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EXACT SOLUTION OF THE MASS TRANSFER EQUATIONS OF GEL FILTRATION CHROMATOGRAPHY BY MEANS OF A FORMAL INVERSION OF THE LAPLACE TRANSFORM, AND THE DERIVATION OF AN EQUATION FOR THE TIME SPENT BY A MOLECULE IN THE GEL PHASE

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SUMMARY

The exact solution of the equations of mass transfer in a gel filtration chromatography column, subject to realistic boundary values and initial conditions, is obtained by means of a formal inversion of the Laplace transform. The time spent by a molecule in the gel phase is also calculated.

INTRODUCTION

The equations describing mass transfer in a gel filtration chromatography column are well known. Their exact solution with realistic boundary values and initial conditions has proved refractory. Use has been made of compartmental analysis[1–4], the Mellin transform[5], the Laplace transform[6], and the numerical Laplace transform[7]. In this paper we obtain the exact solution of the mass transfer equations, subject to realistic boundary values and initial conditions, by means of a formal inversion of the Laplace transform, and we derive an equation for the time spent by a molecule in the gel phase. This latter equation enables one to design an experiment whereby the time spent by a molecule both in the gel phase and in the mobile phase can be obtained from a single experiment.

THEORY

We define the following quantities: let $1/T_1$ be the probability, per unit time, that a molecule of the object species passes from the solution to the gel; $1/T_2$ the probability, per unit time, for the reverse process; C_1 the concentration of the object species in the solution; C_2 the concentration of the object species in the gel;

G the ratio of the gel volume to that of the solution; V the linear velocity of the mobile phase; K the total amount of the object species supplied; and τ the width (in time) of the input pulse. Other quantities will be defined as they arise. Then the rate of transfer of the object species to the gel is C_1/T_1, the rate of the reverse process (per unit volume of solution) is GC_2/T_2, and so the equations of mass transfer per unit volume of solution, neglecting longitudinal diffusion in the mobile phase, are:

$$\frac{\partial C_1}{\partial t} = \frac{GC_2}{T_2} - \frac{C_1}{T_1} - V\frac{\partial C_1}{\partial x} \tag{1}$$

$$G\frac{\partial C_2}{\partial t} = \frac{C_1}{T_1} - \frac{GC_2}{T_2} \tag{2}$$

These have to be solved subject to the following boundary conditions:

$$C_1(0,x) = 0 = C_2(0,x) \qquad (x > 0) \tag{3}$$

$$C_1(t,0) = \begin{cases} K/\tau V & (0 < t < \tau) \\ 0 & (t \geqslant \tau) \end{cases} \tag{4}$$

$$C_2(t,0) = 0 \qquad (t \geqslant 0) \tag{5}$$

Let $T_1(s,x)$ and $T_2(s,x)$ be the Laplace transforms of C_1 and C_2, respectively. Then

$$V\frac{\partial T_1}{\partial x} + \left(\frac{1}{T_1} + s\right)\Gamma_1 - \frac{GT_2}{T_2} = 0 \tag{6}$$

$$G\left(\frac{1}{T_2} + s\right)T_2 - \frac{1}{T_1}T_1 = 0 \tag{7}$$

$$T_1(s,0) = \frac{K}{s\tau V}[1 - \exp(-s\tau)] \tag{8}$$

Eliminating Γ_2 we find

$$V\frac{\partial T_1}{\partial x} + P(s)\,T_1 = 0 \tag{9}$$

where

$$P(s) = \frac{1}{T_1} + s - \frac{1/T_1}{1 + T_2 s} = s\left[1 + \frac{T_2}{T_1}(1 + T_2 s)^{-1}\right] \tag{10}$$

The solution is

$$T_1(s,x) = \frac{K}{s\tau V}[1 - \exp(-s\tau)]\exp\left(-\frac{x\,P(s)}{V}\right) \tag{11}$$

Combining eqns. 10 and 11 we have

$$T_1(s,x) = \frac{K}{s\tau V}[1 - \exp(-s\tau)]\exp\left(-\frac{xs}{V} - \frac{\gamma x}{VT_2}\frac{T_2 s}{1 + T_2 s}\right) \tag{12}$$

where $\gamma = T_2/T_1$.

We introduce the following non-dimensional parameters (L = length of column):

$$\begin{aligned} \alpha &= L/VT_2 & \varepsilon &= \tau/T_2 \\ \beta &= \gamma L/VT_2 & z &= 1 + T_2 s \\ u &= t/T_2 \end{aligned} \tag{13}$$

and we find from eqn. 12 and the inversion theorem (which is valid at times $t > [\tau + x(1+\gamma)/V]$ since then the coefficients of s in the exponents of the integral tend to real positive numbers as $|s| \to \infty$) that, for $c > 0$

$$\begin{aligned} C_1(t, L) &= \frac{K}{\tau V} \frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \frac{ds}{s} \left\{ \exp\left[s\left(t - \frac{x}{\varepsilon}\right)\right] - \right. \\ &\qquad \left. - \exp\left[s\left(t - \tau - \frac{x}{\varepsilon}\right)\right]\right\} \exp\left(-\frac{\beta s T_2}{1 + s T_2}\right) \\ &= \frac{K}{\tau V} \frac{1}{2\pi i} \int_{d-i\infty}^{d+i\infty} \frac{dz}{z-1} \left\{ \exp\left[(u-\alpha)(z-1)\right] - \right. \\ &\qquad \left. - \exp\left[(u - \varepsilon - \alpha)(z-1)\right]\right\} \exp\left(\frac{-\beta(z-1)}{z}\right) \end{aligned} \tag{14}$$

Defining $q(u,\beta)$ to be the function whose Laplace transform (using u,z instead of t,s) is

$$q_1(z, \beta) = \frac{1}{z-1} \exp\left[\frac{-\beta(z-1)}{z}\right] \tag{15}$$

we see that

$$C_1(t, L) = \frac{K}{\tau V} \left[\exp(\alpha - u)\, q(u - \alpha, \beta) - \exp(\alpha + \varepsilon - u)\, q(u - \varepsilon - \alpha, \beta)\right] \tag{16}$$

We find $q(u,\beta)$ as follows:

$$\frac{\partial}{\partial \beta} q_1(z, \beta) = -\exp(-\beta) \frac{1}{z} \exp(\beta/z) \tag{17}$$

By Abramowitz and Stegun (29.3.81)[8], this is the transform of

$$\begin{aligned} \frac{\partial}{\partial \beta} q(u, \beta) &= -\exp(-\beta) I_0(2\sqrt{\beta u}) & (u > 0) \\ &= 0 & (u < 0) \end{aligned} \tag{18}$$

Moreover, for $\beta = 0$ we have

$$q_1(z, 0) = \frac{1}{z-1} \tag{19}$$

giving

$$q(u,0) = \exp(u) \qquad (u > 0;\ 0, u < 0) \tag{20}$$

It follows that

$$\begin{aligned} q(u,\beta) &= \exp(u) - \int_0^\beta \exp(-\lambda)\, I_0(2\sqrt{\lambda u})\, \mathrm{d}\lambda \qquad && (u > 0) \\ &= 0 && (u < 0) \end{aligned} \tag{21}$$

Let us consider the fixed value L of x, and for $k = 0, 1, 2, \ldots$ let us define the kth moment (M_k)

$$M_k = V \int_0^\infty C_1(t, L)\, t^k\, \mathrm{d}t \qquad (k = 0, 1, 2 \ldots) \tag{22}$$

which by eqn. 16 is given by

$$\begin{aligned} M_k = \frac{KT_2^{k+1}}{\tau} \Big\{ & \int_\alpha^{\alpha+\varepsilon} u^k\, \mathrm{d}u - \int_\alpha^\infty u^k \exp(\alpha - u)\, \mathrm{d}u \int_0^\beta \exp(-\lambda)\, I_0\{2[\lambda(u-\alpha)]^{\frac{1}{2}}\}\, \mathrm{d}\lambda + \\ & + \int_{\alpha+\varepsilon}^\infty u^k \exp(\alpha + \varepsilon - u)\, \mathrm{d}u \int_0^\beta \exp(-\lambda)\, I_0\{2[\lambda(u - \varepsilon - \alpha)]^{\frac{1}{2}}\}\, \mathrm{d}\lambda \Big\} \\ = \frac{KT_2^{k+1}}{\tau} \Big\{ & \frac{(\alpha+\varepsilon)^{k+1} - \alpha^k}{k+1} + \\ + & \int_0^\beta \exp(-\lambda)\, \mathrm{d}\lambda \int_0^\infty \exp(-\omega)\, I_0(2\sqrt{\lambda\omega})\, [(\omega + \alpha + \varepsilon)^k - (\omega + \alpha)^k]\, \mathrm{d}\omega \Big\} \end{aligned} \tag{23}$$

where we have taken $u = \omega + \alpha + \varepsilon$ in one integral, and $u = \omega + \alpha$ in another. For $k = 0$ we introduce a parameter a and write

$$\begin{aligned} X(a, \lambda) &= \int_0^\infty \exp(-\omega a)\, I_0(2\sqrt{\lambda\omega})\, \mathrm{d}\omega \\ &= 2 \int_0^\infty \exp(-a y^2)\, I_0(2\lambda^{\frac{1}{2}} y)\, y\, \mathrm{d}y \\ &= \left[\frac{-\exp(-a y^2)}{a} I_0(2\lambda^{\frac{1}{2}} y) \right]_0^\infty + \frac{2\lambda^{\frac{1}{2}}}{a} \int_0^\infty \exp(-a y^2)\, I_1(2\lambda^{\frac{1}{2}} y)\, \mathrm{d}y \\ &= \frac{1}{a} + \frac{2\lambda^{\frac{1}{2}}}{a} \frac{\pi^{\frac{1}{2}}}{2a^{\frac{1}{2}}} \exp\left(\frac{4\lambda}{8a}\right) I_{\frac{1}{2}}\left(\frac{4\lambda}{8a}\right) \end{aligned}$$

by Abramowitz and Stegun (11.4.31)[8]; and

$$X(a, \lambda) = \frac{1}{a} \exp(\lambda / a) \tag{24}$$

by Abramowitz and Stegun (10.2.13)[8].

Now we note that

$$J_k \equiv \int_0^\infty \exp(-\omega)\, I_0(2\sqrt{\lambda\omega})\, \omega^k\, d\omega = (-1)^k \left[\frac{\partial^k}{\partial a^k} X(a, \lambda)\right]_{a=1} \tag{25}$$

so that, in particular

$$J_0 = \exp(\lambda) \tag{26}$$

$$J_1 = (1+\lambda)\exp(\lambda) \tag{27}$$

$$J_2 = (2+4\lambda+\lambda^2)\exp(\lambda) \tag{28}$$

Substituting these into eqn. 23 for the appropriate values of k we find

$$M_0 = \frac{KT_2}{\tau}\left\{\varepsilon + \int_0^\beta \exp(-\lambda)\, d\lambda\, [\exp(\lambda) - \exp(\lambda)]\right\} = K \tag{29}$$

$$M_1 = \frac{KT_2^2}{\tau}\left\{(\alpha + \frac{1}{2}\varepsilon)\,\varepsilon + \int_0^\beta d\lambda\, [(1+\lambda+\alpha+\varepsilon) - (1+\lambda+\alpha)]\right\}$$

and using eqn. 13:

$$M_1 = K\left[\frac{(1+\gamma)L}{V} + \frac{1}{2}\tau\right] \tag{30}$$

whence the mean arrival time ($\bar{t}$) is

$$\bar{t} = M_1/M_0 = \frac{(1+\gamma)L}{V} + \frac{1}{2}\tau \tag{31}$$

Now, analagously to M_k for $k = 0, 1, 2, \ldots$ we form $M_2{}^\dagger$, the second moment, but relative to $\bar{t}$:

$$M_2{}^\dagger = V\int_0^\infty (t-\bar{t})^2\, C_1(t, L)\, dt = M_2 - M_1{}^2/M_0$$

$$= K\left\{\frac{T_2}{\tau}\left(\alpha^2 + \alpha\varepsilon + \frac{\varepsilon^2}{3}\right)\varepsilon + \right.$$

$$+ \int_0^\beta d\lambda\, [(2+4\lambda+\lambda^2+2(\alpha+\varepsilon)(1+\lambda)+(\alpha+\varepsilon)^2) -$$

$$\left. - (2+4\lambda+\lambda^2+2\alpha(1+\lambda)+\alpha^2)\right] - \left[\frac{(1+\gamma)L}{V} + \frac{1}{2}\tau\right]\Big\}$$

$$= K\left\{T_2^2\left(\alpha+\beta+\frac{1}{2}\varepsilon\right)^2 + T_2^2\left(\frac{\varepsilon^2}{12} + 2\beta\right) - T_2^2\left(\alpha+\beta+\frac{1}{2}\varepsilon\right)^2\right\}$$

$$= K\left(\frac{\tau^2}{12} + \frac{2\gamma L T_2}{V}\right) \tag{32}$$

Let us rewrite eqn. 14 as follows:

$$C_1(t,x) = \frac{K}{\tau V}\frac{1}{2\pi i}\int_{f-i\infty}^{f+i\infty}\frac{ds}{s}\left[1-\exp(-s\tau)\right]\exp\left[st-\frac{xP(s)}{V}\right] \tag{33}$$

where $P(s)$ is given by eqn. 10. Eqn. 33 is equivalent to

$$C_1(t,x) = \tau^{-1}\int_0^{\tau}\overset{*}{C}_1(t-u,x)\,du \tag{34}$$

where

$$\overset{*}{C}_1(t,x) = \frac{K}{2\pi iV}\int_{f-i\infty}^{f+i\infty}\exp\left\{s\left[t-\frac{x}{V}\left(1+\frac{\gamma}{1+sT_2}\right)\right]\right\}ds \tag{35}$$

If we can neglect τ compared with the width (in time) of the output pulse, we use eqn. 35 at $x = L$ to estimate the half-width of that pulse. Let us change the variable to φ where

$$Vt - L = \gamma L(1+\varphi)^2 \tag{36}$$

so that $\bar{t}$ corresponds to $\varphi = 0$. Then

$$V dt = 2\gamma L\,(1+\varphi)\,d\varphi \tag{37}$$

and

$$\overset{*}{C}_1(t,L)\,V dt =$$

$$= \frac{2K\gamma L\,(1+\varphi)\,d\varphi}{VT_2}\exp\left[-\beta\,(2+2\varphi+\varphi^2)\right](1+\varphi)^{-1}I_1\left[2\beta\,(1+\varphi)\right] \tag{38}$$

Assuming β to be fairly large, we use the asymptotic formula [Abramowitz and Stegun (9.7.1)[8]]

$$I_1\left[2\beta\,(1+\varphi)\right] \approx \frac{\exp\left[2\beta\,(1+\varphi)\right]}{\left[4\pi\beta\,(1+\varphi)\right]^{\frac{1}{2}}}\left(1-\frac{3}{16\beta\,(1+\varphi)}+\ldots\right) \tag{39}$$

whence for small φ

$$\overset{*}{C}_1(t,L)\,V dt \approx K d\varphi\left(\frac{\beta}{\pi\,(1+\varphi)}\right)^{\frac{1}{2}}\exp(-\beta\varphi^2)\left(1-\frac{3}{16\beta}+\ldots\right) \tag{40}$$

This is a pulse of half-width

$$\Delta\varphi = 2\beta^{-\frac{1}{2}}(\ln 2)^{\frac{1}{2}} \tag{41}$$

corresponding to

$$\Delta t = \frac{2\gamma L}{V}\Delta\varphi = 3.33\,(\gamma L T_2/V)^{\frac{1}{2}} \tag{42}$$

using eqn. 13; or

$$\Delta x = V\mathrm{d}t = 3.33\,(\gamma L T_2 V)^{\frac{1}{2}} \tag{43}$$

If it is possible to measure Δt or Δx (full width at half height) reasonably accurately, this gives T_2, since γ is known from $\bar{t}$ (using eqn. 31), and T_2 is then the only remaining unknown in eqns. 42 or 43. Thus we have, using eqn. 42

$$T_2 = \frac{V}{\gamma L}\,0.09\,(\Delta t)^2 \tag{44}$$

We suggest the following procedure for estimating Δt; it will not be successful unless the output is collected in "buckets" of length h (in time), where h is considerably less than Δt (*i.e.* unless several consecutive "buckets", at least 4 and preferably 5 or 6, are needed to contain say 90% of the total output; and unless τ is about as small (see Appendix 1)).

Let the output be collected in "buckets" of length h (in time), *i.e.* we measure for a range of values n

$$p_n = V\int_{t_n}^{t_n+h} C_1\,(t, L)\,\mathrm{d}t \tag{45}$$

where p_n is the amount of the object species per unit area, $t_n = t_0 + nh$, and t_0 is chosen so that p_0 is the largest of the p_n; thus we are interested in p_n for $n = 0, \pm 1, \pm 2, \ldots, \pm N$ say, where N is moderate (or may even be large, giving increased accuracy, if h is small enough), and is defined by the range including virtually all the output (say 99%).

Then we have

$$M_0 = \sum_{-N}^{N} p_n \tag{46}$$

and, approximately

$$M_1 \approx \sum_{-N}^{N} \left[t_0 + \left(n + \frac{1}{2}\right) h\right] p_n \tag{47}$$

whence

$$\bar{t} = M_1/M_0 \approx t_0 + \frac{1}{2}h + h\left(\sum_{-N}^{N} np_n\right)\Big/\left(\sum_{-N}^{N} p_n\right) \tag{48}$$

Analogously

$$M_2{}^{\dagger} \approx \sum_{m=-N}^{N} \left[t_0 + \left(m + \frac{1}{2}\right) h - \bar{t}\right]^2 p_m = h^2 \sum_{m=-N}^{N} (m - \bar{m})^2 p_m \tag{49}$$

(using m instead of n to avoid confusion) where

$$\bar{m} = \left(\sum_{-N}^{N} np_n\right) \Big/ \left(\sum_{-N}^{N} p_n\right) \tag{50}$$

(note: $\bar{m}$ is not an integer). Thus, if we write for $k = 0, 1, 2, \ldots$

$$Q_k = \sum_{-N}^{N} n^k p_n \tag{51}$$

we have (since we can use m or n as the variable of summation)

$$M_2{}^{\dagger} = h^2 (Q_2 - 2\,\bar{m}\,Q_1 + \bar{m}^2 Q_0) = h^2 (Q_2 - Q_1{}^2/Q_0) \tag{52}$$

since $\bar{m} = Q_1/Q_0$. By choosing the origin t_0 so that p_0 is the largest of the p_n, we have made sure that Q_1/Q_0 is not large and so eqn. 52 is well conditioned. Using eqns. 29 and 32 we have

$$\frac{M_2{}^{\dagger}}{M_0} = \left(\frac{\tau^2}{12} + \frac{2\gamma L T_2}{V}\right) \tag{53}$$

and using eqns. 46, 51 and 52 we have

$$\frac{M_2}{M_0} = h^2 \left[\frac{Q_2}{Q_0} - \left(\frac{Q_1}{Q_0}\right)^2\right] \tag{54}$$

so that

$$\left(\frac{\tau^2}{12} + \frac{2\gamma L T_2}{V}\right) = h^2 \left[\frac{Q_2}{Q_0} - \left(\frac{Q_1}{Q_0}\right)^2\right] \quad (55$$

By eqns. 48 and 55 we have

$$1 + \gamma \approx \frac{V}{L}\left[t_0 + \frac{1}{2}(h - \tau) + h\frac{Q_1}{Q_0}\right] \tag{56}$$

Combining eqns. 55 and 56 we have

$$T_2 = \frac{V}{2\gamma L}\left\{h^2 \left[\frac{Q_2}{Q_0} - \left(\frac{Q_1}{Q_0}\right)^2\right] - \frac{\tau^2}{12}\right\} \tag{57}$$

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APPENDIX 1

The expected error on Q_2/Q_0 caused by a finite h is about $-1/12$. Decreasing h increases the mean value of n^2 and so increases Q_2/Q_0 relative to this fixed error. Alternatively, one can add $1/12$ to Q_2/Q_0, so that eqn. 57 becomes

$$T_2 = \frac{V}{2\gamma L}\left\{h^2\left[\frac{Q_2}{Q_0} + \frac{1}{12} - \left(\frac{Q_1}{Q_0}\right)^2\right] - \frac{\tau^2}{12}\right\} \tag{58}$$

By keeping τ and h as small as practicable, this error is made small compared to Q_2/Q_0.

APPENDIX 2

A simple approach says that any given molecule spends $T_2/(T_1+T_2)$ of its time stationary in the gel phase, and $T_1/(T_1+T_2)$ of its time moving with velocity V. Its average velocity (μ) is therefore

$$\mu = \frac{VT_1}{T_1 + T_2} = \frac{V}{1+\gamma} \tag{59}$$

which is eqn. 31 in another guise. Moreover, it gets stuck on the gel $N = L/VT_1$ times during its passage on average, with standard deviation of the order of $N^{\frac{1}{2}}$, and each time suffers a delay T_2. The output pulse width is therefore of the order of $N^{\frac{1}{2}}T_2$, which is certainly consistent with eqn. 32 although our lengthy analysis is needed to get the exact expression.

APPENDIX 3

In this section we show how other similar models can be put into the form of eqns. 1 and 2 so that our theory can apply. Suppose we have

$$\frac{\partial C_1}{\partial t} = \eta C_2 - \zeta C_1 - V\frac{\partial C_1}{\partial x} \tag{60}$$

$$\frac{\partial C_2}{\partial t} = \xi(\zeta C_1 - \eta C_2) \tag{61}$$

This is the most general conservative form. We define:

$$G = \xi^{-1};\quad T_1 = \zeta^{-1};\quad T_2 = (\xi\eta)^{-1} \tag{62}$$

and divide eqn. 61 by ξ. Then we recover the form of eqns. 1 and 2. Thus we have

$$\gamma = T_2/T_1 = \zeta/\xi\eta;\quad T_2 = (\xi\eta)^{-1} \tag{63}$$

so that the previous method allows us to find these two quantities, but not ξ and η separately.

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