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## THEORY OF TRANSPORT IN LINEAR PARTITION SYSTEMS OCCURRING IN CHROMATOGRAPHIC AND ELECTROPHORETIC MODELS

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## SUMMARY

General formulae for the transport of matter, under the influence of electric or dynamic forces and which distributes linearly between two states, are derived without any restriction about the nature of the partition process. The moments of the concentration profile are calculated from the formulae. In those moments each term is physically clear. From the results the already known formulae for specialized cases of the partition process can easily be derived.

## INTRODUCTION

The following expression governs the transport of matter under influence of flow and diffusion in the unidimensional case

$$\left(\frac{\partial c}{\partial t}\right)_x = -v \cdot \left(\frac{\partial c}{\partial x}\right)_t + D \cdot \left(\frac{\partial^2 c}{\partial x^2}\right)_t \quad (1)$$

or in short notation

$$\partial_t c = -v \cdot \partial_x c + D \cdot \partial_{xx} c$$

where  $v$  is the velocity of the substance. The movement of the substance may be caused by displacement of the fluid in which the substance is dissolved as is the case in chromatography, or by electric forces as in electrophoresis.

The other symbols are as follows:

$c$  = concentration of the substance =  $c(x, t)$ ,

$D$  = diffusion constant;  $D$  is assumed to be a constant.

In chromatography and electrophoresis the moving substance is distributed between a "free" and "bound" state. Distribution may result from the presence of a stabilizing medium to which the substance is sorbed; complex formation with a second solute in excess is another possibility. In all these cases the partition process is assumed to be quasi-linear, which means that (in the absence of transport and

diffusion) the concentrations  $f$  and  $b$  in the free and bound state respectively, are governed by

$$\partial_t f = -l_1 f + l_2 b \quad (2)$$

$$\partial_t b = +l_1 f - l_2 b = -\partial_t f \quad (3)$$

where  $l_1$  and  $l_2$  are the mass transfer coefficients.

With

$$\tau = (l_1 + l_2)^{-1} \quad (4)$$

and the initial condition  $f = f_0$ ,  $b = b_0$  we have

$$f = \left( \frac{\Gamma}{\Gamma + \gamma} \right) (f_0 + b_0) + \tau(l_1 f_0 - l_2 b_0) \cdot e^{-\frac{t}{\tau}} \quad (5)$$

$$b = \left( \frac{\gamma}{\Gamma + \gamma} \right) (f_0 + b_0) - \tau(l_1 f_0 - l_2 b_0) \cdot e^{-\frac{t}{\tau}} \quad (6)$$

where the partition coefficient

$$\gamma = \frac{l_1}{l_2} \quad (7)$$

defines the equilibrium state, which is approached exponentially with a time constant  $\tau$ , while  $f + b$  always equals  $f_0 + b_0$ .

On adding this type of partition term to equation (1), the following equations for the combination of streaming, diffusion and partition are obtained:

$$\partial_t f = -v_f \cdot \partial_x f - (l_1 f - l_2 b) + D_f \cdot \partial_{xx} f \quad (8)$$

$$\partial_t b = -v_b \cdot \partial_x b + (l_1 f - l_2 b) + D_b \cdot \partial_{xx} b \quad (9)$$

When there is only one state  $f$  and no diffusion or partition, it can easily be seen that the concentration peak will move with a velocity  $v_f$ , because  $f(x - v_f t)$  is a solution of the differential equation

$$\partial_t f = -v_f \cdot \partial_x f.$$

From physical considerations it is clear, that after some initial effects the mass centre of  $(f + b)$  will move uniformly with a velocity  $\bar{v}$ .

We will now transform to a new coordinate system moving with this velocity, by putting  $x' = x - \bar{v} \cdot t$ . This makes the mass centre stay at rest with respect to this new system. (A proper choice of the origin of the coordinate system is of course this mass centre.) So

$$\partial_x f = \partial_{x'} f' \quad (10)$$

$$\partial_t f' = \partial_t f + \bar{v} \cdot \partial_x f \quad (11)$$

with similar equations for  $b'$  and  $b$ .

Substitution in eqns. (8) and (9) gives, on omitting primes:

$$\partial_t f = (\bar{v} - v_f) \cdot \partial_x f - (l_1 f - l_2 b) + D_f \cdot \partial_{xx} f \quad (12)$$

$$\partial_t b = (\bar{v} - v_b) \cdot \partial_x b + (l_1 f - l_2 b) + D_b \cdot \partial_{xx} b \quad (13)$$

The assumption of linearity is quite correct even in the presence of a stabilizing medium if partition takes place in the homogeneous phase inside the stabilizing medium. The paper electrophoretic separation of metal ions in a solution of citric acid in excess is a example of such a system.

In chromatographic columns the stabilizing medium itself participates in the partition process. In these heterogeneous systems the assumption of linearity is only approximately fulfilled. This can only lead to results more or less correct depending on the geometry of the system. Furthermore the equations do not include the statistical variations of the transport velocities caused by the grain or fibre structure of the stabilizing medium.

Temperature fluctuations arising from electric resistance differences or from sorption reactions are also neglected, just like micro-scale gravitational turbulences, etc.

Thus, exact solutions to the equations (12) and (13) are of limited value as the equations themselves are open to criticism. Moreover, we get more information than required for practice. We are interested in quantity, displacement, width and skewness of the mass distribution ( $f + b$ ) and the intention is to deduce these variables from the measured signals. These signals are obtained either as a function of time (elution chromatography, gas chromatography) or as a function of place (electrophoresis, thin layer chromatography). There is a relation between the above mentioned variables and the moments of the signals which are defined by the partial integral:

$M_i = \int \xi^i \cdot S(\xi) \cdot d\xi$ , where  $\xi$  is either the time or a coordinate depending on the experimental conditions. These moments are a—preferably linear—function of the respective identically defined moments of the concentration distribution; this function is determined by the instrumental conditions. The moments of the concentration distribution are linear with respect to the above mentioned variables.

Several authors have shown that it is possible to obtain the moments of the concentration distribution directly from equations (12) and (13), and depending on their inclination they calculate either the time moments<sup>1-3</sup> or the place moments<sup>4-8</sup>.

The time and place moments are of course strongly related to each other, as the separation process itself is independent of the method of definition and measurement. The mutual relationship will be discussed later in this paper. Although there is no fundamental difference between the two types of moments, we chose to calculate the place moments as during the calculating procedure the physical meaning is better. The place moments are yielded directly by integration of eqns. (12) and (13). It will be shown that specification of the partition process is not necessary. Only LJUNGGREN<sup>4</sup> has attempted to calculate the place moments in a similar way but he either neglected the thermal diffusion or the electric field. It will be shown that during the calculations the essence of the separation process can be followed step by step and the physical meaning and origin of each term can be easily understood.

#### DEFINITIONS AND MEANING OF MOMENTS

The separating system consists of a column of infinite length with a concentration peak of arbitrary form localized in a small region.

The  $i$ th moments of the concentration distribution are defined by:

$$F_i = \int x^i \cdot f \cdot dx \quad \text{and} \quad B_i = \int x^i \cdot b \cdot dx \quad (14)$$

We then have

$F_0$  is the total amount of substance in the free state;  $F_0$  is a function of time only;

$B_0$  is the total amount in the bound state;

$F_1$  defines the centre of mass in the usual way.

In addition, we define the coordinate  $x_f$  of this mass centre by:

$$F_1 = x_f \cdot F_0 \quad (15)$$

Similar relations hold for  $B_1$ .

It is also well known that the variance  $\mu$  is indicative for the width of a distribution and that this quantity is defined by

$$\mu_f = \frac{\int (x - x_f)^2 \cdot f \cdot dx}{\int f \cdot dx} \quad (16)$$

which makes

$$\mu_f = \frac{F_2}{F_0} - \left(\frac{F_1}{F_0}\right)^2 = \frac{F_2}{F_0} - x_f^2 \quad (17)$$

Analogous relations apply to  $\mu_b$  and  $B_2$ .

The third moments  $F_3$  and  $B_3$  are related to the skewness of the concentration peaks. The discussion of these quantities is given in the corresponding section of this paper.

We shall now calculate the  $i$ th moments. To do so we need the following equations derived from the definition (14):

$$\int x^i \cdot \partial_t f \cdot dx = d_t F_i \quad (18)$$

$$\int x^i \cdot \partial_{x_f} f \cdot dx = \begin{cases} -i F_{i-1} & (i = 1, 2 \dots) \\ 0 & (i = 0) \end{cases} \quad (19)$$

$$\int x^i \cdot \partial_{x_f x_f} f \cdot dx = \begin{cases} i(i-1) F_{i-2} & (i = 2, 3 \dots) \\ 0 & (i = 0, 1) \end{cases} \quad (20)$$

Similar relations hold for the bound state  $b$ .

#### CALCULATION OF THE MOMENTS

##### The zeroth moment

Integrating (12) and (13) along the  $x$ -axis using eqns. (18), (19) and (20) we obtain

$$d_t F_0 = -(l_1 F_0 - l_2 B_0) \quad (21)$$

$$d_t B_0 = + (l_1 F_0 - l_2 B_0) \quad (22)$$

because only the partition terms contribute. This would be expected, as the chemical reaction is the only way of changing the amount of substance in each state.

As (21) and (22) are identical with (2) and (3), with initial conditions  $F_0 = F_{00}$ ,  $B_0 = B_{00}$  and inserting  $c = l_1 F_{00} - l_2 B_{00}$  and

$$F_{00} + B_{00} = M_0 \quad (23)$$

we have

$$F_0 = \left( \frac{1}{1 + \gamma} \right) M_0 - c\tau \cdot e^{-\frac{t}{\tau}} \quad (24)$$

$$B_0 = \left( \frac{\gamma}{1 + \gamma} \right) M_0 + c\tau \cdot e^{-\frac{t}{\tau}} \quad (25)$$

In fact in the partition-only case of (2) and (3)

$$f_0 \hat{=} F_{00} \text{ and } b_0 \hat{=} B_{00}$$

From these equations we conclude that transport and diffusion have no influence on partition. The steady state is exponentially approached with a time constant  $\tau$  and in practice can be said to be reached after  $4\tau$ .

Note that in electrophoresis the electric field is only established after injection of the sample; so the constant  $c$  can be made zero and the steady state is immediately reached.

### The first moment

On multiplying (12) and (13) by  $x$  and integrating along the  $x$ -axis we find that as a result of (20) the diffusion term does not contribute. We then have

$$d_t F_1 = (v_f - \bar{v})F_0 - (l_1 F_1 - l_2 B_1) \quad (26)$$

$$d_t B_1 = (v_b - \bar{v})B_0 + (l_1 F_1 - l_2 B_1) \quad (27)$$

With

$$M_1 = F_1 + B_1 = \kappa_{fb} \cdot M_0 \quad (28)$$

we obtain from these equations for the velocity  $v_{fb}$  of the mass centre of the total amount of substance

$$v_{fb} = d_t \kappa_{fb} = \left( \frac{v_f + \gamma v_b}{1 + \gamma} \right) - \bar{v} + (v_b - v_f) \frac{c\tau}{M_0} \cdot e^{-\frac{t}{\tau}} \quad (29)$$

So from the definition of  $\bar{v}$  in the coordinate transformation it follows that  $\bar{v}$  is equal to the weighted arithmetic mean of  $v_f$  and  $v_b$ :

$$\bar{v} = \left( \frac{1}{1 + \gamma} \right) v_f + \left( \frac{\gamma}{1 + \gamma} \right) v_b \quad (30)$$

It follows from (29), remembering that the origin of the moving coordinate system should be at the mass centre, after the disappearance of the initial effects that:

$$\kappa_{fb} = (v_f - v_b) \frac{c\tau^2}{M_0} \cdot e^{-\frac{t}{\tau}} \quad (31)$$

All further derivations will be made in moving coordinates only. To find the values of  $F_1$  and  $B_1$  we multiply (26) by  $l_1$  and (27) by  $l_2$  and subtract the equations. From this we get after integrating\*:

$$l_1 F_1 - l_2 B_1 = \left( \frac{\gamma}{1 + \gamma} \right) \left( \frac{1}{1 + \gamma} \right) (v_f - v_b) M_0 + (d_1 + d_2 \cdot t) \cdot e^{-\frac{t}{\tau}} \quad (32)$$

Combining this equation with (15), (24) and (28) we obtain for the separate mass centres of the free and the bound states:

\*  $d_1, d_2, d_3, f_1$  and  $f_2$  are constants determined by the initial conditions,  $d_2, d_4, d_6, f_2$  and  $f_4$  are constants determined by the integration.

$$x_f = \left( \frac{\gamma}{1 + \gamma} \right) (v_f - v_b) \tau + (d_3 + d_4 t) e^{-\frac{t}{\tau}} \quad (33)$$

$$x_b = - \left( \frac{1}{1 + \gamma} \right) (v_f - v_b) \tau + (d_5 + d_6 t) e^{-\frac{t}{\tau}} \quad (34)$$

After disappearance of the exponential terms, which quickly occurs, we have:

$$\Delta x = x_f - x_b = (v_f - v_b) \tau \quad (35)$$

$$x_f = (v_f - \bar{v}) \tau \quad (36)$$

$$x_b = (v_b - \bar{v}) \tau \quad (37)$$

and not withstanding:

$$x_{fb} = 0.$$

The shift of the mass centres with respect to each other, acquired during the lifetime of the exponentials and the shift towards the origin are noteworthy. The shifts arise from the fact that when a molecule moves in state "f" its velocity in moving coordinates equals  $(v_f - \bar{v})$ . The mean time for a molecule to be either in state "f" or in state "b" is equal to  $\tau$ . So we can understand both  $x_f$  and  $x_b$  as the mean displacement of one molecule. These are equal to the displacements of the mass centres of the ensembles of molecules.

### The second moment

Following the same procedure as for  $F_1$  and  $B_1$  we get for the second moment:

$$d_t F_2 = 2(v_f - \bar{v}) F_1 - (l_1 F_2 - l_2 B_2) + 2D_f F_0 \quad (38)$$

$$d_t B_2 = 2(v_b - \bar{v}) B_1 + (l_1 F_2 - l_2 B_2) + 2D_b B_0 \quad (39)$$

With  $M_2 = F_2 + B_2$  we obtain after adding the equations:

$$\begin{aligned} d_t M_2 = & 2 \left\{ \left( \frac{1}{1 + \gamma} \right) D_f + \left( \frac{\gamma}{1 + \gamma} \right) D_b \right\} M_0 + \\ & + \frac{2}{\tau} \left\{ \left( \frac{1}{1 + \gamma} \right) (v_f - \bar{v})^2 \tau^2 + \left( \frac{\gamma}{1 + \gamma} \right) (v_b - \bar{v})^2 \tau^2 \right\} M_0 + \\ & + (f_1 + f_2 t) e^{-\frac{t}{\tau}} + (f_3 + f_4 t) e^{-\frac{2t}{\tau}} \end{aligned} \quad (40)$$

Applying (17) we obtain for the variance of the total amount of substance:

$$\mu = \frac{M_2}{M_0} - x_{fb}^2 \quad (41)$$

On integrating (40) and cancelling of the exponentials

$$\begin{aligned}
\mu &= \mu_0 + 2 \left\{ \left( \frac{1}{1+\gamma} \right) D_f + \left( \frac{\gamma}{1+\gamma} \right) D_b \right\} t + 2 \left\{ \left( \frac{1}{1+\gamma} \right) x_f^2 + \left( \frac{\gamma}{1+\gamma} \right) x_b^2 \right\} \frac{t}{\tau} \\
&= \mu_0 + 2 \left[ \bar{D} + \frac{1}{\tau} \left\{ -x_f \cdot x_b \right\} \right] t \\
&= \mu_0 + 2 \left[ \bar{D} + \left( \frac{1}{1+\gamma} \right) \left( \frac{\gamma}{1+\gamma} \right) (v_f - v_b)^2 \tau \right] t \\
&= \mu_0 + 2D' \cdot t \tag{42}
\end{aligned}$$

In this case  $\mu_0$  is the initial variance determined by the width of the concentration peak at  $t=0$ . Note that  $\mu \neq \mu_0$  at  $t=0$  according to the exponentials; their contribution arises from a combination of the mass centre shift away from the origin and a mass partition unequal to  $\gamma$ .

From (42) it is clear that the peak is broadened by diffusion with a coefficient  $\bar{D}$  equal to the weighted mean of  $D_f$  and  $D_b$ . In addition to diffusion there is another broadening effect proportional to the reaction time constant  $\tau$ . This contribution arises from the kinetics of the separation process and has its maximum when  $\gamma = 1$  and is zero for  $\gamma = 0$  and infinity. The contribution can be understood from a random walk model<sup>9</sup>. If  $\gamma$  is the mean length of a step forward and  $z$  is the mean length of a

$$\begin{aligned} d_t M_3 &= 3\{(v_f - \bar{v})F_2 + (v_b - \bar{v})B_2\} + 6(D_f F_1 + D_b B_1) \\ &= 3(v_f - v_b)\tau\{l_1 F_2 - l_2 B_2\} + 6(D_f F_1 + D_b B_1) \end{aligned} \quad (48)$$

On substituting known  $F$ 's and  $B$ 's:

$$d_t M_3 = 6 \left( \frac{1}{1 + \gamma} \right) \left( \frac{\gamma}{1 + \gamma} \right) (v_f - v_b)\tau M_0 \left\{ \frac{x_f^2 - x_b^2}{\tau} + 2(D_f - D_b) \right\} + \text{exponentials} \quad (49)$$

Integrating and cancelling of the exponentials gives

$$\begin{aligned} S &= S_0 + \left\{ 12(v_f - v_b)\tau \left( \frac{1}{1 + \gamma} \right) \left( \frac{\gamma}{1 + \gamma} \right) (D_f - D_b) + \right. \\ &\quad \left. + 6(v_f - v_b)^2 \tau^2 \left( \frac{1}{1 + \gamma} \right) \left( \frac{\gamma}{1 + \gamma} \right) \left( \frac{\gamma - 1}{\gamma + 1} \right) \right\} \cdot t \end{aligned} \quad (50)$$

$S$  is changing linearly with time. The first time-dependant term is a transport-diffusion mixture term, which has its maximum for  $\gamma = 1$ . The second one is a transport-partition term with a maximum for  $\gamma = 3.7$  and  $1/3.7$  and a zero value for  $\gamma = 1$ . We can substitute (50) in (45) and get something like:

$$x_{fb} - x_{\max} = \frac{(S_0 + \beta \cdot t)}{2(\mu_0 + 2D't)} \quad (51)$$

In most practical cases  $S_0 \approx 0$  and so  $(x_{fb} - x_{\max})$  rises from zero to an asymptotic time-independent value  $(\beta/4D')$ , assuming that the concentration distribution is approximately Gaussian. The magnitude of this asymptotic value will be investigated in two extreme cases:

(a) The diffusion determines the width of the concentration profile. Then  $(v_f - v_b)^2 \tau \ll \bar{D}$  and

$$\frac{\beta}{4D'} = \frac{3 \left( \frac{1}{1 + \gamma} \right) \left( \frac{\gamma}{1 + \gamma} \right) (D_f - D_b) (v_f - v_b)\tau}{\bar{D}} \quad (52)$$

The latter function for  $D_b = 0$  and  $\gamma \gg 1$  reaches the extreme

$$\frac{\beta}{4D'} = 3(v_f - v_b)\tau = 3(x_f - x_b) \quad (53)$$

(b) When  $(v_f - v_b)^2 \tau \gg \bar{D}$  the asymptotic value transforms in

$$\frac{\beta}{4D'} = \frac{3}{2} \left( \frac{\gamma - 1}{\gamma + 1} \right) (v_f - v_b)\tau \quad (54)$$

with an extreme value of

$$\frac{\beta}{4D'} = \frac{3}{2} (v_f - v_b)\tau = \frac{3}{2} (x_f - x_b) \quad (55)$$

The importance of these derivations is that the maximum distance between maximum and mean of the concentration profile is comparable with the shift of the mass centres  $f$  and  $b$  during the separation process. The skewness  $(x_{fb} - x_{\max})/\sigma$  diminishes with respect to the root of the time, as it should, which is in agreement with the computer results of VINK<sup>6</sup> for his partition model.



## DISCUSSION

We can now compare the results with formulae published in other papers.

LJUNGGREN<sup>4</sup> made a theoretical study of electrodiffusion by means of moment analysis. He solved the equations after introduction of the moments by Fourier and Laplace transformations but he either neglected the ordinary diffusion or the electric field. He, nevertheless, derived a partition-transport contribution to the diffusion term which agrees with the third term in eqn. (42).

In chromatography there is the restriction  $v_b = 0$  and in addition, our partition coefficient is related to  $R$  by  $\gamma = R/(1-R)$ . Substitution transform the partition-transport term in the well known result<sup>10</sup>

$$\Delta\mu = 2R(1-R) \cdot v \cdot t_a \cdot L \quad (56)$$

VINK<sup>5</sup> made a chromatographic model in which the partition occurs between two rows of unit cells of unequal volume per unit of contact area and separated by a membrane. In this model the partition is controlled by the diffusion in the bound state  $b$ . This makes the kinetic constants equal to

$$l_1 = \frac{2D_2}{V_2 \cdot V_1} \cdot \gamma \quad \text{and} \quad l_2 = \left( \frac{2D_2}{V_1 \cdot V_2} \right) \cdot \left( \frac{V_1}{V_2} \right)$$

Substitution shows agreement between his formulae<sup>7</sup> and equation (42). The first and third term arise from the weighted mean of the diffusion coefficients; the second term in his formulae (34) originates from the exchange between partition and transport.

In KUČERA's paper<sup>1</sup>, the model is assumed to consist of a column filled with small porous grains. In this model the partition occurs in two steps, *viz.* diffusion through the pores followed by sorption on the internal surfaces. First of all confining our attention to the calculation of the time-moments in this paper, and neglecting for a while the extension to a third "phase", we should expect the relation between the  $i$ th time and  $i$ th place moment to be simply

$$i\mu_x = (\bar{v})^i \cdot i\mu_t$$

In this case, however, the broadening of the distribution by diffusion and other effects is neglected during the time the distribution passes the fixed place  $x=L$ . An impression of the correction for this is obtained by dividing a normal distribution into two parts and assuming that the mass points of each part of the distribution lie at distances  $+\sigma$  and  $-\sigma$  from the intersection. Now the elution time for the peak will be  $\Delta t = 2\sigma/\bar{v}$ . During this time the distance  $2\sigma$  grows according to  $\sigma^2 = 2D' \cdot t$  with

$$2\Delta\sigma = \frac{2D'}{\sigma} \cdot \Delta t = \frac{4D'}{\bar{v}}$$

Half the mass shifts this distance making the shift of the mass point of the whole distribution equal to  $2D'/\bar{v}$  or in time coordinates  $\Delta t' = 2D'/\bar{v}^2$ . As the curve is lagging behind the time discrepancy has to be added, giving:

$$i\mu_t = \frac{L}{\bar{v}} + \frac{2D'}{\bar{v}^2} \quad (57)$$

The additional term for the second moment originates from the same effect. The shift in distance relative to the central mass point is  $2D'/\bar{v}$  for each part of the curve. So the contribution to  ${}_2\mu_x$  will be  $(2D'/\bar{v})^2$ . Substitution gives:

$${}_2\mu_x = {}_2\mu_x/\bar{v}^2 + (2D'/\bar{v})^2/\bar{v}^2 = \frac{2D'}{\bar{v}^2} \left( \frac{L}{\bar{v}} + \frac{2D'}{\bar{v}^2} \right) + \frac{4D'^2}{\bar{v}^4} = \frac{2D'L}{\bar{v}^3} + \frac{8D'^2}{\bar{v}^4} \quad (58)$$

Both equations agree with the results of KUČERA<sup>1</sup>. The calculations here are of course not exact but they give an idea of the origin and the physical meaning of the several terms in the time moments.

KUBIN<sup>2</sup> obtained some different results on calculating the same time moments. The discrepancy arises from his initial conditions. He injects a block function with a time width  $t_0$ , which gives a contribution to the first moment of  $\frac{1}{2}t_0$  as a consequence of the mass point shift relative to the origin of the coordinate system. For the same reason the second moment is extended by  $\frac{1}{12}t_0^2$  (the inertia moment of a rectangular box is  $\frac{1}{12}ml^2$ ). The distortion contributions of KUČERA are lost by an approximation in the calculation.

We can now look at what happens when the number of states is extended. For three states we have:

$$\delta_t f = (\bar{v} - v_f) \cdot \delta_x f + D_f \cdot \delta_{xx} f - (l_{12}f - l_{21}b) - (l_{13}f - l_{31}d) \quad (59)$$

$$\delta_t b = (\bar{v} - v_b) \cdot \delta_x b + D_b \cdot \delta_{xx} b - (l_{21}b - l_{12}f) - (l_{23}b - l_{32}d) \quad (60)$$

$$\delta_t d = (\bar{v} - v_d) \cdot \delta_x d + D_d \cdot \delta_{xx} d - (l_{31}d - l_{13}f) - (l_{32}d - l_{23}b) \quad (61)$$

This system extends by one equation, and simultaneously each equation by one exchange term, for each additional state. Similarly to the case with two states, both the time constant  $\tau$  and the factors  $\alpha_i$  determining the partition of total mass  $M_0$  over the three states are only a function of the kinetic constants  $l$ . In general, it is a complicated function when both side and successive reactions exist. An important conclusion is that there is only one time constant  $\tau$  for the whole system.

A characteristic fact from the equations (59)–(61) is that the sum of all kinetic terms is zero. So they always vanish in the calculation of  $M_t$ . Consequently from equations (26) and (27) it follows

$$d_t M_1 = (v_f - \bar{v})F_0 + (v_b - \bar{v})B_0 + (v_d - \bar{v})D_0 \quad (62)$$

This equation is familiar to us from mechanics as it follows immediately from the definition of mass point.

Equation (30) changes in the general case into

$$\bar{v} = \alpha_f \cdot v_f + \alpha_b \cdot v_b + \alpha_d \cdot v_d + \dots \quad (63)$$

In chromatography all velocities are zero except  $v_f$  making the reduced velocity simply equal to the fraction of solute in the mobile phase. This can be stated independently of the column model and is a fundamental law of chromatography<sup>8</sup>.

After introduction of the moving coordinate system we get for the steady state similar to before:  $d_t M_1 = 0$ ,  $x_{fb} = 0$ ,  $F_0, B_0$  etc. = constant. This implies that apart from an exponential term the terms  $(l_{ij}B_1 - l_{ji}D_1)$ , etc. occurring in the extended form of equation (26) will not contain the terms of any order in  $t$ ; this agrees with equation (32). So  $d_t F_1 = d_t B_1 = \dots = 0$ . By comparing the procedure for solution of  $F_0$  and  $F_1$  combined with dimensional analysis it becomes clear how the time constant  $\tau$  depends on the  $l$ -values and how  $\tau$  enters the equations (33) and (34).

It can be verified that equations analogous to (33) and (34) can be derived and from these it can be deduced that the shift of the mass centre of each state is the same as was found before (equations (36) and (37)). So the equation

$$x_i = (v_i - \bar{v})\tau \quad (64)$$

may be stated as a general equation.

In the same way as before we can derive the time derivative of the second moment  $M_2$ . The equation is nearly the same as equation (40). On integrating and cancelling of the exponentials we get an equation like (42). So in general the width of the distribution will be equal to:

$$\mu = u_0 + 2D' \cdot t \quad (65)$$

in which

$$D' = \sum_i a_i D_i + \frac{1}{\tau} \sum_i a_i x_i^2 \quad (66)$$

the summation takes place over all states. A general conclusion now is that:

The laws governing the behaviour of the zeroth, first and second moments can be stated independently of the model and are to be considered as fundamental laws.

With the derived formulae it was possible to verify the general results of KUČERA<sup>1</sup>, for the three state model.

VINK<sup>8</sup> defined a model which was extended to  $n$  states by induction. His formulae for the displacement and width can be derived from the general formulae. The formulae are also applicable to frontal analysis as was shown by VINK<sup>8</sup> by taking the place derivatives.

So far transportation space has been treated as if it were devoid of structure. Such an assumption is certainly wrong in paper electrophoresis and chromatographic columns. In this case homogeneity is disturbed and the velocities become functions of  $x, y, z$ . The nature of the stabilizing medium does not permit a definite statement of these functions. The problem is approximated in a statistical way. This means that:

- (a)  $v_f$  and  $v_b$  are fluctuating statistically;
- (b) the mean values for  $v_f$  and  $v_b$  are lowered differently by obstructivity and tortuosity of the substrate.

The influence on the previously derived results of this is:

- (a) one more term contributing to the formula for the width of the concentration peak;
- (b) slowing down of the mean transport.

A contribution to the skewness is difficult to assess. If there is any contribution at all, it is probably small.

There is still one remarkable thing. It may occur, that a solute divides between

two states in which the velocities are opposite. In that case  $\bar{v}$  will be small, but the solute particles are travelling back and forth.  $x_i$  from equation (64) is the mean travelling distance in state  $i$ . When the time constant  $\tau$  is large,  $x_i$  may become larger than the dimensions of the grains and fibres of the stabilizing medium. In this case the spreading effects may be excessively large, whereas in the opposite case there is no influence at all. This problem may occur in electrophoresis. In chromatography the problem does not exist as all velocities have the same sign.

#### CONCLUSION

It is shown that it is possible to solve the general formulae describing the transport of matter under the influence of electric or dynamic forces and distributing itself linearly between several states. Each term in the formulae for the calculated moments has a distinct physical meaning. The results for the first and the second moment are simple and it is easy to extend the formulae to systems in which one component is distributed between more than two states. For the calculation of the mean velocity and the width we need to know the fraction  $\alpha$  of the solute in each state and the time constant  $\tau$  of the system. If the separate reaction velocity constants  $k_{ij}$  are not known, as is common, the  $\alpha_i$ 's may be determined by chemical analysis or in a more sophisticated way together with  $\tau$  from N.M.R. or E.S.R. experiments. It is in general not necessary to evaluate them from chromatographic or electrophoretic experiments. There is one thing still to note, being the effect on the separation process of taking away the driving forces at the end of a thin-layer chromatographic or an electrophoretic separation. It is easy to understand that the system proceeds in an exponential way to the state in which the mass centres of the free and the bound state(s) coincide, while the distribution as a whole stays at rest. From then on, broadening of the concentration distribution only arises from diffusion.

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