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COMMENTS ON THE THEORETICAL BASIS OF GEL PERMEATION CHROMATOGRAPHY OF POLYMERS*

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SUMMARY

Gel permeation chromatography of polymers is considered a particular type of elution chromatography. Principles of stochasticity and quasi-equilibrium of the process have been utilised, and gel structure has been taken into account. Expressions for probability of sorption and desorption of macromolecules have been obtained. Qualitative differences between the chromatography of polymers and that of low-molecular-weight substances are discussed. On this basis, the dependence of the elution volumes on hydrodynamic size of macromolecules, elution rate, concentration of solution, solvent choice and its temperature have been explained. These dependencies are in agreement with the results of the experiment. The possibility of universal calibration of the chromatograph was confirmed. An expression for dispersion was derived, and the skewing of the chromatographic curves was evaluated as a function of the non-equilibration of the process. The prospect of using gel chromatography as a method for investigating specific properties of macromolecules is discussed.

INTRODUCTION

In working out a theory for GPC of polymers one can proceed from the general concepts of elution dynamics of sorption and chromatography[1,2] modified in accordance with GPC. Special attention should be paid to the stochasticity of the chromatographic process and to its non-equilibrium.

RADUSHKEVITCH was the first to report the possibility of treating chromatography on the basis of a general theory of random processes[3]. Then TUNITSKY AND TCHERNEVA[4] found the distribution function of the particle coordinates with time with regard to the repeated acts of sorption and desorption.

Five years later GIDDINGS AND EYRING came to similar conclusions[5]. Their work was then summarized by MCQUARRIE[6] and quite recently it was applied to the GPC of polymers by CARMICHAEL[7].

Nevertheless, the stochastic approach developed earlier[1-7] does not completely

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describe the dynamics of the chromatographic process, its kinetic features and the peculiarities of the chromatography of macromolecules.

It is possible to fill in this gap if we describe the kinetics of the process by the equation

$$\frac{\partial C(x,t)}{\partial t} = \lambda C(x,t) - \lambda' C'(x,t) \qquad \text{(A)}$$

which is already known[8–10] but also includes as parameters the probabilities of sorption λ and desorption λ' of a macromolecule in unit time.

If the appropriate normalisation is made, the values $C(x,t)$ and $C'(x,t)$ may be considered as probabilities of the occurrence of a macromolecule in the mobile and stationary phases, respectively.

The linearity of the sorption isotherm used here is ensured by the peculiarities of GPC of polymers, where interaction between the eluted macromolecules as well as interaction between them and the sorbent (gel) matrix are absent. The connection between $C(x,t)$ and $C'(x,t)$ and their change with time are determined not only by the kinetic equation but also by the balance equation.

These equations* are valid at any moment for any sections of the column and permit the chromatographic process to be described as a stochastic one without considering the many single acts of sorption and desorption of macromolecules as it was done previously[4–7].

Eqn. A also describes the non-equilibrium of the chromatographic process which superimposes on random wanderings of the molecules their participation in diffusional flow which are continuously set up during the chromatographic process. Both stochasticity and non-equilibrium cause an extension of the "zone" of the eluted substance and hence a spreading of the chromatographic peak. Stochasticity produces spreading owing to the thermal movement of the molecules in each phase (mainly in the mobile phase) and also to stochastic exchange of molecules between the phases (mass exchange) and between capillary flow of the mobile phase (granulation effect). Non-equilibrium causes additional spreading of the chromatographic peak owing to its "curving"** and to the separation of the zone of the eluted substance into two regions. One of them (which belongs to the stationary phase) passes behind the other (belonging to the mobile phase).

THE CHROMATOGRAPHIC BEHAVIOUR OF MACROMOLECULES

The difference in chromatographic behaviour should be attributed to the peculiarities of the polymer solutions. It is known that these peculiarities are due to the fact that the structural units of these solutions are complex autonomous systems and the characteristics of the solution as a whole depend on their behaviour and properties. The sensitivity of the size of macromolecules to the solvent, the solution concentration, its temperature, elution velocity and gradient all have a particular effect on the chromatographic behaviour of polymer solutions. At present these

* A precise solution of this system of equations may be found, for example, in ref. 9.

** "Curving" of the chromatographic peak is understood as a certain mutual displacement of its maximum and mathematical prediction.

peculiarities have been detected in many experimental studies[11–15]. The proposed theoretical scheme interprets the above basic relationships of the chromatographic process in columns with a carrier consisting of gel or porous glass and evaluates them quantitatively.

The stochastic character of a chromatographic process allows it to be described by average probabilities of sorption λ and desorption λ' in a unit time in every elementary section of the column. The expression for λ is the product of $\tilde{\lambda}$ and $\tilde{\tilde{\lambda}}$. In equilibrium conditions $\tilde{\lambda}$ is the probability that the macromolecule reaches the sorbent surface in a unit time as a result of random wandering in the mobile phase. $\tilde{\tilde{\lambda}}$ is the probability that the molecule reaches this surface in front of an "accessible" (*i.e.* a comparatively large and "open") pore. An expression for $\tilde{\lambda}$ may be obtained, if we consider the solution of EINSTEIN-KHOLMOGOROV's differential equation[16,17]

$$\frac{\partial w(x,y,z,t)}{\partial t} = D\frac{\partial^2 w(x,y,z,t)}{\partial x^2} + \frac{\partial^2 w(x,y,z,t)}{\partial y^2} + \frac{\partial^2 w(x,y,z,t)}{\partial z^2} - u\frac{\partial w(x,y,z,t)}{\partial z} \tag{1}$$

under the conditions of chromatographic experiment:

$$\left\{\tilde{\tilde{\lambda}}\, w(x,y,z,t) + (1 - \tilde{\tilde{\lambda}})\left[\frac{\partial w(x,y,z,t)}{\partial x} + \frac{\partial w(x,y,z,t)}{\partial y}\right]\right\} = 0 \tag{2}$$

$$x^2 + y^2 = (r + a)^2$$

Here $w(x,y,z,t)\,dx$, dy, dz is the probability that at the moment t the macromolecule is located in the element of the mobile phase in the column which is determined by the coordinates x, y, z; u is the elution rate, r is the radius of the canals in the mobile phase, a is the depth of permeation into pores characteristic of every type of macromolecule (generally speaking, it is rate-dependent) beginnen from which a molecule may be considered as sorbed, *i.e.*, as entering a pore, and $\tilde{\tilde{\lambda}}$ and $(1 - \tilde{\tilde{\lambda}})$ are the probabilities of appearance of the "absorbing" and "reflecting" boundaries when the macromolecule reaches the sorbent surface.

The value of $\tilde{\lambda}$ inverse to the time corresponding to the maximum solution of eqn. (1) under condition (2) is given by:

$$\tilde{\lambda} = \frac{\zeta_1 D}{(r + a)^2} \exp\left[-\frac{u^2(r + a)^2}{\zeta_2 D^2}\right] \tag{3}$$

where D is the coefficient of translational diffusion of the macromolecules and ζ_1 and ζ_2 are empirical constants (of the order of magnitude 10 to 100) characterising this column and inserted instead of the theoretical values to compensate the inevitable approximation under boundary conditions.

Dependence of $\tilde{\lambda}$ on u obtained from (3) has the following physical meaning. According to Bernoulli's law, hydrostatic pressure and temperature are lower in liquid flow than in the stationary state, and the entropy is higher. This means that the molecules of the solution are partially polarised, their chaotic movement is weakened and the diffusion mobility decreases. This causes an immediate decrease in the sorption probability as compared to the stationary case. Evidently, this effect

is small for solutions of low molecular weight substances and increases sharply for solutions of large macromolecules (owing to low diffusion mobility of the latter). We will assume that $r \sim 10^{-4}$ cm, $u \sim 10^{-2}$ cm/sec, $D \sim 10^{-6}$ cm^2/sec. Then exp $[-u^2(r + a)^2/\zeta_2 D^2] \sim \exp[-0.01] \approx 1$.

In other words under the chosen conditions, examination of the dependence of $\tilde{\lambda}$ on the rate shows that it becomes high for macromolecules with coefficients of translational diffusion lower than 10^{-6} cm^2/sec.

The $\tilde{\tilde{\lambda}}$ value is proportional to the ratio of the surface areas S^{acc} of the entrance openings of the "accessible" pores to the surface area Sg of the whole external gel surface:

$$\tilde{\tilde{\lambda}} \sim S^{acc}/S_g \tag{4}$$

The value S^{acc} may be determined by analogy with ref. 18:

$$S^{acc} = S_p - \int_0^{\infty} d(\bar{\beta}\bar{H}^2) W(\bar{\beta}\bar{H}^2) \int_0^{\bar{\beta}\bar{H}^2} \Psi(s) ds. \tag{5}$$

Here $\bar{H}$ is the distance between the furthest chain segments equal to $\bar{H} = 1{,}4(\bar{h}^2)^{1/2}$ where $(\bar{h}^2)^{1/2}$ is the mean end-to-end distance, $\Psi(s)$ is the density function of the pore distribution by the areas of their entrance openings, β is the empirical constant characterizing the pore geometry, and $W(\bar{\beta}\bar{H}^2)$ is the density distribution function by size for macromolecules of the given molecular weight.

The $\tilde{\tilde{\lambda}}$ value also depends on the elution rate, but for a reason different from that for $\tilde{\lambda}$. The fact is that the macromolecules when flowing are oriented in the direction of the flow rate and are deformed under the action of the stretching and compressing forces of viscous origin connected with the existence of the gradient rate. For real solutions a viscosity value different from zero always produces a transverse velocity gradient in the laminar flow inside the capillary canal. Inhomogeneous packing of the column which is manifested especially on "aging", when the lower part of the column is more densely packed than the upper part, causes the appearance of the longitudinal rate gradient. The results obtained by TAKSERMAN-KROZER[19] permit the evaluation of the deformation of macromolecules in the fields of the transverse and longitudinal rate gradients as follows:

(a) longitudinal gradient field:

$$(\bar{h}^2)_{\parallel} = \frac{3(1-\gamma)}{2(1+\gamma)(1-2\gamma)} \bar{h}_0^2; \tag{6}$$

(b) transverse gradient field:

$$(\bar{h}^2)_{\perp} = \frac{3+2\beta^2}{2} \bar{h}_0^2; \tag{6'}$$

(c) mixed gradient field:

$$(\bar{h}^2)_{\parallel+\perp} = \frac{1}{1+\gamma} \cdot \bar{h}_0^2 + \frac{4-4\gamma+3\gamma^2+8\beta^2}{2(2-\gamma)(1-\gamma-2\gamma^2)} \bar{h}_0^2. \tag{6''}$$

Here $\bar{h}$ is the most probable size of the undeformed macromolecule and $(\bar{h}^2)^{1/2}$ is the mean square size of the deformed macromolecule, β and γ are universal parameters determining the distribution function of macromolecular units in the laminar flow: $\beta = g_x\bar{h}^2/6D$, $\gamma = g_z\bar{h}^2/6D$, here D is the coefficient of molecular diffusion, g_x and g_z are the transverse and longitudinal components of the rate gradient, respectively.

For reasons of simplicity, in the expressions for $(\bar{h}^2)''$, $(\bar{h}^2)'$ and $(\bar{h}^2)''^{+'}$ the rigidity of the macromolecules is neglected. Therefore it should be noted here that macromolecules of the same size but differing in rigidity behave differently with the same flow. In principle this may lead to their chromatographic separation.

Stretching of macromolecules in the gradient flow and their orientation along it results in a decrease in the number of pores accessible for macromolecules according to the size of their entrance pores. In other words, the value of S^{acc} (and λ as well) decreases owing to an increase in the upper limit of the integral in eqn. 5.

It is possible to demonstrate that both the transverse and the longitudinal rate gradients are proportional to the rate itself. For example, in the case of a transverse gradient we have:

$$\frac{du}{dr} = u\frac{2r}{R^2 - r^2},$$

where R is the width of the canal, r is the distance from its axis.

For macromolecules of $\sim 10^3$ Å in size the deformation in the transverse direction under conditions of gel chromatography leads to an increase in size by approximately 10 %.

If we multiply $\tilde{\lambda}$ by $\tilde{\tilde{\lambda}}$ and introduce for every rate the effective areas of the pore entrances:

$$S_{eff}^{acc} = S^{acc} \exp\left[-u^2(r + a)^2/\zeta_2 D^2\right],$$

where S^{acc} is written taking into account the deformation of macromolecules, we have for λ:

$$\lambda = \tilde{\lambda} \cdot \tilde{\tilde{\lambda}} = \zeta_1 D/(r + a)^2 \cdot (S_{eff}^{acc}/S_g). \tag{7}$$

The connection between λ and λ' may be found from the quasi-equilibrium of the chromatographic process*.

$$\mu'[C^{acc}(z,t)] = \mu[C(z + z,t)] \tag{8}$$

where C and C^{acc}, μ and μ' are the concentrations and chemical potentials of macromolecules in the mobile phase in the effective "accessible" volume V_{eff}^{acc} of the stationary phase. In this case, $V_{eff}^{acc} = V^{acc} \exp\left[-u^2(r + a)^2/\zeta_2 D^2\right]$ and V^{acc} is the total volume of pores accessible to macromolecules of a certain type:

$$V^{acc} = V_p - \int_0^{\infty} d(\bar{\alpha}\bar{H}^3)\tilde{W}(\bar{\alpha}\bar{H}^3)\int_0^{\bar{\alpha}\bar{H}^3} \varphi(v)\,dv^{**}$$

* We call the process a quasi-equilibrium one if its deviation from the equilibrium process is small and may be described by the equation: $\mu'(z) = \mu(z) + \Delta\mu(z) \approx \mu(z + \Delta z,t)$, where Δz is a small increment of the coordinate.

** It should be noted that our definition of V^{acc} differs from that of DE VRIES[18]. It cannot be inferred from the DE VRIES' definition of V^{acc} that it is possible to separate monotonously macromolecules by their molecular weight on macroporous adsorbents with narrow pore distribution. Nevertheless this monotonous separation is observed[20]. This possibility is evident from our formula.

Here $\tilde{W}(\bar{\alpha}\bar{H}^3)$ is the density distribution function by volume for macromolecules of the given molecular weight, $\bar{H}$ determines the maximum size of the deformed macromolecules, as above, $\varphi(v)$ is the density function of pore distribution by volume, $\bar{\alpha}$ is a geometric constant. Expanding the right-hand part of eqn. 8 into a series and comparing the result obtained with the equation of the sorption kinetics

$$V_p \frac{\partial C'}{\partial t} = V_0 \lambda C - V_p \lambda' C' \tag{9}$$

which can be written, however, in the form (9') (for equilibrium concentrations C and C' of macromolecules in each phase with volumes V_0 and V_p)

$$V_0 \lambda C(z + \Delta z, t) - V_p \lambda' C'(z,t) = 0, \tag{9'}$$

we find:

$$\frac{\lambda}{\lambda'} = \frac{\gamma}{\gamma'} \cdot \frac{V_{\text{eff}}^{\text{acc}}}{V_p} \exp\left[-\frac{\Delta\mu^\circ}{RT}\right], \tag{10}$$

where γ and γ', μ° and $\mu^{\circ\prime}$ are the activity coefficients and standard chemical potentials in the respective phases.

It is possible to show that the desorption probability is the inverse value of the "lag time" $\tau = \Delta z/u$ which characterises the degree of the non-equilibrium of the process[21].

If we consider eqn. 10 together with the balance equation:

$$\frac{\partial C}{\partial t} = D^* \frac{\partial^2 C}{\partial z^2} - u \frac{\partial C}{\partial z} - \frac{V_p \partial C}{V_0 \partial t} \tag{11}$$

(where D^* is the effective quasidiffusional coefficient[11] characterising the deviation of the actual velocities of macromolecules in the mobile phase from the elution rate u), we obtain an integrodifferential equation completely characterising the process occurring in the chromatographic column filled with a porous sorbent (gel):

$$\frac{\partial C}{\partial t} = D^* \frac{\partial^2 C}{\partial z^2} - u \frac{\partial C}{\partial z} - \lambda C + \lambda\lambda' \int_0^t C(z,t') \cdot \exp\left[\lambda'(t' - t)\right] \mathrm{d}t'. \tag{12}$$

The analysis of the solution of eqn. 12 and of its mathematical prediction, dispersion and skewing under initial and boundary conditions of the elution dynamics of sorption[22] shows that the chromatographic peak does not have a Gaussian shape. The mutual displacement of its maximum and mathematical prediction depends on the degree of the non-equilibrium of the process and is a function of the elution rate,

the gel "porosity" and "geometry", hydrodynamic volumes of the eluted macromolecules and their diffusion mobility.

Eqn. 12 gives the following expressions for the first three moments:

$$\bar{z} = \frac{V_0 u\tau}{V_0 + K_d V_p} + \frac{2K_d V_p}{V_0 + K_d V_p}\tau \qquad \text{(a)}$$

$$u^2\overline{(t-\bar{t})^2} = \sigma^2 \approx \frac{2D^* z}{u}(1 + K_d V_p/V_0)^2 + (2uzK_d V_p/V_0)\tau, \qquad \text{(b)}$$

$$u^3\overline{(t-\bar{t})^3} \approx \sigma u^2 z K_d \frac{V_p}{V_0}\tau^2 + 12D^* z K_d \frac{V_p}{V_0}\left(1 + K_d\frac{V_p}{V_0}\right)\tau + \left(1 + K_d\frac{V_p}{V_0}\right)^3 \times \frac{12D^{*2}z}{u^2} \qquad \text{(c)}$$

The occurrence of the term $+ [(2K_d V_p/V_0)/(1 + K_d V_p/V_0)]\tau$ in (a) means that the non-equilibrium of the process leads to faster elution of the substance from the column as compared to the equilibrium case.

The occurrence of the term $(2uzK_d V_p/V_0)\tau$ in (b) reflects the effect of the non-equilibrium of the process on the width of the chromatographic peak: the peak width increases with τ.

It follows from (c) that the degree of peak asymmetry which is characterised by the ratio:

$$\sqrt[3]{\overline{(t-\bar{t})^3}}/\sqrt{\overline{(t-\bar{t})^2}} \sim \tau^{1/6}$$

also increases with τ.

The retardation in the equilibration between the phases produces an additional transfer of substance from one phase into another owing to the diffusional flow. The value of the latter is determined by the difference in the chemical potential of the components in each phase at the given moment.

These diffusional flows control the exchange of macromolecules between the phases, make it more active and, consequently, the terms proportional to τ appear in the expressions (a, b, c). Naturally, the distribution coefficient K_d also depends on τ. And, indeed, expanding the right-hand part of (8) into a series and limiting ourselves to the two first terms of this expansion, we may obtain the following expression for the distribution coefficient using the equation of the sorption kinetics (9):

$$K_d(z,t) = \frac{\lambda}{\lambda'}\frac{V_p}{V_0}\left[1 + \frac{1}{C}\left(\frac{\partial C(z,t)}{\partial z}u\tau + \frac{\partial^2 C(z,t)}{\partial z^2}\frac{u^2\tau^2}{2}\right)\right] \qquad (13)$$

where λ and λ' are the equilibrium probabilities of sorption and desorption in a unit time. At the peak maximum this expression is simplified:

$$K_d^{\max.}(z,t) = \frac{\lambda}{\lambda'}\frac{V_p}{V_0}\left[1 + \frac{1}{C}\frac{\partial^2 C(z,t)}{\partial z^2}\frac{u^2\tau^2}{2}\right] < \frac{\lambda}{\lambda'}\frac{V_p}{V_0} \qquad (13')$$

and if we neglect the difference between the shape of this peak and the Gaussian one, it is easy to obtain

$$V_e \approx V_0 + V_p \frac{\lambda}{\lambda'} \left(1 - \frac{u^2\tau^2}{\sigma^2}\right) \tag{14}$$

where

$$\frac{V_p}{V_0} \frac{\lambda}{\lambda'} \left(1 - \frac{u^2\tau^2}{\sigma^2}\right) \equiv K_{d\,\text{gauss}}^{\text{max.}} \tag{14'}$$

Thus the distribution coefficient at the maximum of the chromatographic peak (*i.e.* a certain average distribution coefficient) is the lower, the higher is the deviation of the process from the equilibrium conditions. This behaviour of K_d agrees well with the expression for the mathematical definition (a) (which includes the equilibrium distribution coefficient).

It also follows from eqn. 14 that the non-equilibrium of the chromatographic process is one of the reasons of its dependence on the elution rate u (in addition to the dependences considered earlier).

It is interesting to note that the increase in the elution rate in this case (as well as with the effects considered above) also leads to a decrease in the elution volume. Furthermore, this dependence is more appreciable for large macromolecules (τ is high) than for small ones.

The absence of the evident dependence of mathematical expression on the elution rate in (a) suggests that τ should be rate dependent. And, indeed, using eqn. 14 it is easy to show that τ is proportional to the width of the zone of the eluted substance and is inversely proportional to the elution rate:

$$\tau \sim \sigma/u$$

A peculiar feature of gel chromatography of polymers is the dependence of the results obtained on the concentration of the solution. This dependence may be explained as follows. Firstly, the size of macromolecules in solution is very sensitive to concentration. The interaction between the chain segments and between the segments and the solvent molecules lead to effective repulsion between the segments. Owing to this, macromolecules in solution swell and their size increases. On the other hand, mutual repulsion of macromolecules decreases their size. It is clear that the latter effect mainly depends on the concentration of the solution. Quantitatively it is expressed as follows[23]:

$$\alpha = \alpha_0(1 - \varkappa_1 C - \varkappa_2 C^2 - \cdots), \tag{15}$$

where $\varkappa_1 = A_2 M h_1(\alpha_0)$, $\varkappa_2 = A_3 M h_2(\alpha_0)$.

Here C is the polymer concentration in solution, α_0 is the swelling coefficient of macromolecules in a given solvent, α is its value if the concentration effect is taken into account, M is the molecular weight of the polymer, A_2 and A_3 are the second and the third virial coefficients, respectively, which were calculated on paper[23] as well as the functions $h_1(\alpha_0)$ and $h_2(\alpha_0)$. Since $\alpha/\alpha_0 = (\bar{h}/\bar{h}_0)^3$, where $\bar{h}_0$ characterises the size of macromolecules without taking into account the concentration effect, we have

$$\bar{h} \approx \bar{h}_0(1 - \varkappa_1 C - \varkappa_2 C^2)^{1/3}.$$

Inserting the value of $\bar{h}$ obtained in this manner into the expression for S^{acc} and V^{acc}, we will find the increase in these values, and also in the values of λ, K_d and V_e, with an increase in concentration. For a multi-component solution the expressions for the $\varkappa$ coefficients become somewhat more complicated owing to the interaction between the macromolecules of different fractions. However, the overall trend of the concentration dependence remains unchanged.

The influence of the solution concentration upon the course of the chromatographic process is not limited to the above effect. In the case of a multi-component solution osmotic pressure operates between the column phases. Moreover, it was found that macromolecules of different fractions can displace each other from the phase in which they are located. On the whole, this produces an increase in the distribution coefficient of every macromolecular fraction and, hence, an increase in the corresponding elution volumes. In the case of a solution containing two narrow macromolecular fractions the following equations are valid for incompressible macromolecules such as protein molecules:

$$K_{d_1} \approx \frac{V_1^{acc}}{V_p} + V_{A_{1,2}} C_2 \frac{V_1^{acc} + V_2^{acc}}{V_p}; \qquad (16)$$

$$K_{d_2} \approx V_2^{acc}/V_p$$

where indices 1 and 2 are numbers of fractions, v is the molar volume of the solvent, $A_{1/2}$ is the first virial coefficient. The fraction with index 1 consists of molecules smaller than those in fraction 2.

It should be noted that the compressibility of the molecules makes them more liable to enter pores since after compression a macromolecule may penetrate a pore which is smaller than the macromolecule itself. Naturally, this increases the number of accessible pores, their total volume $\tilde{V}^{acc}$ and the distribution coefficient. Thus we have: $\tilde{V}^{acc} = V^{acc} + \Delta V^{acc}$,

$$\Delta V^{acc} = \int_0^\infty dV \tilde{W}_V \int_0^V \varphi(V') \exp\left[-\frac{\Delta F(V) - \Delta F(V')}{RT} \cdot \frac{V'}{CMv}\right] dV' \qquad (17)$$

where $\Delta F(V) - \Delta F(V')$ is the difference in free energies of macromolecules in each phase written as a function of the difference in their size V and V' in these phases, M is the molecular weight of the polymer, C is its concentration and v is the molar volume of the solvent.

Eqn. 17 partially reflects the effect of the solution temperature on the course of the process. The size of macromolecules also depends on temperature[24]:

$$\alpha^5 - \alpha^3 = 2C_m \Psi_1 \left(1 - \frac{\theta}{T}\right) M^{1/2} \qquad (18)$$

Here θ is the critical temperature, Ψ_1, and C_m are constants.

This size increases with temperature, then decreases and then again increases. When the solution temperature is higher than the room temperature, the size of macromolecules increases (as a rule) and their elution volumes decrease.

In conclusion, it should be noted that the development of theoretical concepts in gel chromatography of polymers dates from the work of DE VRIES[18] who demonstrated the dependence of the elution volume on the size ratio of the macromolecules and gel pores. YAU *et al.*[14] then paid attention to the important role of diffusion in the chromatographic process and CARMICHAEL[7] considered its stochastic relationships.

An important feature of our approach is a joint consideration of all the above factors. It became clear that the main factors determining the chromatographic behaviour of macromolecules are their size and diffusion mobility. Moreover, the dependence of the chromatographic separation on the diffusion mobility becomes important only for large macromolecules ($D \sim 10^{-6}$–10^{-7} cm^2/sec). Consequently, the size of macromolecules is the characteristic determining their separation over a broad range of molecular weights (up to 10^5). Thus, it becomes evident that it is possible (and even advisable) to calibrate a gel chromatograph according to the hydrodynamic size of macromolecules in the region of rather low molecular weights. In the region of very large macromolecules the calibration should take into account the molecular diffusion coefficients. The occurrence of two such regions naturally accounts for the existence of two calibration formulae according to molecular weights, *i.e.* MOORE's and MEYERHOFF's formulae.

As an example of the practical application of the above theoretical scheme the following possibilities can be pointed out: the possibility of calculating the shape of the chromatographic peak, the determination of the optimum performance of the chromatograph and the possibility of separating macromolecules according to their specific properties, such as rigidity. This opens up wide possibilities of using gel chromatography for a thorough investigation of polymers.

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