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PHYSICO-CHEMICAL FEATURES OF THE CHROMATOGRAPHY OF MACROMOLECULES

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SUMMARY

The generalized calibration of a column for size-exclusion chromatography (SEC) as a function of K (distribution coefficient of a polymer in the column) on R (ratio of the coil diameter of flexible macromolecular chains to the average pore diameter of the column material) is suggested. Dependences of K on R are considered for chromatographic systems containing column materials with different pore diameters from 5 to 200 nm and surface chemistry (silica-gels, Silochroms and macroporous glasses) and different polymers (polystyrene, polyoxyethylene, polyethylene glycol, dextrans and proteins).

The effect of the composition of the mobile phase and the temperature and internal diffusion of macromolecules on the parameters in the SEC of polymers is discussed.

INTRODUCTION

The retention of macromolecules on column materials under quasi-equilibrium conditions of chromatography is controlled mainly by the sizes and chemical structures of the macromolecules and/or their associates and also by the composition of the mobile phase, the pore distribution and the surface chemistry of the column material. The combination of different parameters of a chromatographic system allows the direction of a separation process to be changed to the distribution of macromolecules according to their sizes or to the distribution of macromolecules according to their adsorption energies. A decrease in the adsorption activity of the column material and/or an increase in the polarity of the eluent leads to a transition from adsorption to size-exclusion chromatography (SEC).

In this paper, the dependences of the distribution coefficients of the macromolecules of polyethylene, polyoxyethylene, polystyrene and proteins on the hydrodynamic diameters of macromolecular coils, molecular weight, the ratio of the pore diameter of the stationary phase to the size of the macromolecules and column temperature are considered. The advantage of the generalized calibration of chromatographic columns for the determination of the molecular parameters of polymers is demonstrated.

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THEORY

The general equation for SEC[1]:

$$V_i = V_0 + KV_s \quad (1)$$

correlates V_i, the retention volume of substance i, with K, the distribution of substance i between two phases and with V_s, the total pore volume of the stationary phase; V_0 is the dead volume, equal to the volume of mobile phase from the injector to the place of recording the maximum concentration in the detector cell, including the volume of mobile phase between the particles of stationary phase and excluding V_s, the total volume of pores accessible to the molecules of components of the mobile phase. Thus, K in ideal SEC reflects the fraction of the pore volume which is accessible for the macromolecules.

If the macromolecules cannot penetrate into the pores because of their size, $K = 0$ and therefore these macromolecules leave the column before macromolecules of smaller size. Molecules that can penetrate to all pores are transported along the column with a velocity equal to the flow-rate of the mobile phase and for these molecules $K = 1$. The accessibility of the pore volume depends on several factors, including molecular weight, the flexibility of the molecular chain, the distribution of the pore volume, temperature and the chemical nature of the mobile phase.

The molecular weight is the most important physical property of substances such as polymers. The correct definition of the molecular weight of polymers with different chemical structures is a very complex problem. SEC in principle allows the molecular parameters of most polymers to be calculated on the basis of the determination of the dependences of K on the molecular weights of narrow fractions of the given polymer and other parametes of the chromatographic system.

In this paper, a generalized principle of column calibration for the SEC of polymers is proposed, together with dependences of these calibrations on the nature of the eluent, pore distribution, surface chemistry and temperature. The advantages of this calibration for the determination of the molecular structure of polymers are demonstrated.

EXPERIMENTAL

The polymers investigated were polystyrenes (Pressure Chemical Co., U.S.A.), polyoxyethylenes (Schuchard, F.R.G.), proteins (Serva, F.R.G.) and narrow fractions of low-pressure polyethylene, prepared by the method described in ref. 2.

Toluene, *p*-xylene, tetrahydrofuran, carbon tetrachloride, water and phosphate buffer (pH 6.5) were used as mobile phases. Columns packed with silica gels, Silochroms and macroporous glasses were used. The measurements were made on the Tsvett 304 (Khimavtomatika, U.S.S.R.), CL-1302 (Scientific Technical Department, U.S.S.R. Academy of Sciences) and Waters-200 (U.S.A.) liquid chromatographs.

RESULTS AND DISCUSSION

Effect of nature of the polymer

Fig. 1 shows the dependences of K on the molecular weights and hydrodynamic

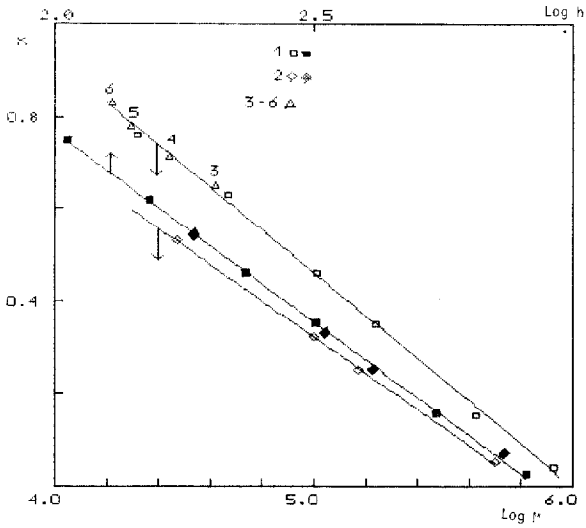

Fig. 1. Dependences of distribution coefficient K for narrow fractions of (1) polystyrene, (2) polyethylene and the globular proteins (3) albumin, (4) α-chymotrypsin, (5) lysozyme and (6) cytochrome *c* on molecular weight and on the hydrodynamic diameter of the macromolecule coil, (h). Stationary phase, Silochrom (d = 60 nm) treated with $(CH_3)_3SiCl$ (1, 2) and 3-amino-$C_3H_6Si(OC_2H_5)_3$ (3–6). Mobile phase, tetrahydrofuran (1), *p*-xylene (2) and buffer 0.05 M Na_2HPO_4–0.1 M NaCl buffer (3–6).

diameters (h) of the macromolecular coils of the different polymers. The hydrodynamic diameters of these coils were calculated by use of the Flory–Fox[3] and Ptitzyn–Eisner[4] equations:

$$h = 2(kM^{1+a})^{1/3}/[\sqrt{6}\varphi_0\,(1 - 2.63\varepsilon + 2.86\varepsilon^2)]^{1/3} \quad (2)$$

where k and a are coefficients of the Mark–Houwink equation, φ_0 is the Flory parameter, equal to $1.86 \cdot 10^{23}$, and $\varepsilon = (2a - 1)/3$. For polystyrene standard solutions in tetrahydrofuran[5]

$$h = 0.027\,M^{0.59} \quad (3)$$

The plot of K *versus* log macromolecular coil diameter is linear and almost independent of the chemical structure of the macromolecular chain. As can be seen from eqn. 2, the chemical nature of the polymer and the interaction between the polymer macromolecule and the solvent molecules can change the shape and size of the macromolecule coil.

Fig. 2 shows plots of K *versus* log M for polystyrene standards introduced into two columns: the packed macroporous silica Silochrom (pore diameter d = 60 nm) with a wide pore volume distribution by pore diameters (standard deviation σ = 20 nm) and macroporous glass (d = 55 nm) with a narrow pore volume distribution by pore diameters (σ = 3 nm). The narrow pore volume distribution by pore diameters leads to a greater slope of the linear part of the calibration line. Hence, for the determination of the molecular parameters of polydisperse polymer or polymers with

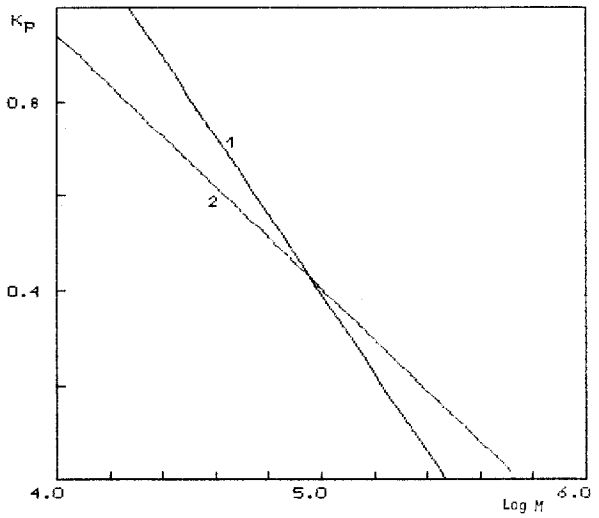

Fig. 2. Effect of pore size distribution on the plot of K for a narrow fraction of polystyrene against the molecular weight. Stationary phase: (1) macroporous glass (d = 55 nm); (2) Silochrom (d = 60 nm). Mobile phase, tetrahydrofuran.

various different molecular weights, M, it is preferable to use a stationary phase with very wide pore volume distribution by pore diameters. In principle it is not difficult to find a stationary phase that will allow the investigation and determination of the molecular characteristics of polymers of different natures.

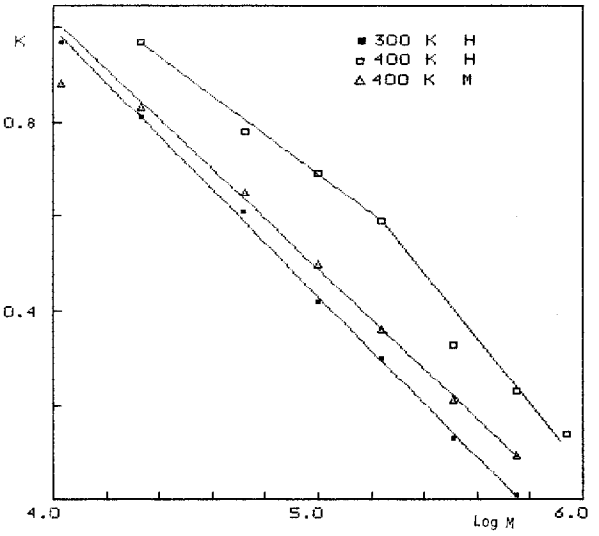

Fig. 3. Effect of surface chemistry and temperature on the plots of K for a narrow fraction of polystyrene. Stationary phase: Silochrom (d = 60 nm) with hydroxylated (H) and modified by $(CH_3)_3SiCl$ (M) surfaces. Mobile phase, p-xylene. Elution temperatures: 300 K and 400 K.

Effect of surface chemistry of the stationary phase

In real SEC there is an uncertainty in the adsorption effects that can misrepresent the parameters of the molecular weight distribution of polymers, defined by chromatographic experiments. Fig. 3 shows examples of the influence of chemical modification of the stationary phase surface (macroporous silica) on the *K versus* log *M* calibration. The replacement of hydroxyl groups on the surface of the stationary phase with trimethylsilyl groups eliminates the specific interaction of the macromolecules with the surface of the silica gel and, as expected, to the displacement of the *K* values to the smaller values of *M*. Hence the modification of the silica surface leads to ideal size-exclusion conditions in the chromatography of polymers.

The chemical structure of the surface is a very important factor in the selectivity of the chromatographic separation of polymer macromolecules. The group separation of oligomers with different functionality is based on the difference in the energy of the specific interactions of structural groups of atoms in molecules with the adsorption sites on the internal surface of porous stationary phase particles. The replacement of hydroxyl groups at the silica surface with amino groups increases the specific interaction of acidic molecules, but there is a decrease in the specific interaction of the oligomer molecules with hydroxyl end-groups and the separation mechanism changes from adsorption to size exclusion. Fig. 4 shows the dependence of *K* on log *M* of polyoxyethylenes with hydroxyl end-groups transported through columns packed with (1) hydroxylated silica gel and (2) amino-silica gel[6]. The transition from adsorption to size-exclusion can be clearly seen.

Chemical modification of the silica gel surface with γ-aminopropyltriethoxysilane decreases the adsorption of the globular proteins albumin, chymotrypsin, lysozyme and cytochrome *c* in neutral and acidic solution and allows their separation by SEC[7]. The nature of polarity of mobile phase, ionic strength and the pH of buffer

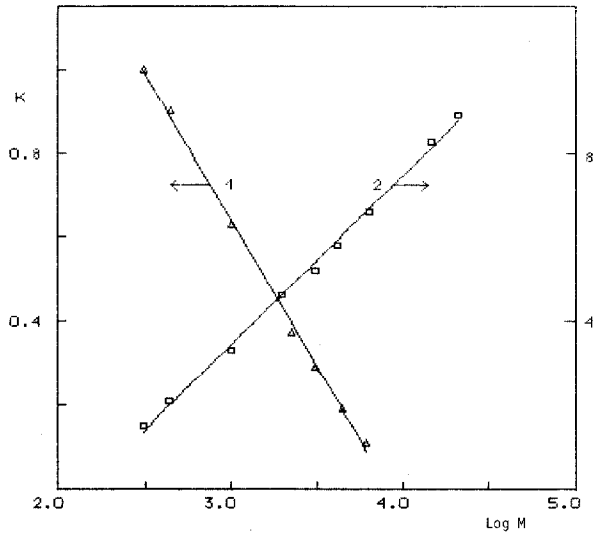

Fig. 4. Dependences of *K* for polyoxyethylene on molecular weight. Stationary phase, silica gels, one (d = 10 nm) with the surface covered with amino groups (1) and the other (d = 50 nm) with a hydroxylated surface (2).

TABLE I

DIFFUSION COEFFICIENTS (D) OF MACROMOLECULES OF POLYSTYRENE INTO PORES OF SILOCHROM (d = 55 nm) AT 293 K

M	D ($\mu m^2/s$)	
	Toluene	CCl_4
9800	60	4.5
97 000	2.0	0.3
198 000	0.36	0.02

solutions may influence the retention and conformation of macromolecules in solution and consequently on the polymer separation mechanism.

Effect of temperature

A change in the temperature in the chromatographic system, which changes the eluent polarity, varies the size and the shape of macromolecules. However, a change in temperature can lead to modifications of the interaction of solvent molecules with the hydroxylated surface of silica. For example, an increase in temperature decreases the sizes of polystyrene molecules in *p*-xylene, which increases the K values. An increase in temperature decrease the adsorption of the solvent and thus promotes an increase in the adsorption of macromolecules of polystyrene, which leads to an increase in the distribution coefficients.

Steric factors have a significant effect on the parameters of molecular structure of polymers calculated from the SEC data. Variations in diffusion coefficients can be more than two orders of magnitude, if the sizes of the macromolecules are close to the pore diameters of the silica, as shown in Table I.

Hence the generalized calibration of distribution coefficients (or the changes in free energy) on the size of macromolecules, or better on the ratio $r = h/d$, allows a single function to be considered that does not depend on the dimensions of the chromatographic column, particle size, specific surface area of the stationary phase or the nature of the mobile phase when the excess adsorption of the polymer is zero. The steric factor has a great effect when the K values is nearly zero.

REFERENCES

1 Yu. A. Eltekov and A. S. Nasansky, *J. Chromatogr.*, 116 (1976) 99.
2 T. A. Romanova and Yu. A. Eltekov, *Kolloid. Zh.*, 36 (1974) 906.
3 P. J. Flory and T. G. Fox, *J. Am. Chem. Soc.*, 73 (1951) 1915.
4 O. B. Ptitzyn and Yu. E. Eisner, *Zh. Fiz. Khim.*, 32 (1958) 2464.
5 W. W. Yau, C. D. Ginnard and J. J. Kirkland, *J. Chromatogr.*, 149 (1978) 465.
6 A. V. Kiselev, T. I. Rjabinina and Yu. A. Eltekov, *Dokl. Akad. Nauk SSSR*, 200 (1971) 1132.
7 Yu. A. Eltekov, A. V. Kiselev, T. D. Khokhlova and Yu. S. Nikitin, *Chromatographia*, 6 (1973) 187.