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Theoretical aspects of the chromatographic analysis of heterogeneous polymers

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ABSTRACT

A method for the chromatographic analysis of heterogeneous polymers is proposed and theoretically confirmed. Such polymers include block and random copolymers, oligomers and polymers with functional groups. The basis of this procedure is the combined use of two main variants of liquid chromatography, size-exclusion and adsorption chromatography. The experimental data are treated by the methods of the theory of distributions. This procedure makes it possible to determine the molecular weight distribution of heterogeneous polymers and their compositional distribution and the distribution of functional groups. The possibility of using the adsorption variant of liquid chromatography under both isocratic and gradient conditions is considered.

INTRODUCTION

The quantitative analysis of heterogeneous polymers should include the determination of their molecular weight distribution (MWD) and composition. In the case of block copolymers this involves the determination of the MWD of each block, and for random copolymers this requires the determination of the weight fraction of each component of the copolymer. For example, for a molecule of a random copolymer with n_A units of component A and n_B units of component B, the molecular weight is

$$M_{AB} = n_A m_A + n_B m_B \tag{1}$$

where m_A is the weight of monomer A and m_B that of monomer B, and the degree of polymerization is

$$N = n_A + n_B \tag{2}$$

More generally, for a copolymer consisting of k components,

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$$M_\Sigma = \sum_{i=1}^{k} n_i m_i = \sum_{i=1}^{k} M_i \qquad (3)$$

where $M_i = n_i m_i$ is the net weight of the ith component in a molecule of the heteropolymer. Complete analysis of the heteropolymer requires that we find the distribution $W_i(M_i)$ for each ith component. The chromatographic behavior of polymers or oligomers with adsorption-active groups is determined by the number of these groups.

Many workers have investigated this problem (*e.g.*, refs. 1–8). Particularly relevant to this study are recent results obtained by Mori[7,8] by means of combined size-exclusion[9] and adsorption[10] chromatography. They used a calibration graph of copolymer composition *versus* retention volume. This procedure is possible only under the unusual circumstances where samples with known composition and molecular weight are available. This paper represents a theoretical attempt to determine the MWD and composition of heteropolymers using the more limited data typically available.

In size-exclusion chromatography (SEC), macromolecules are separated according to their hydrodynamic volume[11]. This separation results from a decrease in the entropy of macromolecules when they pass from the channels of the mobile phase of the chromatographic column into the pores of a stationary phase[12].

In the adsorption form of liquid chromatography (LAC), additional retention is observed. The elution of macromolecules in this instance is determined not only by entropy changes but also the enthalpy changes which they undergo in the interphase transition. The reason for the enthalpy changes is the energetic interaction between the macromolecules and the stationary phase matrix. The magnitude of this interaction is proportional to the number of adsorbing units of the chain. For an adsorbing homopolymer it is a degree of polymerization[13]. In the case of heterogeneous polymers, it is possible that only one kind of unit interacts with the packing. Therefore, the magnitude of this interaction is proportional to the number of such units, n_i, in the macromolecule.

Consequently, the chromatograms obtained by SEC shown distributions of the macromolecules of the sample according to size, whereas in LAC the chromatograms reflect both this size distribution and also the number, n_i, of the adsorbing monomer residues in the macromolecule. As will be show below, if the chromatograms of a heteropolymer obtained by SEC and LAC methods are considered together, distribution theory makes it possible to distinguish the contributions due to the size of macromolecules from those related to their energetic interaction with the packing and hence to obtain distributions according to both molecular weight and compostion.

The problem ultimately reduces to a search for the law of the distribution of a random variable which is a function of two other random variables, the distribution laws of which are known. These three interrelated random variables are the hydrodynamic size, $R_\eta \approx (\bar{M}[\eta])^{1/3}$, of the macromolecules, the net weight, M_i, of the adsorbing monomers, *i.e.*, all monomers of the heteropolymer component taking part in adsorption interaction with the packing matrix, and the chromatographic elution time, t_a ($\bar{M}$ is here an average molecular weight and $[\eta]$ is the intrinsic viscosity of the heteropolymer).

The chromatogram of the sample, $F_1(t_e)$, obtained under pure SEC conditions in which the variable t_e is replaced with R_η [with the aid of the universal calibration dependence[11] $t_e = f(R_\eta)$] yields the distribution $\tilde{F}_1(R_\eta)$ according to the hydrodynamic size of macromolecules R_η (ref. 14).

The distribution in time, t_a, of the elution of macromolecules from the column under the conditions of an adsorption chromatographic experiment is a joint law of the distribution of the variables R_η and M_i (where i corresponds to the adsorbing monomers). This distribution is directly represented by the corresponding chromatogram $F_2[t_a(R_\eta, M_i)]$. Mathematically we can change this classification and consider the variable M as a function of the variables t_a and R_η. Then the law of the distribution of M_i will be the joint distribution of the variables R_η and t_a.

This law is unknown and must be determined, that is, we have to determine the distributions according to the net weight of each ith kind of the adsorbing monomers of the investigated heterogeneous polymers. When it is found, the overall distribution of molecular weight and composition in the sample may be calculated.

THEORETICAL

The interrelationship between the three random variables t_a, R_η and M_i requires the application of conditional distribution laws[15]. From the experimental standpoint, the simplest determination of this law can be carried out for the variable t_a under the condition that R_η takes some fixed values. For this purpose, the heterogeneous sample should be divided into fractions with the aid of SEC. The average molecular size in each fraction will be R_η. Subsequently, each fraction will undergo chromatography on the same column (or a second identical column) but in another eluent, so that the conditions of LAC would be fulfilled successively for each component of the investigated heteropolymer. The family of chromatograms obtained in this manner gives the density of the conditional distribution function $F_2(t_a|R_\eta)$.

The law of joint distribution for a system of two random variables, t_a and R_η, may be expressed through the density of the distribution functions of these variables as follows:

$$F(t_a, R_\eta) = \tilde{F}_1(R_\eta)F_2(t_a/R_\eta) \quad (4)$$

Then the distribution law of a random variable M_i which may be regarded as a function of the values t_a and R_η is given by

$$W_i(M_i) = \frac{\partial}{\partial M_i}\int_{R_1}^{R_2} \mathrm{d}R_\eta \int_{t_0}^{t_a} \mathrm{d}t \cdot F(t,R_\eta) = \int_{R_1}^{R_2} \mathrm{d}R_\eta \cdot \frac{\partial t_a\,(M_i,\, R_\eta)}{\partial M_i} \cdot \tilde{F}_1(R_\eta)F_2(t_a|R_\eta) \quad (5)$$

The function $\tilde{F}_1(R_\eta)$ is obtained from the chromatogram $F_1(t_e)$ by replacing the variables according to the universal calibration of a given chromatographic system, and $W(M)$ is the function density of distribution according to the net weight of the adsorbing monomers.

Before determining the function $t_a = t_a\,(M_i, R_\eta)$, the following remark should be made. When an adsorption interaction between the polymer and the packing exists,it is not always possible to carry out chromatographic experiments under isocratic conditions even if the polymer–packing interaction energies are close to the critical value[10,13]. Hence, it is often necessary to use gradient chromatography[16]. Bearing this in mind, the function t_a (M_i, R_η) will be determined for the more general case of gradient conditions, whereas for isocratic conditions a particular form of this function will be obtained.

The coefficient of interphase distribution of macromolecules may be regarded as their principal characteristic. It is written as the ratio of solution concentrations in the stationary phase, C_{st}, and mobile phase, C_m, of the column and reflects the ratio of probabilities of the existence of molecules in each of these phases[14]:

$$K_d = \frac{C_{st}}{C_m} = \frac{v_m}{v_{st}} \cdot \frac{W_{st}}{W_m} \tag{6}$$

where W_{st} and W_m are the probabilities of the existence of molecules in the stationary and the mobile phases and v_m and v_{st} are the volumes of the mobile phase and stationary phase of the column, respectively. It is easy to show the correctness of eqn. 6, because the number of solute molecules distributed between the phases of the column is as follows:

$$\begin{aligned} n_m &= nW_m \\ n_{st} &= nW_{st} \\ n_m + n_{st} &= n \end{aligned} \tag{7}$$

where n_m and n_{st} are the numbers of solute molecules in the mobile and stationary phase, respectively, and n is the total number of the solute molecules in the column.

Under equilibrium conditions, each of these probabilities W_m and W_{st} is determined in a standard manner by the value of the free energy of the molecules G, the macromolecules being considered as independent thermodynamic systems:

$$W_m = \frac{v_m \exp\,(-G_m)}{v_m \exp\,(-G_m) + v_{st} \exp\,(-G_{st})} \tag{8a}$$

$$W_{st} = \frac{v_{st} \exp\,(-G_{st})}{v_m \exp\,(-G_m) + v_{st} \exp\,(-G_{st})} \tag{8b}$$

where G_m and G_{st} are in units of kT, where k is Boltzmann's constant and T is the absolute temperature.

As the concentration of the solute is $C_m = n_m/v_m$ in the mobile phase and $C_{st} = n_{st}/v_{st}$ in the stationary phase, we obtain for their ratio eqn. 6. Substituting expressions 8 into eqn. 6, we obtain the distribution coefficient K_d as uniquely given by the change in the free energy of macromolecules, $\Delta G = G_{st} - G_m$, during interphase transitions:

$$K_d = \exp\,[-(G_{st} - G_m)] = \exp\,[-\Delta G] \tag{9}$$

The value of ΔG is the sum of the entropy and enthalpy changes which the macromolecules undergo in the course of the chromatographic process:

$$\Delta G = \Delta H - T\,\Delta S \tag{10}$$

When the macromolecules enter the packing pores in the absence of energetic interactions, *i.e.*, in SEC, only the entropy changes. This leads to the following change in the free energy:

$$\Delta G_1 = -T\,\Delta S \tag{11}$$

In the same pores, when an energetic interaction exists (this occurs in LAC), the enthalpy, ΔH, changes simultaneously with the entropy. An additional change in entropy ΔS_2 related to the interaction also occurs. As a result, the interphase free energy of macromolecules undergoes an additional change by the quantity

$$\Delta G_2 = \Delta H - T\,\Delta S_2 \tag{12}$$

The corresponding total change in energy is given by the sum

$$\Delta G = \Delta G_1 + \Delta G_2 \tag{13}$$

Hence, the value of the distribution coefficient may be written as the product of two factors (see, *e.g.*, ref. 17):

$$K_d = \exp(-\Delta G_1)\exp(-\Delta G_2) \equiv k_A k_G \tag{14}$$

Here $k_G = \exp(-\Delta G_1)$ is the distribution coefficient under the conditions of SEC and $k_A = \exp(-\Delta G_2)$ reflects the addition to k_G related to adsorption interaction with the same packing.

In accordance with the adsorption theory of macromolecules[13,18], for homopolymers the value of ΔG_2 is proportional to molecular weight M. Therefore, we may assume that the change in free energy, ΔG_2, in adsorption chromatography is proportional to the net weight, M_i, of the units of the heteropolymer which can interact with the packing matrix. Hence, the fulfillment of the following approximate equality will be considered valid:

$$\Delta G_2 \approx \varepsilon M_i \tag{15}$$

where ε characterizes the value of the polymer–packing adsorption interaction in the given solvent from the standpoint of both enthalpy and entropy changes which the macromolecule undergoes during adsorption.

It is clear that the free energy, ΔG, of the macromolecules on transport into the pore can either increase or decrease. It will increase if the decrease in enthalpy is insufficient to compensate for the decrease in entropy, in this case we have $\Delta G > 0$ and $K_d < 1$. The free energy of the macromolecule will decrease when the decrease in enthalpy is greater than that in entropy; then we have $\Delta G < 0$ and $K_d > 1$. The former case should be classified as SEC complicated by weak adsorptions; the latter is proper LAC.

The situation in which the changes in enthalpy and entropy are equal and mutually compensating deserves particular attention. In this case $\Delta G = 0$ and $K_d = 1$. Hence, regardless of the ratio of the dimensions of the pore and the macromolecule, the latter can exist either in the pore or in the mobile phase with equal probability of an appropriate compensating enthalpy change occurring. This means that when appropriate conditions are chosen, a large macromolecule can enter a small pore, if it can be located in the pore, by changing its "geometry" and "creeping" along its walls. This case corresponds to so-called critical conditions and value ε designated by ε_{cr}.

In 1979, Skvortsov and Gorbunov[19] propounded the use of chromatography under critical conditions for the analysis of block copolymers. According to their idea, the chromatographic system for this purpose should be designed in such a manner that one component of the block copolymer undergoes chromatography by the SEC mechanism and the other component is chromatographed by the LAC mechanism at $\varepsilon = \varepsilon_{cr}$. As a result, the retention time for a block copolymer will be determined only by the size its components, which are not going to have any adsorption interaction with the packing.

Unfortunately, no experimental verification has appeared, perhaps because of the difficulty in obtaining reproducible chromatography under critical conditions. Therefore, in our work an approach is propounded in which the chromatographic experiments have to be carried out under LAC conditions when $|\varepsilon|$ is greater than $|\varepsilon_{cr}|$ but is close to the critical value.

In the general case with gradient chromatographic conditions, it is possible to vary the value of ε according to the linear law

$$\begin{aligned} \varepsilon(t_a) &= \varepsilon_0 - \beta t_a \quad \text{at} \quad t_a \leqslant \varepsilon_0/\beta \\ \varepsilon(t_a) &= 0 \quad \text{at} \quad t_a \geqslant \varepsilon_0/\beta \end{aligned} \tag{16}$$

where β is slope of this dependence and t_a is the retention time under the conditions of the adsorption chromatographic experiment.

Usually this variation of the value of ε is attained by variation of the solvent composition, in which the fraction of the more adsorption-active (with packing) component increases in the solvent with time. Simultaneously, this component of the solvent is chosen so that it is the stronger solvent for the adsorbing units of the heteropolymer. As a result, ε decreases and the rate of motion of the solute along the column increases:

$$U(t_a) = \frac{u_0}{1 + (v_{st}/v_m)K_d(t_a)} \tag{17}$$

where u_0 is the elution rate, and K_d varies according to following law:

$$\begin{aligned} K_d &= k_G \exp[\varepsilon_0 M_i - \beta M_i(t_a - \tau)] \quad \text{at} \quad t_a \leqslant (\varepsilon_0 - \varepsilon_{cr})/\beta + \tau \\ K_d &= k_G \exp(\varepsilon_{cr}) = 1 \quad \text{at} \quad t_a \geqslant (\varepsilon_0 - \varepsilon_{cr})/\beta + \tau \end{aligned} \tag{18}$$

where τ, the lag time, represents the delay between initiating the gradient and arrival of the gradient at the position of the solute in the column at time t_a.

We saw that macromolecules of any molecular weigh M may have a distribution coefficient K_d of unity if the energy of their adsorption interaction is equal to a critical value ε_{cr}. As the value of K_d for a solvent is also unity, then beginning with time $t_a = (\varepsilon_0 - \varepsilon_{cr})/\beta + \tau$, when $\varepsilon(t_a) = \varepsilon_{cr}$, both the solute and solvent begin to move with equal velocity $\bar{u}$:

$$\bar{u} = \frac{u_0}{1 + v_{st}/v_m} \tag{19}$$

For the lag time τ the following expression is correct:

$$\tau = \frac{1}{\bar{u}} \int_0^{t_a} u\,(t)\,\mathrm{d}t \tag{20}$$

where $u(t)$ is determined by eqns. 17 and 18.

Finally, using eqns. 16–20 and the universal calibration $R_\eta = f(t_e)$ in SEC, we can write the following equation connecting the values of the retention time t_a of a solute, the net weight of its adsorbing monomers M_i and the size of the macromolecules R_η:

$$L = \int_0^{t_a} u(t)\,\mathrm{d}t \tag{21}$$

where $L = u_0 t_0$ is the length of the column and t_0 is the column dead time.

A rigorous solution of eqn. 21 is possible only by computer. For an analytical solution, we use the well known approximate expression for retention time in gradient chromatography[20,21]. In our terminology we can write

$$t_a = \frac{1}{\beta M_i} \log\,[2.3\ k_G \beta M_i (t_1/t_0 - 1) \exp(\varepsilon_0 M_i) + 1] + t_e + \tau \tag{22}$$

where t_1 is the retention time in SEC for small molecules which corresponds to a value $K_d = 1$. In addition, we have $(t_1/t_0 - 1) = v_{st}/v_m$.

Eqn. 22 makes it possible to find the function $\partial t(M_i, R)/\partial M_i$ contained in the definition of the density of distribution $W_i(M_i)$ of the net weight of the adsorbing monomers in molecules of the investigated polymer:

$$\frac{\partial t(M_i,\ R_\eta)}{\partial M_i} = \frac{1}{\beta M_i} \left\{ \frac{\varepsilon_{0,i} M_i + 1}{M_i + 1/[2{,}3\ k_G \beta t_e\ \exp(\varepsilon_{0,i} M_i)\ (t_1 - t_0)/t_0]} - \frac{1}{M_i} \log\,[2.3\ k_G \beta t_e M_i \exp(\varepsilon_{0,i} M_i)\,(t_1 - t_0)/t_0 + 1] \right\} \tag{23}$$

Hence, the function of density $W_i(M_i)$ of a heteropolymer is given by

$$W_i(M_i) = \int_{t_a}^{t_0} F_1(t_e) F_{2i} \left(\frac{1}{\beta M_i} \log \left[2.3\, k_G \beta M_i \exp(\varepsilon_{0,i} M_i)\, (t_1/t_0 - 1)\, t_e + 1 \right] + t_e + \tau \,|\, f^{-1}(t_e) \right) \frac{\partial t(M_i, R_\eta)}{\partial M_i} \cdot \mathrm{d}t_e \qquad (24)$$

where F_1 (t_e) is the chromatogram of the sample investigated under SEC conditions, $F_{2,i}(t_a \,|\, R_\eta)$ is the family of chromatograms obtained in LAC for the fractions of the samples eluted in SEC at different fixed times t_e, $f^{-1}(t_e)$ is the function inverse to the universal calibration and $\varepsilon_{0,i}$ is the initial (at the initial moment $t = 0$) characteristic of the energy of interaction between the sorbent and the adsorbing component of the heteropolymer (denoted by subscript i).

The density of the distribution function W_j (M_j) of any other jth kind of adsorbing monomers of the heteropolymer is obtained in a similar manner (in this instance it is necessary to choose an eluent composition such that only the jth kind of monomers will be adsorbed on this packing). The density of the function of the random value M, which is the sum of independent random values M_i[15], is

$$W(M) = \frac{\partial}{\partial M} \int \underset{D}{\cdots} \int \prod_{i=1}^{n} W_i(M_i)\, \mathrm{d}M_i \qquad (25)$$

where D is the range of determination of the function $W_i(M_i)$.

In the case of a two-component copolymer, eqn. 25 becomes

$$W(M) = \frac{\partial}{\partial M} \int \int W_1\,(M_1)\; W_2\,(M_2)\, \mathrm{d}M_1\, \mathrm{d}M_2 = \int W_1\,(M - M_2)\; W_2\,(M_2)\, \mathrm{d}M_2 \qquad (26)$$

DISCUSSION

In order to carry out the above scheme of chromatographic analyses of heteropolymers, preliminary calibration experiments should be made with homopolymers of each of the monomer units contained in the heteropolymer under investigation. The purpose of these experiments is to determine the constants $\varepsilon_{0,i}$ characterizing the polymer–sorbent interaction. For this purpose, the sample of each (ith) homopolymer is first chromatographed under SEC conditions and separated into fractions (8–10 fractions) according to hydrodynamic size. Subsequently, each of these fractions undergoes chromatography under the appropriate LAC conditions with a suitable gradient (*e.g.*, a linear gradient with the coefficient β_i). In other words, for each ith component the functions $F_1(t_e)$ and $F_{2,i}$ (t_a, M_i) contained in eqn. 24 are found. Eqn. 24 is solved with a computer (*e.g.*, by the least-squares method) with respect to the parameter $\varepsilon_{0,i}$, completing the calibration. Now a similar procedure is carried out with the sample being analyzed. It is divided into fractions by SEC, and each fraction is eluted under the LAC conditions k times (according to the number of components of the heteropolymer). These conditions are varied each time precisely repeating those of the corresponding calibration on experiments.

The selection of adequate conditions for the gradient LAC of heteropolymers seems possible, according to the work of Mori[7,8]. The selection of adequate conditions includes the choice of packing, solvent and temperature. This choice is determined by the relationship of energetic interactions in the formation of four pairs of contacts: polymer–solvent, polymer–packing, packing–solvent and solvent–solvent. Contact of a polymer segment with the adsorption surface occurs only if it is energetically more favourable than the interactions of the polymer and the packing with the solvent. In this instance interactions with the solvent should be eliminated, *i.e.*, desolvation of the solvent from both the polymer segments and the adsorption sites of the packing should occur. The greater the preference of polymer adsorption on a given packing at a given temperature, the greater is the number of the adsorption sites that will not be occupied by contacts with the solvent, and the higher is the mean statistical value of the energy of interaction between a polymer segment and the packing. Therefore, by variation of solvent and temperature we can obtain either SEC or LAC for different components of an investigated heteropolymer.

The proposed method contains two assumptions. The first, as mentioned above, is the extension of eqn. 15 to heteropolymers, *i.e.*, the proportionality of the change in the free energy during the adsorption of macromolecules to the net weight of the units of monomer in the macromolecule interacting with the packing matrix. The second assumption is that under the same conditions the proportionality coefficient in eqn. 15 is identical for the heteropolymer and the corresponding homopolymer. In other words, it is assumed that the values of $\varepsilon_{0,i}$ are identical in the calibration and principal experiments. Here the possible change in the magnitude of adsorption interactions between the polymer unit and the packing and the related entropy losses due to the screening of this interaction by units of other components of the heteropolymer are neglected. This screening can lead to a decrease in the effective value of $\varepsilon_{0,i}$ in comparison with that determined in the calibration experiment. The validity of neglecting the possibility of screening the adsorption interaction must be subjected to further experimental tests.

It is clear that both assumptions are correct if the fraction of the adsorbing component of the heteropolymer is not very small in each its macromolecules. Otherwise, these assumptions can be checked by carrying out the chromatographic experiments with different model copolymers (block, graft, etc.). First conditions for SEC and LAC are chosen in these experiments for each homopolymer of the given copolymer, then values of ε are determined using eqns. 14 and 15 under conditions of isocratic chromatography. Subsequently, similar experiments are carried out with the model copolymer, and calculated values of ε should be compared. The same assumptions were also made implicitly by Skvortsov and Gorbunov[19].

CONCLUSIONS

The proposed method of quantitative analysis is suitable for heteropolymers of any structure, including block and random copolymers and polymers and oligomers with functional groups.

This method uses chromatography with one detector. However, it should be noted that the composition and the MWD of heteropolymers can be determined by using chromatography with two detectors, *e.g.*, a refractometer and a spectrophotometer, connected in series. The refractometer is sensitive to all components of the heteropolymer. The spectrophotometer should be tuned to the characteristic wavelength of one component only. The analysis of signals from both detectors permits the determination of the composition, the MWD and some other characteristics of the heteropolymer. Early work in this field was carried out by Anderson *et al.*[22] and Lechermeier *et al.*[23]. If we use a viscometer as the second detector we can determine the MWD of a heteropolymer by means of SEC using universal calibration according to Benoit, but for characterizing the composition of a heteropolymer one should use some additional speculations about the size of its macromolecules.

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