Analysis of Data on Drug Release from Emulsions III

Boundary Effect on Pyridine Release from Water-in-Oil Emulsions as a Function of pH

By TAMOTSU KOIZUMI* and W. I. HIGUCHI

The influence of a membrane separating an unstirred emulsion from a well-stirred sink upon the drug release rate has been studied experimentally and theoretically. Experimental data have been compared to theory. Numerical methods were employed in the analysis with the help of the IBM 7090 computer.

IN THE PREVIOUS paper (1) the release of drug from an amine–amine hydrochloride solute mixture in a water-in-oil emulsion into an aqueous sink was studied experimentally and theoretically. In the treatment of the theory it was assumed that the sink is perfect and that the drug concentration at the emulsion–sink interface is always maintained at zero. This model is shown in Fig. 1. While this assumption was reasonable for the experimental conditions of that study, it may not necessarily be always true when a membrane is used to separate an emulsion from an aqueous sink as is frequently done in studies of this kind.

Another model is proposed in this report. This is illustrated in Fig. 2. The region, $x < -h$, is the aqueous sink which is not perfect for the emulsion because of the membrane of thickness h, which acts as a barrier and separates the emulsion from the sink. Steady-state diffusion may be assumed in the region $-h < x < 0$ characterized by an effective diffusion coefficient, D_m. The region $0 \leq x \leq 1$ is the emulsion for which the effective diffusion coefficient may be calculated by either the Bruggeman or the Wagner-Wiener equation as was done previously (1).

The following appropriate partial differential equations for one-dimensional diffusion may be written:

at $x = 1$ and for all t,

$$\frac{\partial C_e}{\partial x} = 0 \qquad \text{(Eq. 1)}$$

At $0 \leq x \leq 1$ and for $t = 0$,

$$C_e = C_0 \qquad \text{(Eq. 2)}$$

and for $t > 0$,

$$\frac{\partial C_e}{\partial t} = \frac{\partial}{\partial x}\left(D_e \frac{\partial C_e}{\partial x}\right) \qquad \text{(Eq. 3)}$$

At $x = 0$ and for all t,

$$C_m = K_m \cdot C_e \qquad \text{(Eq. 4)}$$

and for $t > 0$,

$$D_e \frac{\partial C_e}{\partial x} = D_m \left\{\frac{(C_m)_{x=0} - (C_m)_{x=-h}}{h}\right\} \qquad \text{(Eq. 5)}$$

At $-h \leq x \leq 0$ and for all t,

$$\frac{\partial C_m}{\partial x} = \frac{(C_m)_{x=0} - (C_m)_{x=-h}}{h} \qquad \text{(Eq. 6)}$$

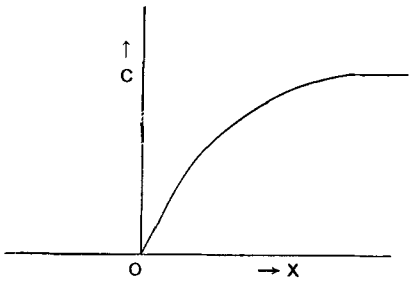

Fig. 1—Boundary conditions used in the previous report.

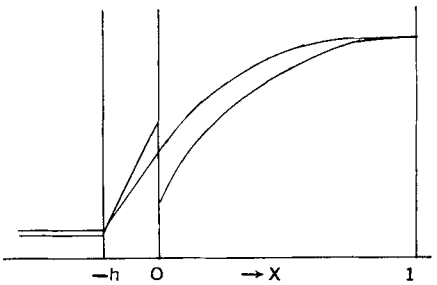

Fig. 2—Boundary conditions used in this report. Key: continuous curve represents the case where the partition coefficient between emulsion and membrane is unity; discontinuous curve is the case where the partition coefficient is not unity.

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At $x = -h$ and for $t = 0$,

$$C_m = 0 \qquad \text{(Eq. 7)}$$

and for $t > 0$,

$$C_m = f(t) \qquad \text{(Eq. 8)}$$

Here C_e is the drug concentration in the emulsion, C_m is the drug concentration in the membrane, D_e is the effective diffusion coefficient which is a known function of C_e, and K_m is the apparent partition coefficient between the emulsion and the membrane. When the sink has a finite volume V_0, then $f(t)$ is given by:

$$f(t) = \frac{Q}{V_0} \qquad \text{(Eq. 9)}$$

where Q is the amount of drug released to the sink in time, t.

For obtaining numerical solutions, Crank (2) recommends the introduction of a new variable, S, when the diffusion coefficient is concentration dependent. This S is defined by Eq. 10:

$$S = \frac{\int_0^{C_e} D_e dC_e}{\int_0^{C_0} D_e dC_e} \qquad \text{(Eq. 10)}$$

With this new variable Eqs. 1 to 4 become Eqs. 11 to 14:

At $x = 1$, for all t,

$$\frac{\partial S}{\partial x} = 0 \qquad \text{(Eq. 11)}$$

At $o \leq x \leq 1$, for $t = 0$,

$$S = 1 \qquad \text{(Eq. 12)}$$

and for $t > 0$,

$$\frac{\partial S}{\partial t} = D_e \frac{\partial^2 S}{\partial x^2} \qquad \text{(Eq. 13)}$$

At $x = 0$, for $t > 0$,

$$\frac{\partial S}{\partial x} = \frac{D_m\{(C_m)_{x=0} - (C_m)_{x=-h}\}}{h \int_0^{C_0} D_e dC_e} \qquad \text{(Eq. 14)}$$

Applying the finite-difference method (3) to Eqs. 11 to 14, one can obtain the following equations:

At $x = 1$,

$$S_{n-1}{}^{+} = (S_n{}^{+} - S_n) \cdot \frac{2}{(D_n{}^{+} + D_n)\frac{\Delta t}{(\Delta x)^2}} + (S_n{}^{+} + S_n) - S_{n-1} \qquad \text{(Eq. 15)}$$

At $0 < x < 1$,

$$S_{i-1}{}^{+} = (S_i{}^{+} - S_i)\frac{2(\Delta x)^2}{(D_i{}^{+} + D_i)\Delta t} + 2(S_i{}^{+} + S_i) - (S_{i+1}{}^{+} + S_{i+1}) - S_{i-1} \qquad \text{(Eq. 16)}$$

At $x = 0$,

$$\frac{-S_2 + 4S_1 - 3S_0}{2(\Delta x)} = \frac{D_m\{(C_m)_{x=0} - (C_m)_{x=-h}\}}{h \int_0^{C_0} D_e dC_e} \qquad \text{(Eq. 17)}$$

Here the range 0 to 1 was divided into a number of equal intervals, Δx, and the time into intervals of Δt. S_{i-1}, S_i, S_{i+1} are the S values at $(i-1)\Delta x$, $i\Delta x$, $(i+1)\Delta x$, respectively, at $t = m\Delta t$, and $S_{i-1}{}^{+}$, $S_i{}^{+}$, $S_{i+1}{}^{+}$ are the corresponding values at $x = (i-1)\Delta x$, $i\Delta x$, $(i+1)\Delta x$ at time $(m+1)\Delta t$.

Similarly, $D_i{}^{+}$ and D_i are effective diffusion coefficients corresponding to the concentration at the point $i\Delta x$ at $t = (m+1)\Delta t$ and $t = m\Delta t$, respectively.

THE COMPUTATION PROCEDURE

The procedure for computation is as follows.

(*a*) Divide the emulsion thickness, 1, into n equal lengths of Δx and divide time into equal intervals of Δt.

(*b*) Set $S_i = 1$ (i = o to n)—initial condition.

(*c*) Choose an arbitrary value (between 0 and 1) of $S_n{}^{+}$.

(*d*) Calculate C_e corresponding to the value of $S_n{}^{+}$ chosen at (*c*). Equation 10 may be used for this calculation.

(*e*) Calculate the effective diffusion coefficient corresponding to the concentration, C_e, just calculated at (*d*). This is $D_n{}^{+}$.

(*f*) Calculate $S_{n-1}{}^{+}$ by Eq. 15.

(*g*) Calculate C_e corresponding to $S_{n-1}{}^{+}$, and then $D_{n-1}{}^{+}$.

(*h*) Calculate $S_{n-2}{}^{+}$ by Eq. 16.

(*i*) Repeat (*g*) and (*h*) until S_0 is calculated.

(*j*) Calculate C_e corresponding to S_0, *i.e.*, $(C_e)_{x=0}$. Calculate $(C_m)_{x=0}$ using Eq. (4), and also calculate $(C_m)_{x=h}$ using Eqs. 8 and 9, where:

$$Q = k \int_0^1 (C_0 - C_e)dx \qquad \text{(Eq. 18)}$$

k = cross-sectional area of the emulsion layer.

(*k*) Calculate separately the lefthand side and the righthand side of Eq. 17 and compare to see whether Eq. 17 holds.

(*l*) If the calculated values of the left and right sides of the equation are consistent within the range of error allowed, the value of Q obtained at (*j*) is the amount of drug released at $t = \Delta t$.

(*m*) If (*l*) is not the case, modify the value of $S_n{}^{+}$ depending on the results of (*k*). Then repeat steps from (*d*) to (*k*), until Eq. 17 holds. When Eq. 17 is obeyed, the most recent value of (Q) is the amount of drug released by $t - \Delta t$.

(*n*) Replace all values of S_i by the new values of $S_i{}^{+}$ obtained, and repeated procedure (*c*) to (*m*) until the desired time ($t = m\Delta t$) is reached. Here m is the number of times step (*n*) is repeated.

THE DETERMINATION OF D_m/h

Figure 3 is the schematic model of the apparatus which was used to determine the D_m/h value.

Compartments 1 and 2 were stirred, and diffusion took place inside the membrane only. At $t = 0$, $C_1 = C_0$ and $C_2 = 0$.

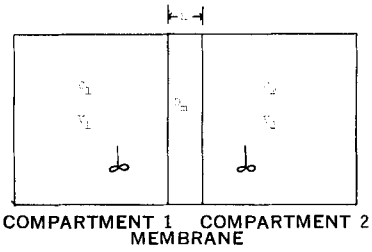

Fig. 3—Schematic model of apparatus for determination of D_m/h.

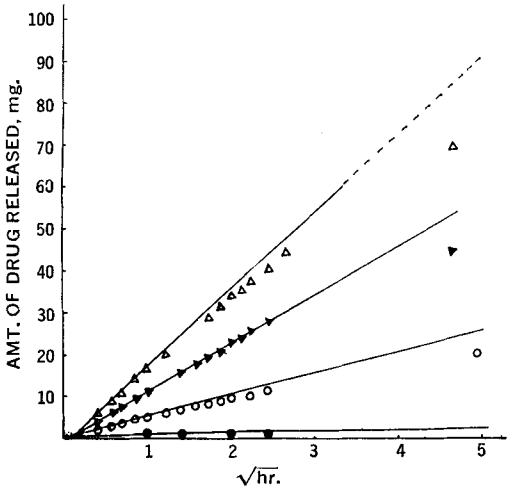

Fig. 4—Comparison of experimental data with theory. Amount of pyridine released from w/o emulsion vs square root of time. Sink is plain water, △, ▼, ○, and ● represent $C_A = 0.063, 0.126, 0.189,$ *and* 0.252 *mole/L., respectively. Solid lines are theoretical value computed by Bruggeman equation.* $V_c = 0.25$; $V_i = 0.75$; $P_o = 0.570$; $D_c = 1.42 \times 10^{-5}$ *cm.²/sec.*; $D_i = 1.19 \times 10^{-5}$ *cm.²/sec.*; $C_o = 0.189$ *mole/L.*; $K_a = 5.89 \times 10^{-6}$ *mole/L.*; $\Delta x = 0.1327$ *cm.*; $\Delta t = 360$ *sec.*; $D_m/h = 3.43 \times 10^{-4}$ *cm./sec.*

Conservation of mass gives Eq. 19 for this situation:

$$V_1C_1 + V_2C_2 = V_1C_0 = (V_1 + V_2)C_0' \quad \text{(Eq. 19)}$$

Assuming steady-state diffusion, Fick's first law gives:

$$\frac{d(V_2C_2)}{dt} = K\left(\frac{D_m}{h}\right)(C_1 - C_2) \quad \text{(Eq. 20)}$$

From Eqs. 19 and 20, Eq. 21 is obtained:

$$\log\left(\frac{C_0^1 - C_2}{C_0^1}\right) = -\frac{K(V_1 + V_2)}{2.303\ V_1 \cdot V_2}\left(\frac{D_m}{h}\right)t \quad \text{(Eq. 21)}$$

where C_1 and C_2 are the drug concentrations in compartments 1 and 2, respectively. V_1 and V_2 are the volumes of compartments 1 and 2, and K is area of the membrane.

When the logarithm of $(C_0^1 - C_2)/C_0^1$ is plotted against time, a straight line will be obtained. The value of D_m/h is then calculated from the slope of the line.

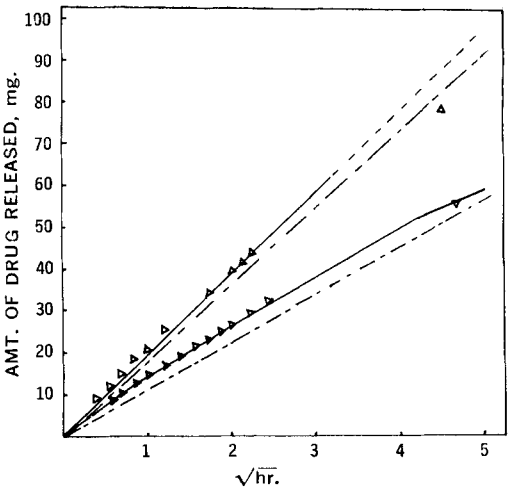

Fig. 5—Comparison of experimental data with theory. Amount of pyridine released from w/o emulsion vs. square root of time. Sink is hydrochloric acid, the concentration of which is equal to C_A. △ *and* ▽ *represent* $C_A = 0.063$ *and* 0.126 *mole/L., respectively. Key: ——, theoretical values computed by this method; - - -, theoretical values computed by the method of the previous report.* $V_c = 0.25$; $V_i = 0.75$; $P_o = 0.570$; $D_c = 1.42 \times 10^{-5}$ *cm.²/sec.*; $D_i = 1.19 \times 10^{-5}$ *cm.²/sec.*; $C_o = 0.189$ *mole/L.*; $K_a = 5.89 \times 10^{-6}$ *mole/L.*; $\Delta x = 0.1327$ *cm.*; $\Delta t = 360$ *sec.*; $D_m/h = 3.43 \times 10^{-4}$ *cm./sec.*

TABLE I—DATA FROM STEADY-STATE DIFFUSION EXPERIMENTS[a]

Time, min.	C_2 (10^{-3} mole/L.)	$(C_0^1 - C_2)/C_0^1$
0	0	1.0000
10	0.078	0.9696
20	0.141	0.9450
30	0.195	0.9240
45	0.287	0.8881
60	0.360	0.8596
90	0.515	0.7992
120	0.661	0.7423
150	0.795	0.6901
180	0.927	0.6386

[a] $C_0 = 5.643 \times 10^{-3}$ mole/L., $V_1 = 100$ ml., $C_0^1 = 2.565 \times 10^{-3}$ mole/L., $V_2 = 120$ ml.

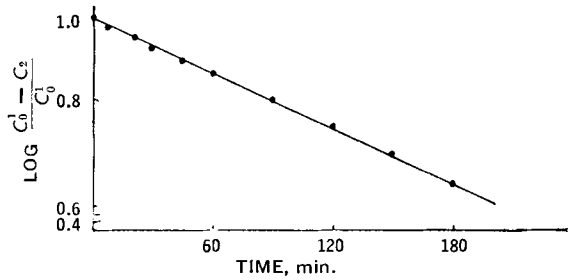

Fig. 6—Plots of log $\left(\frac{C_0^1 - C_2}{C_0^1}\right)$ vs. t. *See text.*

EXPERIMENTAL

The apparatus, the diffusion coefficients in homogeneous solution, and the partition coefficients were the same as reported previously (1). In order to

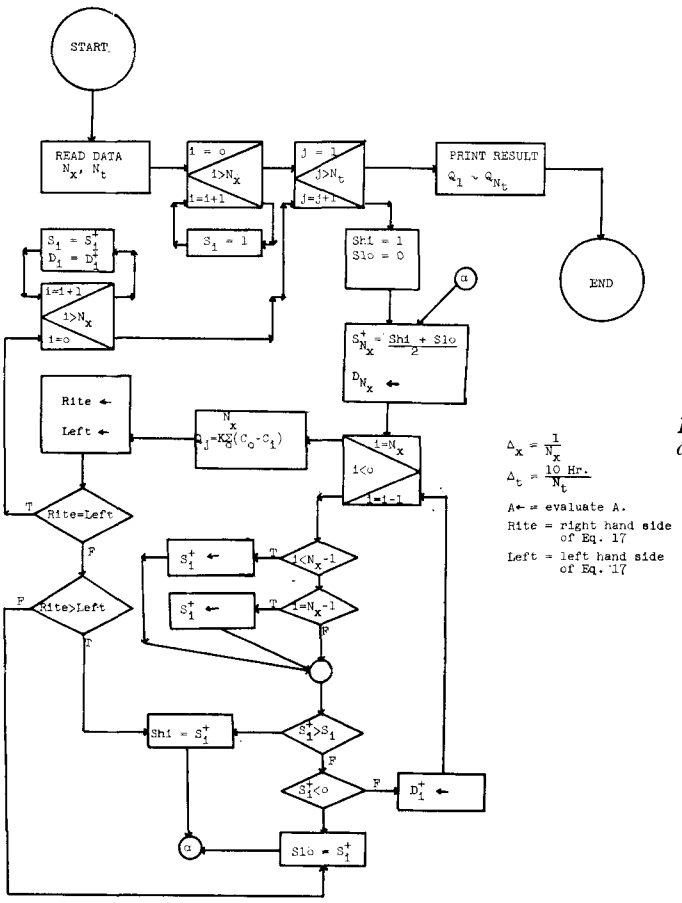

Fig. 7—Flow diagram showing the procedure for the computation of theoretical values.

TABLE II—THEORETICAL VALUES OF THE AMOUNT OF PYRIDINE RELEASED[a]

Time, hr.	C_A = 0.063 mole/L.	C_A = 0.126 mole/L.	C_A = 0.189 mole/L.	C_A = 0.252 mole/L.
0	0	0	0	0
1.0	0.1134591	0.07203754	0.03303765	0.01795251
2.0	0.1650710	0.1043185	0.04752852	0.02280451
3.0	0.2046400	0.1290364	0.05863856	0.02663112
4.0	0.2379512	0.1498451	0.06798362	0.02996549
5.0	0.2672553	0.1681557	0.07620181	0.03368697
6.0	0.2936981	0.1846839	0.08362651	0.03694266
7.0	0.3179419	0.1998395	0.09043241	0.03987066
8.0	0.3403976	0.2138898	0.09677280	0.04255156
9.0	0.3613336	0.2270172	0.1027032	0.04503749
10.0	0.3809442	0.2393349	0.1082879	0.04736435

[a] Expressed as the fraction of total amount in the system. Sink is plain water.

TABLE III—THEORETICAL VALUES OF THE AMOUNT OF PYRIDINE RELEASED[a]

Time, hr.	C_A = 0.063 mole/L.	C_A = 0.126 mole/L.
0	0	0
1.0	0.1305398	0.09348550
2.0	0.1835966	0.1267081
3.0	0.2239798	0.1520191
4.0	0.2579157	0.1732450
5.0	0.2877549	0.1919180
6.0	0.3146762	0.2087835
7.0	0.3393585	0.2242616
8.0	0.3622198	0.2386266
9.0	0.3835348	0.2520417
10.0	0.4034949	0.2646448

[a] Expressed as the fraction of total amount in the system. Sink is hydrochloric acid with concentration equal to C_A.

make the sink more ineffective, plain water was used instead of hydrochloric acid in the compartment B of the cell.

The amount of drug released, Q, was plotted against the square root of time. Results are shown in Fig. 4.

Determination of D_{mh}—The same apparatus as that used in the release experiments was used except both the inside of compartment B and compartment A were stirred, the latter by a screw-type stirrer attached to a motor driven glass rod. The following conditions were used: $C_0 = 5.643 \times 10^{-3}$ M, $V_1 = 100$ ml., $V_2 = 120$ ml., $K = 6.16$ cm.^{2}, and $C_0^1 = 2.565 \times 10^{-3}$ M.

Samples were taken from compartment 2 at predetermined intervals. Concentrations were

determined by UV absorbance. Results are shown in Table I. The logarithm of $(C_0^1 - C_2)/C_0^1$ was plotted against time, and Fig. 6 was obtained. The slope of the straight line was calculated to be 0.0605 hr.$^{-1}$, and D_m/h to be 1.234 cm./hr. or 3.43 $\times$ 10^{-4} cm./sec. This same value was also used under *Appendix* of the previous report (1).

The Computation of Theoretical Values—The computations were executed by the help of the IBM 7090 digital computer. The simplified flow chart is shown in Fig. 7. Results are in Tables II and III and shown in Figs. 4 and 5.

Discussion—The excellent agreement of computed values and experimental results can be seen in Figs. 4 and 5.

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(3) Crank, J., *ibid.*, p. 186.

Keyphrases

Pyridine release from emulsions
Boundary effect on pyridine release as function of pH–w/o emulsions
Membrane effect on pyridine release–w/o emulsions
Diffusion equations, one-dimensional
Computer flow chart—theoretical values of pyridine release

Chemically Defined Medium for the Production of *Fusarium graminearum*

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A chemically defined medium for production of *Fusarium graminearum* has been developed and modified. A series of carbon and nitrogen sources was studied, resulting in the selection of glucose and ammonium succinate as primary substrates. Using the economic coefficient as a criterion for efficiency, the optimum concentrations for glucose and succinic acid were 3 and 0.5 percent, respectively. Growth weight and pH changes as well as glucose, nitrogen, and succinic acid utilization were determined during a typical fermentation period.

FUSARIUM GRAMINEARUM is the imperfect stage of *Gibberella zea* [*G. saubinetti* (Mont.) Sacc.], a plant pathogen causing stalk, root, and ear rot of corn and scab disease on wheat and barley. This organism has been classified in the order Hypocreales of the class ascomycetes. Ingestion of scabbed grains by domestic animals having simple stomachs elicits digestive system disturbances, vomiting, and in extreme cases, death (1–3). Bread made from scabbed grains has been called "intoxicating bread." Recent work on the fungus has resulted in the isolation of an anabolic uterotrophic compound (4, 5). Most investigators concerned with *F. graminearum* metabolites have utilized natural or incompletely defined substances as growth media. These have included potato infusion enriched with dextrose, scabbed barley, and cracked corn (1–5). The present report is concerned with the development of a completely defined chemical medium for the production of the fungus and a study of the growth habits on this medium. With such a medium it is possible to control the total environment of the fungus in order to facilitate extraction, metabolic, and animal toxicity studies.

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EXPERIMENTAL MATERIALS AND METHODS

A culture of *F. graminearum* was maintained by serial transfer on potato dextrose agar (PDA) slants. After initial growth for 1 week in an incubator at 28°, the cultures were stored at 4°. The liquid medium