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THEORY OF CHROMATOGRAPHY OF RIGID ROD-LIKE MACROMOLECULES ON HYDROXYAPATITE COLUMNS

VIII. FURTHER THEORY OF ANALYTICAL CALCULATION OF CHROMATOGRAMS AND FURTHER INVESTIGATIONS ON THE RESOLVING POWER OF THE COLUMNS

TSUTOMU KAWASAKI
6-31-17 Seijo, Setagaya-ku, Tokyo (Japan)
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SUMMARY

Theoretical chromatograms for mixtures of rigid rod-like macromolecules with the same dimensions and the same shape but with different adsorption energies have been calculated analytically for the case when the slope of the linear gradient of the competing ion is small enough. Account has been taken of the fact that the number, x', of sites of hydroxyapatite covered by an adsorbed molecule has a finite value. In Part VII of this series, it was shown that high resolutions can generally be obtained by using a small slope of the gradient. In this paper, we show that the resolving power of the column cannot be improved further if the slope of the gradient of the competing ion is already small enough.

INTRODUCTION

Earlier in this series[1–7], in Part VI[6], we calculated theoretical chromatograms for a mixture of rigid rod-like macromolecules when the elution is carried out with a linear gradient of the competing ion through an analytical method, taking into account the fact that there are repulsive interactions among molecules adsorbed side by side on the surface of hydroxyapatite (HA)[1]. This calculation, however, involves the assumption that, as a macromolecule has large dimensions, an adsorbed molecule covers virtually an infinite number, x', of sites on HA and that the number, x, of adsorption groups of the molecule that react with the crystal sites is also infinite. According to this assumption, the shape of the chromatogram is a right-angled triangle and both the shape of and the elution position on the chromatogram, provided the activity of the competing ion is plotted on the abscissa, are independent of the slope of the gradient (s.o.g.) of the ion. They are also independent of the column length for small loads and these theoretical results explain, at least approximately, the actual chromatograms of DNA (see, for instance, ref. 8). In earlier work[9], it was shown that the elution molarity of T_2 phage, a very large particle with molecular weight *ca.*

$2.6 \cdot 10^8$, is independent of both the column length and the s.o.g. when a small amount of the sample is loaded (see Fig. 1E in ref. 9). The width (as a function of the molarity of the competing ion) of the chromatogram of T_2 phage, however, depends on the s.o.g. (see Fig. 9 in ref. 9), which perhaps is due to such types of molecular diffusion and heterogeneity in flow-rate in the column section as were considered in ref. 10. These effects are negligible when the experimental conditions considered in this paper are used (see Discussion).

In Part VII[7], calculations of theoretical chromatograms were carried out, taking into account of the fact that a macromolecule has finite dimensions. The resolving power of the column for several types of mixtures obtained under different chromatographic conditions (s.o.g., column length and load) was discussed. However, all calculations were performed only numerically and they involved a rough approximation (see Fig. 5 in ref. 7).

In this paper, in order to obtain more precise informations on the resolving power of the column than in ref. 7, we attempt to calculate the theoretical chromatograms of model molecules with finite dimensions through an analytical method. In order to simplify the calculation, however, we treat only the case of a mixture of rod-like molecules with the same dimensions and the same shape but with different adsorption energies. We also treat the case when the s.o.g. is very small (see Theoretical), which, according to the theoretical considerations in ref. 7, must be the experimental conditions that give the maximum resolving power to the column. In an earlier paper[11], it was shown experimentally that, for tropocollagen (heterogeneous molecules homogeneous both in shape and in dimensions (see refs. 3, 4 and 11), the resolution of the column increases markedly with a decrease in the s.o.g. (see Figs. 1, 2 and 6 in ref. 11).

On the other hand, a series of theoretical investigations on HA chromatography with small loads were carried out[10,12,13] and the experimental parameters such as the number, x', of crystal sites covered by an adsorbed molecule and the adsorption energy per molecule were estimated by the analysis of experimental data[12,13]. For small loads, the mutual interaction among macromolecules is negligible and the development of a component in the mixture is carried out independently of the presence of the other components on the column, while in Parts I–VII of this series[1–7] and in this paper, we treat the case when a large amount of macromolecules with large dimensions are loaded. In this case, the resolution of the column is amplified by the repulsive interaction among macromolecules adsorbed on the crystal surfaces or by the mutual displacement effect, which means that the maximum resolution can generally be achieved with a heavy load*.

THEORETICAL

It can be understood, by following the same procedure as for the derivation of eqn. 19 or 19a in ref. 10 that, when the s.o.g. is so small that the total volume of the eluent in which macromolecules are involved is much larger than the total inter-

* When the sample is heterogeneous in both molecular length and adsorption energy per unit molecular length, the maximum resolution is not always achieved with a heavy load (see ref. 7).

stitial volume of the column, the evolution of a mixture of molecular species 1, 2, $\cdots$, ϱ', $\cdots$, ϱ on the column can be expressed by the simultaneous differential equations:

$$-\left\{\frac{\mathrm{d}\left[\frac{B_{(\varrho')}}{1-B_{(\varrho')}}\cdot\chi_{(\varrho')}\right]}{\mathrm{d}V}+\frac{\mathrm{d}\chi_{(\varrho')}}{\mathrm{d}V}\right\}=\frac{B_{(\varrho')}}{1-B_{(\varrho')}}\cdot\chi_{(\varrho')} \tag{1}$$

($\varrho' = 1, 2, \cdots, \varrho$) where $B_{(\varrho')}$ is a measure of the proportion of species ϱ' that exist in solution; $\chi_{(\varrho')}$ is the relative amount of species ϱ' adsorbed on the crystal surfaces in the whole column, being unity when all effective surfaces of the crystals are saturated only by species ϱ'; and V is the elution volume measured in such units that the total interstitial volume of the column is unity. It should be noted that the right-hand side of eqn. 1 shows the amount of species ϱ' in solution and that the first and second terms on the left-hand side show the changes in the amounts in solution and in the adsorbed phase, respectively. However, when the molecule is large enough for the transition of the value of $B_{(\varrho')}$ from 0 to 1 to occur virtually stepwise with an increase in the parameter χ [see eqn. 6; see also Figs. 1, 2 and 3 in ref. 7, in which $G_{(\varrho')} = 1 - B_{(\varrho')}$], then it could be considered that the evolution of molecules on the column is carried out only due to the decrease in the amount of adsorbed molecules that occurs caused by the increase in the activity of the competing ion. This can be understood if it is recalled that, provided the dimensions of the molecule are infinite, the value of χ at which there is the transition in $B_{(\varrho')}$ gives the value of $\chi'_{(\varrho')}$ defined by eqn. 69 in Part II[2] or the maximum possible value of χ that can be realized, at a given activity of the competing ion, by the molecules with an adsorption energy less than or equal to the energy of species ϱ' and that the value of $\chi'_{(\varrho')}$ is not influenced by molecules in solution (see ref. 7; see also the footnote on p. 274 in ref. 10). Hence, eqn. 1 reduces to

$$-\frac{\mathrm{d}\chi_{(\varrho')}}{\mathrm{d}V}=\frac{B_{(\varrho')}}{1-B_{(\varrho')}}\cdot\chi_{(\varrho')} \tag{2}$$

($\varrho' = 1, 2, \cdots, \varrho$), which is the equation already proposed as eqn. 16 in ref. 6.

When the elution is carried out with a linear gradient of the competing ion, it is convenient to rewrite eqn. 2 by using, instead of V, the relative activity, y, of the competing ion defined by eqn. 58 in ref. 2 and by introducing a parameter that indicates the value of the s.o.g.:

$$\eta=\frac{\mathrm{d}y}{\mathrm{d}V} \tag{3}$$

as

$$-\frac{\mathrm{d}\chi_{(\varrho')}}{\mathrm{d}y}=\frac{1}{\eta}\cdot\frac{B_{(\varrho')}}{1-B_{(\varrho')}}\cdot\chi_{(\varrho')} \tag{4}$$

($\varrho' = 1, 2, \cdots, \varrho$), where η is constant for a linear s.o.g., and it should be noted that the right-hand side of eqn. 4 expresses the amount of species ϱ' in solution in the column interstices when the activity of the competing ion is y and that it can be considered as the contribution of species ϱ' [denoted by $f_{(\varrho')}$] to the total chromatogram. Hence, we can write

$$f_{(\varrho')}(y)=-\frac{\mathrm{d}\chi_{(\varrho')}}{\mathrm{d}y}=\frac{1}{\eta}\cdot\frac{B_{(\varrho')}}{1-B_{(\varrho')}}\cdot\chi_{(\varrho')} \tag{5}$$

Now, in order to solve the simultaneous equations in eqn. 4, we introduce the parameter

$$\chi = \sum_{\varrho''=1}^{\varrho} \chi_{(\varrho'')} \tag{6}$$

in which the maximum value of χ is unity. It was mentioned in ref. 7 that the parameter $B_{(\varrho')}$ can be considered as a function of χ when all molecules have the same dimensions and the same shape. In this case, by using eqn. 3 in ref. 7 [in which $G_{(\varrho')} = 1 - B_{(\varrho')}$] and the third equation on p. 278 in ref. 7 for the approximate expression of the second term of the denominator of the right-hand side of eqn. 3 in ref. 7, we have

$$\frac{B_{(\varrho')}}{1 - B_{(\varrho')}} = [\beta_3 \sigma p(\chi)]^{-1} \cdot \exp\left\{\frac{u_{(1)}}{kT} [w_{(\varrho')} - y - \Xi \sqrt{\chi}]\right\} \tag{7}$$

where β_3 is a parameter related only to the properties of the column; σ is the symmetry factor of the molecule (assumed to be the same for all molecules); $u_{(1)}$ [$= -x_{(1)} \varepsilon_3 = -x'_{(1)} \xi_{(1)} \varepsilon_3$; see ref. 2] is the adsorption energy per molecule of species 1 provided that it is adsorbed in the isolated state; $w_{(\varrho')}$ [$= \xi_{(\varrho')}/\xi_{(1)} = u_{(\varrho')}/u_{(1)}$; see eqn. A3 in Appendix II in ref. 6 and eqn. 11 in ref. 2] is the adsorption energy of species ϱ' measured in the units of energy of species 1; Ξ is a parameter with a positive value that describes the repulsive interactions among adsorbed macromolecules (see Results of calculations of chromatograms); and $p(\chi)$ is the probability that, when a new molecule is added at random on to the crystal surface, the proportion χ of which is already occupied by molecules, it is not superimposed on the already adsorbed molecules (see ref. 1). $p(\chi)$ can be expressed approximately as

$$p(\chi) \approx 1 - \chi \tag{8}$$

If χ is expressed as a function of only $\chi_{(\varrho')}$, where ϱ' indicates a particular value of 1, 2, $\cdots$, ϱ, then each equation in eqn. 4 can be integrated independently. For this purpose, if an equation for species ϱ'' in eqn. 4 is divided by another for species ϱ', then

$$\frac{\mathrm{d} \log \chi_{(\varrho'')}}{\mathrm{d} \log \chi_{(\varrho')}} = E_{(\varrho', \varrho'')} \tag{9}$$

is obtained where

$$E_{(\varrho', \varrho'')} = \exp\left\{-\frac{u_{(1)}}{kT} \cdot [w_{(\varrho')} - w_{(\varrho'')}]\right\} \tag{10}$$

Eqn. 9 can easily be integrated to give

$$\chi_{(\varrho'')} = \chi^*_{(\varrho'')} \cdot \left[\frac{\chi_{(\varrho')}}{\chi^*_{(\varrho')}}\right]^{E_{(\varrho', \varrho'')}} \tag{11}$$

where $\chi^*_{(\varrho')}$ and $\chi^*_{(\varrho'')}$ are the initial values of $\chi_{(\varrho')}$ and $\chi_{(\varrho'')}$, respectively. Hence, by using eqn. 6, χ can be expressed as a function of $\chi_{(\varrho')}$ as

$$\chi = \sum_{\varrho''=1}^{\varrho} \chi^*_{(\varrho'')} \cdot \left[\frac{\chi_{(\varrho')}}{\chi^*_{(\varrho')}}\right]^{E_{(\varrho', \varrho'')}} \tag{12}$$

where, for convenience, we assign the values 1, 2, $\cdots$, ϱ to ϱ' and ϱ'', following the decrease in the absolute value of the adsorption energy of the molecule. It should be noted that, if the absolute value of $-u_{(1)}/kT \cdot [w_{(\varrho')} - w_{(\varrho'')}]$ is large for any pair of different ϱ' and ϱ'', then the value of $E_{(\varrho',\varrho'')}$ is either zero or infinity when $\varrho' \neq \varrho''$, and that $E_{(\varrho',\varrho'')}$ is unity if $\varrho' = \varrho''$ (see eqn. 10). Hence eqn. 12 reduces to

$$\chi = \chi_{(\varrho')} + \sum_{\varrho''=1}^{\varrho'-1} \chi^*_{(\varrho'')} \tag{13}$$

because, in general, $\chi_{(\varrho')}/\chi^*_{(\varrho')} \leqslant 1$*. Eqn. 13 means that a molecule with a large absolute value of the adsorption energy is adsorbed in preference to a molecule with a smaller adsorption energy, which is the case treated in Parts I–VI[1–6].

Now, introducing eqn. 7 into eqn. 4, each equation in eqn. 4 can be integrated independently as

$$\int_{y^*}^{y} \exp\left[-\frac{u_{(1)}}{kT}\, y\right] \mathrm{d}y = \beta_3\, \sigma\, \eta \cdot \exp\left[-\frac{u_{(1)}\, w_{(\varrho')}}{kT}\right] \cdot \int_{\chi_{(\varrho')}}^{\chi^*_{(\varrho')}} \chi^{-1}{}_{(\varrho')} \cdot p(\chi) \cdot \exp\left[\frac{u_{(1)}}{kT} \cdot \Xi \cdot \sqrt{\chi}\right] \mathrm{d}\chi_{(\varrho')} \tag{14}$$

where y^* is the initial value of y, virtually equal to zero in many experiments. As the value of $-u_{(1)}/kT$ is large for a "retained" molecule (see Appendix I in ref. 1), the left-hand side of eqn. 14 becomes

$$\int_{y^*}^{y} \exp\left[-\frac{u_{(1)}}{kT}\, y\right] \mathrm{d}y \approx -\frac{1}{u_{(1)}/kT} \cdot \exp\left[-\frac{u_{(1)}}{kT}\, y\right] \tag{15}$$

In order to calculate another integral in the right-hand side of eqn. 14, it should be noted that, as $-u_{(1)}/kT$ is large, the term $\exp[u_{(1)}/kT \cdot \Xi \cdot \sqrt{\chi}]$ decreases very rapidly with an increase in $\chi_{(\varrho')}$ or χ (see eqn. 12), so that the integrand of the integral of the right-hand side of eqn. 14, *i.e.*, $\chi^{-1}{}_{(\varrho')} \cdot p(\chi) \cdot \exp[u_{(1)}/kT \cdot \Xi \cdot \sqrt{\chi}]$, must also decrease rapidly with an increase in $\chi_{(\varrho')}$. Hence, only the maximum value of the integrand in the range $[\chi_{(\varrho')}, \chi^*_{(\varrho')}]$ is of importance for the integration or the value of the integral is virtually proportional to the maximum value of the integrand. Hence, introducing a proportionality constant K, we have

$$\int_{\chi_{(\varrho')}}^{\chi^*_{(\varrho')}} \chi^{-1}{}_{(\varrho')} \cdot p(\chi) \cdot \exp\left[\frac{u_{(1)}}{kT} \cdot \Xi \cdot \sqrt{\chi}\right] \mathrm{d}\chi_{(\varrho')} \approx \approx K \cdot \chi^{-1}{}_{(\varrho')}\, p\left\{\chi\left[\chi_{(\varrho')}\right]\right\} \cdot \exp\left\{\frac{u_{(1)}}{kT} \cdot \Xi \cdot \sqrt{\chi\,[\chi_{(\varrho')}]}\right\} \tag{16}$$

Now, by substituting eqns. 15 and 16 into eqn. 14,

$$y = w_{(\varrho')} - \Xi\, \sqrt{\chi} + \frac{1}{u_{(1)}/kT}\left\{\log \frac{\chi_{(\varrho')}}{p(\chi)} - \log\left[-\frac{u_{(1)}}{kT}\right] - \log\left(\beta_3\, \sigma\, \eta\, K\right)\right\} \tag{17}$$

is obtained and the differentiation of eqn. 17 leads to

$$\frac{\mathrm{d}y}{\mathrm{d}\chi_{(\varrho')}} = -\frac{\Xi}{2\sqrt{\chi}} \cdot \frac{\mathrm{d}\chi}{\mathrm{d}\chi_{(\varrho')}} + \frac{1}{u_{(1)}/kT}\left[\frac{1}{\chi_{(\varrho')}} - \frac{1}{p(\chi)} \cdot \frac{\mathrm{d}p(\chi)}{\mathrm{d}\chi_{(\varrho')}}\right] \tag{18}$$

* When $\chi_{(\varrho')}/\chi^*_{(\varrho')} = 1$, $\chi_{(\varrho')}$ in eqn. 13 is equal to $\chi^*_{(\varrho')}$.

As, on the other hand, the relationship

$$\frac{\mathrm{d}\chi}{\mathrm{d}\chi_{(\varrho')}} = -\frac{\mathrm{d}p(\chi)}{\mathrm{d}\chi_{(\varrho')}} = \sum_{\varrho''=1}^{\varrho} \frac{\chi^*_{(\varrho'')} \cdot E_{(\varrho',\,\varrho'')}}{\chi^*_{(\varrho')} E_{(\varrho',\,\varrho'')}} \cdot \chi_{(\varrho')}^{E_{(\varrho',\,\varrho'')}-1} \tag{19}$$

is obtained from eqns. 8 and 12, then upon introduction of eqns. 19 and 8 into eqn. 18 and eqn. 18 into eqn. 5, eqn. 5 becomes

$$f_{(\varrho')} = \left\{ \frac{\Xi}{2\sqrt{\chi}} \sum_{\varrho''=1}^{\varrho} \frac{\chi^*_{(\varrho'')} \cdot E_{(\varrho',\,\varrho'')}}{\chi^*_{(\varrho')} E_{(\varrho',\,\varrho'')}} \cdot \chi_{(\varrho')}^{E_{(\varrho',\,\varrho'')}-1} - \frac{1}{u_{(1)}/kT} \left[\frac{1}{\chi_{(\varrho')}} + \right. \right.$$

$$\left. \left. + \frac{1}{1-\chi} \cdot \sum_{\varrho''=1}^{\varrho} \frac{\chi^*_{(\varrho'')} \cdot E_{(\varrho',\,\varrho'')}}{\chi^*_{(\varrho')} E_{(\varrho',\,\varrho'')}} \cdot \chi_{(\varrho')}^{E_{(\varrho',\,\varrho'')}-1} \right] \right\}^{-1} \tag{20}$$

in which it should be recalled that χ is a function of only $\chi_{(\varrho')}$ expressed by eqn. 12. Now, eqns. 20 and 17 describe $f_{(\varrho')}$ as a function of y, using $\chi_{(\varrho')}$ as an intermediate parameter.

Finally, it should be noted that the theoretical chromatogram depends only slightly on the value of the parameter K (see eqn. 16), as the value of y in eqn. 17 depends only slightly on $\beta_3 \sigma \eta K$ or K when $-u_{(1)}/kT$ is large. This means that the exact estimation of K is unnecessary for the calculation of theoretical chromatograms. In fact, if $-u_{(1)}/kT = \infty$, K can have any finite value.

RESULTS OF CALCULATIONS OF CHROMATOGRAMS

In this section, we present the results of calculations of theoretical chromatograms obtained through eqns. 20 and 17. In all calculations, it is assumed that $\Xi = 0.5$, as in refs. 1–7, and that $\log(\beta_3 \sigma \eta K) = -15$, *i.e.*, $\beta_3 \sigma \eta K \approx 3 \cdot 10^{-7}$ (for this assumption, see below). If we assume further that Ξ has the same physical meaning as in ref. 7, then $\Xi = 0.5$ means that $-[u_{(1)}/kT]/x' = 1/15$, where x' is the number of crystal sites covered by an adsorbed molecule*. The values of x' and $-u_{(\varrho')}/kT$ can be estimated as 300 and about 20, respectively, for any species of tropocollagen (molecular weight = 300,000)[7], so the value of $-[u_{(\varrho')}/kT]/x'$ for tropocollagen is about 1/15. As the molecule of DNA is, in general, larger than that of tropocollagen, x' for DNA must be greater than 300. The value of $-[u_{(\varrho')}/kT]/x'$ or $-[u_{(1)}/kT] \cdot w_{(\varrho')}/x'$ for DNA, where $\varrho' = 1, 2, \cdots, \varrho$, can be considered to be about equal to or more than twice the value for tropocollagen, as the elution molarity of DNA is about equal to or more than twice the molarity of tropocollagen**. Therefore, if the parameter $w_{(\varrho')}$ for DNA is defined as the ratio of the adsorption energy of DNA to that of the hypothetical standard molecule (with $\varrho' = 1$) that has the same value of

* It should be recalled that Ξ is proportional to the ratio of the interaction energy per unit molecular length to the adsorption energy per unit molecular length of the standard molecule with $\varrho' = 1$ and that the interaction energy per unit molecular length is assumed to be independent of the type of the molecule (see Appendix II in ref. 6). In ref. 7, it has been assumed that $\Xi = 0.5$ when the adsorption energy per unit molecular length of the standard molecule or $-[u_{(1)}/kT]/x'$ is equal to 1/15. It is evident that the molecular length is proportional to x' for a rod-like molecule.

** The elution molarity is roughly proportional to $\xi_{(\varrho')}$ or to $-[u_{(\varrho')}/kT]/x'$ when x' is large enough[1,2].

$-[u_{(\varrho')}/kT]/x'$ as that for tropocollagen and the same x' value as that for DNA itself, then the value of $w_{(\varrho')}$ is about equal to or more than 2. Eqns. 20 and 17 show, however, that it is only the elution position of the chromatogram that is related essentially to the value of $w_{(\varrho')}$ itself and that it is the factor $E_{(\varrho',\varrho'')}$ or the difference in the adsorption energies between different components in the mixture that affects the shape of the chromatogram. Therefore, the theoretical chromatogram obtained by assuming that $-[u_{(1)}/kT]/x' = 1/15$ and that $x' > 300$ can be applied to DNA except for the elution position (see also ref. 7). In order to simplify the discussion, we have summarized in Table I all of the data corresponding to our calculated chromatograms.

Fig. 1 illustrates the simplest theoretical chromatograms for the case of a single molecular species (see Table I). The solid curves are the chromatograms obtained when the column is initially saturated by molecules or when $\chi^*_{(1)} = 1$. Eqns. 20 and 17 show that the left-hand part of the chromatogram is removed when the value of $\chi^*_{(1)}$ decreases, so the right-hand sides of three dotted lines with half the areas of the three total chromatograms show the chromatograms obtained when the load is halved. It can be seen in Fig. 1 that the shape of the chromatogram tends to that shown in Fig. 1 in ref. 6 when x' approaches infinity. It should be recalled that the chromatogram is generally displaced to the left with a decrease in the s.o.g. (see ref. 7; this is evident also from eqn. 17). As the elution position on the chromatogram of a molecule with $x' = 3{,}000$ in Fig. 1 is about equal to that shown in Fig. 10a in ref. 7, the value of $\beta_3\,\sigma\,\eta\,K$ assumed in all calculations in this paper corresponds roughly to the s.o.g., $g^* = 0.0025$, in ref. 7 when $x' = 3{,}000$. The dependence of the elution position on the s.o.g. is so small, however, that virtually an identical chromatogram is obtained

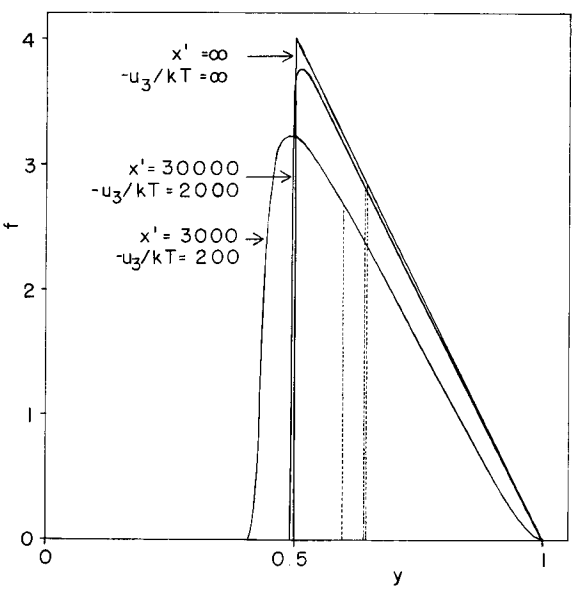

Fig. 1. Theoretical chromatograms for a single type of macromolecule. u_3 is written instead of $u_{(1)}$ for the adsorption energy of a molecule, as in refs. 1–7 we used this symbol for a single-component system.

TABLE I

SUMMARY OF DATA CORRESPONDING TO CALCULATED CHROMATOGRAMS

Figure No.	x'	*Species 1*			*Species 2*			*Species 3*			*Remarks*
		$-u_{(1)}/kT$	$w_{(1)}$	$\chi^*_{(1)}$	$-u_{(2)}/kT$	$w_{(2)}$	$\chi^*_{(2)}$	$-u_{(3)}/kT$	$w_{(3)}$	$\chi^*_{(3)}$	
1	3,000	200	1	1 or 1/2							The case of a single molecular species
	30,000	2,000	1	1 or 1/2							
	∞	∞	1	1 or 1/2							
2	3,000	200	1	1/2	180	0.9	1/2				The case of two molecular species
3	3,000	200	1	1/2	198	0.99	1/2				
4	3,000	200	1	1/2	199.8	0.999	1/2				
5	30,000	2,000	1	1/2	1800	0.9	1/2				
6	30,000	2,000	1	1/2	1980	0.99	1/2				
7	30,000	2,000	1	1/2	1998	0.999	1/2				
8	30,000	2,000	1	1/2	1999.8	0.9999	1/2				
9	3,000	200	1	1/4	198	0.99	1/4				
10	3,000	200	1	1/3	198	0.99	1/3	196	0.98	1/3	The case of three molecular species

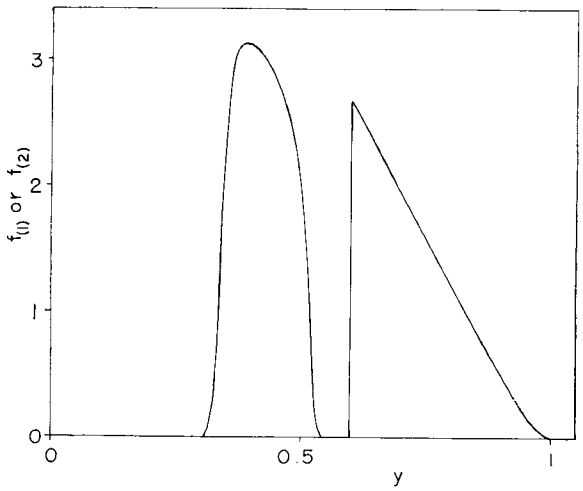

Fig. 2. Theoretical chromatograms for a two-component system.

in spite of considerable fluctuations in the value of $\beta_3 \sigma \eta K$ or K. When $x' = 30{,}000$ (see Figs. 5–8), the chromatogram is virtually independent of the value of K.

Figs. 2–4 and 5–8 show how the chromatographic separation is reduced with a decrease in the difference in adsorption energies between different species when $x' = 3{,}000$ and when $x' = 30{,}000$, respectively. In all of the figures, it was assumed that the column is initially saturated with molecules (see Table I). It can be seen that the chromatographic separation between two components is complete in Figs. 2, 5 and 6, which is the case when $-u_{(1)}/kT - [-u_{(2)}/kT] = -u_{(1)}/kT \cdot [w_{(1)} - w_{(2)}] \geqslant 20$. Figs. 3, 4, 7 and 8 are the cases when the separation is incomplete. It can be seen that the chromatographic separations of two components occur virtually in the same manner in Figs. 3 and 7 and in Figs. 4 and 8, respectively, which shows that the efficiency of the chromatographic separation depends only on the factor $-u_{(1)}/kT \cdot [w_{(1)} - w_{(2)}]$

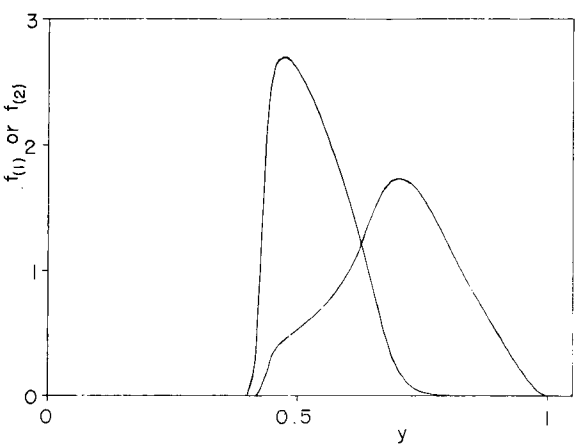

Fig. 3. Theoretical chromatograms for a two-component system.

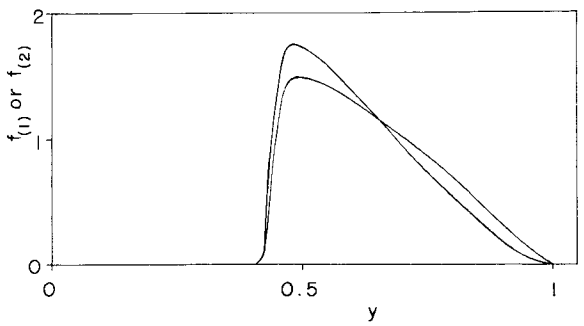

Fig. 4. Theoretical chromatograms for a two-component system.

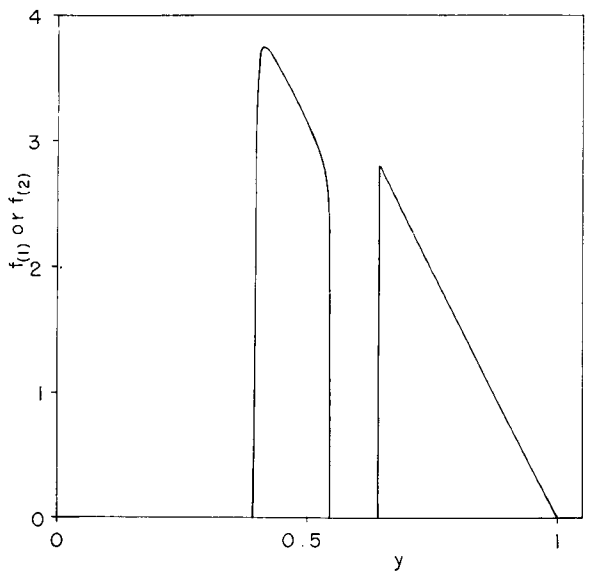

Fig. 5. Theoretical chromatograms for a two-component system.

or $E_{(1,2)}$ (see eqn. 10), as, in Figs. 3 and 7 and in Figs. 4 and 8, the values of $-u_{(1)}/kT \cdot [w_{(1)} - w_{(2)}]$ are 2 and 0.2, respectively (see Table I). This conclusion can easily be derived directly from eqns. 20 and 17 and we reached the same conclusion also in ref. 7.

It appears, however, that the chromatographic separation is much better according to the numerical calculation in ref. 7 than according to the present calculation. Compare, for instance, Figs. 7 and 8 with Figs. 29a and 29b in ref. 7, respectively. The samples loaded on the column and the experimental conditions except for the s.o.g. are the same in Fig. 7 in this paper and Fig. 29a in ref. 7, and in Fig. 8 in this paper and Fig. 29b in ref. 7, respectively. The s.o.g. in Figs. 7 and 8 is about 100 times smaller than that in Figs. 29a and 29b in ref. 7, so the chromatographic separations in Figs. 7 and 8 should be better than those in Figs. 29a and 29b in ref. 7. It can be seen, however, that the chromatographic separations in Figs. 29a and 29b in ref. 7

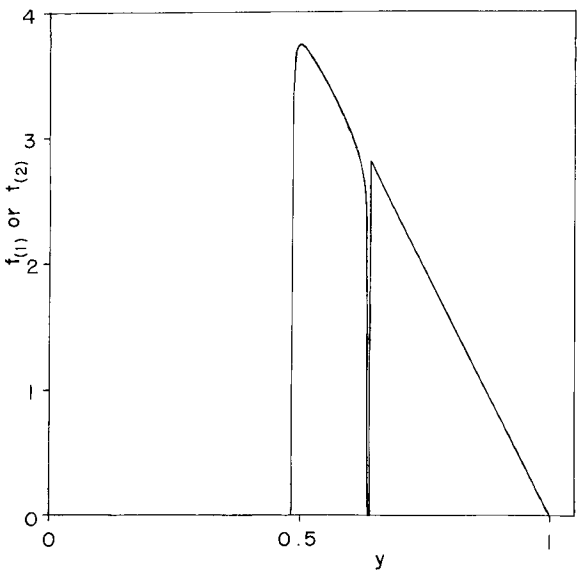

Fig. 6. Theoretical chromatograms for a two-component system.

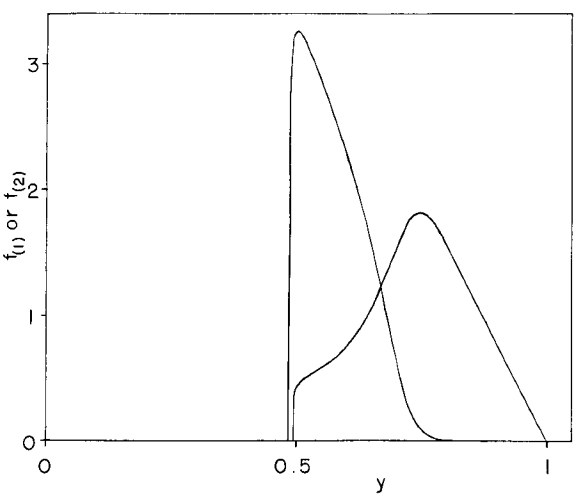

Fig. 7. Theoretical chromatograms for a two-component system.

are much better than those in Figs. 7 and 8 here. This result may be due to the rough approximation in Fig. 5 in ref. 7 that was used for the numerical calculations of Figs. 29a and 29b in ref. 7, as the approximation of Fig. 5 in ref. 7 reduces the range of χ in which two molecular species have $G_{(\varrho')}$ values between 0 and 1 at the same value of χ.

Fig. 9 is the chromatogram for the same mixture as that in Fig. 3 when the load is halved (see Table I). It can be seen that the effect of the repulsive interactions among adsorbed molecules or the mutual displacement effect is reduced and that the width of the chromatogram is decreased.

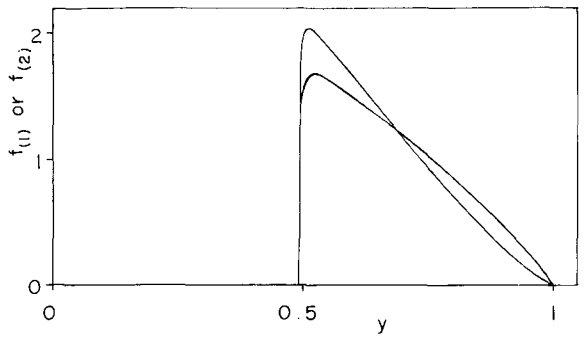

Fig. 8. Theoretical chromatograms for a two-component system.

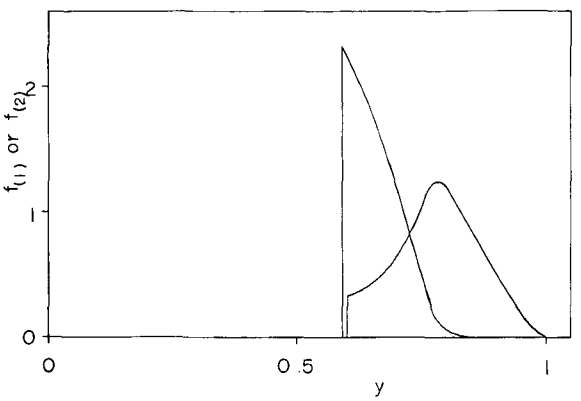

Fig. 9. Theoretical chromatograms for a two-component system.

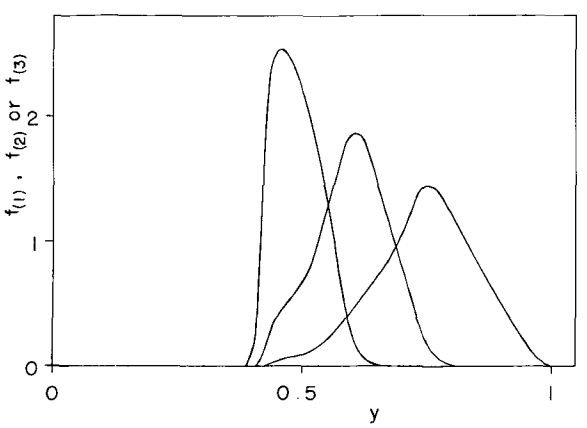

Fig. 10. Theoretical chromatograms for a three-component system.

Finally, Fig. 10 illustrates an example of a theoretical chromatogram for a mixture of three molecular species with the same dimensions as those of molecules in Figs. 2–4 and 9 (see Table I).

DISCUSSION

Most of the conclusions reached through the analytical calculations are qualitatively the same as those reached in ref. 7. However, it was shown in ref. 7 that the resolving power of the column can generally be improved by reducing the s.o.g., while the calculations in this paper show that the chromatographic resolution, when the s.o.g. is small enough, is related only to the factor $E_{(\varrho',\varrho'')}$ (see eqn. 10) and that the reduction in the value of $\beta_3\,\sigma\,\eta\,K$ or the s.o.g., η, brings about only a displacement of the position of the total chromatogram (see Results of Calculations of Chromatograms). This means that the resolving power of the column cannot be improved further by reducing the s.o.g. if it is already small enough.

In earlier work[10], it was mentioned that the diffusion of molecules in the small size range on the column and the heterogeneity in the flow-rate in different parts of the column section must, in general, be taken into consideration in order to explain the shape of the experimental chromatogram. However, if the s.o.g. is so small that the total volume of the eluent in which any component of the mixture is involved is much larger than the total interstitial volume of the column, then these two effects are evidently negligible. It is also evident that the resolution of the column increases with a reduction in these effects, so the conclusion that good resolution of the column can be achieved by using a small value of the s.o.g. is valid, even when these effects are taken into account.

Finally, we have sometimes observed a chromatogram of DNA with a shoulder when a large amount (relative to the volume of the column) of the sample is loaded on the column. If different parts of the original chromatogram are re-chromatographed independently, they appear in the same positions and again show shoulders. If a small amount of the sample is loaded, however, the beginning of the chromatogram migrates towards its end and the shoulder disappears[14]. Now, it can be shown by using eqns. 20 and 17 that the shape of the theoretical chromatogram is sensitive to the shape of the function $p(\chi)$ (see eqn. 8) in its left-hand part unless the value of x' is very large. In this paper and ref. 7, theoretical chromatograms have been calculated assuming that $p(\chi)$ is expressed as eqn. 8, which seems to be a good approximation if the sample molecules are homogeneous enough in both dimensions and shape. If not, however, $p(\chi)$ should be written as $p_{(\varrho')}(\chi_{(1)}, \cdots, \chi_{(\varrho)})$ (see ref. 2). In our opinion, the shoulder may be observed when the preparation of DNA is considerably heterogeneous in molecular length and when its average molecular weight is not so large.

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