov, V. V. and Kusayev, A. I., *Reaktsionno-sposobnyye Oligomery* (Reactive Oligomers). Khimija, Moscow, 1985.
5. Gorbunov, A. A., Solovyova, L. Ya, Pasechnik, V. A. and Lukyanov, A. Ye, *Vysokomolekulyarnye Soedineniya, Seriya A*, 1986, **28**, 1859.
6. Bakos, D., Bleha, T., Ozima, A. and Berek, D., *Journal of Applied Polymer Science*, 1979, **23**, 2233.
7. Campos, A., Soria, V. and Figueruelo, J. E., *Makromolekulare Chemie*, 1979, **180**, 1961.
8. Gorbunov, A. A. and Skvortsov, A. M., *Vysokomolekulyarnye Soedineniya, Seriya A*, 1986, **28**, 2170; 2453.
9. Skvortsov, A. M. and Gorbunov, A. A., *Journal of Chromatography*, 1986, **358**, 77.
10. Gorbunov, A. A. and Skvortsov, A. M., *Doklady AN SSSR*, 1987, **294**, 396.
11. Gorbunov, A. A. and Skvortsov, A. M., *Vysokomolekulyarnye Soedineniya, Seriya A*, 1988, **30**, 3.
12. Tennikov, M. B., Nefyodov, P. P., Lazareva, M. A. and Frenkel, S. Ya., *Vysokomolekulyarnye Soedineniya, Seriya A*, 1977, **19**, 657.
13. Belenkii, B. G., Valchikhina, M. D., Vakhtina, I. A., Gankina, E. S. and Tarakanov, O. G., *Journal of Chromatography*, 1976, **129**, 115.
14. Gorshkov, A. V., Much, H., Becker, H., Pasch, H., Evreinov, V. V. and Entelis, S. G., *Journal of Chromatography*, 1990, **523**, 91.
15. Pasch, H., Much, H., Schulz, G. and Gorshkov, A. V., *LC-GC International*, 1992, **5**, 38.
16. Trathnigg, B., Thamer, D., Yan, X. and Kunugasa, S., *Journal of Liquid Chromatography*, 1993, **16**, 2439.
17. Trathnigg, B., Thamer, D., Yan, X., Maier, B., Holzbauer, H.-R. and Much, H., *Journal of Chromatography*, 1994, **665**, 47.
18. Trathnigg, B., Maier, B. and Thamer, D., *Journal of Liquid Chromatography*, 1994, **17**, 4285.
19. Boldyrev, A. G., Dumpis, Yu. Ya., Malko, Ye. I., Fyodorova, N. M., Gorbunov, A. A., Solovyova, L. Ya., Pisarevsky, Yu. S., Vorobyov, V. P., Vinogradov, Ye. L., Pasechnik, V. A. and Morozov, S. V., in *Khromatograficheskiye i Filtratsionnyye Metody Ochistki i Kontsentrirovaniya Biologicheskikh Preparatow* (Chromatographic and Filtration Methods for Purifying and Concentrating Biological Preparations). Glavmikrobioprom, Moscow, 1983, p. 19.
20. Gorbunov, A. A., Solovyova, L. Ya. and Pasechnik, V. A., *Journal of Chromatography*, 1988, **448**, 307.
21. De Gennes, P. G., *Reports on Progress in Physics*, 1969, **32**, 187.
22. Gorbunov, A. A. and Skvortsov, A. M., *Advances in Colloid and Interface Science*, 1995, **62**(1), 31.
23. Fleer, G. J., Cohen Stuart, M. A., Scheutjens, J. M. H. M., Cosgrove, T. and Vincent, B., *Polymer at Interface*. Chapman, Hall, 1993.