PII: S0032-3861(97)10225-7

# The effects of substrate interactions in the liquid chromatography of polymers

## Marios K. Kosmas*, Efthymios P. Bokaris and Elena G. Georgaka

*Chemistry Department, University of Ioannina, Ioannina, Greece*

We present a study of the elution behaviour of polymers through a liquid chromatographic column and its dependence on the interactions with the substrate. An earlier method based on statistical thermodynamics is employed to describe chains in the presence of interacting cubic pores in terms of the probability to find the chains in and far from the pores. The partition coefficient of the chains between the mobile and the stationary phases, as a function of the polymer molecular weight, the average size of the pores and a polymer–substrate interaction parameter, is determined and compared with the results of supportive experiments. Two series of chromatographic experiments with two columns with substrates different in nature but with the same pore size, indisputably shows the importance of the polymer–surface interactions. Due to the change of the nature of the substrate an alternation of the mode of elution of the same poly(methyl methacrylates) in the same solvent is observed. © 1998 Published by Elsevier Science Ltd. All rights reserved.

(Keywords: polymer; liquid; chromatography)

## Introduction

Liquid chromatography of polymer solutions through porous substrates is one of the most important current techniques for the identification and separation of polymers, not only according to their nature but also according to their molecular weight[1]. A column packed with a porous material is used and polymers in solution pass through the porous matrix and elute at the other end of the column. Strictly speaking, both static and dynamic effects govern the partition of the chains between the mobile and stationary phases and the retention process[2]. However, a complete study of the phenomenon presupposes a connection of the macroscopic with the microscopic parameters and has to start from the understanding of the static effects. Besides studies of separations and the successful comparison of the experimental and theoretical results indicate that the static effects play the dominant role. Two partners are involved in the elution process, the polymeric chains in the presence of the solvent and the substrate. As far as polymers are concerned, the hydrodynamic volume of the chains which is expressed by the size $R$ of the coils in the solution under flow, has already been used as a calibration parameter, and the elution behaviour of linear chains, and polymers of other architectures, has been studied[3]. The partition of the polymeric chains between the solution phase and the space inside the pores makes the morphology of the second partner another significant factor for the determination of the elution behaviour of chains. A basic characteristic of this morphology is the average size $D$ of the pores of the substrate and it is of importance whether the mean size $R$ of the coils is larger or smaller than $D$. The size $D$ competes with $R$ in determining the probability of finding the coils inside the pores and the comparison of the two sizes, expressed by the ratio $D/R$, has been found to be directly related to the separating power of the chromatographic column[4]. The importance of the size of the pores has also been seen in previous studies of chains in different restricted

geometries of slabs, cylinders and spheres by means of statistical thermodynamics[5]. These studies of the effect of the geometry of the pores mainly describe the entropic contribution to the macroscopic behaviour, without considering the contribution from the interactions with the substrate. The combined effects from the restriction of the pores plus the interactions with the substrate remain to be investigated.

The average interactions between the substrate and the polymers depending on the medium solvent is a third equally important factor which affects the elution behaviour of chains. Three different modes of elution which lead to different dependences on molecular weights have been detected by changing the solvent and the mean interactions between the surface and the monomers. They have been explained by means of the existence of the critical conditions of adsorption corresponding to a region of negligible mean interactions between the polymer and the substrate[6]. The interactions between the polymeric units and the surface can on average be repulsive or attractive leading to desorbed or adsorbed states of the chains respectively. An interaction parameter

$$u_a = \int dz\{1 - \exp[(-V_a(z))/KT]\}$$

has been defined that describes the intensity of these interactions in a large region of molecular parameters[7]. It depends on the temperature $T$ and the average molecular potential $V_a(z)$ between the units of the chains and the substrate in the presence of the solvent, and obtains positive or negative values for repulsions or attractions, respectively. To a first approximation $u_a$ is equal to an interaction parameter $X$ between the units of the chain and the substrate, it is related to $\Delta H$ of adsorption and can be determined from experiments of adsorption. We have used this parameter in order to describe chains in the vicinity of an interacting surface and it was shown, by means of the partition function $C_z$ of the system, that varying $u_a$ from negative to positive values a nice description of the macroscopic behaviour of the chains, going from an adsorbed to a desorbed state, can

---

* To whom correspondence should be addressed

be provided. The average number of adsorbed units

$$v = -\frac{\partial \ln C_z}{\partial u_a}$$

for example, changes from a large value in the adsorbed state to a vanishing one in the desorbed state, while the average size of the coils changes in this transition from that of a two-dimensional adsorbed chain to that of a three-dimensional desorbed one[7].

We present in this paper an effort to extend the study of chains interacting with a surface made by means of a microscopic model and methods of statistical thermodynamics, to the description of the elution behaviour of polymers in the case of liquid chromatography. In the latter the interacting substrate has pores where the chains can enter and interact. The problem to be solved includes chains in spaces of restricted geometry which also interact with the surface of these spaces, and aims to the simultaneous description of both effects. The probability to find the chains inside the pores depends on these interactions and determines the partition coefficient and the elution behaviour. The results of a supportive experiment are also presented and show that with a change of the nature of the substrate, while keeping all the rest of the factors the same, the $u_a$ parameter changes leading not only to a shift of the results but also to the change of the mode of elution. Comparison and fitting of the theoretical results with experiments determines the values of the $u_a$ parameter and opens a possibility of a successful study of polymers of other kinds and architectures. An interpretation of their chromatographic behaviour in terms of their microscopic molecular characteristics will be possible.

*The model*

The configurational partition function of a flexible polymer chain in the presence of an impenetrable interacting surface, represented by the *XY* plane, is proportional to the probability of finding the chain in the volume under study. It has been calculated before and is given in[7]

$$C_z = V\{1 - kF(U)\},$$

$$F(U) = \left[\frac{2}{\pi^{1/2}} - \frac{1}{U} + \frac{1}{U}\exp(U^2)\,\mathrm{erfc}(U)\right],$$

$$k = \frac{2lN^{1/2}}{D6^{1/2}}, \quad U = \frac{u_a(6N)^{1/2}}{2}, \tag{1}$$

where $\mu_0$ and $l$ are the molecular weight and the length of each segment while $N = M/\mu_0$ represents the number of the segments of the chains and is proportional to the molecular weight $M$ of the polymer. $D$ is the maximum distance from the interacting surface being equal to the width of the available volume $V$. Equation (1) corresponds to the known reflecting boundary conditions with a maximum of the zeroth order probability to find polymeric units at the surface, meaning that in the absence of the $u_a$ interactions a lot of monomers are permitted to be found at the substrate[7]. This, together with the variation of the value of $u_a$, can produce all interesting experimental situations from a full depletion of the chains for $u_a \rightarrow \infty$ to a complete adsorption for $u_a \rightarrow -\infty$.

The substrate of a chromatographic column has a large number of pores with a variety of sizes and shapes. A microscopic model description beyond the average pore size $D$ requires also a proper simulation of the shapes of the
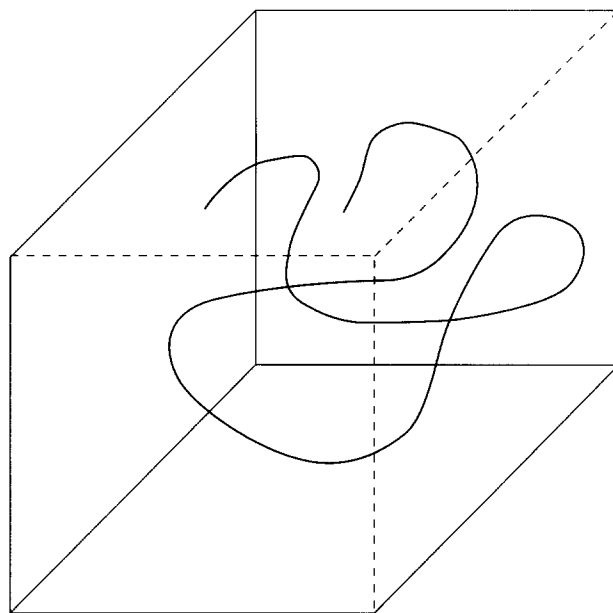


**Figure 1** A cubic pore of size *D* and an interacting interior surface

pores. An analytic solution in the frame of statistica thermodynamics could then lead to a complete compariso of theoretical and experimental results. Because of th complexity of the structure of the real pores a detaile description of the system is impossible. In order t overcome this problem we choose in this work representa tive cubic pores shown in *Figure 1*, for which an exac analytical solution can be given. The edge of the cubes i equal to $D$, and represents the average size of the pores. Th interior solid angle of the cube consists of thre impenetrable interacting planes, while the remaining thre planes are penetrable so that the chains can enter the cub and interact with its internal surface. The solution to thi problem is an extension of the one-dimensional cas described by $C_z$, to the three-dimensional space with thre interacting planes. The problem is separable along the thre equivalent perpendicular dimensions and the configuration a partition function $C = C_x C_y C_z$ is equal to the third power o $C_z$. The partition coefficient between the stationary an mobile phase expresses the ratio of the probabilities to fin the chains in the spaces of the two phases and is equal to th ratio

$$K = C_s/C_m = (\mu_0^N/C_m)K_o, \quad K_o = \{1 - kF(U)\}^3, \tag{2}$$

of the partition functions $C_s = C$ and $C_m$ in these tw spaces[8]. Though this attempt to describe the large variet of pores of a real system with solid angles cannot be exact, i yields a first simple approximation which connects elutio volumes with the interesting parameters of the molecula weight $M = \mu_0 N$ of the polymers, the interaction paramete $u_a$ between the polymer units and the substrate, and the forn and the size $D$ of the pores. The benefit of this analytica solution based on the proper description of a molecula model is its capability of application in a vast range o values of molecular parameters. The size of the pores o the substrates can be determined by gas adsorption measure ments[9] and in the comparison with the performe experiments their values are taken as those given by th manufacturers. The segment length $l$ of PMMAs can als be determined by experimental methods, like viscosimetry and the manner of its determination is discusse elsewhere[10]. The molecular weights of polymers ar

determined by g.p.c. using a calibration curve found by means of standard samples of polymers of known molecular weights and narrow distributions. Proper values of the remaining $u_a$ parameter are sought via the comparison with the experiments.

Before the comparison and fitting with the experimental results and the determination of the interaction parameter $u_a$, an analysis of equation (2) is in order. We can show that the three different modes of elution in liquid chromatography can be recovered from equation (2) by just varying the interaction parameter $u_a$. Opposite dependences of $K$ on $N$ for positive and negative $u_a$ values can be produced and all three known experimental behaviours can be explained. In the simplest case of a $\theta$ solvent, for example, where $C_m = \mu_0^N$, $K$ becomes equal to $K_0$ of equation (2). For positive $u_a$ and net repulsions between the polymeric units and the substrate, the function $F(U)$ is positive, the exclusion mode is dominant and a decrease of the elution volume and the partition coefficient with $N$ is taken from equation (2). We thus recover the behaviour of a large category of systems of polymers solvents and substrates, such as that of silica, where the chains elute with the exclusion mode and a reduction of $K$ on $N$ is observed[11]. For $u_a$ negative and net attractions, $F(U)$ becomes negative, the adsorption mode dominates and an opposite behaviour of the elution volume on the molecular weight is produced. The partition coefficient $K$ increases with the number $N$ of the segments and the molecular weight describing the adsorption mode observed also in many systems[12]. For zero intensity of interactions $u_a = 0$, $F(U) = 0$ and the critical region of no dependence on the molecular weight is taken[6]. The above analysis of the $\theta$ state concerns cases without excluded volume and can represent ideal chains or chains in denser states in the presence of many other chains. Though the chains in the adsorbed states inside the pores can be considered, to a large extent, to be in a dense state without excluded volume, in the solution of a good solvent outside the pores, they are not ideal and expand. The partition function of the ideal chain $C_m = \mu_0^N$ in the mobile phase must then be replaced with $C_m = \mu^N N^\alpha$ where $\mu$ is a good solvent chemical potential and $\alpha$ is a characteristic critical exponent. If $u$ is the excluded volume parameter representing the intensity of interactions between the monomers, the partition function has been found to behave like $C_m = \mu_0^N N^{2u} \exp(-2uN)$ at the area of the fixed point of the expanded state[13]. This modifies $K$ of equation (2) which is divided by $C_m$, corresponding in multiplying with a factor of $N^{-\alpha} \exp(bN)$ which must be taken into account when the solvent used is a good solvent.

## Experimental

*Instrumentation.* The g.p.c. results were obtained by using a Waters Model 510 pump with a Rheodyne 7125 sample injector (20 $\mu$l injection volume) and an LKB differential refractometer Model 2142 as a detector. Two columns are used: (A) a commercial Supelcosil LC-301 column (25 cm $\times$ 6.2 mm ID, pore size 300 Å, particle diameter 5 $\mu$m), with the packing material based on silica with a coverage of trimethylsilyl groups; (B) the second column was packed by a slurry procedure[8], using 2 g of silicagel (purchased from MZ Analysentechnik, pore size 300 Å, particle diameter 5 $\mu$m) in an h.p.l.c. stainless steel tube (25 cm $\times$ 4.2 mm ID). The value of the average diameter of the pores used, common to both columns, is $D = 300$ Å and is that given by the manufacturers.

The eluents used were cyclohexane (Merk), h.p.l.c. purity grade and methyl ethyl ketone (MEK) (Lab-Scan) analytical grade. MEK and cyclohexane were purified by filtration through 0.2 $\mu$m Nylon 66 membranes (Supelco). A mixture of MEK 70% and cyclohexane 30% was selected as elution solvent for both series of experiments in order to achieve the adsorption mode for the PMMA samples in case B.

*Preparation of PMMA samples.* Six samples of poly-(methyl methacrylate) (PMMA) were used with molecular weights in the range from 5500 to 90 000, the first five were prepared by anionic polymerisation while the sixth sample with the largest molecular weight was purchased from Rohm GmBH chemische Fabrik. The preparation was done by polymerising MMA at a low temperature ($-70°C$) using *sec*-BuLi/1,1-diphenylethylene as initiator. The 1,1-diphenylethylene was introduced prior to the addition of MMA in order to avoid the side reactions by decreasing the nucleophilicity of the active sides. The molecular weight characterisation of the PMMA samples was made by using a polystyrene standard (Supelco) calibration curve, THF as an eluent and Supelco LC-1 and LC-301 columns in series. The weight-average molecular weights $\bar{M}_n$ and their molecular weight distribution ratio $DP = \bar{M}_w/\bar{M}_n$ were calculated as: $\bar{M}_n = 5500$ (DP = 1.13), 6500 (1.10), 7500 (1.04), 21 000 (1.05), 30 000 (1.06) and 90 000 (1.15). As expected for anionic polymerisation, the distributions of the molecular masses of the samples are very narrow. The concentrations of the injected polymer solutions were 12.7 mg ml$^{-1}$ and the g.p.c. measurements were conducted at 25°C in a flow rate of 1 ml min$^{-1}$.

*Calculations.* The elution volumes $V$ of the PMMA samples were calculated from the initial points of injection to the middle of the distance between the intercepts on the baseline of the two tangents at the two points of inflection of the chromatogram curves[14]. Each measurement was performed at least twice and no shifts between the two values were observed. The values of the partition coefficient $K = (V - V_m)/V_s$ were found from the elution volumes $V$ and the volumes of the mobile and stationary phases $V_m$ and $V_s$, respectively. The volumes $V_m$ and $V_s$ were experimentally determined by using a high-molecular weight polystyrene standard (Supelco) with $M = 900 000$ for $V_m$ and toluene for $V_m + V_s$. They are equal to $V_m = 4.10$ ml, $V_s = 1.75$ ml for column A and $V_m = 2.80$ ml, $V_s = 1.15$ ml for column B, and the values of $K = (V - V_m)/V_s$ are given in *Table 1*. The length of the statistical segment of PMMA is taken equal to $l = 13.3$ Å, a value determined by means of viscosimetry, as is explained and used in Ref. 10. This includes $13.3/2.5 = 5.3$ monomers of MMA and has a molecular weight $\mu_0 = 5.3 \times 100 = 530$. The numbers

**Table 1** The partition coefficients $K$ of poly(methyl methacrylate) of various molecular weights $N = \bar{M}_n/\mu_0$ in the two columns of different substrates of silica covered with trimethylsilyl groups (A) and pure silica (B), respectively

| $N$ | $K$ | |
| --- | --- | --- |
| | Column A | Column B |
| 10.377 | 0.886 | 1.217 |
| 12.264 | 0.857 | 1.261 |
| 14.151 | 0.829 | 1.304 |
| 39.623 | 0.600 | 2.522 |
| 56.604 | 0.514 | 3.565 |
| 169.81 | 0.143 | |
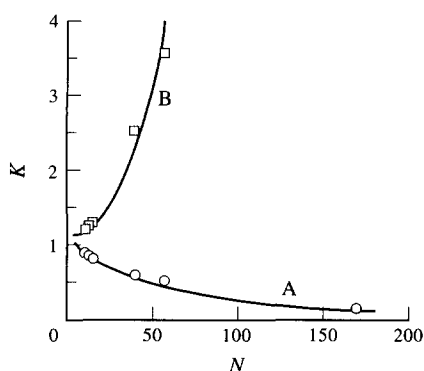
**Figure 2** The two theoretical graphs based on equation (2) are superimposed on the experimental data in the two columns of substrates of different nature. Two different modes of elution are followed: (A) the exclusion mode (O) with $u_a = 0.100$ and (B) the adsorption mode (□) with $u_a = -0.135$

$N = \bar{M}_n/\mu_o$ of the segments of the chains are taken from the ratio of the molecular weights $\bar{M}_n$ and the molecular weight $\mu_o$ of the segment, and are also given in *Table 1*.

## Results and discussion

The aim of the supportive experiments is to show the importance of the interactions of the polymers with the substrate and the influence they can have on the interaction parameter $u_a$. In the two series of experiments performed the same polymers and the same solvent are used, and the two columns, A and B, had two substrates different in nature but of the same pore size. We managed, in this way, to demonstrate that by changing only the nature of the substrate and keeping all the rest of the parameters the same, significant changes can be brought on the $u_a$ parameter and the elution behaviour of chains. We did not obtain only a shift of the results but a change of the mode of elution as well. The two different behaviours in the cases of the two different substrates are depicted in *Figure 2*. Two theoretical graphs based on equation (2) are sought for the best fitting which must have a common good solvent prefunction of the structure $cN^{-\alpha}\exp(bN)$ as we have explained above. Plotting $K$ versus $N$ for various values of the parameters $c$, $\alpha$, $b$ and $u_a$, and superimposing on the two sets of experimental points a nice fitting is achieved with the forms $K = 1.2N^{-0.077}\exp(-5 \times 10^{-3}N)K_o$ with $u_a = 0.100$ for column A and $K = 1.2N^{-0.077}\exp(-5 \times 10^{-3}N)K_o$ with $u_a = -0.135$ for column B. The solvent used is a good solvent for PMMA, and this can be seen from the exponent $\alpha$ which takes a positive value. On the other hand the $u_a$ parameter obtains both a positive and a negative value. For the case of column A the interactions between the surface and the polymers are repulsive, $u_a$ is positive, and we are in an exclusion mode. In the case of column B the interactions between the substrate and the polymers become attractive, the $u_a$ parameter reaches a negative value and the mode of elution changes into an one of adsorption.

Though in the present work we focus on the polymer rather than on the porous matrix, and provide an analyti dependence of the macroscopic chromatographic behaviou of polymers on their molecular weights and the interaction with the substrate, the effort touches the characterisation c the matrix by means of polymers as well. It describes simple way of studying both the size and the shape of th pores but also the intensity of the average interaction between the polymeric units and the substrate.

## References

1. Basedow, A. M., Ebert, K. H., Ederer, H. J. and Fosshag, E.J *Chromatography*, 1980, **192**, 259; Lesec, J., *J. Liquid Chromatogr* 1985, **8**, 875; Chance, R. R., Baniukiewicz, S. P., Mintz, D., Ve Strate, G. and Hadjichristidis, N., *Int. J. Polymer Analysis c Characterization*, 1995, **1**, 3.
2. DiMarzio, E. A. and Guttman, C. M., *Macromolecules*, 1970, : 131; *J. Phys. Chem.*, 1989, **93**, 7004.
3. Benoit, H., Grubisic, Z., Rempp, P., Decker, D. and Zilliox, J. G *J. Chim. Phys.*, 1966, **63**, 1507; Grubisic, Z., Rempp, P. and Benoi H., *J. Polym. Sci. B*, 1967, **5**, 753; Potschka, M., *Anal. Biochem* 1987, **162**, 47; Tennikov, M. B., Gorbunov, A. A. and Skvortsov, A M., *J. Chromatogr.*, 1990, **509**, 219; Sanayei, R. A., Pang, S. an Rudin, A., *Polymer*, 1993, **34** 2320.
4. Gorbunov, A. A. and Skvortsov, A. M., *Polymer Sci. USSR*, 199( **32**, 567; *Polymer*, 1991, **32**, 3001.
5. Casassa, E. F., *J. Polym. Sci. B*, 1967, **5**, 773; Casassa, E. F. an Tagami, Y., *Macromolecules*, 1969, **2**, 14; Kosmas, M., Ten Brinke G. and Hadziioannou, G., *J. Phys. II France*, 1994, **4**, 501.
6. Gorshkov, A. V., Much, H., Becker, H., Pasch, H., Evreinov, V. V and Entelis, S. G., *J. Chromatogr.*, 1990, **523**, 91; Zimina, T. M Kever, J. J., Melenevskaya, E. Yu. and Fell, A. F., *J. Chromatogr.* 1992, **593**, 233; Pasch, H., *Polymer*, 1993, **34**, 4095; Pasch, H. Brinkmann, C. and Gallot, Y., *Polymer*, 1993, **34**, 4100; Pasch H., Rode, K. and Chaumien N., *Polymer*, 1996, **37**, 4079.
7. Kosmas, M. K., *Makromol. Chem. Rap. Commun.*, 1981, **2**, 563 ,Stratouras G. and Kosmas, M., *J. Chem. Phys.*, 1991, **95**, 4656 Stratouras, G. K. and Kosmas, M.K., *J. Chem. Phys.*, 1995, **102** 2239.
8. Giddings, J.C., Kucera, E., Russell, C.P. and Myers, M.N., *J. Phys Chem.*, 1968, **72**, 4397.
9. Unger, K. K., Rouquerol, J. and Avnir, D., *Pure Appl. Chem.*, 1994 **66**, 1739; Studies in surface science and catalysis 87, in *Character ization of Porous Solids III*, ed. J. Rouquerol, R. Rodriquez, K.S.W Sing and K.K. Unger. Elsevier, 1994.
10. Dondos, A. and Casassa, E. F., *Polymer*, 1993, **34**, 3747; Dondos A., *Polymer*, 1996, **37**, 3093.
11. *Adv. Poly. Chem.*, 1970, **24**, 343; Regnier, F. E. et al., *Analytica Biochem.*, 1980, **103**, 1; Snyder et al., *J. Chromatogr.*, 1983, **255** 163; Pasch, H. and Rode, K., *Macromol. Chem. Phys.*, 1996, **197** 2691.
12. Engelhardt, H., Czok, M., Schultz, R. and Schweinheim, E. *J. Chromatogr.*, 1988, **458**, 79; Pasch, H., Brinkmann, Much, H and Just, U., *J. Chromatogr.*, 1992, **623**, 315.
13. Kosmas, M.K., *J. Phys. A: Math. Gen.*, 1981, **14**, 931.
14. Engelhardt, H., *High Performance Liquid Chromatography* Springer-Verlag, Berlin, 1979.