The Effect of Intermediates upon the Transport Properties of Polymerizing Systems. I. Monomer, Trimer, and Nonamer[*]

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ABSTRACT: A numerical investigation of the equations governing the asymptotic transport behavior of a model system involving rapid equilibration between monomer, trimer, and nonamer predicts that three boundaries may be found for certain values of the equilibrium constants. The positions of the minima, as well as of the intermediate boundary, are functions of the equilibrium constants governing the reaction. This predicted behavior contrasts with that of monomer-*single* polymer systems, where maximally only two boundaries can occur, the position of the minimum being a function only of the degree of polymerization (G. A. Gilbert, *Discussions Faraday Soc. 20*, 68 (1955).

The studies of reactions among biological macromolecules is fundamental in providing a chemical basis for biological processes. The transport properties of one class of such reactions, protein polymerization, has been extensively investigated both experimentally and theoretically (for a review of the relevant literature to 1963, see Nichol *et al.*, 1964; Winzor and Scheraga, 1963, 1964; Ackers and Thompson, 1965; Bethune, 1965a,b; Belford and Belford, 1962, 1964; Oberhauser *et al.*, 1965; Makino and Rogers, 1965). The general theory covering moving-boundary transport experiments upon such systems where reequilibration is instantaneous was first elaborated by Gilbert (1955) to account for the anomalous sedimentation behavior of chymotrypsin at low ionic strength (Massey *et al.*, 1955).

The solution of the relevant asymptotic equations, formulated in terms of monomer and a *single* higher polymer, showed that the model chosen could account for the experimental observations, *i.e.*, above a certain protein concentration two boundaries were formed, the area and velocity of the slower remaining constant if the concentration was increased further, while both the area and velocity of the faster boundary increased. Extensive theoretical investigations have been carried out only for this monomer–single polymer model, and here it is found that the maximum number of boundaries, two, will be formed only if the degree of polymerization is greater than two, the position of the minimum between these being a function only of the degree of polymerization (Gilbert, 1955).

Thus for more complex models, involving more than one polymer, no predictions of the possible number of boundaries or the positions of any minima between these has been made. While general equations have been formulated for these systems (Rao and Kegeles, 1958; Gilbert, 1959), their complexity has precluded a general analytical investigation of inflection points in the boundary curves. Numerical calculations have been carried out in two cases, for successive dimerizations (Gilbert, 1959), where it was predicted that only one boundary should exist, and for a system containing monomer, dimer, and trimer, where two boundaries were shown to occur (Bethune and Kegeles, 1961). Here the pattern for only one set of the relevant parameters was investigated.

Both of these systems, however, involved the presence of significant amounts of dimer, and on the basis of the simple model, might not be expected to exhibit extra boundary formation. A more favorable model system to investigate involves successive trimerizations, where each reaction alone could give rise to two boundaries. This paper details the results of a numerical investigation of the asymptotic equations formulated for the model system monomer–trimer–nonamer.[1]

Theory

The relevant asymptotic equations are formulated as by Gilbert (1959) and Rao and Kegeles (1958). The starting point is the continuity equation

$$\frac{\partial(C_m + C_t + C_n)}{\partial t} + V_m\frac{\partial C_m}{\partial x} + V_t\frac{\partial C_t}{\partial x} + V_n\frac{\partial C_n}{\partial x} = 0 \qquad (1)$$

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[1] A similar examination of the system monomer–dimer–trimer over a wide range of the relevant parameters is in process (J. L. Bethune, L. W. Nichol, D. L. Oberhauser, and P. J. Grillo, unpublished data).

where C_m, C_t, and C_n refer to the mass concentrations of monomer, trimer, and nonamer, respectively, and V_m, V_t, and V_n refer to the velocities of the monomer, trimer, and nonamer, respectively, along the x axis, and t is time.

Definition of two equilibrium constants, $K_t = C_m{}^3/C_t$ and $K_n = C_m{}^9/C_n$, allows eq 1 to be reformulated as

$$\frac{\partial C_m}{\partial t}\left(1 + \frac{3C_m{}^2}{K_t} + \frac{9C_m{}^8}{K_n}\right) + \left(V_m + \frac{3V_tC_m{}^2}{K_t} + \frac{9V_nC_m{}^8}{K_n}\right)\frac{\partial C_m}{\partial x} = 0 \quad (2)$$

Definition of a parameter, δ, where $\delta = ((\partial x/\partial t) - V_m)/(V_n - V_m)$, and application of the relations between partial derivatives allows eq 2 to be written

$$\delta = \frac{\dfrac{3V_t{}'}{K_tV_n{}'}C_m{}^2 + \dfrac{9}{K_n}C_m{}^8}{1 + \dfrac{3}{K_t}C_m{}^2 + \dfrac{9}{K_n}C_m{}^8} \quad (3)$$

where $V_t{}' = V_t - V_m$ and $V_n{}' = V_n - V_m$. Since the total concentration

$$C^0 = C_m + C_t + C_n = C_m + \frac{C_m{}^3}{K_t} + \frac{C_m{}^9}{K_n} \quad (4)$$

Then

$$\frac{\partial C}{\partial x} = \frac{\partial C_m}{\partial x}\left(1 + \frac{3C_m{}^2}{K_t} + \frac{9C_m{}^8}{K_n}\right) \quad (5)$$

where $\partial C/\partial x$ represents the gradient of the total mass concentration. $\partial C_m/\partial x$ is found from eq 3 resulting in

$$Z = V_n{}'t\frac{\partial c}{\partial x} = \frac{C_m{}^3\left(\dfrac{3R}{K_t} + \dfrac{9C_m{}^6}{K_n}\right)^2}{\delta^2\left[\dfrac{6R}{K_t} + \dfrac{72C_m{}^6}{K_n} - \delta\left(\dfrac{6}{K_t} + \dfrac{72C_m{}^6}{K_n}\right)\right]} \quad (6)$$

where $\partial\delta/\partial x = 1/V_nt$, since $\partial x/\partial t$ may be written as x/t (Nichol *et al.*, 1964), and $R = (V_t{}'/V_n{}')$.

In the formulation for monomer, dimer, and trimer (Rao and Kegeles, 1958; Bethune and Kegeles, 1961), the resultant quadratic equation, corresponding to eq 3, could be solved analytically, as would also be possible for a system containing no polymer of higher degree than five, allowing closed solutions describing the asymptotic gradient pattern. Here, however, since eq 3 involves the eighth power of C_m, no closed solution is possible and a numerical investigation was, therefore, carried out.

Method of Numerical Computation

The logical structure of the program written to evaluate the gradient pattern was formulated as follows: for a given set of the relevant parameters (*i.e.*, C^0, the initial total concentration, K_t and K_n, the equilibrium constants, and R, the velocity ratio), the initial value of C_m, which is also the value in the plateau region, was determined using eq 4. Equation 3 was then employed, using this value of C_m, to give the maximum value δ can attain for the given set of parameters. This maximum value of δ, δ_{max}, was divided by 100 and the corresponding values of C_m and Z found from eq 3 and 6. The calculation is repeated at successive increments of $\delta_{max}/100$ up to δ_{max}.

This leaves one point to be evaluated, that at $\delta = 0$. From eq 3, for $\delta = 0$, the only physically satisfactory condition is $C_m = 0$. Also from eq 3

$$\frac{\delta C_m}{\partial\delta} = \frac{1 + \dfrac{3}{K_t}C_m{}^2 + \dfrac{9}{K_n}C_m{}^8}{\dfrac{6}{K_t}C_m(R - \delta) + \dfrac{72}{K_n}C_m{}^7(1 - \delta)}$$

and as $\delta \rightarrow 0$, $C_m \rightarrow 0$; therefore $\partial C_m/\partial\delta \rightarrow \infty$. But

$$\frac{\partial C}{\partial x} = \frac{\partial C\partial C_m\partial\delta}{\partial C_m\partial\delta\partial x} = \left(1 + \frac{3C_m{}^2}{K_t} + \frac{9C_m{}^8}{K_n}\right)\frac{\partial C_m}{\partial\delta}\frac{1}{V_n{}'t}$$

Then

$$\lim_{\delta\rightarrow 0} Z = \lim_{\delta\rightarrow 0}\left(1 + \frac{3C_m{}^2}{K_t} + \frac{9C_m{}^8}{K_n}\right)\frac{\partial C_m}{\partial\delta} \rightarrow \infty$$

since here it is legitimate to take the limits of the individual factors. Therefore, if $C > 0$, there is always one boundary present at the origin of the coordinate system.

Indeed, examination of Gilbert's equations (Gilbert, 1955) for monomer–single polymer systems indicate that $\lim_{\delta\rightarrow 0} Z$ is finite only when dimer is present. Thus

$$C_m = \left(\frac{1}{nK_n}\frac{\delta}{1 - \delta}\right)^{\frac{1}{n-1}}$$

and

$$C_p = K_n\left(\frac{1}{nK_n}\frac{\delta}{1 - \delta}\right)^{\frac{n}{n-1}}$$

where K_n is the equilibrium constant for the nth polymer, p. It is obvious that, for $n = 2$

$$\lim_{\delta\rightarrow 0}\frac{\delta C_m}{\delta x} = \frac{1}{2K_2V_p{}'t}$$

$$\lim_{\delta\rightarrow 0}\frac{C_p}{\partial x} = 0$$

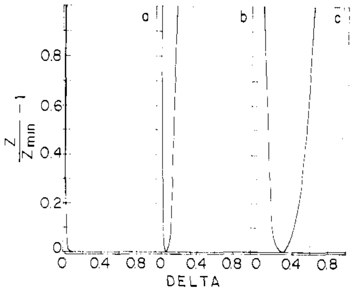

FIGURE 1: Effect of predominance of either polymer. (a) $K_t = 10^{-5}$ g^2/l.^{2}, $K_n = 10^{-10}$ g^8/l.^{8}, and $C^0 = 10^{-3}$ g/l. C^0 is below the level at which the second boundary appears. (b) Same as in a, but $C^0 = 10$ g/l., trimer emphasized. (c) $K_t = 10^5$ g^2/l.^{2}, $K_n = 10^{-20}$ g^8/l^8, $C^0 = 10^{-1}$ g/l, nonomer emphasized. The Z values have been normalized to those at the minimum. The dashed vertical lines, b and c, designate δ_{max} for that concentration.

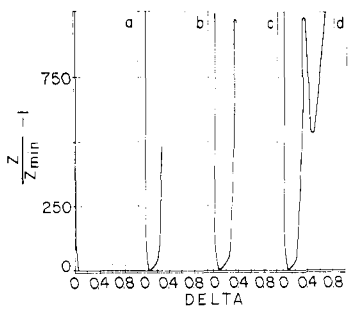

FIGURE 2: Effect of increasing concentration, significant amounts of both trimer and nonomer being present. $K_t = 10^{-5}$ g^2/l.^{2} and $K_n = 10^{-14}$ g^8/l.^{8}. (a) $C^0 = 10^{-3}$ g/l. (b) $C^0 = 3 \times 10^{-2}$ g/l. (c) $C^0 = 3 \times 10^{-1}$ g/l. (d) $C^0 = 10$ g/l. The Z values have been normalized to those at the minimum. The dashed lines indicated δ_{max} for that value of C^0.

While for $n > 2$

$$\lim_{\delta \to 0} \frac{C_m}{\partial x} \to \infty$$

$$\lim_{\delta \to 0} \frac{\partial C_p}{\partial x} = 0$$

For the monomer, dimer, trimer system it was found

$$\lim_{\delta \to 0} \frac{\delta C}{\delta x} = \frac{1}{2K_2V_p't}$$

(Rao and Kegeles, 1958), *i.e.*, the limiting result is that obtained for a dimerizing system alone. Formulation of the relevant equations for the system monomer, trimer, and tetramer for which a closed solution also may be written, yields

$$\lim_{\delta \to 0} \frac{\delta C}{\delta x} \to \infty$$

Therefore, only if dimer is present is a finite value found for the limit of the gradient at $\delta = 0$. The present calculations were also carried out for three additional values of δ, namely, $\delta_{max}/10^3$, $\delta_{max}/10^4$, and $\delta_{max}/10^5$. In all cases Z increased rapidly over this range of δ.

A FORTRAN IV program using the above logical flow pattern was written for an IBM 7094 digital computer. The program incorporates a double precision Newtonian root-finding subroutine, with appropriate logical criteria for convergence to the required single positive root of eq 3. This subroutine was tested utilizing equations formulated from known roots. The program also incorporates subroutines which list the positions of maxima and minima, as well as plotting routines. The main program has been checked by selecting random values of δ in different patterns and hand calculating the corresponding natures of Z. No errors were found to the limits to which the hand calculations were carried out, usually three significant figures.

Results and Discussion

The significant parameters are C^0, the initial concentration, K_t and K_n, the dissociation constants for trimer and nonamer, respectively, and the reduced velocity ratio, R (*i.e.*, V_t'/V_n'). The ranges over which they have been examined are, for C^0, 10^{-3} to 10^3 g/l.; for K_t, 10^{-6} to 10^4 g^2/l.^{2}; and for K_n, 10^{-20} to 10^{-10} g^8/l.^{8}. The majority of calculations were performed using a value of 0.325 for R, calculated assuming spherical molecules. A few calculations have been carried out using other values of the ratio. A total of 3000 sets of the parameters have been investigated.

Effect of Changes in C^0 at Fixed Values of K_t, K_n, and R. Figure 1 shows the effect of increasing concentration when the values of the equilibrium constants are chosen to emphasize trimer (Figure 1b) or nonamer (Figure 1c). There are maximally only two boundaries and the minimum is at the expected position (Gilbert, 1955), *i.e.*, at 0.06 for trimer and 0.29 for nonamer. When, however, significant amounts of both polymers are present, a third maximum may appear in the gradient curve (Figure 2). Here, at the lowest concentration investigated, only one boundary appears, at $\delta = 0$ (Figure 2a). As the concentraiton is increased, the pattern appears to develop into the type illustrated in Figure 1, with a second maximum appearing at $\delta = 0.33$ (Figure 2b). With further increase in concentration, however, the gradient again becomes negative (Figure 2c), defining an intermediate inflection point in the boundary curve, and, as the concentration is still further increased, a third boundary appears (Figure 2d).

It should be emphasized here that the system is in equilibrium and all three species are present in their equilibrium concentrations at all positions. Moreover, while boundaries appear in the positions roughly associated with monomer and nonamer, no identification of boundaries with species is possible; indeed, the intermediate boundary is not at a position associated with any of the species present.

As in the monomer–single polymer situation, however, the boundary at $\delta = 0$ is not affected by an increase in concentration, with respect to either position or area. Remarkably, this is true also of the second, intermediate boundary, once it, too, is fully formed. Again, as in the single polymer case (Gilbert, 1955), when the concentration has reached a value which allows the minimum positions to be reached, further increase in concentration appears only under the third, rapid boundary, and the value of $\delta_{\max}$, *i.e.*, the velocity of this boundary, increases with concentration. Thus, for a system of this type, as the total concentration is increased, first one, then two, and finally three boundaries may be seen. A further increase in concentration, however, will reveal that the areas under the first *two* boundaries are constant, as are their velocities, while both the area and velocity of the third boundary increase together.

Effect of Changes in K_t and K_n at Fixed Values of C^0 and R. While in the monomer–single polymer case the position of the minimum between two boundaries is a function only of the degree of polymerization (Gilbert, 1955), this is no longer the case when more than one polymer occurs (Figure 3). Here the position of both minima, as well as that of the intermediate maximum, are dependent upon the values assigned to the equilibrium constants, but *not* upon C^0. An examination of the individual results reveals that certain patterns occur (Table I). Thus, over the range that calculations have been performed, the maximum value that the intermediate boundary can attain, on the δ scale, is 0.33. As K_n and K_t are varied, the lowest value attained is 0.11. At any fixed value of K_n, decreasing K_t, *i.e.*, increasing the relative concentration of trimer, raises the position of the intermediate maximum up to a limiting δ value of 0.34. Similar effects are seen for the position of the second minimum. Thus, the functional relationship between the values of K_t and K_n and those of δ at which these inflection points occur tends to a limiting form. The position at which the first minimum occurs, however, is much less sensitive to variations in these parameters. Thus, in terms of the monomer–single polymer model, $\delta_{\min}$ for trimer should occur at 0.17 on a scale in which V_t is the largest possible attainable value. On the scale used here, where V_n is the largest attainable value, this corresponds to a value of 0.06 and, indeed, one inflection point occurs regularly near this value. However, $\delta_{\min}$ for nonamer alone should appear at 0.29 and neither the intermediate maximum nor the second minimum occurs here. Thus, the positions of the other two inflection points are not related to those of the simpler model.

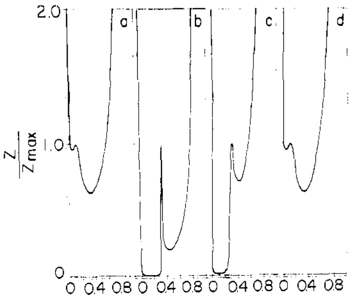

FIGURE 3: Effect of varying K_t and K_n. (a) $K_t = 10^{-3}$ g^2/l.^{2} and $K_n = 10^{-12}$ g^8/l.^{8}. (b) $K_t = 10^{-5}$ g^2/l.^{2} and $K_n = 10^{-12}$ g^8/l.^{8}. (c) $K_t = 10^{-5}$ g^2/l.^{2} and $K_n = 10^{-18}$ g^8/l.^{8}. (d) $K_t = 10^{-5}$ g^2/l.^{2}, $K_n = 10^{-20}$ g^8/l^8. The Z values have been normalized to those of the intermediate maximum.

TABLE I: Effect of Changes in K_t and K_n on the Positions of Inflection Points.[a]

K_t	K_n	$\delta_{\min 1}$	$\delta_{\max 2}$	$\delta_{\min 2}$
10^{-5}	10^{-20}	0.06	0.11	0.33
10^{-5}	10^{-19}	0.05	0.21	0.35
10^{-5}	10^{-18}	0.05	0.28	0.38
10^{-5}	10^{-17}	0.05	0.30	0.40
10^{-5}	10^{-16}	0.06	0.31	0.43
10^{-5}	10^{-15}	0.06	0.32	0.43
10^{-5}	10^{-10}	0.06	0.33	0.44
10^{-3}	10^{-12}	0.06	0.11	0.33
10^{-4}	10^{-12}	0.06	0.31	0.42
10^{-5}	10^{-12}	0.06	0.32	0.44

[a] $R = 0.325$. The concentration at which all three boundaries appear is a function of the values assigned to these constants. Here are listed values of K_t and K_n for which three boundaries appear at any concentration in the range 10^{-3} to 10^3 g/l.

Effect of Variation of R, with Fixed Values of K_t, K_n, and C^0. The position of the intermediate maximum is also a function of the reduced velocity ratio (Figure

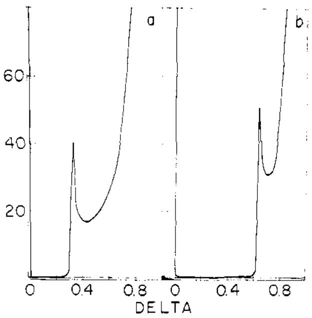

FIGURE 4: Effect of variation in the reduced velocity ratio, R. $K_t = 10^{-5}$ g^2/l.^{2} and $K_n = 10^{-13}$ g^8/l.^{8}. (a) $R = 0.325$. (b) $R = 0.649$.

4). The effect is directly predictable, since an increase in the ratio, *i.e.*, the velocities of trimer and nonamer approach one another, is accompanied by a shift in the position of the inflection points toward higher results of δ, the magnitude of the areas under the different boundaries changing concomitantly. While the value of this ratio will not be a variable in any single system when examined by *one* technique, such changes may become important when the system is examined by different techniques, *e.g.*, sedimentation and gel filtration, where the factors determining the value of R may be quite different, resulting in the appearance of different patterns. This, of course, is also true for the monomer–single polymer model.

While no experimental systems are presently known which can be used to test the theoretical predictions developed here, they may be used immediately to detect the possible occurrence of such systems. Thus, if sedimentation velocity experiments are performed as a function of concentration, theory predicts that first one, then two, and finally three boundaries will be found, that neither the areas under the two slower boundaries or their velocities will increase with increasing concentration, but that both the area and velocity of the third fast boundary will do so. Moreover, if any of the equilibrium constants are functions of temperature (*i.e.*, if $\Delta H \neq 0$), the position of the intermediate boundary and the second minimum will vary with temperature, since they are functions of these constants. These experimental approaches would be sufficient to define the system as one in which these reactions occur.

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