ROLE OF ADSORPTION IN LIQUID CHROMATOGRAPHY OF MACROMOLECULES AND POTENTIAL OF LIQUID CHROMATOGRAPHY IN ASSESSING ADSORPTION OF MACROMOLECULES ONTO SOLID SURFACES

Dušan Berek
Polymer Institute of the Slovak Academy of Sciences,
842 36 Bratislava, SLOVAKIA

Abstract: Many liquid chromatographic (LC) separations of macromolecules are influenced or directly based on adsorption of solutes on column packing. In the case of well known size exclusion chromatography (SEC), adsorption effects are usually unwanted and therefore suppressed. Still they appear in many SEC systems and may badly affect precision of results obtained. In other LC methods applicable to high polymers, adsorption is deliberately combined with exclusion. The aim is to discriminate complex polymer systems which exhibit more than one single distribution of their molecular characteristics. The main goals of such combinations include either a controlled increase or a full suppression of separation selectivity according to one molecular characteristics. Most important so far known exclusion-adsorption compensation methods allowing to suppress dependence of LC retention volumes on polymer molar mass are reviewed. The discussion is accomplished with a presentation of newly developed full adsorption – desorption (FAD) method which can be combined with various LC procedures. A very useful combination represents the on-line FAD/SEC procedure which enables also to study adsorption and desorption phenomena in the systems solid surface – solvent – macromolecules.

INTRODUCTION

Attractive interactions between a solute and a solid surface as rule bring about accumulation and often specific arrangement of solute molecules on the surface. The resulting process is called solute adsorption and it affects many areas of science, technology and everyday life. This explains large interest of researchers in the study of adsorption phenomena.

Adsorption of macromolecules differs in numerous aspects from that of small molecules. This is mainly caused by existence of simultaneous contacts among several building units of a macromolecule and the surface of adsorbent. This multiple attachment of macromolecules on adsorbent surface is co-responsible for the steepness of the "high affinity adsorption isotherms" for many polymer-solvent-adsorbent systems and also for complicated adsorption dynamics of macromolecules.

Liquid chromatography (LC) belongs to the areas where adsorption of macromolecules on solid surfaces plays a very important role. In some liquid chromatographic systems, the differential migration of macromolecules along LC column may be controlled exclusively by the differences in solute adsorption onto the column packing. In other words, separation mechanism is based on adsorption phenomena.

Alternatively, solute adsorption represents just one of two or several different separation mechanisms which simultaneously affect polymer retention in LC. In these cases we speak about (an intentional) coupling of separation mechanisms or about (an incidental) mixed separation mechanism. Consequently, retention of macromolecules in the LC columns may be influenced by adsorption processes either in a controlled way or unwantedly. For example, in the former case the size exclusion chromatographic (SEC) separation selectivity can be either enhanced or intentionally suppressed from the point of view of analyte molecular sizes so that another parameter can be determined without interference. On the other hand, selectivity and/or efficiency of SEC separation may be adversely affected in latter case and the data processing becomes more complicated due to simultaneous presence of more than one separation mechanism.

Numerous physico-chemical methods have been applied to the study of **polymer adsorption on solid surfaces.** They include also liquid chromatography which can produce valuable information on the adsorption – desorption processes.

In this contribution, we shall briefly discuss both the role of adsorption in liquid chromatography of macromolecules and the potential of liquid chromatography in the dynamic study of polymer adsorption. We shall stress the aspect of liquid chromatography as a method for separation and characterizations of polymers, as well as a tool for assessing adsorption of macromolecules.

1. Adsorption in liquid chromatography

The effects of adsorption in liquid chromatography of macromolecules can be easily visualized with plots of log polymer molar mass vs. corresponding retention volume. Such dependences are called calibration curves and are widely applied in SEC. The typical examples of calibration curves are schematically represented in Fig. 1.

Curve 1 in Fig. 1 corresponds with the situation when the effective segmental adsorption energy of macromolecules with the column packing surface, ε, is negligible. To reach this "ideal size exclusion chromatographic" behavior, the adsorption energy between solvent molecules and packing surface must fairly exceed that between polymer segments and column packing. We have a "strong" solvent – in contrast with a "weak" solvent, which exhibits only a low affinity toward column packing surface. The solvent strength does not necessarily correlate with the thermodynamic quality of solvent toward polymer that is with the ability of given solvent to

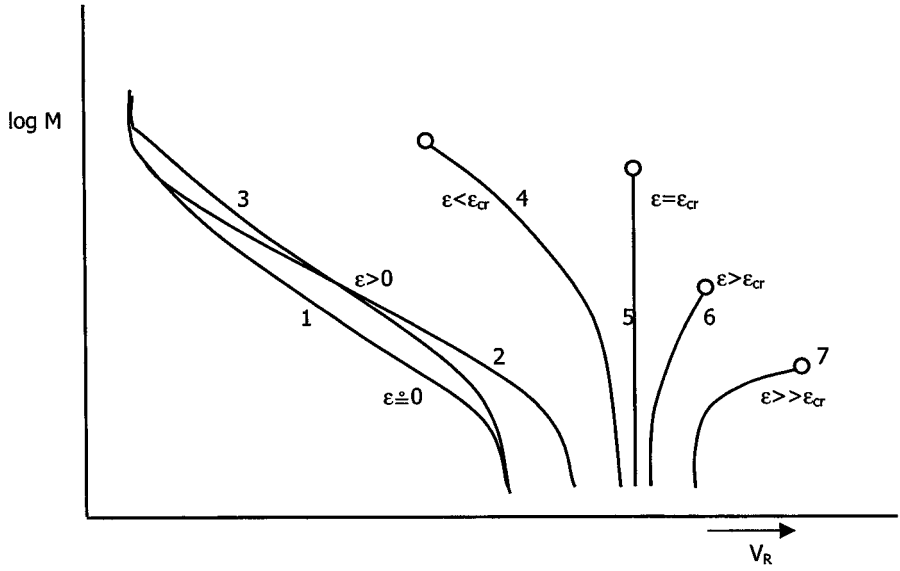


Figure 1 Dependences of log (polymer molar mass M) vs. retention volumes (V_R) in liquid chromatography with porous column packings. Representation of exclusion and adsorption effects. For detailed explanations see the text.

solvate macromolecules. The terms "thermodynamically" good (for polymer) and "strong" (as to its interaction with column packing) solvent are often confused in literature. For example, thermodynamically good solvent may either promote or prevent adsorption of macromolecules on a given solid surface. Similarly, a nonsolvent added to a polymer solution may promote either adsorption or desorption of macromolecules. Further, a strong solvent may prevent adsorption of one polymer but at the same time, if can still promote adsorption of another polymer on the same surface and at the same temperature. Therefore, we proposed the terms: adsorption promoting liquid – "adsorli" and desorption promoting liquid – "desorli" – for a given polymer – adsorbent system and at a given temperature. In other words, an adsorli for a polymer P1 and adsorbent A1 may be a desorli for another polymer P2 and/or for for another adsorbent A2 and/or at a different temperature - and vice versa.

In "ideal SEC", macromolecules are separated exclusively on the base of entropic effects and we have for polymer retention volume (V_R)

$$V_R = V_0 + K_D V_p , \qquad (1)$$

where K_D is exclusion distribution coefficient and V_p is volume of column packing pores or more generally the volume of mobile phase from which macromolecules are partially or fully excluded. Theoretically, macromolecules can be excluded also from a part of interstitial mobile phase volume, V_0, especially from that in contact with outer surface of column packing particles.

If a weak interaction does exist between eluted macromolecules and column packing, that is if ε is positive but rather small, the entropic separation mechanism still prevails and V_R's increase with decreasing polymer molar mass (M). A slight increase of polymer retention volumes is observed in comparison with $\varepsilon \sim 0$ (curves 2 and 3 in Fig. 1), especially for macromolecules with lower molar masses (curve 2). In these cases, the SEC separation selectivity, may be improved (Ref. 1) but a direct comparison of retention volumes is no more possible among different polymer-eluent couples (Ref. 2) - even if hydrodynamic volumes instead of molar mass values are considered for macromolecules (universal SEC calibration concept) (Ref. 3). We can write (Ref. 4)

$$V_R = V_0 + K_D V_p + K_A V_A , \qquad (2)$$

where K_A is adsorption distribution coefficient and V_A the effective pore volume or, more precisely the volume of (quasi) stationary phase in which adsorption takes place (Ref. 5).

For a simplified situation where the net adsorption effect does not depend on polymer molar mass we have (Ref. 6).

$$V_R = V_0 + K_D K_p V_p , \qquad (3)$$

where K_p is a parameter characterizing adsorption of macromolecules on the column packing surface. Eq. 3 holds for many systems comprising polystyrene – divinylbenzene SEC column packing (Ref. 6) and can be schematically represented by curve 3 in Figure 1. The shifts of retention volumes represented by curve 2 were observed e.g. with silica gel and porous glass SEC column packings (Refs. 2,4,7).

Further rise in the effective segmental adsorption energy leads to a strong shift of V_R for high polymer molar masses (curve 4). As rule, sample recoveries drop with increasing molar masses and macromolecules may be fully retained within column, especially above their exclusion limit. This is stressed by the sign (O) on the corresponding calibration curves.

When ε reaches its "critical value" ($\varepsilon = \varepsilon_{cr}$), macromolecules are eluted within the same retention volume V_{Rcr} irrespectively of their molar mass (curve 5), $K_D = 1$ and

$$V_{Rcr} \cong V_0 + V_p . \qquad (4)$$

This is the situation when adsorption and exclusion effects mutually compensate and we speak about the point of exclusion-adsorption transition (PEAT) (Ref. 8). At PEAT, macromolecules assume a specific conformation (Ref. 9) and they "do not see" the structure of column packing (Ref. 10).

Further increase of the effective segmental adsorption energy ($\varepsilon > \varepsilon_{cr}$) brings about prevailing effect of adsorption over exclusion on polymer elution. The retention volumes of macromolecules increase with their rising molar masses. The limiting molar mass is further decreased at which polymer becomes fully retained within column (curves 6 and 7 in Fig. 1). For high ε values, the retention volumes of analytes may very strongly depend on M (curve 7) and the isocratic separation of macromolecules, except for oligomers, becomes experimentally non-feasible.

The reason for retention behavior as shown in Fig. 1, curve 7 lies again in the multiple attachment of macromolecules on the adsorbent surface. Even a very small change in the effective segmental adsorption energy which otherwise hardly affects the adsorption behavior of a single monomeric unit, strongly influences adsorption and retention characteristics of the whole macromolecule composed of numerous monomeric units (Ref. 5). In other words the solvent strength range which is available for a control of ε and thus for retention adjustment in polymer liquid chromatography remains often too small to be experimentally feasible. Macromolecules exhibit an "on-off" elution behavior in an isocratic mode (too low retention vs. full retention). This is the reason why gradient elution must be applied in most interactive LC procedures for high polymers.

Let us discuss the practical aspects of adsorption in particular procedures of polymer liquid chromatography:

In **size exclusion chromatography**, the operators try to diminish adsorption of samples applying the so-called non-interactive column packings and strong, single or mixed eluents, that is efficient desorlis for given polymers. Sometimes, adsorption of a sample can be suppressed by increased temperature of experiment. However, the extent of polymer adsorption on solid surfaces often increases with temperature and therefore this latter approach must be carefully tested. Polystyrene/divinylbenzene macroreticular gels are considered noninteractive when compared with silica gels and with some other commercially available column packings for nonaqueous SEC (gel permeation chromatography). As we have recently shown (Ref. 11) this is not necessarily the case for all PS/DVB commercial SEC columns. The elution behavior corresponding to calibration curves 2-4 and even to curve 7 in Fig. 1 has been observed for medium polar poly(methyl methacrylate) samples applying weak eluents of low polarity.

The molar mass independent polymer retention (LC PEAT – curve 5 in Fig. 1) can be applied for discrimination of polymer blend constituents and for separation of many block- and graft-copolymers. One kind of polymer chains leaves the LC PEAT column irrespective of their M approximately in the total volume of liquid within column. Chemically different, less adsorbed polymer chains, however, elute at V_R's which correspond to their size in solution. The latter chains can be characterized applying SEC separation mechanism – independently of accompanying, more adsorbed chains, which elute under PEAT conditions and are "chromatographically invisible" (Ref. 12). In the case of oligomers, LC PEAT behavior

of macromolecular chains can be combined with adsorption based retention of their functional groups. In this way, macromolecules can be separated according to their functionality (Refs. 13,14).

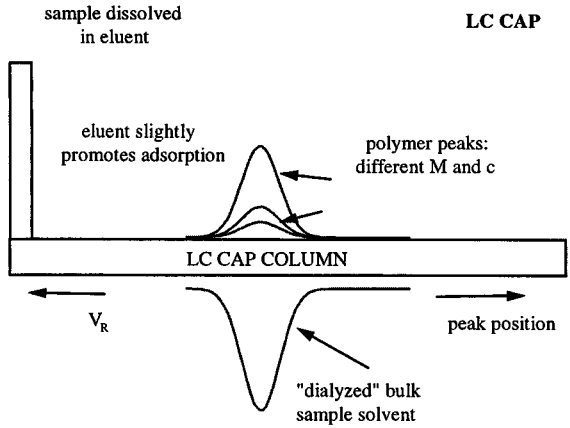
So far, four approaches to LC PEAT were elaborated. The original procedure proposed by Belenkii's group in Skt. Petersburg (Refs. 15-18) applies eluent as sample solvent. The eluent strength is controlled by mixing appropriate adsorli(s) and desorli(s). This approach is called LC under critical conditions or **liquid chromatography at the critical adsorption point** (LC CAP). LC CAP represents a very attractive method to molecular characterization of various complex polymers exhibiting multiple distributions of their properties. For example, LC CAP was utilized to separation of binary polymer blends, block- and graft- copolymers and functionalized oligomers (Refs. 19-24).

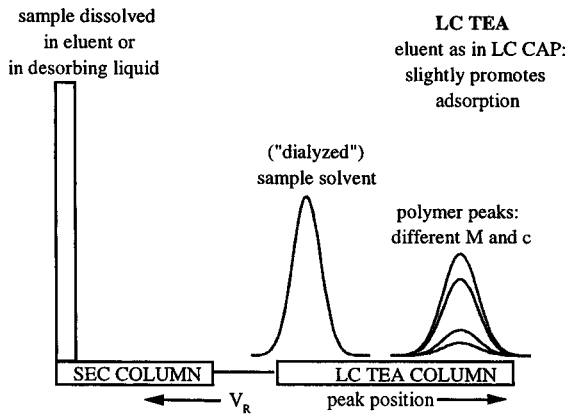
In LC CAP, macromolecules travel along the column being surrounded with their initial solvent. Due to preferential solvation of macromolecules with one component of a mixed solvent the intermolecular, bulk mixed solvent differs in composition from the intramolecular solvent which is situated in the vicinity of macromolecular chains. This may cause severe detection problems when applying nonspecific detectors like differential refractometer. The bulk solvent which was "dialyzed on molecular level" can be separated from solvated macromolecules by adding a narrow pore column before the LC CAP column. As result the detection problems are mitigated and the control of polymer retention is easier. This approach is called **LC at theta exclusion-adsorption** (LC TEA) (Ref. 25).

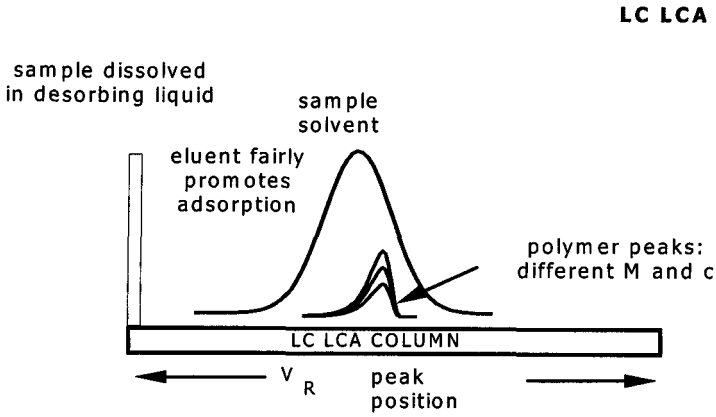
Further two LC PEAT procedures can be designated also "the barrier approaches". Here again polymer adsorption is combined with the exclusion processes. Exclusion accelerates progression of macromolecules along the column in comparison with the eluent molecules. The non-permeable adsorptive "barrier" of a low molecular substance, e.g. of an efficient adsorli, however, moves slowly because it permeates the column packing pores and therefore it retards elution of macromolecules. The adsorptive barrier can be formed either by an adsorli eluent (a continuous barrier) or by a narrow zone of an adsorli (a local barrier). In the former case, polymer is injected in a desorli solvent into an adsorli eluent. Sample solvent prevents polymer adsorption but macromolecules cannot leave and outrun the desorli zone due to the eluent barrier. This method is called **liquid chromatography under limiting conditions of adsorption** (LC LCA) (Refs. 26-29). Alternatively, eluent may be a desorli for macromolecules which are slowed down by a small volume zone of an adsorli, e.g. by the initial sample solvent (**liquid chromatography under limiting conditions of desorption** (LC LCD) (Ref. 30). Similarly to LC CAP, macromolecules elute from both LC LCA and LC LCD systems independently of their molar mass.

The LC CAP, LC TEA, LC LCA and LC LCD approaches are schematically depicted in Fig. 2. LC LCA and LC LCD were successfully applied to discrimination of binary polymer blends and it is anticipated that they will be applicable also to separation of various copolymers.

The all four LC PEAT procedures can be further combined with another LC method such as SEC. Thus we arrive at **a two dimensional polymer separation** which enables to simultaneously but independently determine two distribution functions of complex polymers, for example their molar mass distribution and chemical composition distribution. A successful two-dimensional separation was presented by Kilz et al. (Ref. 31) for selected polyesters by an on-line combination of LC CAP with SEC.







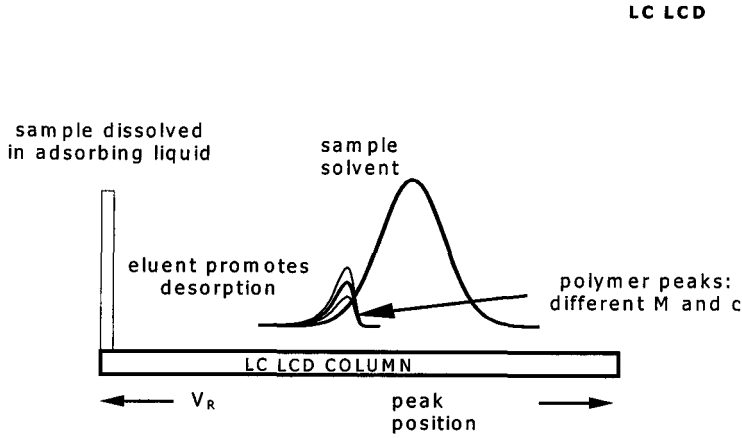


Fig. 2: Schematic representation of elution principles for liquid chromatography at the critical adsorption point (LC CAP), liquid chromatography at theta exclusion-adsorption (LC TEA), liquid chromatography under limiting conditions of adsorption (LC LCA) and liquid chromatography under limiting conditions of desorption (LC LCD). For detailed explanations see the text.

A promising method for separation of polymer blends and random copolymers is eluent gradient **liquid adsorption chromatography** (LAC) (Refs. 32-37). Polymer sample is injected into an adsorli eluent to be fully retained near the inlet of an adsorptive LC column flushed with an adsorli eluent. Next, a gradient is applied with increasing amount of desorli so that macromolecules successively start moving – in dependence on their adsorption strength i.e. on their chemical composition – while their molar mass often does not affect their retention. In LAC, the eluent gradient probably assumes the role of a continuous barrier with changing adsorbing - desorbing strength. Macromolecules which are partially or fully excluded from the packing pores tend to outrun small molecules of eluent. However, the actual progression of macromolecules is controlled with their adsorption which depends mainly on composition. Consequently, macromolecules with different compositions find their appropriate positions within eluent gradient. This position does not depend on polymer molar mass (Ref. 38).

Adsorption based polymer retention can be controlled also by temperature adjustment. This can be done especially effectively in the vicinity of the critical adsorption point. The corresponding approach was proposed by Chang et al. (Refs. 39-41) in his **temperature gradient interaction** polymer liquid **chromatography** (TGIC). TGIC allows highly selective separation of macromolecules according to their molar masses (Ref. 39) and also efficient discrimination of polymer species differing in their adsorptivity within column packing (Ref. 40).

An interesting opportunity for polymer characterization represents the complete retention of macromolecules followed with their controlled stepwise release. We speak about **a full adsorption-desorption** (FAD) approach (Refs. 42-45). All steps are done in a dynamic LC–like system (Fig. 3). The FAD column traps macromolecules of various kinds from an adsorli solvent. Next, polymer species are at a time or successively desorbed either by means of one single desorli or with a series of displacers formed with adsorli/desorli mixtures of various compositions. Various physico - chemical methods can be used to monitor concentration and properties of desorbed macromolecules. Very advantageous is the on-line coupling of the FAD column with the SEC apparatus. The latter determines amount, molar mass and molar mass distribution of macromolecules released from the FAD column. Numerous polymer blends were discriminated and their constituents independently characterized with the FAD/SEC combination including six-component polymer systems (Refs. 46,47). It is anticipated that the coupling of

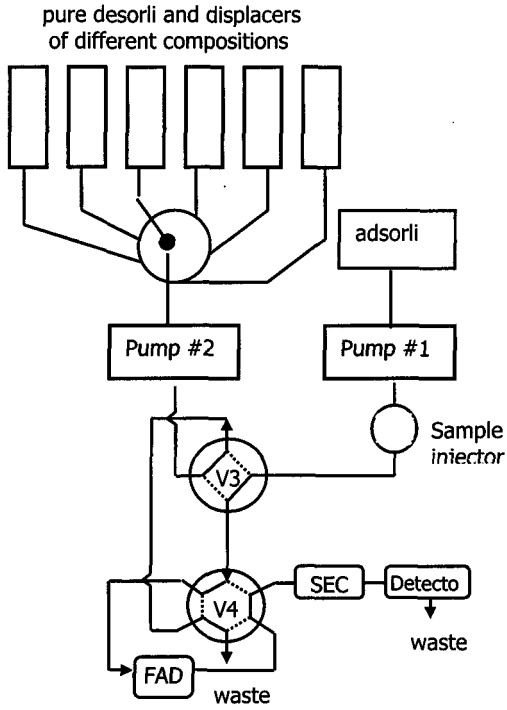


Fig. 3: Scheme of the full adsorption – desorption/SEC assembly. For detailed explanations see the text.

FAD with SEC, LC CAP, LC LCA, LC LCD or with LAC procedure will allow also an on-line separation and molecular characterization of various copolymers (refs. 48,49). An important advantage of the FAD procedure is its ability to reconcentrate diluted polymer solutions. Macromolecules are retained within FAD column practically independently of their concentration in solution. This means that large volumes of very diluted polymer solutions can be transported through FAD column to trap essentially all polymer species. The retained macromolecules are released within a narrow zone of displacer in the course of an eluent switching (Ref. 50) or a pulsed desorli application (Ref. 51). In this way, FAD approach can be utilized for effluent reconcentration in the multi-dimensional LC of macromolecules. FAD columns can serve for trapping, storing and reconcentration of fractions leaving columns in the course of multidimensional liquid chromatography of complex polymers. For example the combination LAC/FAD/SEC is expected to bring a major breakthrough into molecular characterization of statistical

copolymers. The diluted fractions from an LAC column can be retained within a set of FAD columns. These fractions would contain macromolecules with different molar masses but with similar chemical compositions. The LAC fractionation can be repeated and corresponding fractions combined. In the next step, LAC fractions are successively released into an SEC system for further characterization applying a strong desorli. The SEC separation is done isocratically with desorli eluent and this fact substantially simplifies effluent detection: even application of both refractive index devices and "absolute" detectors which monitor molar mass of macromolecules leaving SEC system is to be anticipated. Subsequently, data processing will be rather straightforward. The on-line combination of LAC/FAD/SEC brings several advantages when compared with a direct LAC/SEC coupling. The LAC overloading is avoided, similarly as tedious off-line procedure proposed for example by Mori (Ref. 52) and Schunk (Ref. 53).

The FAD procedure enables also studying various basic processes of polymer adsorption and desorption under dynamic conditions.

2. Dynamic adsorption and desorption of macromolecules on-line studied by liquid chromatography.

Several authors determined concentration and molecular characteristics of macromolecules in supernatant solutions by means of the off-line SEC in the course of the static adsorption experiments (Refs. 54,55). Generally, such measurements are very time and material consuming. Therefore, we proposed dynamic assessment of polymer adsorption using the above described FAD assembly (Fig. 3). Polymer solution in an appropriate adsorli solvent is injected into the FAD column. The nonadsorbed part of sample is directly forwarded into SEC column for characterization. After the adsorption step has been completed, a displacer (e.g. an adsorli/desorli mixture with adjusted composition) is forwarded into the FAD column to desorb either the entire retained polymer or its fraction. Alternatively, FAD column temperature can be varied. The desorbed polymer is monitored by means of an on-line SEC system which has been preequilibrated with displacer of the same composition. If necessary the displacer composition or temperature is changed in several steps until polymer desorption is completed.

In this way, we were able to dynamically study the adsorption and desorption processes in several model systems. For example, we determined amount and molar mass of macromolecules retained within FAD column packing and monitored exchanges of smaller or weakly adsorbed macromolecules for larger or strongly adsorbed species (Refs. 44,45).

Our measurements have shown that attachment and detachment of macromolecules on the nonporous solid surfaces are very fast processes. It seems that the transport phenomena within porous and not properly stirred systems, as well as the conformational rearrangements and exchanges of adsorbed

macromolecules may be responsible for slow establishment of adsorption equilibria often presented in literature (Refs. 54, 55).

Some typical examples of our dynamic measurements of adsorption and desorption processes are displayed in Figs. 4-6 (Refs. 44,45). The results illustrate and quantitatively characterize effect of polymer molar mass and chemical composition as well as adsorli nature and temperature on the adsorption extent.

Important advantages of the described dynamic FAD approach over the static adsorption-desorption measurements include a very good precision and repeatability, high versatility, experimental simplicity and speed, as well as low consumption of adsorbent, polymer sample and solvents. Future studies will show coherence of dynamic and static studies of polymer adsorption and desorption onto/from solid surfaces. In any case, the dynamic FAD approach combined with various liquid chromatographic procedures produces precise, reliable and valuable data on dynamic polymer adsorption and desorption. Thus FAD is anticipated to successfully extend the existing arsenal of methods assessing these interesting and important processes.

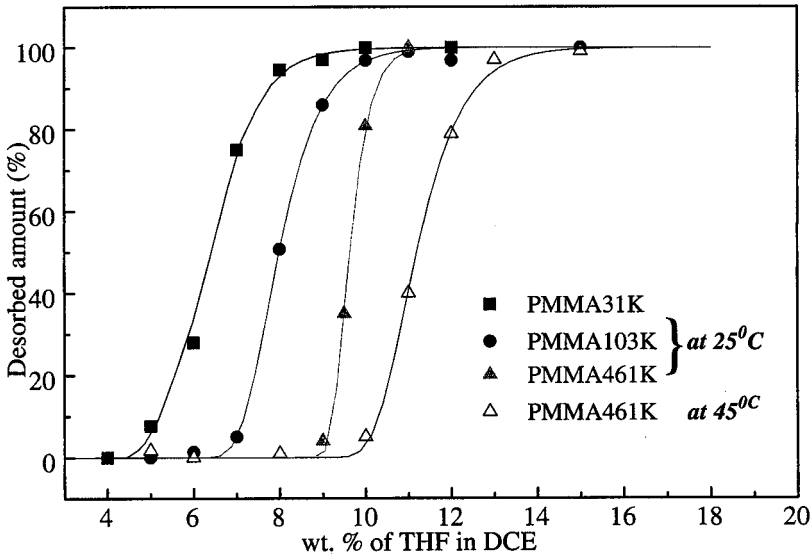


Fig. 4: Typical dynamic integral desorption isotherms for model PMMAs measured by means of the FAD arrangement depicted in Figure 3. The effect of polymer molar mass and temperature is evidenced. Adsorli was dichloroethane (DCE) and desorli was tetrahydrofuran (THF). FAD column 45x2 mm was packed with nonporous silica (Ref. 44).

	M_w	M_n	M_w/M_n
------ 3rd injection of PMMA57K	20.5	13.0	1.60
····· 4th	26.3	14.6	1.80
5th	34.6	18.6	1.86
—— 9th (ADC is saturated)	56.1	30.3	1.85

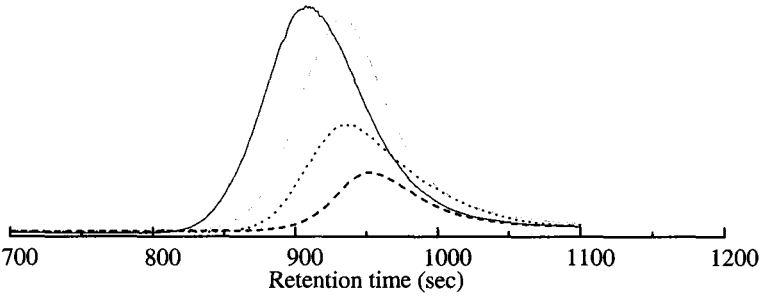


Fig. 5: *Evidence of preferential adsorption of poly(methyl methacrylate) (PMMA) on nonporous silica packed into FAD column 150x3.3 mm. FAD and SEC eluent was chloroform. Temperature was 25 °C. SEC traces and calculated averages of MM are shown. Injected amount of polymer was 0.05 mg. First two portions of polymer were fully retained. Macromolecules with lowest MM were successively displaced and eluted in the course of FAD column saturation. Eigth injection caused full saturation of FAD column and ninth injection produced an SEC trace which was practically identical with the initial one that is without presence of the FAD column. Molar masses of non-retained species were calculated from the SEC traces using calibration with narrow PMMA (Ref. 44).*

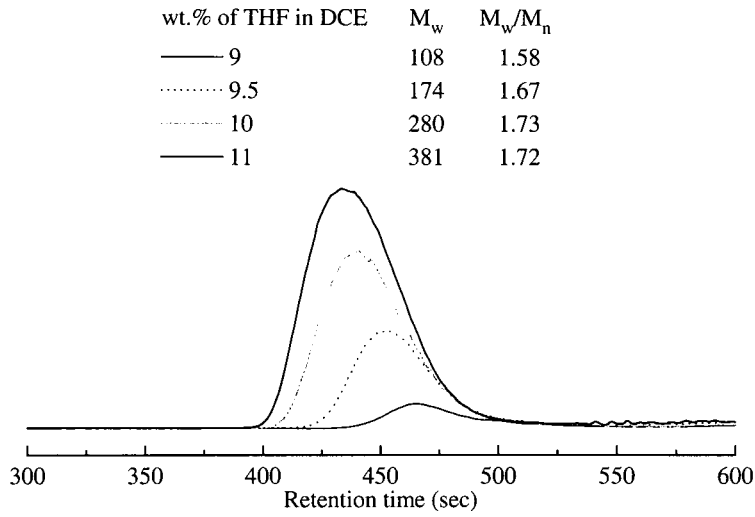


Fig. 6: Preferential desorption of PMMA's with lower MM evidenced by SEC. Experimental conditions as in Figure 5. Molar masses of desorbed species were calculated from the SEC traces. PMMA was fully desorbed with displacer containing 11% of THF and the corresponding SEC trace is identical to that obtained without presence of the FAD column (Ref. 44).

ACKNOWLEDGEMENT

This work was supported with the Slovak Grant Agency VEGA, project No. 2/4012/97.

The author thanks to Mrs. J. Tarbajovska for technical assistance and to Dr. S.H. Nguyen for cooperation on the full adsorption-desorption procedure.

REFERENCES

(1) D. Berek, D. Bakoš, *J. Chromatogr.* 91, 237 (1974)

(2) D. Berek, D. Bakoš, T. Bleha, L. Šoltés, *Makromol. Chem.* 176, 391 (1975)

(3) Z. Grubisic, P. Rempp, H. Benoit, *J. Polym. Sci. B* 5, 753 (1967)

(4) D. Bakoš, T. Bleha, A. Ozimá, D. Berek, *J. Appl. Polym. Sci.* 23, 2233 (1979)

(5) G. Glöckner, *Gradient HPLC of Copolymers and Chromatographic Crossfractionation,* Springer, Berlin, 1991

(6) J.V. Dawkins, M. Hemming, *Makromol. Chem.* 176, 1796 (1975)

(7) T. Spychaj, D. Berek, *Polymer* 20, 1108 (1979)

(8) D. Berek, *Macromol. Symp.* 110, 33 (1996)

(9) A.M. Skvortsov, A.A. Gorbunov, D. Berek, B. Trathnigg, *Polymer* 39, 423 (1998)

(10) A.A. Gorbunov, A.M. Skvortsov, *Adv. Colloid Interface Sci.* 62, 31 (1995)

(11) D. Berek, *Interactive Properties of Polystyrene/Divinylbenzene and Divinylbenzene – Based Commercial Size Exclusion Chromatography Column,* in: C.-s. Wu, Ed., Column Handbook for Size Exclusion Chromatography, Academic Press, N. York, 1999

(12) A.M. Skvortsov, A.A. Gorbunov, *Vysokomol. Sojedin. (Moscow)* 21, 339 (1979)

(13) S.G. Entelis, V.V. Evreinov, A.V. Gorshkov, *Adv. Polym. Sci.* 76, 129 (1986)

(14) G. Schulz, H. Much, H. Krüger, G. Wehrsted, *J. Liq. Chromatogr.* 13, 1745 (1990)

(15) B.G. Belenkii, E.S. Gankina, M.B. Tennikov, L.Z. Vilenchik, *Dokl. Akad. Nauk SSSR (Moscow)* 231, 1147 (1976)

(16) B.G. Belenkii, M.D. Valchikhina, I.A. Vakhtina, E.S. Gankina, O.G. Tarakanov, *J. Chromatogr.* 129, 115 (1976)

(17) M.B. Tennikov, P.P. Nefedov, M.A. Lazareva, S.Yu. Frenkel, *Vysokomol. Sojed. (Moscow)* A 19, 657 (1977)

(18) B.G. Belenkii, E.S. Gankina, M.B. Tennikov, L.Z. Vilenchik, *J. Chromatogr.* 147, 99 (1978)

(19) H. Pasch, B. Trathnigg, *HPLC of Polymers,* Springer Berlin, 1998

(20) E. Gankina, B. Belenkii, I. Malakhova, E. Melenevskaya, V. Zgonnik, *Chromatographia* 4, 199 (1991)

(21) T.M. Zimina, J.J. Kever, E.Y. Melenevskaya, A.F. Fell, *J. Chromatogr.* 593, 233 (1992)

(22) D. Braun, E. Esser, H. Pasch, *Int. J. Polym. Anal. Charact.* 4, 501 (1998)

(23) R. Murgašová, I. Capek, E. Lathová, D. Berek, Š. Florián, *Eur. Polym. J.* 34, 659 (1998)

(24) H. Pasch, H. Much, G. Schulz, A.V. Gorshkov, *LC GC International* 5, 38 (1992)

(25) D. Berek, *Liquid chromatography of macromolecules at the point of exclusion – adsorption transition,* IN: Proc. 7th EPF Symp. Polym. Mat., Szczecin 1998, p. 23

(26) D. Berek, *Coupled Procedures in Liquid Chromatography of Macromolecules,* IN: Proc. Simposio Latino-American des Polímeros, SLAP 96, Mar del Plata 1996, p. 147

(27) D. Berek, *Macromol. Symp.* (Symposium Kusatsu, 1997), in press

(28) D. Berek, D. Hunkeler, *J. Liq. Chrom. Rel. Technol.,* in press

(29) A. Bartkowiak, D. Hunkeler, D. Berek, T. Spychaj, *J. Appl. Polym. Sci.* 69, 2549 (1998)

(30) D. Berek, *Macromolecules* 31, 8517 (1998)

(31) P. Kilz, R.-P. Krüger, H. Much, G. Schulz, *ACS Adv. Chem. Ser.* 247, 223 (1995)

(32) S. Teramachi, A. Hasegawa, Y. Shima, M. Akatsuka, M. Nakayama, *Macromolecules* 12, 992 (1979)

(33) S. Teramachi, *Macromol. Symp.* 110, 217 (1996)

(34) S. Mori, *Macromol. Symp.* 110, 87 (1996)

(35) H. Sato, K. Ogino, T. Darwint, I. Kiyokawa, *Macromol. Symp.* 110, 177 (1996)

(36) S. Mori, *Trends Polym. Sci.* 2, 208 (1994)

(37) H. Sato, K. Ogino, S. Marno, M. Sasaki, *J. Polym. Sci., B, Polym. Phys. Ed.* 29, 1073 (1991)

(38) D. Berek, *Macromolecules* 32, 3671 (1999)

(39) H.C. Lee, T. Chang, *Polymer* 37, 5747 (1996)

(40) H.C. Lee, T.H. Chang, *Macromolecules* 29, 7294 (1996)

(41) W. Lee, H.C. Lee, T.H. Chang, *Macromolecules* 31, 344 (1998)

(42) M. Jančo, D. Berek, T. Prudskova, *Polymer* 36, 3295 (1995)

(43) M. Jančo, T. Prudskova, D. Berek, *Int. J. Polym. Anal. Charact.* 3, 319 (1997)

(44) D. Berek, S.H. Nguyen, *Macromolecules* 31, 8243 (1998)

(45) S.H. Nguyen, D. Berek, *Colloids Surf. A: Physicochem. Eng. Aspects,* in press

(46) S.H. Nguyen, D. Berek, *Chromatographia* 48, 65 (1998)

(47) S.H. Nguyen, D. Berek, *Colloid. Polym. Sci.* 277, 318 (1999)

(48) D. Berek, S.H. Nguyen, J. Pavlinec, *J. Appl. Polym. Sci.,* submitted

(49) D. Berek, S.H. Nguyen, G. Hild, *Eur. Polym. J.,* accepted

(50) S.H. Nguyen, D. Berek, O. Chaintore, *Polymer* 39, 5127 (1998)

(51) S.H. Nguyen, D. Berek, *J. Polym. Sci. A, Polym. Chem.* 37, 267 (1999)

(52) S. Mori, *Anal. Chem.*, 58, 303 (1986)

(53) T.C. Schunk, *J. Chromatogr. A,* 661, 215 (1994)

(54) G.J.Fleer, M.A. Cohen Stuart, J.M.H.M. Scheutjens, T. Cosgrove, *Polymers at Interfaces,* Chapman and Hall, London 1993

(55) M. Kawaguchi, *Colloid Interface Sci.* 32, 1 (1990)