As the results, it became evident that impurities, especially low molecular weight polyethylene glycol, permeate rapidly, but permeation of nonionic surfactant molecules is negligible.

To obtain the magnitude of interaction between benzocaine or sodium salicylate and Tween 80, equilibrium dialysis experiments were successfully carried out employing the cellulose membrane.

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54. **Hisao Matsumoto,**[*1,*2] **Hisakichi Matsumura,**[*1] **and Sadao Iguchi**[*3] :

Studies with Static Dialysis Method on the Release of Drugs from Nonionic Surfactant Solutions. II.[*4] Dialysis of Benzocaine Saturated in Tween 80 Solutions.

(*Faculty of Pharmaceutical Sciences, Fukuoka University*[*1] *and Faculty of Pharmaceutical Sciences, Kyushu University*[*3])

According to the theory of micelle formation, it is considered that drugs in surfactant solutions are partitioned between "micellar phase" and surrounding "aqueous phase", and pointed out that efficiency of phenolic preservatives used in nonionic surfactant solutions is displayed not by the partitioned to the micellar phase but only by the remained in the aqueous phase.[1] Therefore it is recognized that the preservatives must be added to maintain their concentrations above the effective level in the aqueous phase.

If drugs in the aqueous phase in the surfactant solution are removed rapidly by some causes, it will be naturally expected that the drug molecules in the micellar phase distribute to the aqueous phase when sufficient time or stirring is supplied. Without these conditions, it is not yet thoroughly investigated whether such transfer of the drug molecules rapidly occurs or not.

In the past literature, Allawala[2] evidenced utilizing characteristics of bactericidal action of iodine that such transfer occurs instantaneously. But there remain some questions whether it was carried out without any stirring or not. Nishida[3] reported as the results of dialysis experiments of short time period that transfer of chlorobutanol from micellar phase did not occur even if moderate stirring was added and the drug in the aqueous phase rapidly removed by the dialysis.

At this time, the authors investigated these phenomena in more detail by prolonging the dialysis period and utilizing the static dialysis method which is not supplied any stirring. For it is considered these mechanism must have close relation to the absorption of various drugs through biologic membrane from solutions containing nonionic surfactants.

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[*4] Part I. H. Matsumoto, H. Matsumura, S. Iguchi : This Bulletin, **14**, 385 (1966).
[1] M. Matsumoto, M. Aoki : *Ibid.*, **10**, 251 (1962); W.P. Evans : J. Pharm. and Pharmacol., **16**, 323 (1963); A.G. Mitchell : *Ibid.*, **16**, 533 (1964).

In this paper, dialysis of benzocaine from its saturated solutions was investigated as a preliminary experiment, and the rapid transfer from micellar phase to aqueous phase was confirmed.

Prior to the dialysis experiments, theoretical equations of dialysis were derived as follows.

When two solutions in different concentration of a solute are in contact each other with a membrane whose area is A and the concentration gradient across the membrane is linear, from the Fick's equation, flux of the solute is given by

$$\frac{dX}{dt} = -PA\frac{\varDelta c}{\varDelta l} \tag{1}$$

where $\varDelta l$ is thickness of the membrane and $\varDelta c$ is concentration difference in the aqueous phase between inner and outer solutions.

When volume of the outer fluid is large enough to neglect the drug concentration in it at any time, $\varDelta c$ is given by

$$-\varDelta c = \frac{1}{V}(x_0 - X_t) \tag{2}$$

where V is volume of the inner solution, x_0 is amount of the solute in the inner solution at the beginning and X_t is amount of the solute dialyzed after time t.

Substitution of Eq. (2) into Eq. (1), integration and evaluation of constant give

$$\ln\frac{x_0}{x_0 - X_t} = \left(\frac{AP}{\varDelta lV}\right)t \tag{3}$$

Apparent permeability coefficient, P', is defined as follows :

$$P' = \left(\frac{A}{\varDelta lV}\right)P \tag{4}$$

Then, Eq. (3) becomes

$$\ln\frac{c_0}{c_t} = P't \tag{5}$$

where c_0 and c_t is concentration of the solute in the inner solution at the beginning and after time t respectively.

If area of the membrane and volume of the inner solution are kept in constant at any time, the value of P' becomes constant and Eq. (5) indicates the first order relationship. Therefore half life of inner concentration becomes

$$T_{½} = \frac{\ln 2}{P'} = 0.693\left(\frac{\varDelta l}{P}\right)\left(\frac{V}{A}\right) \tag{6}$$

Eq. (6) indicates that the smaller the volume of the inner solution is or the larger the membrane area is, the shorter the half life of the inner concentration of the solute becomes.

When volume of the outer fluid is limited to nV, concentration difference in the aqueous phase between inner and outer solutions becomes

$$-\varDelta c = \frac{x_0 - X_t}{V} - \frac{X_t}{nV} = \frac{1}{V}\left[x_0 - \left(\frac{n+1}{n}\right)X_t\right] \tag{7}$$

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2) N. A. Allawala, S. Riegelman : J. Am. Pharm. Assoc., Sci. Ed., **42**, 267 (1953).

3) M. Nishida, *et al.* : Yakuzaigaku, **22**, 257 (1962).

Substitution of Eq. (7) into Eq. (1), integration and evaluation of constant give

$$\frac{n}{n+1}\ln\frac{\left(\frac{n}{n+1}\right)x_0}{\left(\frac{n}{n+1}\right)x_0-X_t}=\left(\frac{AP}{\Delta lV}\right)t=P't \qquad (8)$$

where P' is apparent permeability coefficient and it is coincident with previous Eq. (4).

Experimental

Materials——Potassium chloride was extra pure reagent grade. The other reagents were the same as used in the preceding paper.*4

Dialysis Apparatus and Procedure——Unit A : Deionized water (25 ml.) in a test tube (2.7 cm. in diameter and 10 cm. in length) was used as the outer apparatus. Unit B : Deionized water (400 ml.) in a beaker (500 ml.) was used as the outer apparatus.

Cellulose membrane bags and the inner apparatus were the same as used in the preceding paper in both units. Dialysis experiments were carried out at 30° as described in the preceding paper.

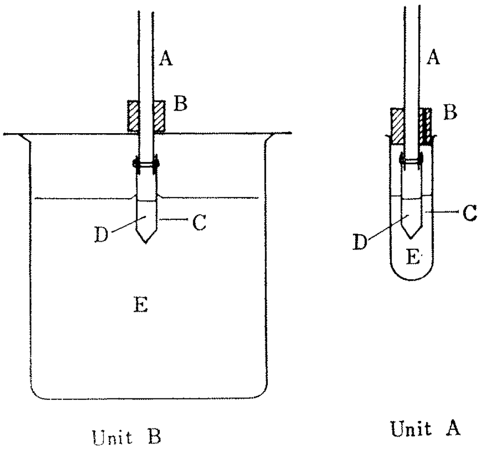

Fig. 1. Dialysis Apparatus

A : Glass tube
B : Rubber stopper
C : Cellulose membrane bag
D : Inner solution
E : Outer fluid

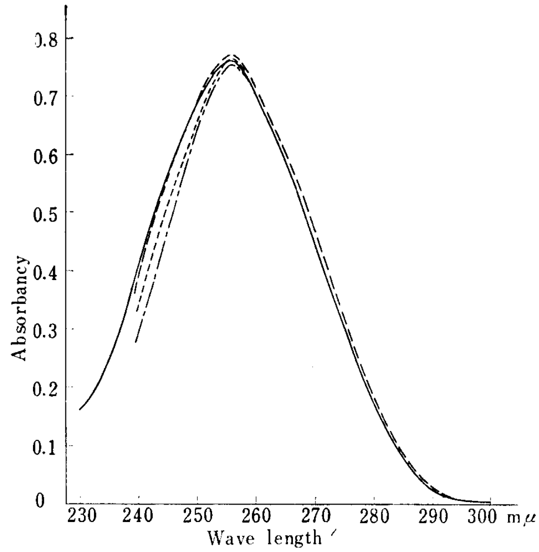

Fig. 2. Absorption Spectra of Methylparaben in the Presence of Tween 80

Solvent : 0.01N HCl, aqueous solution
Reference : Tween 80 was added to the same concentration with sample solutions
Methylparaben : $0.5\times10^{-4}M$
Tween 80 : ———— 0% ------- 0.4%
— — — 0.2% ------ 0.6%

Thickness and Contact Area of the Cellulose Membrane——Thickness, Δl, was measured with a thickness gauge (minimum graduation, 2μ) and it indicated 42 μ after dialysis experiments. Area, A, was measured with following manner. A sample solution (4 ml.) was pipetted into the inner apparatus and immersed in the outer fluid to the same surface level of these solutions. The cellulose bag was cut at that level and spread to form a rectangular sheet. Then its area was measured and indicated 17.7 cm^2.

Determination of Half Life of Potassium Chloride Solution——A sample solution (4 ml.) of 0.1M KCl was dialyzed to 25 ml. of deionized water using unit A for 30 min. at 30° and apparent permeability coefficient, P', was calculated with Eq. (8). Mean value of five times experiments was 4.26×10^{-2}(min^{-1}). Then its half life in the inner solution was calculated with Eq. (6).

Determination of Methylparaben——Aliquot portions of a sample solution were transferred into measuring flask and 1 ml. of 0.1N HCl and distilled water was added to make up to 10 ml., and the absorbancy was measured at 256 mμ. The calibration curve indicated a straight line through origin. Additive property of the absorbancy in the presence of Tween 80 was confirmed, therefore methylparaben concentration could be determined by subtracting the absorbancy owing to Tween 80 (Fig. 2).

Results and Discussion

Applicability to the Theoretical Equations

In these experiments, static dialysis method, which permits rapid dialysis and is not given any stirring, is adopted. Then applicability to the theoretical equation (3), (5) or (8) was tested using $10^{-3}M$ methyl *p*-hydroxybenzoate (methylparaben) standard solution as inner solutions.

Fig. 3 shows linear relationship as the results of calculation with Eq. (3) or (8), and their each slope values correspond to apparent permeability coefficient, P'. These values should be coincident in the every case at the ideal condition, but some deviations are observed in Fig. 3. Then the effect of volume of the outer fluid, nV, was tested and the results are given in Table I. The effect of immersing depth of the cellulose bag was also tested. If it was immersed too deep into the outer fluid, it will be flattened and the contact area of the membrane may be enlarged. As given in Table II, the effect was negligible even if the depth of immersion is a little changed.

Diameter of the outer container also affected on P' as shown in Table III. As various experimental conditions effected to the value of P', it was necessary to compare the results of dialysis at the same condition.

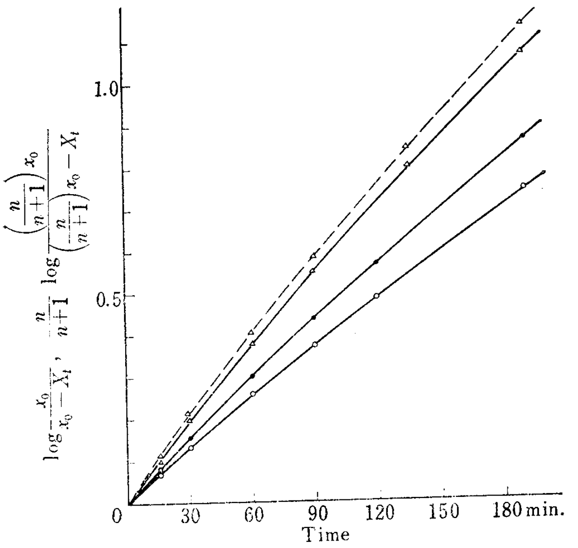

Fig. 3. Nearly Linear Relationships between Calculated Values with Eq. (3) or (8) and Dialysis Period

Inner solution : 4 ml. of methylparaben solution ($10^{-3}M$) in all cases

Outer fluid :

—○— 25 ml. of water in 2.7 cm. diameter test tube

—●— 25 ml. of water in 3.3 cm. diameter test tube

—△— 400 ml. of water in 500 ml. beaker

--△-- 400 ml. of water in 500 ml. beaker and stirred only the outer fluid occasionally

Table I. Effect of Volume of the Outer Fluid, nV, on Apparent Permeability Coefficient, P'

n	3.75	5.00	6.25	7.50	10.0
$P'(\times 10^{-2})$	1.02	1.08	1.13	1.18	1.20

Table II. Effect of Diameter of the Container of Outer Fluid on Apparent Permeability Coefficient, P'

Diameter (cm.)	2.7	3.3	4.5
$P'(\times 10^{-2})$	1.12	1.63	1.77

Uniformity of Cellulose Membrane and Reproducibility

Permeability of each cellulose bag was measured using $10^{-3}M$ methylparaben

Table III. Effect of Immersing Depth of the Cellulose Bag on Apparent Permeability Coefficient, P'

Immersing depth[a] (cm.)	0	0.4	1.0	1.5	2.0
$P'(\times 10^{-2})$	1.22	1.26	1.35	1.38	1.47

a) from the same surface level of inner and outer fluids.

standard solution as the inner solution with unit A. The values of P' of the twenty bags were calculated after 60 minutes dialysis. The bags ranging without 2.5% from the mean value were removed.

Reproducibility of P' was tested by ten times repeated dialysis and changes of the values were within 2% from the mean value. Then the cellulose bags could be used many times if they were stocked immersing in deionized water.

Dialysis of Potassium Chloride

Dialysis apparatus used in this study was constructed to shorten the half life of the inner solute by enlarging the ratio of the membrane area to volume of the inner solution. For this purpose, the dialysis membrane was employed in the form of small bags. Half life of 0.1 M potassium chloride in the inner solution with this apparatus was 16.3 minutes and it was much shorter than that of Nakagaki's apparatus[4] (about 160 min.[*5]).

Next, according to Nakagaki and cowakers,[4] permeability coefficient, P, and membrane constant, $f=P/D$, were calculated, and they became 6.75×10^{-7} (cm^2/sec.) and 3.38×10^{-2} respectively. These values well agreed with Nakagaki's report obtained with the same membrane. Therefore it may be considered that dialysis with this apparatus also took place successfully.

Effect of Tween 80 on the Dialysis of Sodium Salicylate

To ascertain the effect of surfactant on membrane permeability characteristics, dialysis of sodium salicylate from Tween solutions of varying concentration was investigated.

Sodium salicylate was dissociated form in these solutions (pH was about 6.0) and had not any interaction with the surfactant.[*4] After 60 minutes dialysis with unit A, the values of P' were calculated and shown in Table IV. It indicates the values of P' are almost unaffected by the addition of Tween 80 and the cellulose membrane permeability remains constant.

Table IV. Effect of Tween 80 on Apparent Permeability Coefficient, P', of Sodium Salicylate

Concn. of Tween 80 (%)	0	1	2	4	6	8
$P'(\times 10^{-2})$	0.83	0.84	0.79	0.77	0.80	0.76

Dialysis of Benzocaine Saturated in Tween 80 Solutions

As an example of drugs which interact with surfactant micelles, ethyl *p*-aminobenzoate (benzocaine) was solubilized to saturate Tween 80 solutions of varying concentration and they were dialyzed to 25 ml. of deionized water. In all these saturated

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[*5] Calculated with the additional data from private communication.

4) M. Nakagaki, N. Koga, S. Iwata: Yakugaku Zasshi, 82, 1138 (1962).

solutions, amount of free benzocaine in each true aqueous phase is considered to be equal. Therefore amout of dialysis of benzocaine in the definite period must be equal if transfer of benzocaine from micellar phase to aqueous phase or direct dialysis from the micellar phase to the outer fluid did not occur.

Amounts of benzocaine dialyzed into the outer fluid with the lapse of time are plotted in Fig. 4, and it shows amounts of the dialysis increase with increasing concentration of Tween and solubility of benzocaine. A horizontal split line indicates the equilibrium value of benzocaine in the outer fluid which was calculated assuming that only free benzocaine distributed between inner and outer fluids. But this line is crossed over by the actually dialyzed benzocaine after 55 to 75 minutes. These results suggested that benzocaine in the micellar phase was dialyzed into the outer fluid through any route.

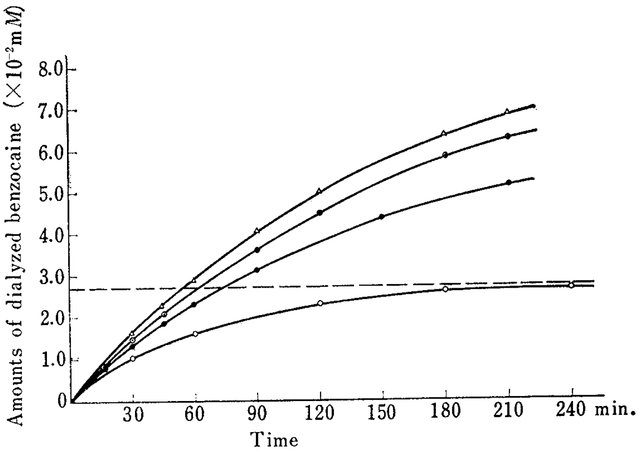

Fig. 4. Amounts of Benzocaine Dialyzed from Its Saturated Solutions

Inner solution : 4 ml. of saturated solution with benzocaine
○ in aqueous solution
● in 2% Tween 80 solution
⊙ in 4% Tween 80 solution
△ in 6% Tween 80 solution
Outer solution : 25 ml. of deionized water

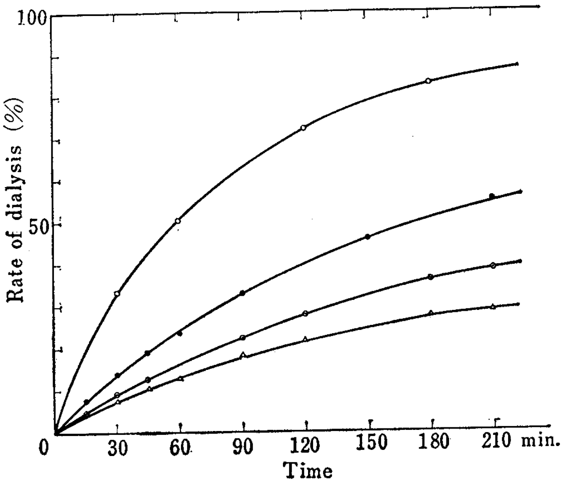

Fig. 5. Rate of Dialyzed Benzocaine to the Initial Amount of Benzocaine Saturated in the Inner Solution

—○— in aqueous solution
—●— in 2% Tween 80
—⊙— in 4% Tween 80
—△— in 6% Tween 80

Rate of dialyzed benzocaine to the initial amount of benzocaine satulated in the inner solution is plotted in Fig. 5. It decreases apparently with increasing concentration of Tween. Bound benzocaine in the micellar phase increases with increasing concentration of Tween as determined in the preceeding paper*4 and their magnitude was expressed as ratio, *r* (tolal to free). Therefore it indicates that free benzocaine in the aqueous phase mainly participates in the dialysis.

On the other hand, marked change of the rate of dialysis in the presence of Tween indicated that Eq. (8) was not applicable any more. Then the decrease of the dialysis rate in the presence of the surfactant was assumed tentatively to the result of decrease of apparent dialysis coefficient, P'', which changed corresponding to the concentration of Tween, and Eq. (9) was considered from Eq. (8) as follows :

$$\frac{n}{n+1}\log\frac{\left(\frac{n}{n+1}\right)x_0}{\left(\frac{n}{n+1}\right)x_0-X_t}=0.4343P''t \qquad (9)$$

Then above results were calculated with Eq. (9) and plotted in Fig. 6. Straight line relationships were obtained and indicated Eq. (9) was well applicable, and the slope values corresponded to P''.

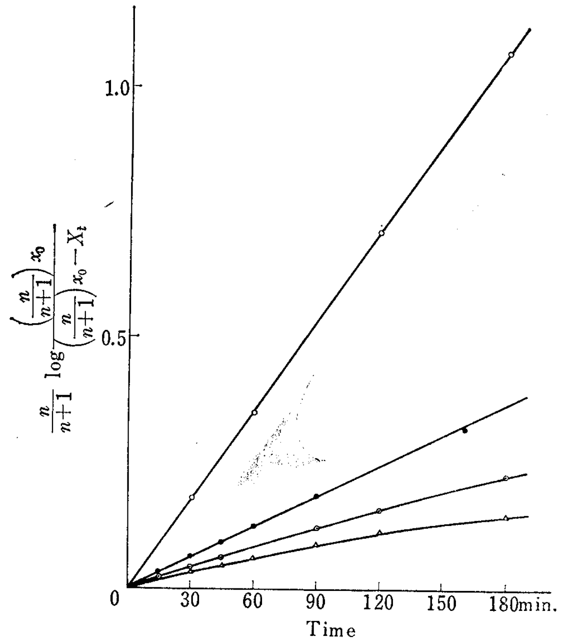

Fig. 6. Linear Relationships between Calculated Values with Eq. (9) and Dialysis Period

—○— in aqueous solution
—●— in 2% Tween 80
—⊙— in 4% Tween 80
—△— in 6% Tween 80

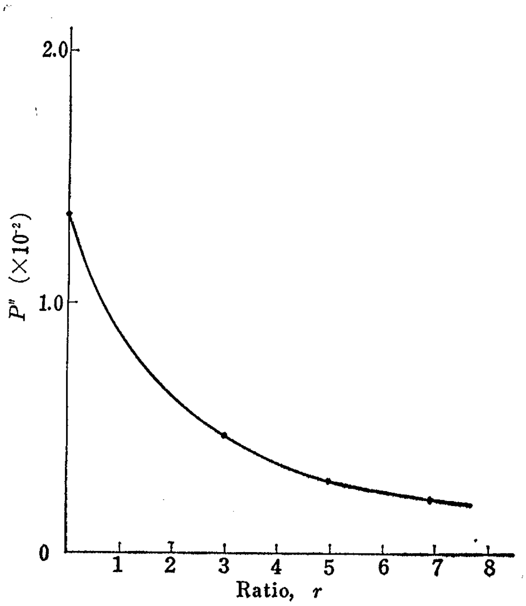

Fig. 7. Relationship between Apparent Dialysis Coefficient, P'', and Ratio, r

Fig. 6 shows these slope values markedly decrease with increasing concentration of Tween 80. This relationship is plotted in Fig. 7 and it indicates P'' is inversely proportional to the ratio, r.

As evidenced with the above studies, drug molecules in the micellar phase also dialysis into the outer fluid through any route, and amount of dialysis from saturated solutions with benzocaine increases with increasing concentration of Tween. In order to ascertain the direct dialysis from the micellar phase contributes or not, following experiments were attempted.

As an inner solution, yellow AB, which is thought to be soluble only in the micelles, was solubilized into 4% Tween 80 solution and 4 ml. of the solution in a cellulose bag was allowed to stand for 48 hours immersing in 25 ml. of toluene which solves yellow AB easily. But dialysis of yellow AB was not observed entirely. When 25 ml. of 4% Tween 80 solution was used as the outer fluid, dialysis of yellow AB was not recognized. From these results, it may be considered that direct dialysis from micelles into outer fluid is negligible.

Therefore it is concluded that drug molecules in the micellar phase are transferred to the aqueous phase rapidly corresponding to the decrease of concentration of the drug in the aqueous phase even if any stirring is not given, and then they participate in the dialysis to the outer fluid.

The authors wish to thank Dr. Goto for many helpful discussions.

Summary

Two kinds of dialysis apparatus were constructed and deviced to permit rapid

dialysis of drug molecules. Applicability of theoretical equations of dialysis was tested and proved to be well applicable even if any stirring was not supplied.

But it was found that results of dialysis must be compared and discussed among the same condition of dialysis.

Dialysis from saturated solutions of benzocaine in Tween 80 solutions was studied and it was concluded that drug molecules in the micellar phase are transferred to the aqueous phase rapidly corresponding to decrease of the concentration of the drug in the aqueous phase even if any stirring is not supplied, then they participate in the dialysis to the outer fluid.

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55. Hisao Matsumoto[*1,*2]: Studies with Static Dialysis Method on the Release of Drugs from Nonionic Surfactant Solutions. III.[*3] Rapidity of Transfer of Drug Molecules from Micellar to Aqueous Phase.

(*Faculty of Pharmaceutical Sciences, Fukuoka University*[*1])

In the preceding paper,[*3] rate of dialysis of benzocaine was markedly decreased by the addition of Tween 80, and it was considered provisionally that it will be due to decrease of apparent dialysis coefficient, P'', which indicated inverse proportionality to the ratio, r.

But membrane permeability itself is not changed with nonionic surfactant. Therefore decrease of dialysis rate must be attributed to the decrease of drug concentration in the aqueous phase in the surfactant solution.

It was also evidenced that drug molecules solubilized in the micellar phase are rapidly transferred into the aqueous phase even if any stirring is not supplied, and that drugs only in this aqueous phase participate in dialysis.

In this report, theoretical equations were derived assuming that micellar to aqueous phase transfer of drug molecules occurs instantaneously corresponding to decrease of drug concentration in the aqueous phase, and the applicability was investigated.

When a drug is partitioned between micellar and aqueous phase with ratio, r (total to free), in a surfactant solution, and amount of dialyzed drug after time t is expressed as X_t, amount of drug in the aqueous phase in the inner solution, x_t' becomes

$$x_t' = \frac{x_0 - X_t}{r} \qquad (1)$$

When unit A is used and volume of the outer fluid is limited to nV, concentration difference of the drug in the aqueous phase between inner and outer fluids, Δc, is given by

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