J. Pharm. Pharmacol. 1995, 47: 1–7
Received December 10, 1993
Accepted September 6, 1994

The Analysis of Drug Release from Diluted Water/oil/water Emulsions by a Model of the Rupture of Oil Membrane

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Abstract

The release behaviour of theophylline encapsulated in the inner aqueous phase of a water/oil/water emulsion was investigated by two methods. A cellulose tube containing a sample of the emulsion was placed in a rotary basket and was stirred in a dissolution medium (Method A), or the w/o/w emulsion was dispersed in a dissolution medium and the system was stirred by a paddle, allowing the drug to permeate into a cellulose tube placed in the dispersing medium (Method B).

In Method A, the drug release rate from the emulsion decreased with increase in the concentration of sodium chloride co-formulated with the drug in the inner aqueous phase. The drug release rate in the dissolution test medium No. 1 or No. 2 of the JP XII was greater than that in purified water and was increased with the ionic strength of the dissolution medium. The drug was released more rapidly in Method B than in Method A, because the emulsion was destroyed more easily using the former method. As this destruction of emulsion structure occurred immediately after dilution with dissolution medium, the influence of the dissolution medium on the release profile could not be detected using Method B.

The experimental data of drug release were satisfactorily explained by the destruction model of the oil membranes of the water/oil/water emulsions.

There have been several applications of water-in-oil-in-water emulsions (w/o/w emulsions) in pharmaceutics, including lymphatic delivery (Fukushima et al 1987), controlled release systems (Benoy et al 1972; Mishra & Pandit 1990), replacements for red blood cells (Borwanker et al 1988; Zheng et al 1992) and use in treating drug overdose (Morimoto et al 1979). To establish practical uses of w/o/w emulsions, stability of the system and biocompatibility of oil and surfactant must be shown, and this is the subject of this report, using theophylline as a model drug.

Theoretical Background

Analysis of drug release from w/o/w emulsions by destruction of the oil membrane of the emulsion droplet

In the previous study, we reported that the destruction of the oil membrane of a w/o/w emulsion was a factor in the leakage of solutes from the inner aqueous phase during storage (Kawashima et al 1992). According to this finding, we assumed a model of drug release from a w/o/w emulsion contained in a cellulose tube as shown in Fig. 1.

When the oil membrane rupture follows first-order kinetics, the decreasing rate of inner aqueous droplets is described by equation 1:

$$V_1 = V_0 e^{-kt} \quad (1)$$

The change in the inner volume of aqueous droplets follows a first-order decay where, V_1 is the volume of inner aqueous phase at time, t, k is the rate constant (min^{-1}), and V_0 is the initial volume of the inner aqueous phase at the start of the release test.

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The drug released by the rupture of the membranes transfers through the cellulose membrane to the acceptor phase according to Fick's law:

$$V_3\, dC_3/dt = D \cdot S(C_2 - C_3)/h \quad (2)$$

where, D, S and h are the diffusion coefficient of the drug in the pore of cellulose tube ($cm^2\ min^{-1}$), the effective total cross-section area (cm^2) of the pores and the thickness (cm) of the membrane of the tube, respectively.

The parameters, C_2, C_3 and V_3 are the concentrations of the drug in the outer aqueous phases in the donor and the acceptor phases and the volume of the acceptor phase, respectively.

The parameters, X and Y, are defined according to equations 3 and 4, respectively. Equation 5 is derived from equation 4:

$$X = V_1 + V_2 = \frac{V_s(V_i + V_e)}{V_i + V_o + V_e} \quad (3)$$

$$Y = \frac{C_1 V_s V_i}{V_i + V_o + V_e} \quad (4)$$

$$C_1V_1 + C_2V_2 + C_3V_3 = Y \quad (5)$$

where, V_2 is the volume of the outer aqueous phase in the donor phase, C_1 is the concentration of drug in the inner aqueous phase, and V_i, V_o, V_e are the volumes of the inner aqueous, the oil phase, and the outer aqueous phases, respectively.

Equation 6 is derived from equations 1, 2, 3 and 5:

$$\ln V_0 - kt = \ln Q \quad (6)$$

where,

$$Q = X - Z(Y - C_3V_3 - C_1X)/\{V_3\, dC_3/dt - Z(C_1 - C_3)\} \quad (7)$$

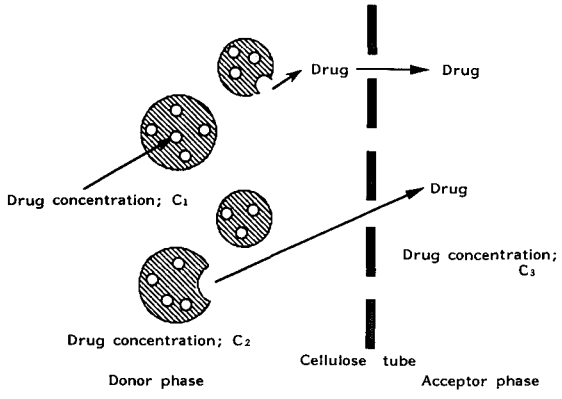

FIG. 1. Schematic model of drug release from a w/o/w emulsion.

and

$$Z = D \cdot S/h \tag{8}$$

The initial volume percentage of the inner aqueous phase at the start of the release test, α (%), is described as follows:

$$\alpha = 100C_1V_0/Y \tag{9}$$

Therefore, parameters, α and k, are obtained by plotting ln Q against t according to equation 6.

Materials and Methods

Materials

The oil used was Triester F-810, the triglyceride of a medium chain fatty acid. The lipophilic and hydrophilic surfactants used were hexaglyceryl condensed polyricinolate (Hexaglyn PR-15, HGCR; HLB = 3·2) and polyoxyethylene hydrogenated caster oil-60 (HCO-60; HLB = 14·0), respectively. The oil and surfactants are specified in the Japanese official formulary of food additives. These materials were supplied by Nikko Chemical Co. (Tokyo, Japan) and used without further purification. All other chemicals were reagent grade. Theophylline was used as a model drug.

Preparation of w/o/w emulsions

A w/o/w emulsion was prepared using a two-step emulsifying procedure. Twenty millilitres aqueous solution containing 0·5 w/v % theophylline and 0–0·2 w/v % sodium chloride was dropped into 20 mL oil phase, Triester F-810 containing 5 w/v % HGCR, agitated using a Chemy B-150 stirrer (Tokyo Rikakikai Co., Tokyo, Japan) at the rate of 1680 rev min^{-1} (first emulsification stage). The resultant w/o emulsion was dropped into 40 mL 4 w/v % HCO-60 aqueous solution agitated using a magnetic stirrer. The system was homogenized using a high-speed homogenizer, Physcotron NS-50 (Nition'i-Rikakikai Co., Chiba, Japan) for 1 min and the w/o/w emulsion was prepared (second emulsification stage).

Release test of theophylline from the w/o/w emulsion

Method A. Five millilitres of the w/o/w emulsion sample was placed in a seamless cellulose tube (Viskase Sales Co., USA) and was placed in a rotary basket. The basket was rotated in 900 mL dissolution medium (acceptor phase) at 100 rev min^{-1} and the concentration of theophylline in the acceptor phase was measured spectrophotometrically at 270 nm (Fig. 2).

Method B. Five millilitres of a w/o/w emulsion was dispersed in 880 mL dissolution medium (donor phase). A seamless cellulose tube containing 20 mL dissolution medium (acceptor phase) was placed in the donor phase and the concentration of theophylline transferred into the acceptor phase was measured spectrophotometrically.

The acceptor phase solution was circulated through the cell of the spectrophotometer at the rate of 30 mL min^{-1} by an autosampler Model W (Toyama Sangyo Co., Osaka, Japan) in both methods. The dissolution medium was purified water, the disintegration test solutions No. 1 (pH = 1·2) and 2 (pH = 6·8) specified in the JP XII. The ionic strengths of the test solutions No. 1 and 2 were 0·10 and 0·12, respectively.

Determination of the parameter, Z (D · S/h)

Five millilitres of aqueous solutions of theophylline (0·1, 0·3 and 0·5 w/v %) were placed in the donor phase to determine the parameter Z by Methods A and B. The parameter, Z in equation 8, which represents the index of the rate of drug permeation through the cellulose tube, was determined by equation 11 which was derived from equations 2 and 10:

$$C_2V_2 + C_3V_3 = C_0V_2 \tag{10}$$

where, V_2 and V_3 are volumes of donor and acceptor phases, respectively, and C_2 and C_3 are the concentrations of theophylline in the corresponding phases, respectively. The parameter, C_0 is the initial concentration of theophylline in the donor phase:

$$P = Zt \tag{11}$$

where,

$$P = \{V_2V_3/(V_2 + V_3)\} \times \ln\{C_0V_2/(C_0V_2 - C_3V_2 - C_3V_3)\} \tag{12}$$

The parameter Z is the slope of regression line of the plots of P against t forced through the origin.

Volume percent of inner aqueous phase in the w/o/w emulsion

Ten millilitres of the w/o/w emulsion was placed in a seamless cellulose tube (Viskase Sales Co., USA) and dialysed against 90 mL purified water for 20 h. The optical micrographs of w/o/w emulsions taken before and after dialysis revealed no significant changes in the emulsion structure during dialysis. The concentration of theophylline dialysed into the medium was determined spectrophotometrically at 270 nm. The volume percentage of the inner aqueous phase in the w/o/w emulsion, β (%), was calculated from equation 13 (Kawashima et al 1991):

$$\beta = 100\{1 - C_dV^*/(C_1 - C_d)V_i\} \tag{13}$$

where, C_d is the concentration of theophylline dialysed in the medium and V^* is defined by equation 14:

$$V^* = V_e + V_d(V_i + V_e + V_o)/V_s \tag{14}$$

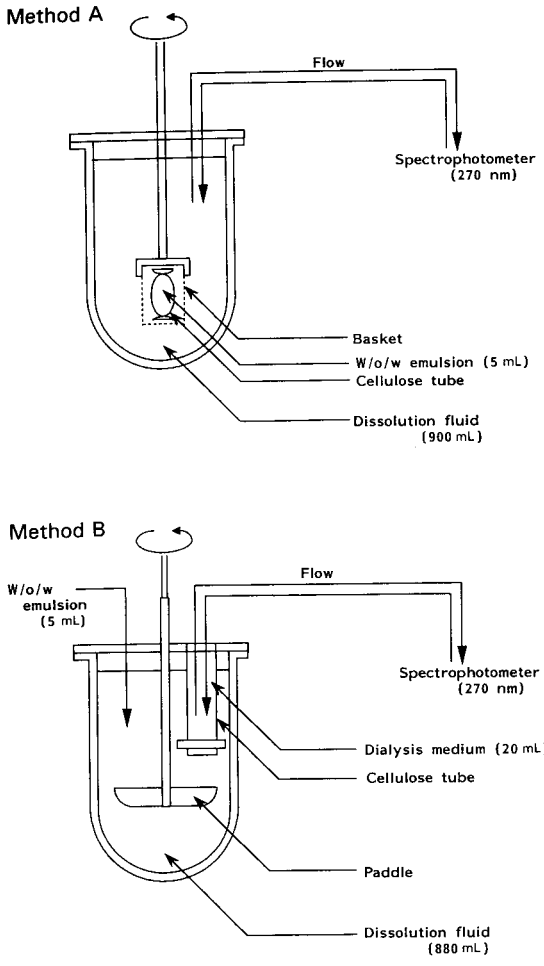

FIG. 2. Apparatus for determining release of drug from a w/o/w emulsion.

where V_d and V_s are the volumes of dialysis medium and dialysed emulsion, respectively.

Results and Discussion

Structure of the w/o/w emulsion

Fig. 3 shows the optical microphotograph of the w/o/w emulsion diluted four times with purified water. Multiple emulsion droplets contained many small inner aqueous droplets dispersed within them. The mean diameters of w/o/w emulsion droplets were 6·6–10·3 μm. Volume percentages of inner aqueous phase trapped in the w/o/w emulsions were 39–72%. Although the phase separation of aqueous phase was observed after 1 day storage of w/o/w emulsion without sodium chloride in the inner aqueous phase, the separation could not be detected with the salt-co-encapsulating emulsion (0·05–0·2 mol L^{-1}) even after five days storage.

Determination of the parameter Z

Fig. 4 shows the relationship between P and t using Method A.

A good linear relationship was found, irrespective of the concentration of drug in the donor phase. The regression line

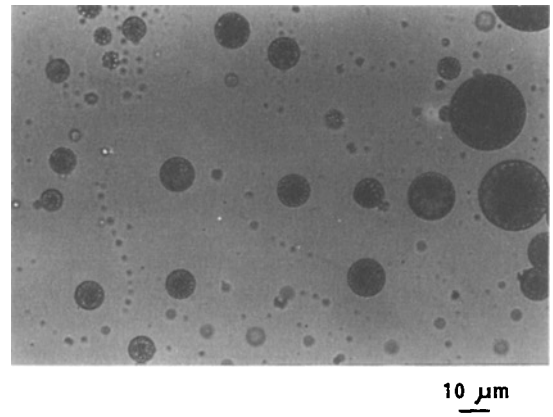

FIG. 3. Optical micrograph of w/o/w emulsion. Concentration of sodium chloride co-encapsulated with theophylline in the inner aqueous phase was 0·05 M.

was significant by regression analysis ($F_0 = 1759$, $P < 0·01$). The values of Z, the slopes of the regression lines, were 0·109 and 0·288 mL min^{-1} in Methods A and B, respectively.

Drug release behaviour from the w/o/w emulsion (Method A) Effect of the concentration of salt co-encapsulated in the inner aqueous phase with theophylline on the release of the drug. Fig. 5 shows the release profiles of theophylline from the w/o/w emulsions using Method A. Fig. 6 shows the plot of t vs ln Q of the data in Fig. 5. The release rate constants and the initial percentages of theophylline entrapped in the inner aqueous phases at the start of the release tests are shown in Table 1.

The increase in the concentration of sodium chloride in the inner aqueous phase prolonged the release of theophylline from the w/o/w emulsion (Fig. 5). The good linear relationships between ln Q and t found in Fig. 6 (regression lines were significant by regression analyses, $P < 0·05$) and Table 1 (correlation coefficient, −0·977–−0·990) indicated the validity of the assumption that the rupture of the oil membranes determined the release rate

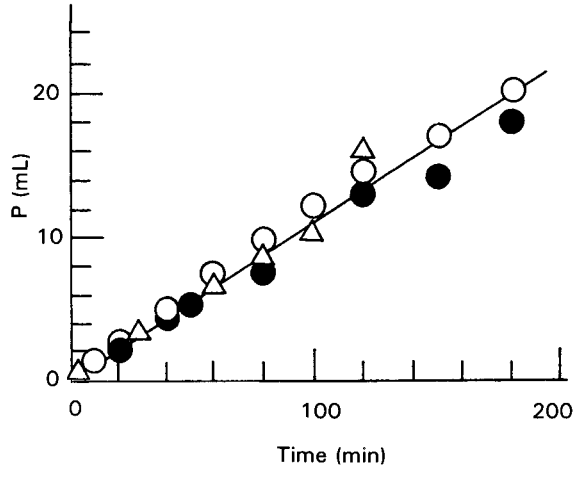

FIG. 4. Determination of the parameter, Z, using Method A. Initial concentration of theophylline in the donor phase: △ 0·1, ● 0·3, ○ 0·5 M.

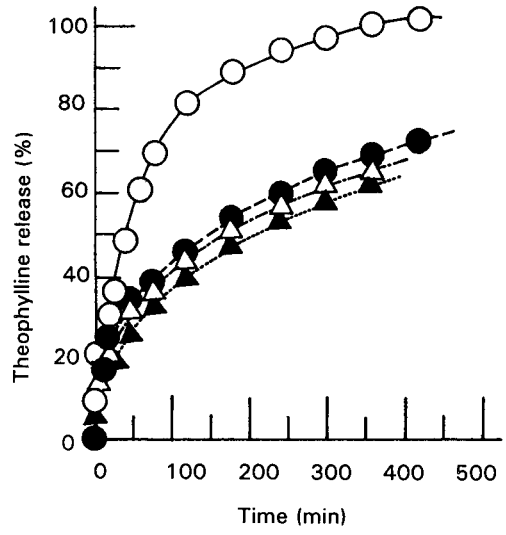

FIG. 5. Effect of concentration of sodium chloride in the inner aqueous phase on the release of theophylline from w/o/w emulsions using Method A. Concentration of sodium chloride in the inner aqueous phase: ○ 0, ● 0·05, △ 0·10, ▲ 0·20 M. Dissolution medium: purified water.

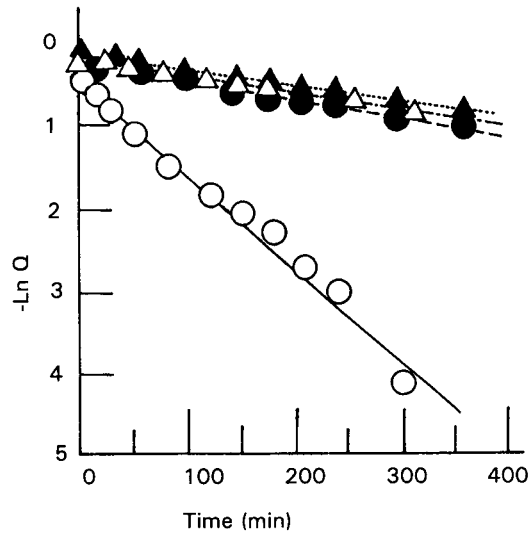

FIG. 6. Replotted data of Fig. 5 based on equation 6. Concentration of sodium chloride in the inner aqueous phase: ○ 0, ● 0·05, △ 0·10, ▲ 0·20 M. Dissolution medium: purified water.

of the drug from w/o/w emulsions (Fig. 1). Coincidence of the initial percentages of the drug with the percentage of aqueous phase entrapped in the w/o/w emulsion confirmed the analysis of the data by the present methods to be reasonable.

The increase in the concentration of sodium chloride formulated with theophylline in the inner aqueous phase increased the initial trap percentage of drug and decreased the release rate. These results agreed well with our previous work that a w/o/w emulsion containing a high concentration of sodium chloride in the inner aqueous phase had a large encapsulation efficiency and was stable during storage (Kawashima et al 1992). The mechanism of increasing the initial percentage of drug and prolonging the drug release with high concentrations of salt in the inner aqueous phase may be explained as follows. The salt encapsulated in the inner aqueous phase dehydrated the bound water molecules from the polar headgroups of the surfactant molecules aligned at the inner aqueous-oil interface. The curvature of the interface of the inner aqueous droplet decreased and the direct interaction of polar headgroups of the surfactant and salt made the surfactant film layer rigid. Therefore, the initial percentage of the drug increased and the rupture of oil membrane of the emulsion decreased.

Table 1. Analysis of dissolution behaviour of theophylline from w/o/w emulsion.

Concn sodium chloride in inner aqueous phase (M)	Release rate constant (min^{-1})	Initial percentage of theophylline entrapped in inner aqueous phase	Correlation coefficient	Volume percentage of inner aqueous phase trapped in the w/o/w emulsion
—	0·0111	47·4	−0·983	39·1
0·05	0·0025	68·4	−0·990	64·5
0·1	0·0022	68·5	−0·977	69·0
0·2	0·0021	73·4	−0·981	71·2

Dissolution medium: water.

Table 2. Effect of the dissolution medium and the concentration of sodium chloride in the inner aqueous phase on the release behaviour of theophylline from w/o/w emulsion.

Dissolution medium	Concn sodium chloride in the inner aqueous phase (M)	Release rate constant (min^{-1})	Initial percentage of theophylline trapped in the inner aqueous phase	Correlation coefficient
JP XII No. 1 fluid	—	0·0113	49·0	−0·982
	0·05	0·0107	50·0	−0·989
	0·1	0·0035	63·5	−0·973
	0·2	0·0022	73·5	−0·970
JP XII No. 2	—	0·0140	44·5	−0·983
	0·05	0·0134	53·8	−0·982
	0·1	0·0070	62·0	−0·981
	0·2	0·0018	73·7	−0·975

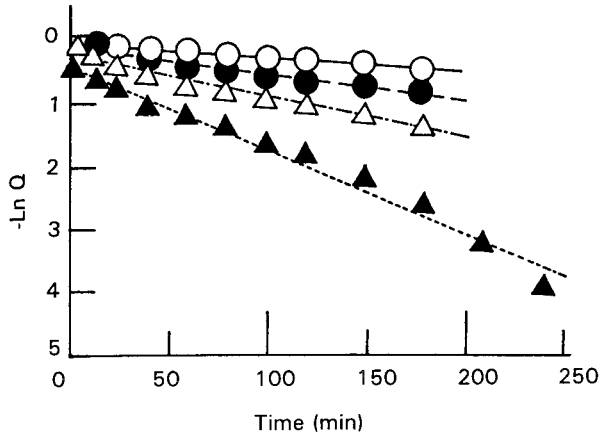

FIG. 7. Effect of ionic strength of dissolution fluids on the release of theophylline from w/o/w emulsions (Method A). Concentration of sodium chloride in the inner aqueous phase: 0·05 M. Ionic strength of the dissolution medium: ○ 0·02, ● 0·05, △ 0·08, ▲ 0·11. Release rate constant and initial percentage of theophylline entrapped in the inner aqueous phase: ○ 0·0021 min^{-1} and 74·7%, ● 0·0052 min^{-1} and 78·4%, △ 0·0073 min^{-1} and 64·5%, ▲ 0·0134 min^{-1} and 53·8%. Correlation coefficient: ○ −0·986, ● −0·972, △ −0·978, ▲ −0·982.

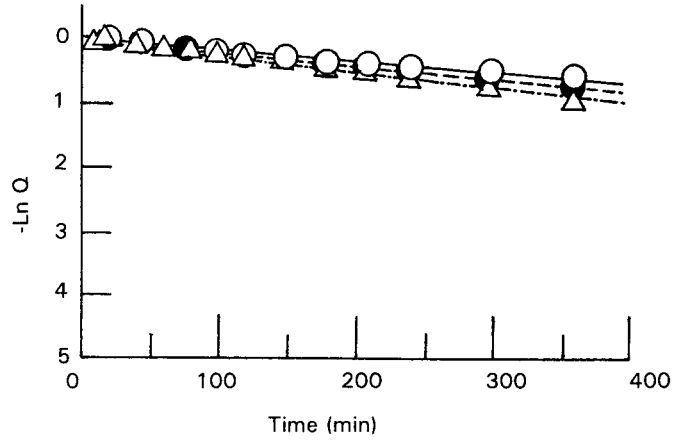

FIG. 8. Effect of pH of dissolution medium on release of theophylline from w/o/w emulsions (Method A). Concentration of sodium chloride in the inner aqueous phase: 0·05 M. Ionic strength of the dissolution medium: 0·02. pH of the medium: △ 5, ● 6, ○ 8. Release rate constant and initial percentage of theophylline entrapped in the inner aqueous phase: △ 0·0017 min^{-1} and 77·6%, ● 0·0016 min^{-1} and 75·1%, ○ 0·0014 min^{-1} and 75·5%. Correlation coefficient: △ −0·980, ● −0·994, ○ −0·969.

Effect of the types of dissolution medium on the release of theophylline from w/o/w emulsions

Table 2 shows the initial trap percentages of theophylline in the w/o/w emulsion and the rate constants found in the dissolution test solutions No. 1 and 2 specified in JP XII. Compared with the values in Table 1, it was found that the release rate constant in the No. 1 or No. 2 solution was greater than that in purified water, whereas the initial trap percentages of drug were little changed. An increase in the concentration of sodium chloride in the inner aqueous phase prolonged the drug release in the No. 1 and No. 2 solutions as found in the purified water in Table 1. The initial rapid drug release from w/o/w emulsions in the test was small using Method A, since the initial trap percentages of the drug were little influenced by the dissolution media. The coincidence of the values of initial trap percentages of the drug in Tables 1 and 2 with the values of the trap percentages of aqueous phase, and the fact that the former values were not influenced by the dissolution media indicated the validity of the present analysis methods.

To explain the difference in the release rate constants between the dissolution media, further investigations were carried out.

The effect of ionic strength of the dissolution medium on the release of theophylline from the w/o/w emulsion was studied in the No. 2 solutions diluted at various volume ratios of the solution with distilled water; the dilution did not influence the pH of the medium (Fig. 7).

The effect of pH of the dissolution medium was also studied. Phosphate buffers were used as the dissolution media. The ionic strength of the solutions was 0·02. The results are shown in Fig. 8. The concentration of sodium chloride formulated with theophylline in the inner aqueous phase of the w/o/w emulsion was 0·05 mol L^{-1} in all the experiments reported in Figs 7, 8.

The release of theophylline from the w/o/w emulsion was influenced remarkably by the ionic strength of the dissolution medium, whereas the pH value of the medium had little effect. The drug release rate increased with an increase in the ionic strength of the medium. This result was expected from our previous study showing that the addition of salt or sugar in the outer aqueous phase made the w/o/w emulsion unstable (Kawashima et al 1992). The solutes in the outer aqueous phase dehydrated the water molecules bound to the outer water–oil interface. The dehydrated interface made the oil membrane unstable against hydrodynamic stress, resulting in the rupture or the coalescence of the membrane.

Drug release from the w/o/w emulsion to simulate in the gastrointestinal tract

Most of the drug release studies from w/o/w emulsions were investigated using Method A. When the w/o/w emulsion is administered, the emulsion would be diluted with digestive fluid. Therefore, Method B in Fig. 2 is assumed to be more suitable to simulate the drug release behaviour from the w/o/w emulsion in the gastrointestinal tract after its administration.

Table 3 shows the results of the release tests of theophylline from the w/o/w emulsion with Methods A and B. Fig. 9 shows the release profiles of theophylline with Method B.

Table 3. Comparison of release rate constant and initial percentage of trapped theophylline using Methods A and B.

pH of dissolution medium	Rate constant (min^{-1})		Initial percentage of trapped theophylline	
	Method A	Method B	Method A	Method B
5	0·0017	0·0337	77·6	28·7
6	0·0016	0·0349	75·1	27·6
8	0·0014	0·0288	75·5	24·2

Concentration of sodium chloride in inner aqueous phase: 0·1 M. Ionic strength of dissolution medium (KH_2PO_4/K_2HPO_4 buffer solution): 0·02.

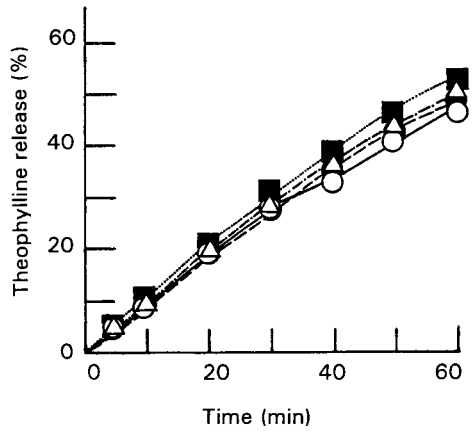

FIG. 9. Release profiles of theophylline from w/o/w emulsion (Method B). Concentration of sodium chloride in the inner aqueous phase: 0·05 M. Ionic strength of the dissolution medium: 0·02. pH of the medium: △ 5, ● 6, ○ 8. ■: Release profile of theophylline from 0·5 w/v % aqueous solution into water.

As shown in Table 3, the rate constants obtained using Method B were greater than those for Method A. The initial percentages of theophylline encapsulated in the emulsion in Method B were smaller than those in Method A. These findings suggested the immediate rupture of the oil membrane of the w/o/w emulsion droplets when dispersed in the dissolution medium. The membrane structure was destroyed and theophylline was released immediately. Therefore, the release profiles of theophylline from the w/o/w emulsions showed a similar pattern with that of the drug from the aqueous solution as shown in Fig. 9.

To confirm the hypothesis that the w/o/w emulsion structure was destroyed by the dilution of the emulsion, the trapped percentages of aqueous phase in the w/o/w emulsions diluted 10–100 times with water were measured. The result is shown in Table 4. The percentage of inner aqueous phase entrapped in the w/o/w emulsion remarkably decreased on diluting the emulsion.

The original multiple w/o/w emulsion droplet contained several small inner aqueous droplets as shown in Fig. 1. The number of the inner aqueous droplets in the multiple emulsion droplet decreased with dilution of the emulsion and finally the multiple droplet contained only one inner aqueous droplet as found in the previous study (Kawashima et al 1992). The drug encapsulated in the inner aqueous phase might leak due to the destruction of multiple droplet structure caused by thinning of the oil membrane on dilution. The surfactant adsorbed on the oil membrane will desorb on dilution, resulting in the thinning of the oil membrane.

Table 4. Effect of the dilution of w/o/w emulsion on the volume percentage of inner aqueous phase trapped in the w/o/w emulsion.

Concn sodium chloride in the inner aqueous phase (M)	Rate of dilution[a]	Volume percentage of inner aqueous phase trapped in the w/o/w emulsion
0·05	1[b]	60·5
0·05	10	32·1
0·05	100	1·7
0·10	1[b]	69·0
0·10	10	24·7
0·10	100	0·0
0·20	1[b]	70·0
0·20	10	18·3
0·20	100	0·0

[a]Volume of diluted emulsion (= volume of original w/o/w emulsion + added water)/volume of original w/o/w emulsion. [b]Original w/o/w emulsion without dilution.

The effect of the volume of a w/o/w emulsion tested on the release behaviour using Method B was investigated (Table 5). The drug release rate and the initial percentage of trapped theophylline were independent of the sample volume of the emulsion in the test. The changes in the volume ratio of the emulsion to the dispersing medium significantly affected the trap percent of aqueous phase in the w/o/w emulsion (Table 4). The multiple droplet structure was destroyed by diluting the system more than 10 times as shown in Table 4; this destruction occurred immediately after dilution. Thus, we conclude that in Method B, the sample volume of the w/o/w emulsion did not influence the release profile in the release test (Table 5). Experiments were carried out with purified water and the JP XII—No. 1 fluid used as the dissolution media in Method B. A significant difference in the release profile of these media and of the JP XII—No. 2 fluid could not be detected because the destruction of the w/o/w emulsion structure occurred immediately after dilution.

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Table 5. Effect of sample volume of w/o/w emulsion on the release of theophylline using Method B.

Sample volume of w/o/w emulsion (mL)	Volume of dissolution medium (%)		Rate constant (min^{-1})	Initial percentage of entrapped theophylline	Correlation coefficient
	Donor	Acceptor			
5	880	20	0·035	27·2	−0·925
48	760	20	0·022	32·6	−0·919
98	760	20	0·028	30·1	−0·946

Dissolution medium: JP XII No. 2 fluid. Concentration of sodium chloride in inner aqueous phase: 0·1 M.

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