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Achievements and uses of critical conditions in the chromatography of polymers

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SUMMARY

A method for the analysis and separation of macromolecules is proposed, based on the effect of the chromatographic “invisibility” of some chain fragments, namely blocks of a certain type in block copolymers, grafted chains in graft copolymers and linear elements of macromolecules with rings. When certain critical conditions for such fragments of polymer molecules are created neither the length nor the molecules weight distribution of these molecules influences the retention volume, and this effect is treated as a chromatographic “invisibility” of the elements involved.

Results of the modern theory of chromatography and the direct simulation of the equilibrium behaviour of macromolecules in a chromatographic column have been taken into account and conclusion is drawn that this method of “invisibles” provides an efficient separation of macrocycles and linear polymers and the separation of macromolecules according to the number of functional groups. The method of “invisibles” also makes it possible to separate two-block copolymers according to the size of the “visible” block only, and to separate grafted polymers according to the backbone length. The scope of the application of the method to chromatographic analyses of copolymers is discussed.

INTRODUCTION

The fundamental existence of so-called critical conditions is well known in polymer chromatography. Under these conditions, the entropy losses of a macromolecule within a pore and the enthalpic effects due to adsorption of the chain units on the pore walls are exactly compensated. Under the critical conditions, when passing from the solvent volume into the pores, the free energy change for a macromolecule is found to be zero (the distribution coefficient $K = 1$). The first experimental work was published by Tennikov and co-workers^{1,2} and subsequently the results were confirmed for different polymers and various adsorbents^{3,4} and a theoretical foundation was elucidated⁵.

We use the expression that macromolecules become "invisible", in order to emphasize that, under critical conditions, the retention volumes of homopolymer macromolecules of any molecular weight (MW) become equal to the retention volume of solvent molecules, in such a way as to make them indistinguishable from each other and from the solvent. The idea of chromatographic "invisibility" has been used⁶ with the aim of building up a theory of a two-block copolymer separation according to the lengths of one "visible" block only, provided that the copolymer was exposed under the conditions critical for the other "invisible" block. The method of "invisibles" is, in a way, analogous to the well known procedure⁷ of copolymer analysis by means of light scattering, where a solvent is chosen that has a refractive index identical with that of one of the copolymer components. This results in the optical invisibility of this component and makes the measurement of the radius of gyration of the visible component possible. In previous work⁶ only one case was considered, namely that of a long two-block copolymer with block sizes greater than the pore width; this results in the application limits of the method of "invisibles" in chromatographic analysis being unclear.

Critical conditions can be created experimentally by means of particular choices of mixed eluent, temperature or pH (when aqueous eluents are used) variations, etc. Critical conditions for many polymer-adsorbent-solvent systems have been found experimentally⁸. All operations under conditions of critical chromatography are carried out using standard chromatographic equipment.

The aim of this work was to clarify whether a polymer chain (or its fragment) can be treated as "invisible" when the chromatography is carried out under critical conditions. Further, we shall show that, for this reason, it is necessary for the "invisible" part of the chain to have a free end, as in the cases of end blocks in three-block copolymers, side grafts to the backbone in graft copolymers, etc. However, in general, neither the central block, the backbone of a graft copolymer nor multi-block (both regular and statistical) copolymer fragments can be treated as "invisible" species.

LINEAR BLOCK COPOLYMERS

The distribution coefficient, K , is equal to the ratio of the statistical sum of the chain within the pore to that in the solvent volume. Let one chain end be fixed at a distance x from a certain pore wall and $Z(x)$ this chain statistical sum. For a slit-like pore of width D , we have

$$K = D^{-1} \int_0^D Z(x) dx \quad (1)$$

The function $Z(x)$ can be represented by

$$Z(x) = \int_0^D Z(x, x') dx' \quad (2)$$

where $Z(x, x')$ is the statistical sum of the chain with the two ends fixed at x and x' . The function $Z(x, x')$ for the Gaussian flexible chain under critical conditions is equal to⁹

$$Z_{cr}(x, x') = D^{-1} \left\{ 1 + 2 \sum_{m=1}^{\infty} \cos\left(\frac{\pi mx}{D}\right) \cos\left(\frac{\pi mx'}{D}\right) \exp\left[-\left(\frac{\pi mR}{D}\right)^2\right] \right\} \quad (3)$$

where $R = (Nb^2/6)^{1/2}$, the average radius of gyration of the chain consisting of N units each of length b .

Under the critical conditions for all values of x with any R and D :

$$Z_{cr}(x) = 1 \quad (4)$$

This becomes evident after the substitution of eqn. 3 into eqns. 2 and 1. Consequently,

$$K_{cr} = 1 \quad (5)$$

Now, let us consider a two-block copolymer, AB, and introduce statistical sums $Z_A(x)$ and $Z_B(x)$ for block A and B, respectively. Then the following is valid for the copolymer distribution coefficient, K_{AB} :

$$K_{AB} = D^{-1} \int_0^D Z_A(x)Z_B(x) dx \quad (6)$$

If the critical conditions are attained for block B it follows, from eqn. 4, that $Z_B(x)|_{cr} = 1$ and the distribution coefficient of the copolymer coincides with that of block A:

$$(K_{AB})_{crB} = D^{-1} \int_0^D Z_A(x) dx = K_A \quad (7)$$

Hence the block B under critical conditions becomes chromatographically “invisible” in this two-block copolymer. Eqn. 7 is valid and, consequently, the method of “invisibles” applies for two-block copolymers at arbitrary macromolecule and pore sizes and also at any copolymer composition.

The distribution coefficient for a three-block copolymer B_1AB_2 is

$$K_{B_1AB_2} = D^{-1} \int_0^D \int_0^D Z_{B_1}(x)Z_A(x, x')Z_{B_2}(x') dx dx' \quad (8)$$

Provided that the critical conditions are created for both of the end blocks, B_1 and B_2 , using eqn. 4 we obtain

$$(K_{B_1AB_2})_{crB} = D^{-1} \int_0^D \int_0^D Z_A(x, x') dx dx' = K_A \quad (9)$$

and, consequently, the method of "invisibles" is also valid.

When the critical conditions are created for the inner block, A, the sum $Z_A(x, x')|_{cr}$ is described by eqn. 3. If the block A size is assumed to be greater than the pore width ($R_A > D/\pi$), it is sufficient to take only the first term in the series of eqn. 3, and this leads to $Z_A(x, x')|_{cr} \approx D^{-1}$. The following equation for the distribution coefficient of such a copolymer can be written:

$$(K_{B_1AB_2})_{crA} \approx K_{B_1}K_{B_2} \quad (10)$$

It should be noted that the only characteristics that are present on the right-hand side of eqn. 10 are those of "visible" blocks, B_1 and B_2 .

In a similar way, one can write the following equation for the multi-block copolymer $A_1B_1A_2B_2 \dots A_nB_n$:

$$(K_{\text{multiblock}})_{crA} \approx K_{B_1}K_{B_2} \dots K_{B_n} \quad (11)$$

provided that the critical conditions for component A are created and the sizes of the "invisible" blocks $A_1, A_2, \dots A_n$ are greater than the value of D . If the sizes of all "visible" blocks B_i also exceed the pore sizes, then the chromatographic behaviour of such copolymers is determined by the total length of all "visible" blocks B only. Note that eqn. 11 is also valid in the case of statistical copolymers with a low content of the "visible" component B.

The method of "invisibles" makes it possible to carry out separations for grafted copolymers according to the backbone length, provided that the critical conditions are created for the grafted copolymer component. This variant of the method is rigorous for the model of flexible chains at any pore width (both narrow and wide). However, when the critical conditions are attained for the backbone units, the chromatographic "invisibility" of these units takes place in narrow pores only. If this condition is satisfied, the distribution coefficients of grafted copolymers are described by eqn. 11 as for linear copolymers. The chromatographic separation in this instance will take place according to the MW of the whole grafted part of the polymer.

MACROMOLECULES WITH FUNCTIONAL GROUPS

One of the important applications of the method of "invisibles" is in the analysis of functionally active polymers by means of critical chromatography. Usually mono- or difunctional molecules are dealt with, one or two end units of which differ in their adsorption properties from the other non-functional units. Problems of the chromatographic separation of such functional molecules, under critical conditions, have been rigorously considered⁸⁻¹⁰ and we shall subsequently discuss the scope of application of the method of "invisibles" to these systems and illustrate the efficiency of the method when separation is performed according to the number of functional groups.

When critical conditions are created, normal non-functional units become "invisible"; therefore, for macromolecules without functional groups $K_{cr} = 1$, independent of the pore width, whereas the distribution coefficient of the monofunctional chain coincides with that of the functional group itself and is equal to⁹

$$K_{1_{cr}} = 1 + \frac{2\delta}{D} (q - 1) \quad (12)$$

where δ is the width of the layer near the wall. Getting into this layer causes an energy gain, $-\epsilon_f$ for a functional unit and $-\epsilon_{cr}$ for a non-functional unit, and $q = \exp[-(\epsilon_f - \epsilon_{cr})]$. Energy values here and further are expressed in units of kT (k = Boltzmann's constant, T = absolute temperature). Note that if there is only one functional group in the polymer molecule, the distribution coefficient of the chain is described by eqn. 12, independent of this group position. This is why non-functional units in monofunctional molecules can be treated under the critical conditions as "invisible" species at any pore width.

According to previous work¹⁰, the following is valid for difunctional molecules in narrow pores:

$$K_2 \approx K_1^2 \quad (13)$$

In a similar way, molecules with S functional groups have been shown⁸ to satisfy

$$K_{S_{cr}} \approx (K_{1_{cr}})^S \quad (14)$$

provided that the distance between such groups is greater than the pore size.

Note that macromolecules with functional groups can be treated as a special case of two-block copolymers with the "visible" blocks being very short. Eqns. 13 and 14 are obviously the consequences of eqns. 10 and 11; hence all the conclusions concerning the correctness and the scope of application of the method of "invisibles" given in the above section are still valid for functional macromolecules also.

Fig. 1 shows the theoretical chromatograms calculated for a mixture of equal parts of mono- and difunctional molecules and for molecules without functional groups, their molecular weight distributions (MWD) being the same. The MWD was simulated as a Gaussian function with polydispersity $M_w/M_n = 1.25$. To imitate the experimental conditions, the broadening due to the polydispersity of the samples and the instrumental broadening were taken into account, the latter being assumed to be represented by $\sigma_v = 0.02V_R$. The volumes of both the mobile and stationary phases are assumed to be equal to unity. As can be seen from Fig. 1a, when adsorption-active groups are situated at the ends of the molecule and gel permeation chromatographic (GPC) conditions for the remaining chain units are created, the chromatogram of such a sample looks like a single non-separated peak. An analogous situation also takes place when adsorption of non-functional groups occurs (Fig. 1c). In these instances, macromolecules of different length and functionality are mixed in the exit zone of the chromatographic peak.

Fig. 1b corresponds to the critical conditions created for non-functional chain units. Under these conditions, the retention volume is no longer dependent on MW

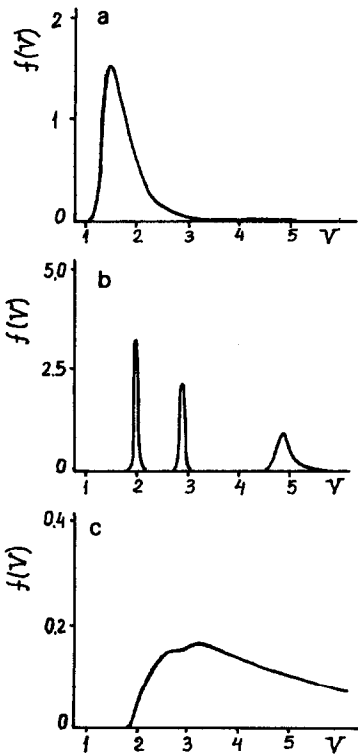


Fig. 1. Pattern of chromatograms for a mixture of equal parts of di-, mono- and non-functional polymers. Polydispersity, $M_w/M_n = 1.25$; ratio of average macromolecular radius to pore width, $R/D = 0.29$; adsorption energy of functional groups, $-\varepsilon_f = 2$. For the internal (non-functional) chain units, the following conditions are attained: (a) GPC conditions, $\varepsilon = 0$; (b) critical conditions, $-\varepsilon = -\varepsilon_{cr} = 0.182$; (c) adsorption conditions, $-\varepsilon = 0.25 > -\varepsilon_{cr}$.

(and consequently on MWD) and separation of macromolecules is carried out exclusively according to the number of functional groups.

RING MACROMOLECULES AND MACROCYCLES

It follows from the theory of the chromatography of ring macromolecules^{11,12} that the distribution coefficient of macrocycles is equal to

$$K_{c,cr} \approx \begin{cases} 2\sqrt{\pi} \cdot \frac{R}{D} & \text{if } R \gg D \\ 1 + \sqrt{\pi} \cdot \frac{R}{D} & \text{if } R \ll D \end{cases} \quad (15)$$

As can be seen from eqn. 15, the distribution coefficient of cyclic macromolecules always exceeds unity under critical conditions, and unlike the case with linear chains,

K_{cr} depends on the ratio of the macromolecule and the pore diameters. Such a dependence is conditioned by specific topology effects, which change the shape of the distribution of polymer chain units near the pore walls of the adsorbent. The density of units of linear macromolecules under critical conditions is known to be constant at any distance from the pore walls⁵. On the other hand, according to the exact theory, the density of units of ring macromolecules near walls is greater than that inside a pore. The increase in distribution coefficient is related to this circumstance. This problem has been treated in detail elsewhere^{11,12}.

Therefore, even perfectly uniform cyclic macromolecules cannot be considered to be "invisible" when they are exposed under critical conditions. This is why it is possible to separate linear polydisperse chains from ring macromolecules using the method of "invisibles".

In order to demonstrate the efficiency of the method of "invisibles" when separating linear and ring polydisperse polymers let us reconsider our "theoretical chromatograph". Fig. 2 shows the chromatograms calculated for a mixture of equal parts of linear and ring polymers, their average MWs being the same. Their polydispersities are also assumed to be identical and to be $M_w/M_n = 1.25$. In the GPC

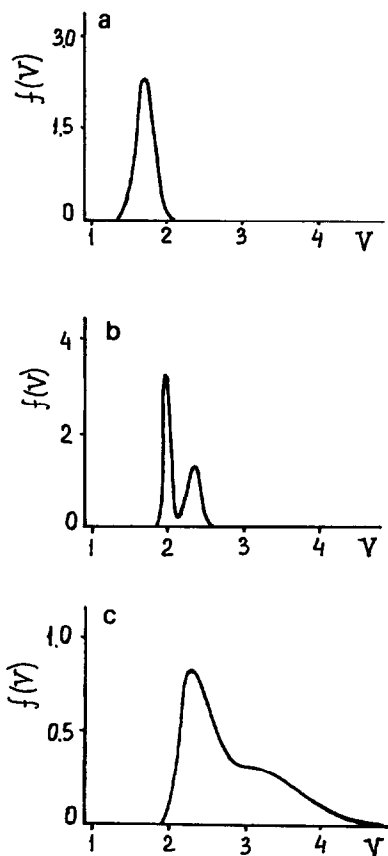


Fig. 2. Chromatograms calculated for a mixture of polydisperse flexible macrocycles with analogous linear molecules. $M_w/M_n = 1.25$; $R/D = 0.2$. (a) $\epsilon = 0$; (b) $-\epsilon = -\epsilon_{cr}$; (c) $-\epsilon > -\epsilon_{cr}$.

mode (Fig. 2a), and also under conditions of adsorption chromatography, the separation of macromolecules takes place according to both the sizes and topology of the macromolecules. This results in the presence of a single broadened peak in the chromatogram. Application of the method of "invisibles", *i.e.*, chromatography under critical conditions, allows us to eliminate the broadening due to the linear polymer polydispersity and enables the macromolecules to be separated according to their topology (Fig. 2b). When one part of a chain contains macrocycles, whereas another is linear, the latter becomes "invisible" under critical conditions. In this case, the separation takes place according to the sizes of the cyclic parts of the macromolecule only. The method of "invisibles" for such a system is a rigorous one and could be useful for the problems of the detection and isolation of partially cyclic macromolecules with intramacromolecular cross-linkages.

DISCUSSION

The method of "invisibles" in polymer chromatography is exactly rigorous for a number of polymer systems that contain a single continuous "visible" chain fragment. In particular, monofunctional macromolecules, two-block copolymers, three-block copolymers with the central block being investigated, partially cyclic macromolecules and graft copolymers with "invisible" side-chains (Fig. 3) belong to such systems. The most convenient realization of the method of "invisibles" in such systems would be obtained using wide-pore adsorbents.

For other systems, *e.g.*, copolymers with several "visible" fragments joined together with "invisible" inserts (Fig. 4), the method of "invisibles" becomes rigorous when using narrow-pore adsorbents only, the pore sizes being smaller than those of all the chain elements under the critical conditions. For the realization of the method of "invisibles" in such systems, it is important to have narrow-pore adsorbents with a uniform pore surface, and also to be able to select conditions that are both critical for one of the copolymer components and at the same time do not cause strong adsorption of the other "visible" component. This can be achieved by choosing a suitable eluent, variations of temperature and adsorbent surface modification.

The theoretically predicted efficiency of the method of "invisibles" for functionally active macromolecules has been demonstrated experimentally in detail for a large number of polymer systems, and a review was published by Entelis *et al.*⁸. Some other predictions of the theory, in particular the possibility of the application of

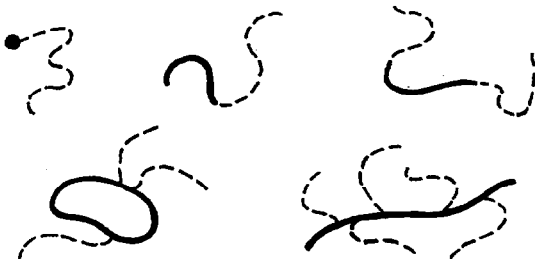


Fig. 3. Types of macromolecules for which the method of "invisibles" is valid in arbitrary pores. Here and in Fig. 4 "invisible" elements under critical conditions are shown as dashed lines.

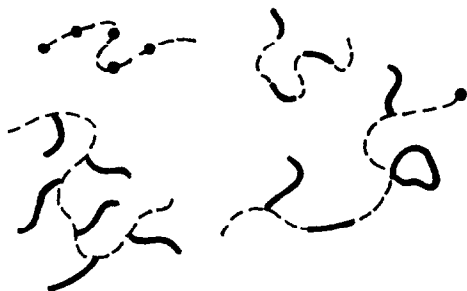


Fig. 4. Types of macromolecules for which the method of "invisibles" is valid only when applied to narrow-pore adsorbents. Dashed lines as in Fig. 3.

the "invisibility" method to the analysis of both block and graft polymers and to the separation of linear and cyclical macromolecules, have not received experimental corroboration to date.

The performance of the model chromatographic experiments according to the method of "invisibles", using well characterized copolymer samples with exactly known structures, and the subsequent comparison of the results of these experiments with the predictions of the theory, seem to be of interest. For example, when there is a series of two-block copolymers differing from each other in the length of one of the blocks, say A-type, whereas the other block, B, is the same, and when the critical conditions are created for the blocks of A-type, all such copolymers leave from the chromatographic column with equal retention volumes; this would be expected according to the theory. In contrast, when block B is made "invisible" in such a system, the copolymers are then separated according to the length of block A.

It would also be of interest to investigate the chromatographic behaviour of both linear and ring polymers with the same chemical structure. It follows from the theory that, under the critical conditions, all linear polymers have a distribution coefficient $K_{1r} = 1$, independent of their length and polydispersity, whereas for cyclic polymers, according to eqn. 15, $K_{cr} > 1$ and they are more strongly retained the greater is their molecular weight, *i.e.*, they behave as in adsorption chromatography.

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REFERENCES

- 1 M. B. Tennikov, P. P. Nefedov, M. A. Lazareva and S. Ya. Frenkel, *Vysokomol. Soedin., Ser. A*, 19 (1977) 657.
- 2 A. M. Skvortsov, B. G. Belenky, E. S. Gankina and M. B. Tennikov, *Vysokomol. Soedin., Ser. A*, 20 (1978) 678.
- 3 A. V. Gorshkov, V. V. Evreinov and S. G. Entelis, *Zh. Fiz. Khim.*, 57 (1983) 2665.
- 4 A. A. Gorbunov, L. Ya. Solovyova, V. A. Pasechnik and A. Ye. Lukyanov, *Vysokomol. Soedin., Ser. A*, 28 (1986) 1859.
- 5 A. A. Gorbunov and A. M. Skvortsov, *Vysokomol. Soedin., Ser. A*, 28 (1986) 2453.
- 6 A. M. Skvortsov and A. A. Gorbunov, *Vysokomol. Soedin., Ser. A*, 21 (1979) 339.
- 7 M. Leng and M. Benoit, *J. Chem. Phys.*, 59 (1962) 929.

- 8 S. G. Entelis, V. V. Evreinov and A. V. Gorshkov, *Adv. Polym. Sci.*, 76 (1986) 129.
- 9 A. A. Gorbunov and A. M. Skvortsov, *Vysokomol. Soedin., Ser. A*, 26 (1984) 949.
- 10 A. M. Skvortsov and A. A. Gorbunov, *Vysokomol. Soedin., Ser. A*, 22 (1980) 2641.
- 11 A. A. Gorbunov and A. M. Skvortsov, *Vysokomol. Soedin., Ser. A*, 26 (1984) 2062.
- 12 A. A. Gorbunov and A. M. Skvortsov, *Vysokomol. Soedin., Ser. A*, 28 (1986) 2447.