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COMPARISON OF DIMENSIONS OBTAINED BY SIZE-EXCLUSION CHROMATOGRAPHY AND X-RAY DIFFRACTION OF RIGID MOLECULES

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SUMMARY

Homo- and copolymers of monomers having vinyl groups with large, rigid side chains were investigated by size-exclusion chromatography and small-angle X-ray diffractometry in the solid state. The distributions of the molecular dimensions in dilute solutions and the distributions of the inhomogeneities of electron density in solid materials showed many similarities, and on the basis of these findings some conclusions are drawn concerning the molecular structure in the solid state and in dilute solutions, and also on the nature of the separation mechanism in size-exclusion chromatography.

INTRODUCTION

Size-exclusion is said to occur in gel permeation chromatography (GPC) when dissolved macromolecules are excluded from the pores of the packing beads according to their molecular size. Separations on rigid gels showing no adsorption characteristics have been assumed to be based on this mechanism, as have theoretical considerations concerning the separation mechanism of GPC. Also, different recommended "universal calibrations" have been based on the hydrodynamical dimensions of the dissolved macromolecules using hypothetical hydrodynamic equations for macromolecular solutions^{1,2}. Nevertheless, the only evidence of the validity of these methods was that the calibrating points for different materials fell on the same curve.

In the present study we have determined the average dimensions and also the distribution of the hydrodynamic sizes of dissolved macromolecules via size-exclusion chromatography, and compared these values to the sizes of the local inhomogeneities of electron density in the bulk material. The polymers comprised vinyl chains having long, rigid side groups, therefore no significant expansion was expected upon dissolution in an organic solvent. On the basis of the comparison of the data obtained by the two methods, some conclusions can be drawn on the reliability of the methods and on the solution properties of these rigid macromolecules.

EXPERIMENTAL

Three types of polymers were tested: phenyl-, methoxy-, ethoxy-, propoxy- and butoxyphenyl esters of *p*-acryloyloxybenzoic acid (FAB, MFAB, EFAB, PFAB and

BFAB respectively); copolymers of cholesterylvinyl succinate and *p*-methoxy-*p'*-acryloyloxy-azoxybenzene (CVS/MAAB) of different molar ratios; copolymers of *N*-acryloylpiperidine and cholesteryl acrylate of different molar ratios (AP/CA). The preparations of the materials were described elsewhere^{3,4}.

Two GPC systems were used for the determination of the distribution of molecular sizes in solutions. A Waters GPC-200 apparatus, equipped with five Styragel columns of nominal porosity $3 \cdot 10^4$, 10^4 , $3 \cdot 10^3$, 10^3 and 10^2 nm, respectively, was employed for the FAB samples at 130°C with 1,2,4-trichlorobenzene as solvent. The other instrument was home-made, and employed 10^4 , 10^3 and $3 \cdot 10^2$ nm Styragel columns with tetrahydrofuran as solvent at ambient temperature. The sample concentration was 5 g/l in each case, and 2 ml of each sample solution were injected.

Figs. 1 and 2 show Benoit's universal calibration for the high-temperature and room-temperature GPC systems respectively. In the case of the high-temperature system the curves were obtained by running narrow fractions of polyethylene, polypropylene, poly(propylene oxide) and polystyrene of known molecular mass, while at ambient temperature poly(vinyl chloride) and homo- and equimolar copolymers of styrene and ethyl, *n*-butyl and *n*-octyl methacrylate were used. The intrinsic viscosities of the fractions were measured in an Ubbelohde-type capillary viscometer under conditions pertinent to the GPC measurements. The characterization of the calibrating fractions has been given elsewhere⁵.

The distribution and average sizes of the inhomogeneities in electron density of the solid materials were computed from the course of the intensity curve of diffracted

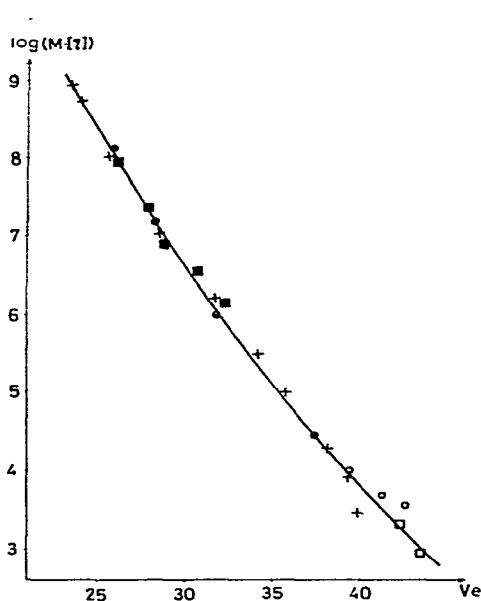


Fig. 1. Universal calibration for high-temperature measurements. Symbols: +, polystyrene; ■, polyethylene; ●, polypropylene; ○, poly(propylene oxide); □, *n*-paraffin.

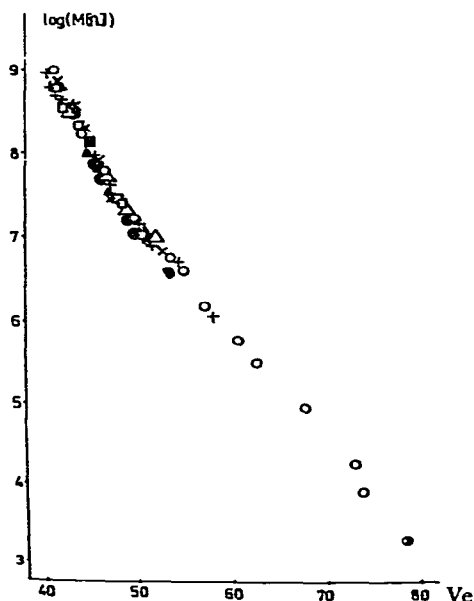


Fig. 2. Universal calibration for low-temperature measurements. Symbols: ○, polystyrene; △, poly(vinyl chloride); □, poly(ethyl methacrylate); ●, poly(butyl methacrylate); ▲, poly(octyl methacrylate); +, styrene-ethyl methacrylate copolymer; ×, styrene-butyl methacrylate copolymer; ■, styrene-octyl methacrylate copolymer; ●, squalane.

X-rays in the vicinity of the incident beam. The diffractograms were recorded by a Rigaku-Denki low-angle diffractometer, using pellets of 2 cm in diameter and 0.7–1.5 mm in thickness. Nickel-filtered Cu-K radiation was applied.

THEORETICAL

According to the Flory–Fox equation, used in Benoit's calibration

$$\langle R_0^2 \rangle^{3/2} \alpha^3 = [\eta] \cdot M / \varphi = 10^{F(V)} / \varphi \quad (1)$$

where $[\eta]$ is the intrinsic viscosity in cm^3/g , M is the molecular mass, φ is Flory's universal constant having a magnitude of $2.5 \cdot 10^{23}$ (ref. 6), $F(V)$ is the universal calibration curve according to Benoit, R_0 is the unperturbed radius of gyration of the dissolved molecules and α is the linear expansion coefficient. Thus, $\langle R_0^2 \rangle^{3/2} \alpha^3$ gives the hydrodynamic volume of the molecules. For the hydrodynamic radius of gyration:

$$\langle R^2 \rangle^{1/2} = \alpha \cdot \langle R_0^2 \rangle^{1/2} = [10^{F(V)} / \varphi]^{1/3} \quad (2)$$

The chromatogram—the concentration of the eluted solvent as a function of the elution volume—can be transformed into molecular size distribution using

$$\log \langle R^2 \rangle^{1/2} = F(V)/3 = F'(V) \quad (3)$$

for each point of the elution volume scale, having a chromatogram, $C(V)$

$$w[\langle R^2 \rangle^{1/2}] = \frac{C(V)}{d[F'(V)]/d[\langle R^2 \rangle^{1/2}]} \quad (4)$$

where $w[\langle R^2 \rangle^{1/2}]$ is the distribution of molecular sizes according to mass. Using N equidistant points of the chromatogram, having heights C_i , the average dimensions for the number average are

$$R_n = \frac{\sum_i C_i \cdot \langle R_i^2 \rangle^{-1}}{\sum_i C_i \cdot \langle R_i^2 \rangle^{-3/2}} \quad (5)$$

for the mass average

$$R_m = \frac{\sum_i C_i \cdot \langle R_i^2 \rangle^{1/2}}{\sum_i C_i} \quad (6)$$

and for the most probable dimensions:

$$\langle R^2 \rangle^{1/2} = \frac{[\sum_i C_i \cdot \langle R_i^2 \rangle^{1/2}]}{[\sum_i C_i \cdot \langle R_i^2 \rangle^{3/2}]} \quad (7)$$

The X-ray intensity data vs. scattering angle functions (scattering curve) reflect the dimensions of the electron-density inhomogeneities in the irradiated material.

According to Guinier and Fornet⁷, the part of the scattering curve in the vicinity of the direct beam can be replaced by a Gaussian curve, *i.e.*, the logarithm of the intensity vs. the square of the scattering angle gives a straight line. The slope of this straight line is proportional to the radius of gyration of the inhomogeneities

$$\log I_{\theta} = \log I_0 - (16\pi^2/3\lambda^2) R_g^2 \theta^2 \quad (8)$$

where θ is the scattering angle, I_{θ} and I_0 are the intensities of the scattered radiation at angles θ and 0 respectively, λ is the wavelength of the irradiation and R_g is the radius of gyration of the inhomogeneities.

The rough scattering curves were smoothed using nine points for calculation of each smoothed value. The same procedure was employed for the background-scattering, determined from the intensity with the sample not in the scattering position. The difference between these curves were then plotted as described above.

The method of Brill *et al.*⁸ makes it possible to determine the diameters of spherical particles or equivalent spheres, if the scattering curve is known. Accordingly, for the $\varrho(d)$ distribution of dimensions

$$\varrho(d) = Ad^{-2} \int [h^4 \cdot I(h) - C] \psi(h, d) dh \quad (9)$$

where h is a parameter of the reciprocal lattice, related to the scattering angle by $h = (4/\lambda) \cdot \sin \theta/2$. $I(h)$ is the intensity function, A is a normalizing parameter and $C = \lim_{h \rightarrow \infty} [h^4 I(h)]$. For the function $\psi(h, d)$:

$$\psi(h, d) = (1 - 8\bar{h}^2 \bar{d}^2 \cos hd) - [4 - 8\bar{h}^2 \bar{d}^2 (\sin hd)/hd]$$

The rough scattering curves were first smoothed and corrected for the background as described above, and then corrected for the finite beam-width with the help of the slit-correction described by Kratky *et al.*⁹.

RESULTS AND DISCUSSION

Fig. 3 shows the distribution functions for the gyration radii of two CVS/MAAB systems obtained from GPC measurements in tetrahydrofuran at room temperature, *i.e.*, these distributions reflect the situation in dilute solution. The electron-density inhomogeneities obtained from the small-angle X-ray scattering data by using eqn. 9 are illustrated in Fig. 4. Comparison of the two figures shows that the two methods yield dimensions of similar order. The region of the distributions shown is the same in both figures, and also the bimodal character in case 2 is seen for both distribution types.

In Fig. 5 the distributions are given for the AP/CA copolymer systems obtained by GPC at room temperature in tetrahydrofuran, while Fig. 6 shows the distribution of the electron-density fluctuation calculated from the X-ray distribution for this series. The character and the region of the distribution curves in these figures exhibit many similarities and suggest that the two methods give similar information.

It is worth comparing the numerical values of the average dimensions. In Fig. 7

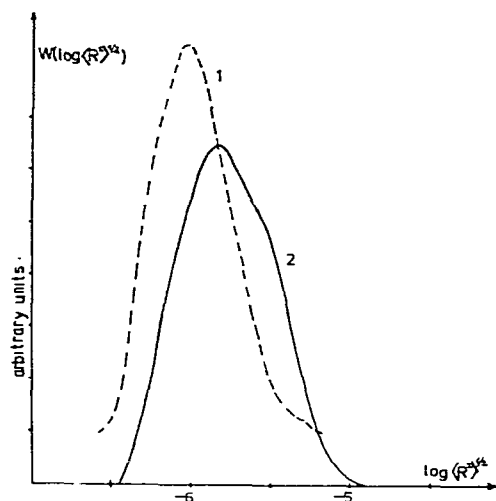


Fig. 3. Hydrodynamic size distribution for CVS/MAAB copolymers.

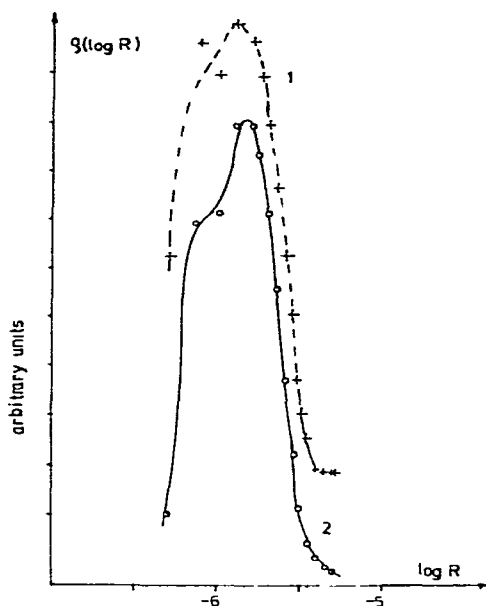


Fig. 4. Distribution of electron-density inhomogeneities in CVS/MAAB copolymers.

the size distributions are shown for FAB polymers, obtained by GPC at elevated temperature. The Guinier plots for these materials are given in Fig. 8, and the calculated average sizes are listed in Table I. From Fig. 8 it is seen that the extrapolation

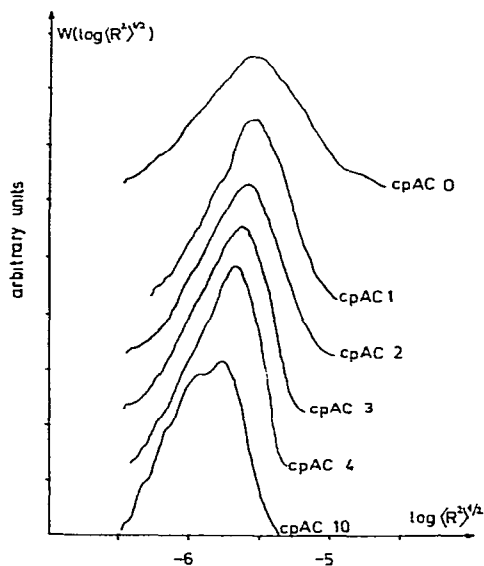


Fig. 5. Hydrodynamic size distribution for AP/CA copolymers.

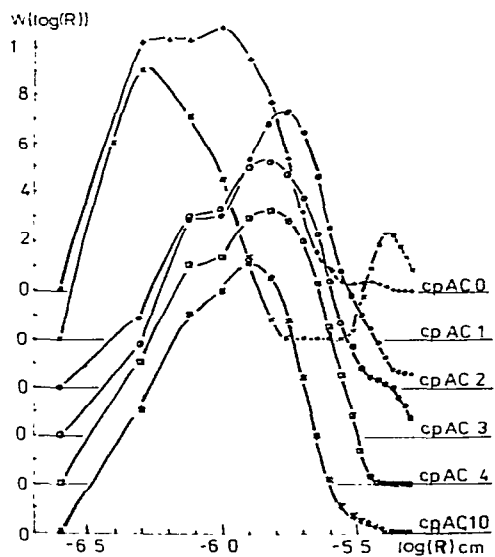


Fig. 6. Distribution of electron-density inhomogeneities in AP/CA copolymers.

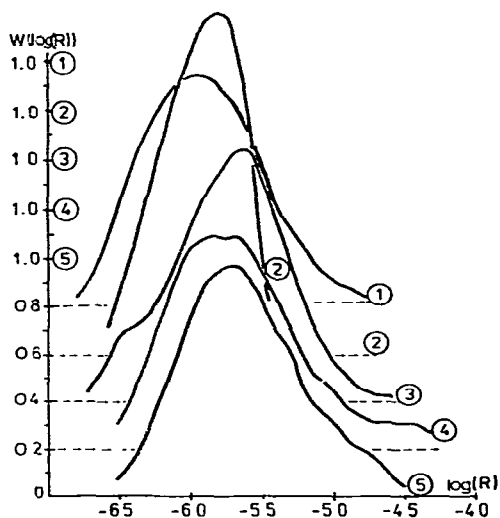


Fig. 7. Hydrodynamic size distributions for FAB polymers.

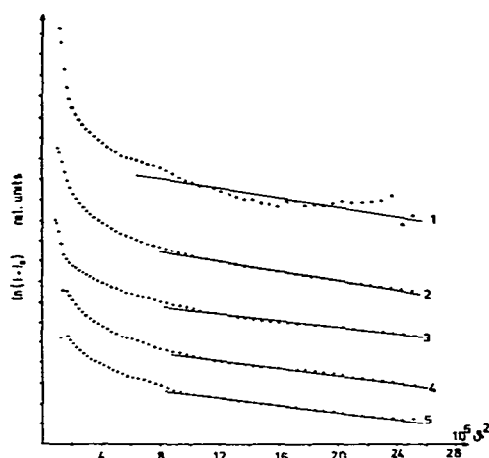


Fig. 8. Guinier plots for FAB polymers.

was done from the asymptote of the scattering curve. Similar extrapolation is often used in studies of the light scattering of dilute polymer solutions¹⁰ and yields $\langle R^2 \rangle^{1/2}$. In this work the $\langle R^2 \rangle^{1/2}$ values obtained by X-ray scattering and by exclusion chromatography are of similar magnitudes.

It can be concluded that the results obtained by small-angle X-ray scattering of the solid materials and by the chromatographic method applied to dilute solutions reflect similar physical properties. Therefore, it seems very probable that the X-ray scattering in these cases yields the dimensions of the individual molecules, which are similar to those in dilute solutions.

Some consideration must be made on the assumptions used in the calculations. The use of the universal calibration of Benoit is based on the hydrodynamical behaviour of random coil molecules in dilute solutions. The validity of this calibration has been confirmed for rod-like molecules¹¹ and for polyelectrolytes¹². Thus, it can

TABLE I

DIMENSIONS (IN nm) MEASURED FOR *p*-ALKOXYPHENYL *p*-ACRYLOYLOXYBENZOATE POLYMERS

SAXS = Small-angle X-ray scattering.

Alkyl group	GPC			SAXS
	$\langle R^2 \rangle^{1/2}$	R_m	R_n	R_g
H	3.78	18.7	3.13	6.6
Methoxy	5.37	13.2	4.58	6.8
Ethoxy	4.37	26.3	3.46	6.4
Propoxy	6.14	46.9	5.13	6.5
Butoxy	6.64	38.9	5.41	6.1

be assumed that, also for the present rigid molecules, chromatography occurs according to the radius of gyration.

The small-angle X-ray scattering method was tried also for dilute solutions, *i.e.*, assuming no interference of the beams reflected by different inhomogeneities and spherical particles. The bulk samples were prepared by precipitation from dilute solutions. Because of the rigidity of the molecules, interpenetration of the particles during precipitation is unlikely, *i.e.*, there are individual macromolecular aggregates in the bulk having the dimensions of the single molecules. Since the molecules have large rigid side groups, the number of conformational variations is small; solvent molecules cannot penetrate into the inner volume of the cylindrical molecules, thus the dimensions of the bulk molecules are similar to those of the dissolved particles.

As regards the possible interference of the reflected beams, Kratky *et al.*⁹ showed that when interference occurs there is a plateau in the innermost region of the scattering curve. In this case the extrapolation can be done only in the region outside the plateau. We did not record any plateau, and the extrapolation was not performed in the region where such plateaux are usually found. It must be emphasized that, both in X-ray scattering and chromatography, spherical particles are assumed, and quasi-dimensions are obtained.

Taking into consideration the above problems and the fact that the calculations were executed for three independent series with fifteen samples, it seems very probable that GPC yields the same dimensions as does X-ray diffraction of the solid materials. This means that the parameter obtained from separation, is the molecular dimension, which in our case was equal to the dimension of the molecular aggregates in the solid material. Therefore, the size exclusion expression is valid also in case of very rigid macromolecules.

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