Biophysical Chemistry, 38 (1990) 231–239
Elsevier

BIOCHE 01513

# Kinetic model for membrane transport

## 2. Time lag and overshoot

Kimiko Makino [a], Hiroyuki Ohshima [a] and Tamotsu Kondo [a,b]

[a] *Faculty of Pharmaceutical Sciences and Institute of Colloid and Interface Science, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162 and* [b] *Research Institute for Biosciences, Noda-shi, Chiba 287, Japan*

Received 18 October 1989
Revised manuscript received 10 May 1990
Accepted 29 May 1990

Membrane transport; Time lag; Overshoot

A lag time during the period of variation in solute concentration in the receiver phase and overshoot in that in the membrane phase have been predicted to occur with a kinetic model for membrane transport which takes into account both the membrane volume and the partitioning kinetics (Makino et al., Biophys. Chem. 35 (1990) 85). The duration of the lag time becomes longest when the donor and receiver phases have the same volume. This maximum grows in length with increase in the partition coefficient, tending to be proportional to the volume fraction of the receiver phase. Moreover, it displays an increase in length with decreasing membrane volume fraction. Overshoot occurs only when the volume fraction of the receiver phase is greater than that of the donor. Overshoot is observed during the earlier stages of membrane transport when the partition coefficient is smaller or the volume fraction of the receiver phase is larger.

## 1. Introduction

A number of investigations have been reported [1,2] on the overshoot phenomenon in which the concentration of the solute transitorily exceeds its equilibrium value in a membrane system. In the preceding paper [3] *, we presented a simple kinetic model for the full time course of membrane transport from the source solution phase (solution 1) to a receiver phase (solution 2) through a membrane (see fig. 1). Overshoot during the time course of solute concentration in the membrane was observed under certain conditions. Furthermore, a lag period was demonstrated for the increase in solute concentration in the receiver

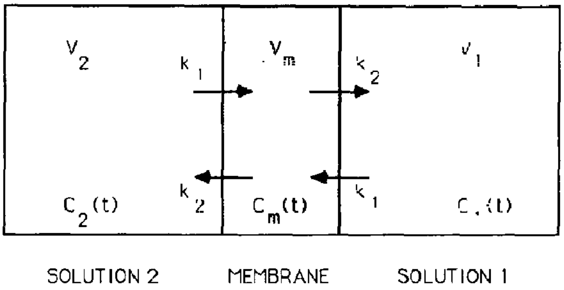

Fig. 1. A membrane of volume $V_m$ separating two solutions, 1 and 2, whose volumes are $V_1$ and $V_2$, respectively. $\phi$ and $\phi_m$, respectively; volume fractions of solution 2 and membrane, $C_1(t)$, $C_2(t)$ and $C_m(t)$, solute concentrations of the respective phases; $k_1$ and $k_2$, rate constants.

Correspondence address: T. Kondo, Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan.

* *Note*: In Ref. 3, on p. 87, in the legend to fig. 2 (b), $e^{-f\alpha}$ (curve 3) and $e^{-g\alpha}$ (curve 4) should read $e^{-f\alpha t}$ (curve 3) and $e^{-g\alpha t}$ (curve 4), respectively. On p. 90, in eq. 45, $C_m(t)$ should read $C_m(t)/C_0$. On p. 94, the legend to fig. 8 should include the final part of the last sentence: when $\phi = 0.6$, $\phi_m = 0.2$ and $K = 1$.

solution phase (solution 2). In this model, both the finite volume of a membrane and the partitioning kinetics were taken into account without assuming the process of partitioning to attain equilibrium. With this model, when the membrane, which is permeable to the solute, separates two solutions, 1 and 2, the time course of the variation in solute concentration in the respective phases is described as follows.

$$V_1 \frac{\mathrm{d}C_1(t)}{\mathrm{d}t} = -k_1 C_1(t) + k_2 C_\mathrm{m}(t) \tag{1}$$

$$V_2 \frac{\mathrm{d}C_2(t)}{\mathrm{d}t} = -k_1 C_2(t) + k_2 C_\mathrm{m}(t) \tag{2}$$

$$V_\mathrm{m} \frac{\mathrm{d}C_\mathrm{m}(t)}{\mathrm{d}t} = -k_1 (C_1(t) + C_2(t)) - 2k_2 C_\mathrm{m}(t) \tag{3}$$

where $V$ and $C$ represent the volume and the solute concentration in the respective phases as indicated by the subscripts 1, 2 and m corresponding to the donor phase (solution 1), receiver phase (solution 2) and membrane. Note that $C_\mathrm{m}$ is the solute concentration averaged over the membrane phase. $k_1$ denotes the rate constant relating to transport of solute from the solution into the membrane phase, $k_2$ corresponding to that from the membrane phase to the solution.

The initial conditions are as follows.

$$C_1(0) = C_0 \tag{4}$$

$$C_2(0) = 0 \tag{5}$$

$$C_\mathrm{m}(0) = 0 \tag{6}$$

Subject to the initial conditions (eqs 4–6) the time course of the solute concentration in solution 1, solution 2 and the membrane is given as follows.

$$\begin{aligned}\frac{C_1(t)}{C_0} = {} & \frac{1-\phi-\phi_\mathrm{m}}{1+(K-1)\phi_\mathrm{m}} \\ & + [g(\phi + FK\phi_\mathrm{m}) \exp(-P_1\gamma t) \\ & -f(\phi + GK\phi_\mathrm{m}) \exp(-P_2\gamma t)] \\ & \times [(g-f)(1+(K-1)\phi_\mathrm{m})]^{-1}\end{aligned} \tag{7}$$

$$\begin{aligned}\frac{C_2(t)}{C_0} & = \frac{1-\phi-\phi_\mathrm{m}}{1+(K-1)\phi_\mathrm{m}} \\ & \quad \times \left(1 - \frac{g \exp(-P_1\gamma t) - f \exp(-P_2\gamma t)}{g-f}\right)\end{aligned} \tag{8}$$

$$\begin{aligned}\frac{C_\mathrm{m}(t)}{C_0} & = \frac{K(1-\phi-\phi_\mathrm{m})}{1+(K-1)\phi_\mathrm{m}} \\ & \quad \times \left(1 - \frac{gF \exp(-P_1\gamma t) - fG \exp(-P_2\gamma t)}{g-f}\right)\end{aligned} \tag{9}$$

with

$$\alpha = \frac{k_1}{2}\left(\frac{1}{V_1} + \frac{1}{V_2}\right) = \frac{1-\phi_\mathrm{m}}{2(1-\phi-\phi_\mathrm{m})\phi}\gamma \tag{10}$$

$$\gamma = k_1/(V_1 + V_2 + V_\mathrm{m}) \tag{11}$$

$$P_1 = \alpha f/\gamma \tag{12}$$

$$P_2 = \alpha g/\gamma \tag{13}$$

$$\phi = V_2/(V_1 + V_2 + V_\mathrm{m}) \tag{14}$$

$$\phi_\mathrm{m} = V_\mathrm{m}/(V_1 + V_2 + V_\mathrm{m}) \tag{15}$$

$$K = k_1/k_2 \tag{16}$$

$$\begin{aligned}f = 1 + \frac{2\phi(1-\phi-\phi_\mathrm{m})}{K\phi_\mathrm{m}(1-\phi_\mathrm{m})} - \Bigg(\left(1 - \frac{2\phi}{1-\phi_\mathrm{m}}\right)^2 \\ + \left(\frac{2\phi(1-\phi-\phi_\mathrm{m})}{K\phi_\mathrm{m}(1-\phi_\mathrm{m})}\right)^2\Bigg)^{1/2}\end{aligned} \tag{17}$$

$$\begin{aligned}g = 1 + \frac{2\phi(1-\phi-\phi_\mathrm{m})}{K\phi_\mathrm{m}(1-\phi_\mathrm{m})} + \Bigg(\left(1 - \frac{2\phi}{1-\phi_\mathrm{m}}\right)^2 \\ + \left(\frac{2\phi(1-\phi-\phi_\mathrm{m})}{K\phi_\mathrm{m}(1-\phi_\mathrm{m})}\right)^2\Bigg)^{1/2}\end{aligned} \tag{18}$$

$$F = 1 - \frac{(1-\phi_\mathrm{m})f}{2(1-\phi-\phi_\mathrm{m})} \tag{19}$$

$$G = 1 - \frac{(1-\phi_\mathrm{m})g}{2(1-\phi-\phi_\mathrm{m})} \tag{20}$$

where $\phi$ and $\phi_m$ designate the volume fractions of solution 2 and the membrane, respectively. We have replaced $\alpha f$ and $\alpha g$ in eqs 16–18 of our previous article [3], which correspond to eqs 7–9 in the present paper, with $P_1\gamma$ and $P_2\gamma$, respectively. Furthermore, we use scaled time $\gamma t$ instead of $t$. At equilibrium ($t \to \infty$), eqs 7–9 give

$$C_m(\infty) = KC_1(\infty) = KC_2(\infty) \tag{21}$$

and eqs 1–3 satisfy

$$V_1C_1(t) + V_2C_2(t) + V_mC_m(t) = V_1C_0 \tag{22}$$

The purpose of the current article is to explain the time lag associated with the increase in solute concentration in solution 2 and the overshoot of the solute concentration in the membrane by means of this model.

## 2. Basic equations

The lag period observed during the course of increase in the solute concentration in solution 2 can be defined as the time interval between $t = 0$ and $t = t_L$ within which $C_2(t)/C_0$ displays a point of inflection.

From eq. 2,

$$\frac{d^2C_2(t)}{dt^2} = -\frac{2\alpha(1-\phi-\phi_m)}{(1-\phi_m)} \times\left[\frac{dC_2(t)}{dt} - \frac{1}{K}\frac{dC_m(t)}{dt}\right] \tag{23}$$

with eqs 8 and 9,

$$t_L = \frac{\ln(g/f)}{\alpha(g-f)} \tag{24}$$

$$\gamma t_L = \frac{2\phi(1-\phi-\phi_m)}{(1-\phi_m)}\frac{\ln(g/f)}{(g-f)} \tag{25}$$

Overshoot in $C_m(t)/C_0$ occurs at $t = t_0$, when the slope of $C_m(t)/C_0$ is zero.

From eq. 9,

$$t_0 = \frac{\ln(F/G)}{\alpha(f-g)} \tag{26}$$

$$\gamma t_0 = \frac{2\phi(1-\phi-\phi_m)}{(1-\phi_m)}\frac{\ln(F/G)}{(f-g)} \tag{27}$$

Since the value of $g$ is always greater than that of $f$, $F$ is always greater than $G$. Therefore, only when $G < F < 0$ can $t_0$ be positive which indicates that overshoot can be observed solely when the volume of solution 2 is greater than that of solution 1, i.e., $1-2\phi-\phi_m < 0$.

## 3. Results and discussion

We have carried out an investigation aimed at the elucidation of the behavior characteristic of the lag time and overshoot phenomenon observed during the period of variation in solute concentration in solution 2 and in the membrane, respectively. The results obtained are shown in figs 2–17, where the scaled time $\gamma t$ is used, since $\gamma t$ does not depend on the values of the volume fraction $\phi$ or those of $\phi_m$.

We have defined the lag time in the time course of the solute concentration in solution 2 as the time interval from $t = 0$ to $t = t_L$ as expressed by eq. 24. This equation contains $\alpha$, $\ln(g/f)$ and $(g-f)$ which depend on $K$, $\phi$ and $\phi_m$.

When solutions 1 and 2 have equal volumes ($V_1 = V_2$), the time lag is found to be the longest as seen in fig. 2. When $V_1 = V_2$ $(1 - 2\phi - \phi_m = 0)$,

$$\gamma t_L = \frac{K\phi_m}{2}\ln\left(1 + \frac{1-\phi_m}{K\phi_m}\right) \tag{28}$$

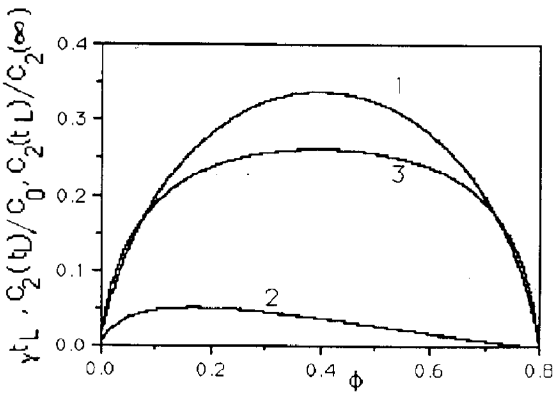

Fig. 2. Effects of $\phi$ on $\gamma t_L$ (curve 1), $C_2(t_L)/C_0$ (curve 2) and $C_2(t_L)/C_2(\infty)$ (curve 3), when $K = 10$ and $\phi_m = 0.2$.

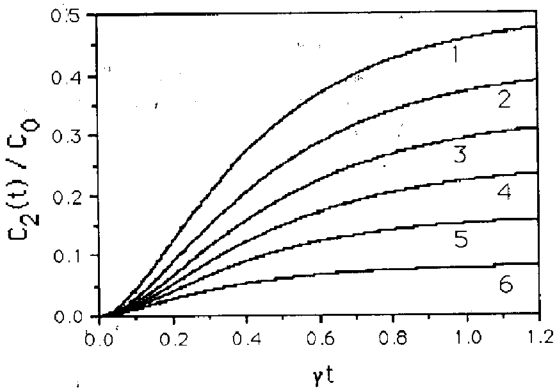

Fig. 3. Effects of $\phi$ on $C_2(t)/C_0$ when $K = 2$, $\phi_m = 0.2$ and $\phi = 0.2$ (curve 1), 0.3 (2), 0.4 (3), 0.5 (4), 0.6 (5), 0.7 (6).

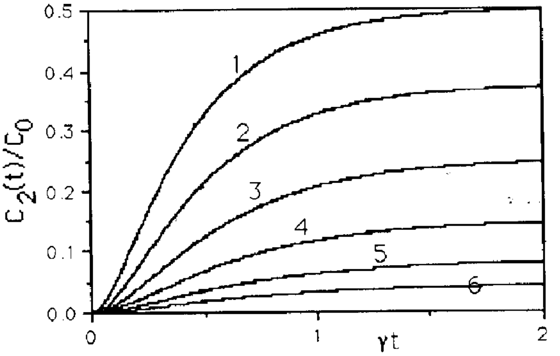

Fig. 5. Effects of $K$ on $C_2(t)/C_0$ when $\phi = 0.2$, $\phi_m = 0.2$ and $K = 2$ (curve 1), 4 (2), 8 (3), 16 (4), 32 (5), 64 (6).

In the case shown in Fig. 2, $C_2(t_L)/C_2(\infty)$ also has a maximum at $1 - 2\phi - \phi_m = 0$, where

$$\frac{C_2(\infty)}{C_0} = \frac{1 - \phi - \phi_m}{1 + (K - 1)\phi_m} \tag{29}$$

Therefore, $C_2(t_L)/C_0$ also displays a maximum at a particular value of $\phi$. This occurs as a result of the fact that, on $\phi$ increasing, $C_2(\infty)/C_0$, which is given in eq. 29, decreases, as is apparent in fig. 3.

Fig. 4 demonstrates the effects of the partition coefficient, $K$, on $\gamma t_L$, $C_2(t_L)/C_0$ and $C_2(t_L)/C_2(\infty)$. Here, $k_2$ is varied while $k_1$ is held constant. When $K \to 0$, $\gamma t_L$ also tends to zero, and for $K$ increasing in value, $\gamma t_L$ becomes greater, indicating that the time lag lengthens. However, $C_2(t_L)/C_0$ and $C_2(t_L)/C_2(\infty)$ attain a maximum at a certain value of $K$. As described in the preceding paper [3], during the initial stages of membrane transport, $C_2(t)/C_0$ is proportional to $t^2$.

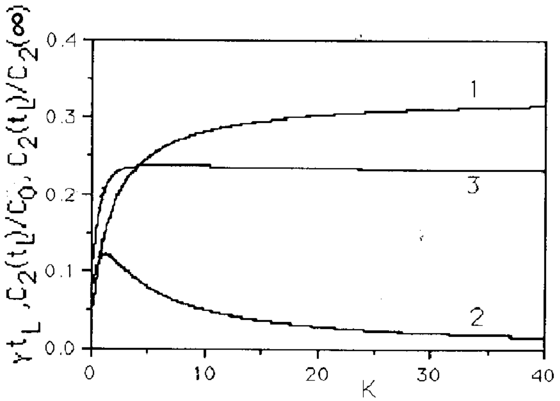

Fig. 4. Effects of $K$ on $\gamma t_L$ (curve 1), $C_2(t_L)/C_0$ (curve 2) and $C_2(t_L)/C_2(\infty)$ (curve 3), when $\phi = 0.2$ and $\phi_m = 0.2$.

$$\frac{C_2(t)}{C_0} = \frac{2\phi(1 - \phi - \phi_m)^2}{(1 - \phi_m)^2 \phi_m} \frac{1}{K} \alpha^2 t^2 \tag{30}$$

Therefore, during the early stages of membrane transport, the curvature of $C_2(t)/C_0$ at $t = 0$ diminishes as $K$ increases, resulting in an increase in $\gamma t_L$. In the case of $K$ increasing, $\gamma t_L$ becomes greater although $C_2(\infty)/C_0$, defined in eq. 29, decreases (see fig. 5). Therefore, $C_2(t_L)/C_0$ attains a maximum at a particular value of $K$. Fig. 5 illustrates the effects of $K$ on $C_2(t)/C_0$. For $K$ increasing, $C_2(t)/C_0$ and $C_2(\infty)/C_0$ decrease, while $\gamma t_L$ increases. In the limiting case where $K \to \infty$, $\gamma t_L$ is expressed as:

$$\gamma t_L = \frac{\phi(1 - \phi - \phi_m)}{(1 - 2\phi - \phi_m)} \ln \frac{1 - \phi - \phi_m}{\phi} \tag{31}$$

In the case where $V_1 = V_2$, $\gamma t_L$ is given by eq. 28, and when $K \to \infty$, $t_L$ becomes proportional to $\phi$, viz.,

$$\gamma t_L = \phi, \quad 0 < \phi < 0.5 \tag{32}$$

except at $\phi = 0.5$. Note that as $\phi \to 0.5$, $\gamma t_L$ tends to zero. Therefore, when $V_1 = V_2$ and $K$ is large, the time lag increases in duration with increase in

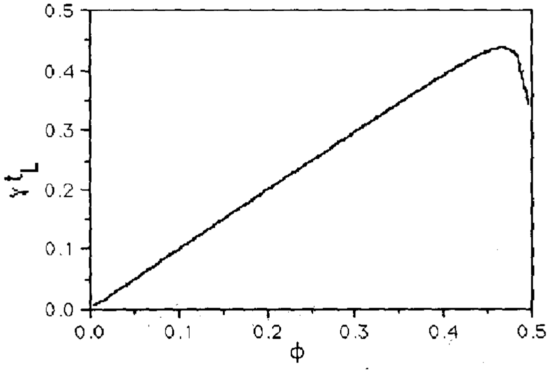

Fig. 6. Effects of $\phi$ on $\gamma t_L$ when $K = 100$ and $V_1 = V_2$.

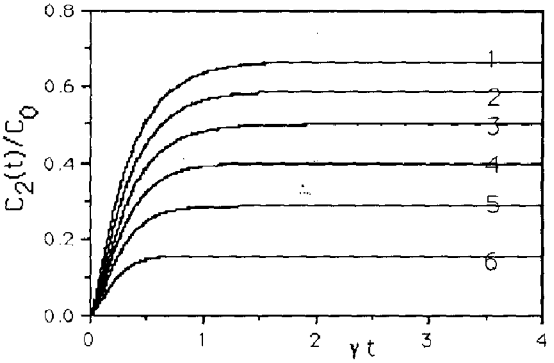

Fig. 8. Effects of $\phi_m$ on $C_2(t)/C_0$ when $K = 0.5$, $\phi = 0.2$ and $\phi_m = 0.2$ (curve 1), 0.3 (2), 0.4 (3), 0.5 (4), 0.6 (5), 0.7 (6).

the volume fraction of solution 2 (that of the membrane decreases), as shown in fig. 6.

Fig. 7 shows the effect of $\phi_m$ on the lag period for the case where $\phi$ is constant. $\gamma t_L$ displays a maximum at a certain value of $\phi_m$ when $K$ and $\phi$ are constant. As $K$ increases, with $\phi$ remaining constant, the value of $\phi_m$ at which $\gamma t_L$ reaches a maximum decreases and this maximum value of $\gamma t_L$ increases. When $\phi_m$ is large, $\gamma t_L$ is not affected by $K$. However, in the case where $\phi_m$ is small, $\gamma t_L$ is strongly dependent on $K$. As described before (see fig. 4), $\gamma t_L$ is markedly affected by $K$ when $K$ is small. Fig. 8 shows the effect of $\phi_m$ on $C_2(t)/C_0$, when $K = 0.5$ and $\phi = 0.2$.

Consequently, the time lag depends on the volume fraction of both solution 2 and the membrane and on the partition coefficients. Data obtained from the dependence of the lag period on the volume fraction of solution 2 show that the lag time is longest when solutions 1 and 2 are equal in volume. This maximum value of $\gamma t_L$ becomes greater with increasing $K$. Moreover, in this situation, as the volume fraction of membrane diminishes, the time lag lengthens correspondingly. Except when the volumes of solutions 1 and 2 are identical, the time lag becomes shorter in duration for increase in the extent of deviation of the volume fraction of solution 2 from that for equal volumes of solutions 1 and 2, while it is lengthened when the partition coefficient increases. As judged on the basis of the influence of the membrane volume fraction on the time lag, the lag period is longest at a certain value of the membrane volume fraction depending on the value of partition coefficient.

Overshoot is evident in the time course for variation in $C_m(t)/C_0$ when the volume of solution 2 is larger than that of solution 1, as described above. It is apparent from eq. 26 that $t_0$ is governed by $K$, $\phi$ and $\phi_m$.

Fig. 9 depicts the effect of $K$ on $\gamma t_0$, $C_m(t_0)/C_0$, and $C_m(t_0)/C_m(\infty)$. $C_m(t_0)/C_m(\infty)$ can be evaluated as the height of overshoot. With increase in $K$, $\gamma t_0$ also increases. Similarly, $C_m(t_0)/C_0$ also increases, although $C_m(t_0)/C_m(\infty)$ falls toward unity, as $K$ increases, since $C_m(\infty)/C_0$ increases, tending toward unity, as $K$ increases, as shown in fig. 10. That is, as $K$ decreases, overshoot appears

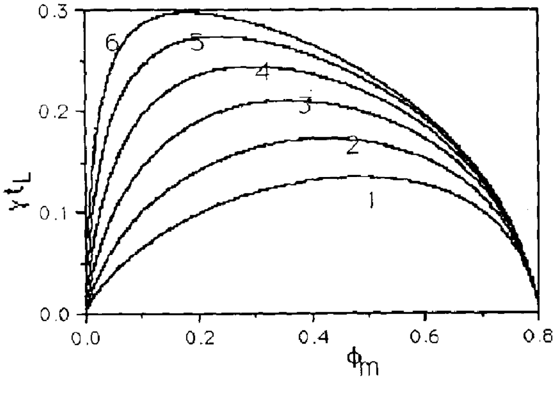

Fig. 7. Effects of $\phi_m$ on $\gamma t_L$ when $\phi = 0.2$ and $K = 0.5$ (curve 1), 1 (2), 2 (3), 4 (4), 8 (5), 16 (6).

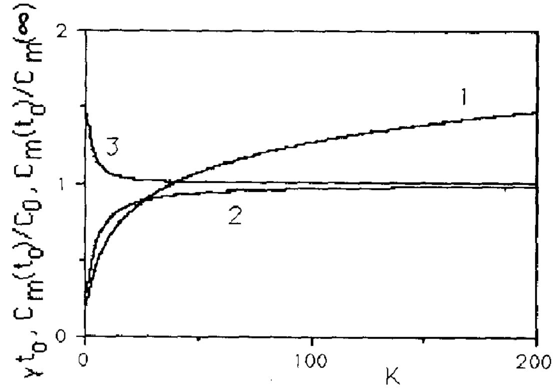

Fig. 9. Effects of $K$ on $\gamma t_0$ (curve 1), $C_m(t_0)/C_0$ (curve 2) and $C_m(t_0)/C_m(\infty)$ (curve 3), when $\phi = 0.6$ and $\phi_m = 0.2$.

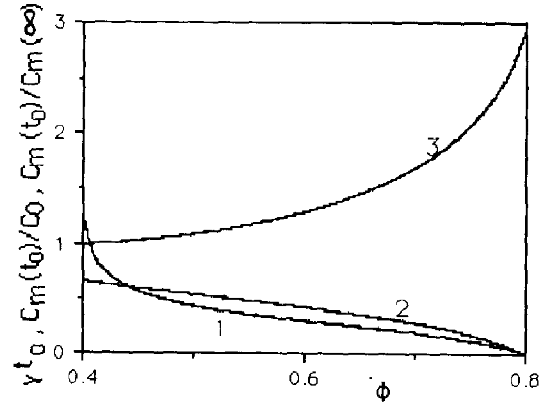

Fig. 11. Effects of $\phi$ on $\gamma t_0$ (curve 1), $C_m(t_0)/C_0$ (curve 2) and $C_m(t_0)/C_m(\infty)$ (curve 3), when $K = 2$ and $\phi_m = 0.2$.

during the earlier stages of membrane transport and its height, $C_m(t_0)/C_m(\infty)$, becomes greater.

Fig. 11 demonstrates the effects of $\phi$ on $\gamma t_0$, $C_m(t_0)/C_0$ and $C_m(t_0)/C_m(\infty)$ with $\phi_m$ held constant. When the volume fraction of solution 2 approaches that of solution 1, $\gamma t_0$ tends to infinity, showing no occurrence of overshoot for this situation. Furthermore, as $\phi$ increases, $\gamma t_0$ decreases, tending to zero, and $C_m(t_0)/C_0$ diminishes, although $C_m(t_0)/C_m(\infty)$ increases, since $C_m(\infty)/C_0$ falls with increasing $\phi$, as shown in fig. 12. Therefore, as the volume fraction of solution 2 becomes greater, overshoot occurs during the initial stages of membrane transport and is of greater height as compared with the equilibrium value, given as $C_m(t_0)/C_m(\infty)$.

Fig. 13a and b illustrates the effects of $\phi_m$ and $K$ on $\gamma t_0$. The cases where $\phi < 0.5$ and $\phi > 0.5$ must be considered separately, since when $\phi < 0.5$, $\phi_m$ lies between $1 - 2\phi$ and $1 - \phi$, while for $\phi > 0.5$, $\phi_m$ can be between 0 and $1 - \phi$. When $\phi < 0.5$ (fig. 13a), as $\phi_m$ increases from $1 - 2\phi$ to $1 - \phi$, $\gamma t_0$ decreases from infinity to zero. As $K$ increases, $\gamma t_0$ increases at each value of $\phi_m$, as shown in fig. 13a, although when $\phi_m$ is large, $\gamma t_0$ does not depend markedly on $K$. That is, when $\phi < 0.5$, overshoot occurs during the earlier stages of membrane transport as $\phi_m$ increases and $K$ decreases. Fig. 14a and b shows the effects of $\phi_m$ on $\gamma t_0$ and $C_m(t_0)/C_0$ when $\phi = 0.4$ at $K = 5$ (a) and $K = 1$ (b). The height of the overshoot as

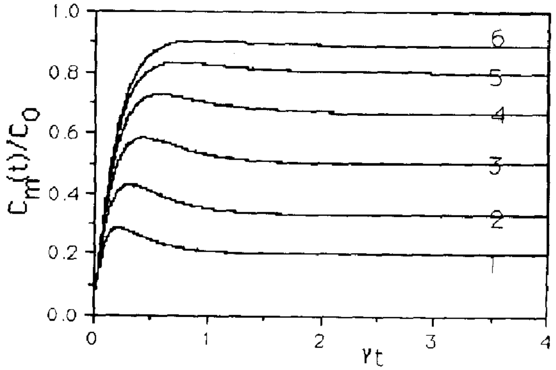

Fig. 10. Effects of $K$ on $C_m(t)/C_0$ when $\phi = 0.6$, $\phi_m = 0.2$ and $K = 1$ (curve 1), 2 (2), 4 (3), 8 (4), 16 (5), 32 (6).

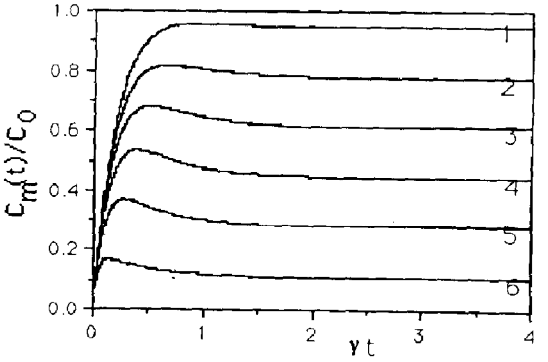

Fig. 12. Effects of $\phi$ on $C_m(t)/C_0$ when $K = 5$, $\phi_m = 0.2$ and $\phi = 0.46$ (curve 1), 0.52 (2), 0.58 (3), 0.64 (4), 0.70 (5), 0.76 (6).

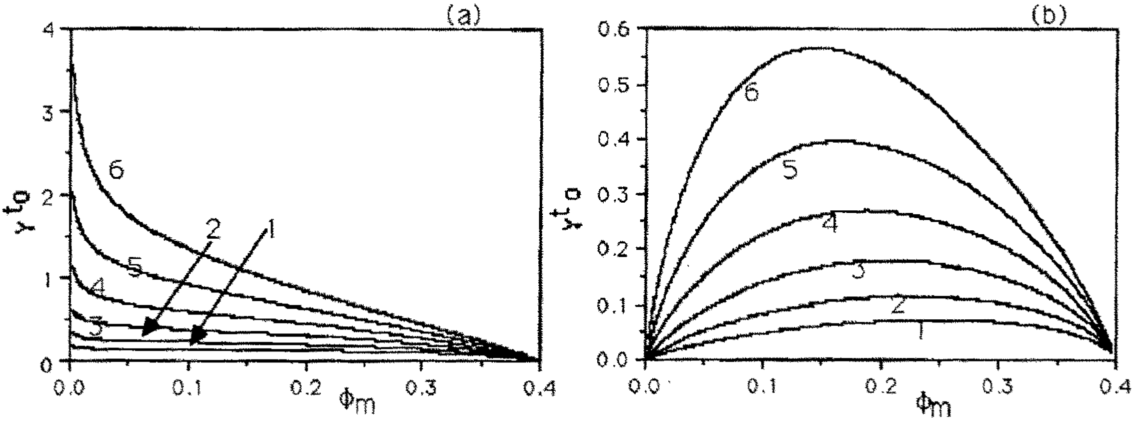

Fig. 13. Effects of $\phi_m$ and $K$ on $\gamma t_0$ when $\phi = 0.4$ (a), 0.6 (b) and $K = 0.2$ (curve 1), 0.4 (2), 0.8 (3), 1.6 (4), 3.2 (5), 6.4 (6).

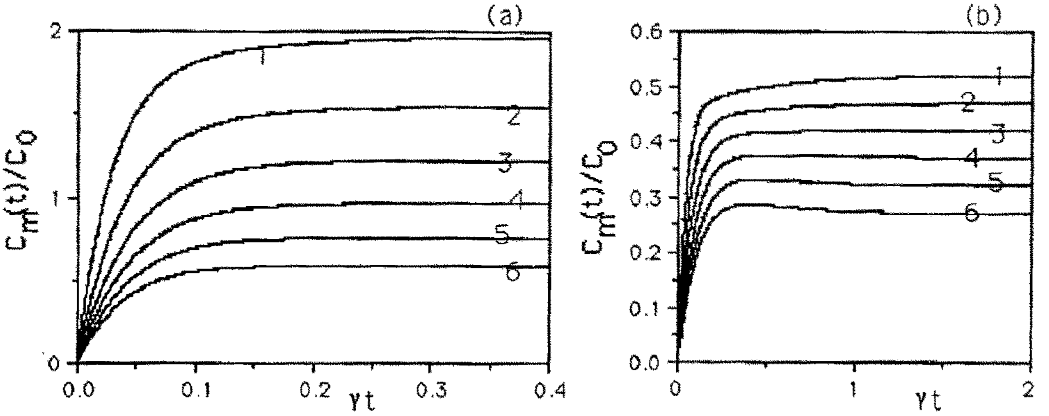

Fig. 14. Effects of $\phi_m$ on $\gamma t_0$ and $C_m(t)/C_0$ when $K = 5$ (a) and $K = 1$ (b), and $\phi_m = 0.08$ (curve 1), 0.13 (2), 0.18 (3), 0.23 (4), 0.28 (5), 0.33 (6) at $\phi = 0.4$.

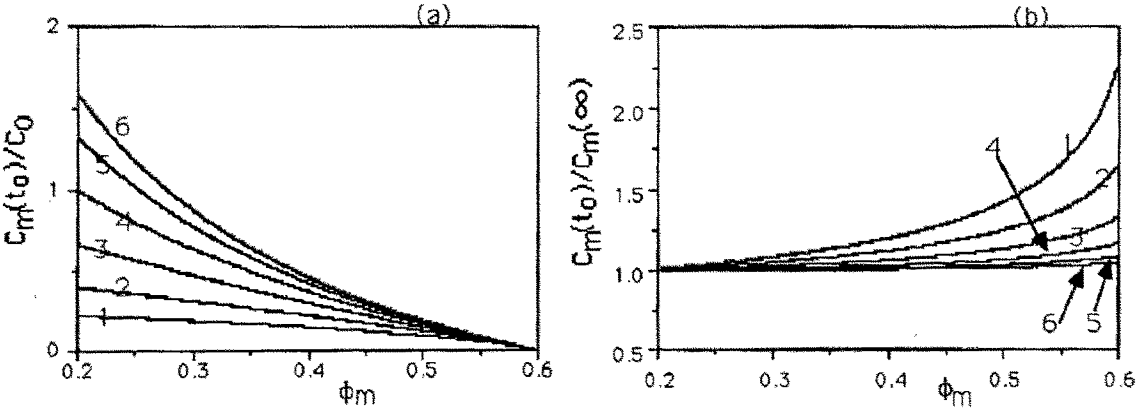

Fig. 15. Effects of $\phi_m$ and $K$ on $C_m(t_0)/C_0$ (a) and $C_m(t_0)/C_m(\infty)$ (b) when $\phi = 0.4$ and $K = 0.5$ (curve 1), 1 (2), 2 (3), 4 (4), 8 (5) and 16 (6).

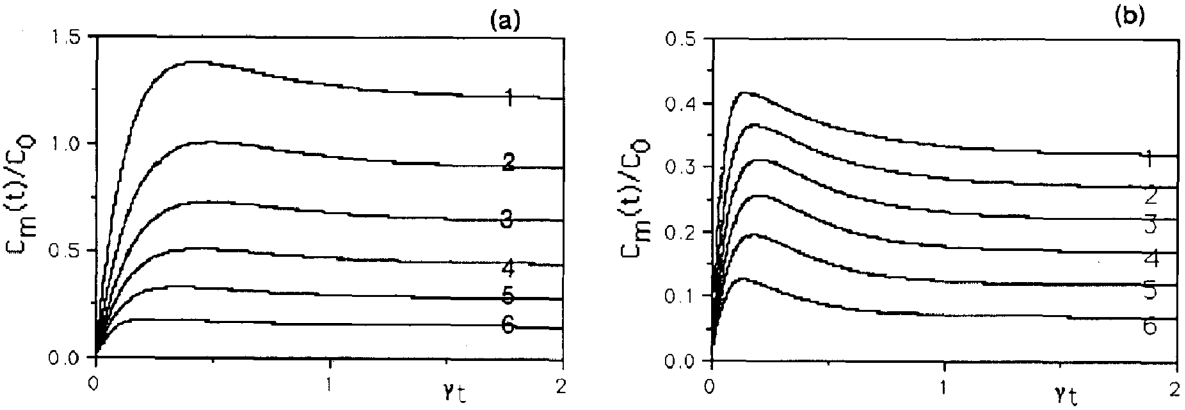

Fig. 16. Effects of $\phi_m$ on $\gamma t_0$ and $C_m(t)/C_0$ when $K = 5$ (a) and $K = 1$ (b), and $\phi_m = 0.08$ (curve 1), 0.13 (2), 0.18 (3), 0.23 (4), 0.28 (5), 0.33 (6) at $\phi = 0.6$.

compared with each equilibrium value, $C_m(t_0)/C_m(\infty)$, grows with increasing $\phi_m$ and $K$ decreases as shown in fig. 15b, since $C_m(\infty)/C_0$ decreases as $\phi_m$ increases and $K$ decreases. When $\phi > 0.5$ (fig. 13b), $\gamma t_0$ attains a maximum at a particular value of $\phi_m$. The value of $\phi_m$ at which $\gamma t_0$ exhibits the maximum decreases as $K$ increases, although when $K$ is small, $\gamma t_0$ is less strongly affected by $\phi_m$ than in the case where $K$ is large. Fig. 16a and b shows the effects of $\phi_m$ on $\gamma t_0$ and $C_m(t_0)/C_0$, when $\phi - 0.6$ at $K = 5$ (a) and $K = 1$ (b). Also, as shown in fig. 17, $C_m(t_0)/C_0$ decreases and $C_m(t_0)/C_m(\infty)$ increases as $K$ decreases. $C_m(t_0)/C_0$ diminishes as $\phi_m$ increases, although $C_m(t_0)/C_m(\infty)$ reaches a minimum at a certain value of $\phi_m$, since $C_m(\infty)/C_0$ decreases as $\phi_m$ increases. From the above, it is obvious that when $\phi > 0.5$, overshoot occurs during the earlier stages of membrane transport when the volume fraction of the membrane is either very small or very large. The time interval before overshoot occurs is longest in duration at a certain value of $\phi_m$, depending on $K$, and this maximum value of $\gamma t_0$ increases as $K$ increases. This time interval becomes much more strongly dependent upon $K$ as $K$ increases. When $\phi < 0.5$, overshoot occurs in the initial stages of membrane transport

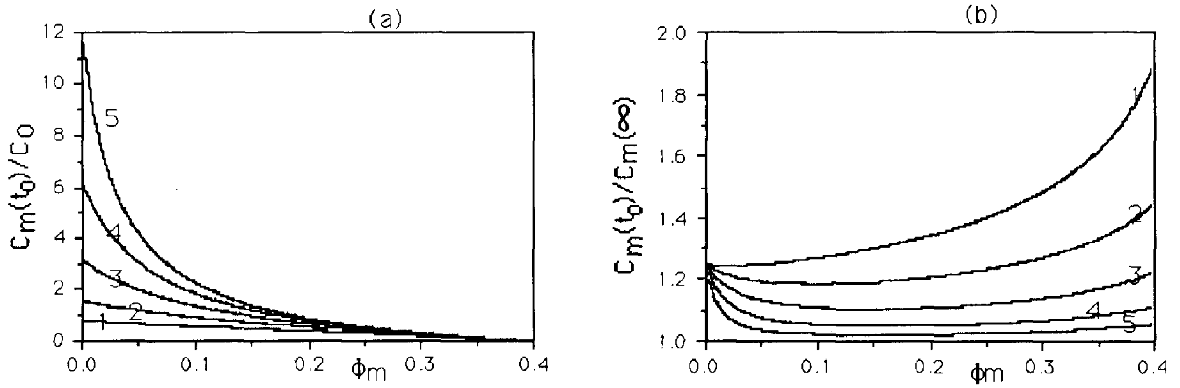

Fig. 17. Effects of $\phi_m$ and $K$ on $C_m(t_0)/C_0$ (a) and $C_m(t_0)/C_m(\infty)$ (b) when $\phi = 0.6$ and $K = 1.6$ (curve 1), 3.2 (2), 6.4 (3), 12.8 (4) and 25.6 (5).

as the membrane volume fraction increases. Overshoot occurs in the earlier stages of membrane transport when the partition coefficient is small. Also, a larger volume fraction for solution 2 induces the occurrence of overshoot in the earlier stages of membrane transport.

## References

1 R. Neihof and K. Soliner, J. Phys. Chem. 61 (1957) 159.
2 N. Takeguchi, I. Horikoshi and S. Tanaka, Bull. Chem. Soc. Jap. 48 (1975) 3044.
3 K. Makino, H. Ohshima and T. Kondo, Biophys. Chem. 35 (1990) 85.