DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 14(9), 1137-1154 (1988)

GRISEOFULVIN-HYDROGENATED SOYA PHOSPHOLIPID COPRECIPITATES

Toshiaki Nishihata
Pharmaceutical Chemistry Department,
University of Kasnas, 2065 Constant Avenue,
Lawrence, Kansas 66046, USA

Yoshiko Chigawa and Akira Kamada
Faculty of Pharmaceutical Sciences, Osaka
University, 1-6 Yamadaoka, Suita, Osaka 565,
Japan

Kiyoshi Sakai
Osaka University of Pharmaceutical Sciences,
1-10-65 Kawai, Matsubara, Osaka 580, Japan.

Koichi Mastumoto, Kazuhoko Shinozaki,
and Yujin Tabata
Nikko Chemicals Co., 1-4-8 Bakurocho,
Nihonbashi, Chuo-ku, Tokyo 103, Japan.

ABSTRACT

The dissolution of griseofulvin was accelerated significantly by formulation as a griseofulvin-hydrogenated soya phospholipid coprecipitate. The transport of griseofulvin across both cellulose membranes and rat intestinal sac was also accelerated by the use of coprecipitates. X-ray diffraction data

suggest that the decrease in crystallinity of griseofulvin in the coprecipitate accelerated the dissolution of griseofulvin. The possible aggregation (possible small micelle formation) of phospholipid including griseofulvin in water may also increase the apparent solubility of griseofulvin from the coprecipitate. The increase in surface area of coprecipitate and pure griseofulvin by including bulking (core) substances further accelerated the dissolution of griseofulvin and resulted in rapid transport of griseofulvin through cellulose membranes.

INTRODUCTION

Dissolution rates are known to be often rate-limiting in the absorption of drugs from the gastrointestinal tract (1). For poorly soluble drugs, a reduction of particle size (2) and chemical modification, e.g. prodrugs (3, 4) have been attempted to improve dissolution behavior.

There are many studies concerning the use of solid dispersion and coprecipiates to accelerate drug dissolution (5-14). The dissolution rates of poorly water-soluble drugs has been increased by their prior dispersion in a water soluble carrier such as polyethylene glycol. Coprecipitates of -carotene and a

water soluble polymer resulted in a colloidal dispersion when exposed to water (15). Coprecipitates of nifedipine and polyvinylpyrrolidone showed an increased solubility of the drug probably due to a colloidal dispersion, and an increased intestinal absorption of nifedipine was observed both in rats and dogs (14).

In the present study, a coprecipitate of griseofulvin (a lipophilic drug) with hydrogenated soya phospholipids caused an acceleration of apparent griseofulvin dissolution in buffered-saline. Phospholipids have some surfactant character and can form membranes in which various substances can become involved (16,17). Therefore, preparation of coprecipitate of griseofulvin with phospholipid was examined. Further, the utility of bulking (core) substances to increase the effective surface area was also examined to accelerate the dissolution of griseofulvin.

MATERIALS AND METHODS

Griseofulvin was supplied by Fujisawa Pharmaceutical Industry (Osaka, Japan). Hydrogenated soya phospholipid (phospholipid) was supplied by Nihon Surfactant Co. (Tokyo, Japan). The phospholipid content

was more than 98 %, of which phosphatidylcholine was about 30 % and phosphatidylethanolamine was about 70 %, and the iodine value was about 3 %. Other reagents used were of analytical grade and were obtained commercially. Cellulose membrane tubes used were obtained from Spectrum Medical Industries Inc. (Los Angels, USA): molecular weight cutoff value of the membrane of tube-1 (15.9 mm diameter) is 12,000-14,000, and that of the membrane of tube-2 (11.5 mm diameter) is 3,500.

Preparation of the coprecipitate was performed as follows. Suitable amounts of drug and phospholipid (in the weight ratios as shown Table 1) were weighed and dissolved in chloroform. The solvent was evaporated under reduced pressure to obtain a hard lipid film. The lipid film as a residual solid was pulverized with a motar and pestle, and the particles having size of 30 to 75 μm were collected. When lactose or calcium phosphate was used as a bulking (core) substance, a suitable amounts of lactose and calcium phosphate (particle size: 10 to 30 μm) was suspended in the chloroform solution used to dissolve drug and/or phospholipid. After evaporation, the fraction having a particle size of 30 to 75 μm was collected. The particle size of grisefulvin used in the control experiment as pure griseofulvin was either less than 10

TABLE 1

Codes and Constituents in Formulation Examined.

Formulation	Weight Ratio of Constituents			% w/w of Griseofulvin in Formulation[a]
	drug[b]	phospholipid	core[c]	
Drug alone				
A-1 (particle size less than 10 μm)	1	0	0	100
A-2 (particle size: 30 to 75 μm)	1	0	0	100
Physical Mixture				
PM-0.5	1	0.5	0	67.4±3.1
PM-2	1	2.0	0	34.1±1.6
PM-7.5	1	7.5	0	11.2±0.6
Coprecipitate				
C-0.5	1	0.5	0	65.7±1.0
C-2	1	2.0	0	32.6±0.6
C-7.5	1	7.5	0	11.1±0.9
Griseofulvin with Core Compound				
L-2	1	0	2.0*	29.1±2.1
L-7.5	1	0	7.5*	10.1±0.7
Coprecipitate with Core Compound				
LC-0.5	1	0.5	7.5*	10.2±0.9
LC-2	1	2.0	7.5*	8.2±0.4
CPC-0.5	1	0.5	7.5**	10.0±0.8
CPC-2	1	2.0	7.5**	8.1±0.2

Note of TABLE 1: a, the content of gliseofulvin was measured three times from each formulation (each value represents the mean ± S.D., n=3); b, drug represents griseofulvin; c, core compound with * and ** are lactose and calcium phosphate, respectively.

μm or 30 to 75 μm. The particle size of physical mixture of griseofulvin and phospholipid was also less than 10 um. The codes and constituents in the formulations examined in this study are listed in Table

1. The content of griseofulvin in each formulation was measured and summarized in Table 1.

To characterize griseofulvin-phospholipid coprecipitate briefly, solid samples were analysed by X-ray powder diffractomtery according to a method described previously (14).

The dissolution of griseofulvin in each formulation was determined in 200 ml of buffered-saline (buffer: 0.05 M sodium phosphate buffer, pH 7.4) maintained at 37°C in a cylindrical beaker with a slightly concave bottom. An appropriate amount of test sample containing 200 μmoles griseofulvin equivalent was weighed and placed in 200 ml of the buffered-saline and shaken at at 100 cycles/min. At various suitable time intervals, 1 ml of the sample solution was pipetted through a Millipore filter (0.22 um pore size) for assay.

Transport of griseofluvin through cellulose membrane: A 5 cm length of tube-1 and 10 cm length of tube-2 were used for the tranport study of griseofluvin. The cellulose membrane tube containing 5 ml of the buffered-saline and a test sample with 5 umoles griseofulvin-equivalent was placed in 20 ml of saline in a test tube at 37°C. Two hundred ul of solution from outside the tube was collected at designated time intervals to assay griseofulvin.

Transport of grisefulvin across rat small intestinal sac was performed using approximately 15 cm small intestine which was excised from Wistar rats (weighing 200 to 225 g) after decapitation. Small intestinal sac containing 5 ml of the buffered-saline and test sample with 5 μmoles of griseofuliven equivalent was placed in 20 ml of Krebs-Ringer solution at 37°C. The amounts of griseofluvin in outside solution (serosal side) was determined at 0.5 h and 1 h.

Solubility of griseofulvin was determined at 37°C after incubation for 48 h, and sample was collected through a Millipore filter (pore size; 0.22 μm).

Assay of griseofulvin was carried out by high performance liquid chromatography: a reverse-phase column (RP-18, 4.6 i.d. x 15 cm) was used. The mobile phase was a mixture of acetonitrile and water (3:7 v/v), operated at flow rate of 1.0 ml/min. The column effluent was monitered at 285 nm. The column was maintained at 25°C. The assay sensitivity limit was 0.5 uM.

Statistcal analyses were performed using Student's t-test.

RESULTS

X-ray diffraction patterns of pure griseofulvin, phospholipid and coprecipitates in various weight

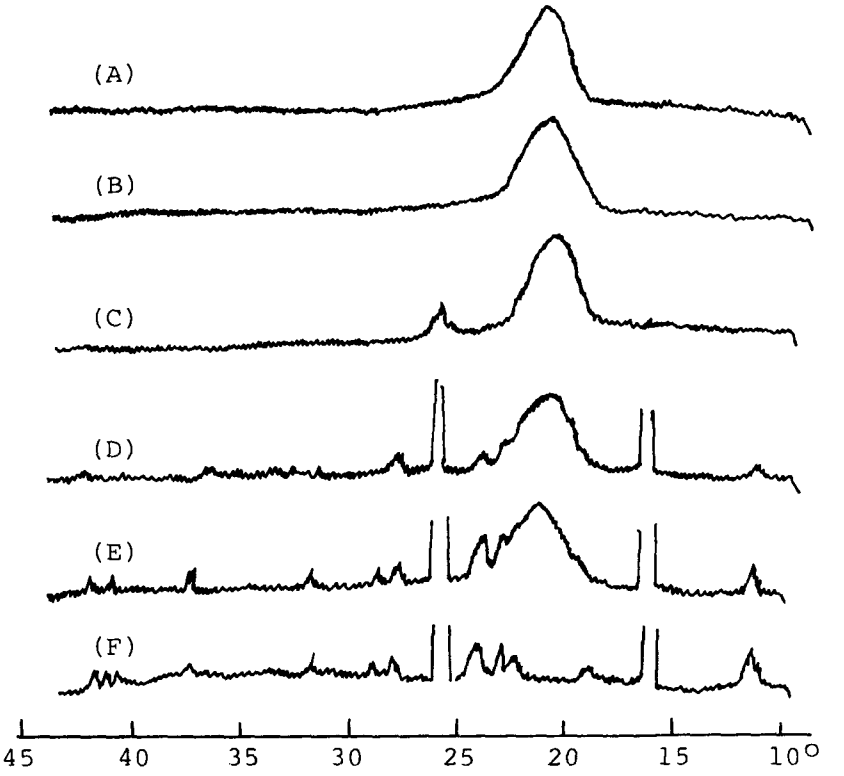

FIG. 1

X-Ray Diffraction Patterns for (A) phospholipid alone, (B) 1:7.5 w/w coprecipitate of Code C-7.5, (C) 1:2 w/w coprecipitate Code C-2, (D) 1:0.5 w/w coprecipitate of Code C-0.5, (E) 1:2 w/w physical mixture of Code PM-2, and (F) griseofulvin alone.

ratios are shown in Fig. 1. Pure griseofulvin and phospholipid exhibited crystalline and amorphous forms, respectively. A marked difference in X-ray diffraction patterns in Code C-2 and Code C-7.5 were observed in comparison with pure griseofulvin (Code A-1). The data indicate a significant decrease in crystallinity for Code C-2 (Fig. 1C) as well as the lack of crystallinity in the coprecipitate of Code C-7.5 (Fig. 1B).

In the dissolution study, 200 ml of the medium was used against 200 umoles of griseofulvin. This amount of griseofulvin was greater than the amount required to saturate the medium (solubility of griseofulvin in the buffered-saline was 48.6±3.1 μM, n=3). The small volume of medium was used to investigate any increase in apparent solubility of griseofulvin from the various formulations.

As shown in Table 2, a reduction of particle size of griseofulvin resulted in rapid dissolution of griseofulvin in saline (Code A-1 compared to Code-2), as reported previously (2). The coprecipitate of griseofulvin accelerated the apparent dissolution of griseofluvin significantly, and an apparent supersaturated concentration was observed for Code C-2 and C-7.5 (Table 2). The concentration of griseofulvin (for the coprecipitate of Code-2) reached a constant value at 2h, which was about 1.5 times higher than the solubility of griseofulvin (48.6±3.1 μM, n=3). The system containing either a physical mixture or pure griseofulvin showed identical behavior (Table 2). When the solubility of griseofulvin was determined after incubation at 37°C for 48 h, the solubility of grieseofulvin in the buffered-saline, in a 0.01 %w/v phospholipid solution, and in 0.05 %w/v phospholipid solution (did not obtain clear soultion at a

TABLE 2

Apparent Dissolution of Griseofulvin from Various Formulations as A Function of Time at 37°C.

Code	Concentration of Griseofulvin, μM				
	Time after Immersion, min				
	15	30	60	120	360
A-1	14±4	21±3	28±5	32±4	40±4
A-2	7±2*	11±4*	12±3*	20±3*	36±5
PM-0.5	14±3	22±6	28±2	31±2	38±6
PM-2	14±2	21±4	27±5	32±4	39±4
PM-7.5	12±2	21±3	27±4	31±2	37±4
C-0.5	39±3*	44±3*	50±4*	57±6**	60±3**
C-2	86±12**	77±6**	75±6*	72±5**	72±6**
C-7.5	101±17**	84±14**	70±5**	63±5**	63±4**
L-2	22±5	32±5*	38±4	41±3*	44±4
L-7.5	45±6*	51±5*	50±2*	50±3*	50±3*
LC-0.5	98±19**	96±12**	95±11**	74±7**	73±4**
LC-2	131±16**	125±11**	99±17**	90±5**	86±6**
CPC-0.5	60±9**	72±8**	76±6**	72±9**	73±6**
CPC-2	108±11**	101±16**	89±8**	75±7**	74±5**

A sample containing 200 umoles griseofulvin equivalent was placed in 200 ml of buffered-saline.
Eavh value represents the mean ± S.D. (n=6). * and **, $p<0.05$ versus A-1; **, $p<0.05$ versus the saturated concentration of griseofluvin alone in bufferd-saline of 48.6±3.1 μM.

concentration of more than 0.05 %w/v) was 48.6±3.1 μM, 50.4±4.2 μM, and 53.9±2.6 μM, respectivcely (n=3). Since these data showed that phospholipid dissolving in buffered-saline could hardly affect the solubility of griseofulvin, it is suggested that the supersaturated concentration of griseofulvin (Table 2) is due to the formulation as a coprecipitate.

The effect of lactose or calcium phosphate as a bulking (core) substance on the dissolution of griseofulvin was examined. An increase in the ratio of lactose to griseofulvin alone or to coprecipitate accelerated the dissolution of griseofulvin (Table 2). The solubility of griseofulvin in saline, in a 1 %w/v lactose solution, and in a 2 %w/v lactose solution were 48.6±3.1 μM, 50 .7±1.9 μM and 51.4±2.2 μM, respectively (n=3). These data also show that lactose in the formulation could hardly affect the solubility of griseofulvin.

The use of calcium phosphate as a bulking substance also accelerated the dissolution of griseofulvin as did lactose (Table 2).

A reduction of particle size of pure griseofulvin (Code A-1 compared to A-2) accelerated the apparent transport of griseofulvin through cellulose membranes (Fig. 2). The transport of griseofulvin in the formulation of Code L-7.5 (pure griseofluvin including lactose as a bulking substance) through the cellulose membrane of both tube-1 and tube-2 was also accelerated significantly, in comparison to those in formulation of Code A-1 (Fig. 2). The relatively slow transport through the membrane of tube-2 is due to small pore size of the membrane.

The transport of griseofulvin through the cellulose membrane of both tube-1 and tube-2 was also

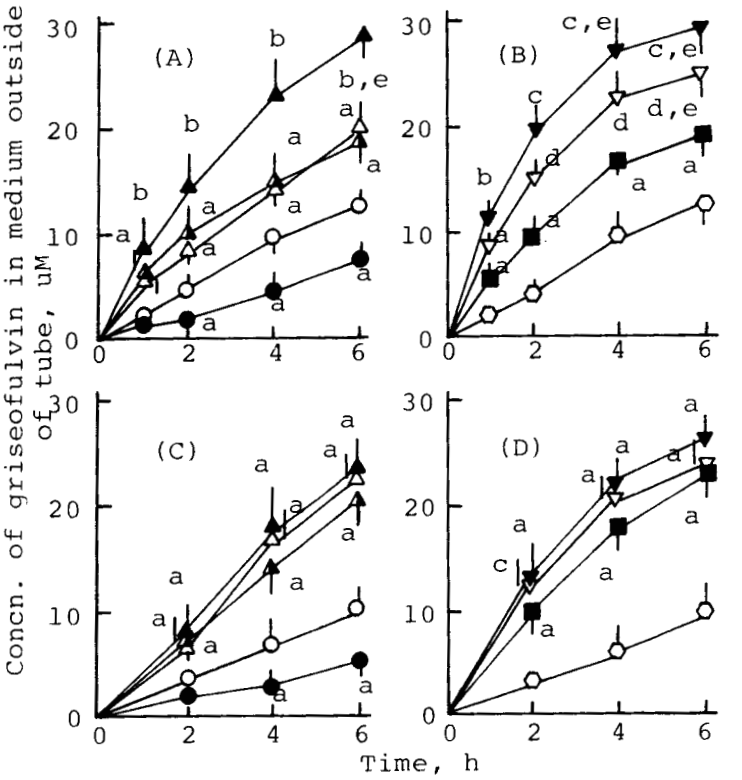

FIG. 2

Transport of griseofulvin in various formulations through the cellulose membrane of tube-1 (A and B) and of tube-2 (C and D) (amount equivalent to 5 μmols of griseofulvin with 5 ml saline in the tube). Transport of griseofulvin was represented by the concentration of griseofulvin in the solution outside of the tube.
Key in (A and C): ○, A-1; ●, A-2; △, C-0.5; ▲, C-2; and ◭, C-7.5.
Key in (B and D): ⬡, PM-2; ■, L-7,.5; ▽, LC-0.5; and ▼, LC-2.
Each value represnts the mean ± S.D. (n=4). a, b, c, and d, $p<0.05$ versus A-1; b, c, and d, $p<0.05$ versus C-0.5; e, $p<0.05$ versus the saturated concentration of griseofulvin in saline (46.8±3.2 μM).

TABLE 3

Transport of Griseofulvin across Small Intestine using the Rat Small Intestinal Sac Method (5 μmoles of griseofulvin equivalent with 5 ml of the buffered-saline was placed inside the sac, and 20 ml of the buffered-saline was the volume outside of the sac).

Code	Amounts of Griseofulvin Transported, nmoles	
	0.5 h	1 h
A-1	16.4±6.5	46.1±7.1
PM-2	21.6±4.2	59.9±8.9
C-2	56.2±9.7*	146.2±16.9*
L-7.5	27.4±8.1**	65.1±10.3**
LC-2	79.5±11.3*	186.5±21.9*
CPC-2	72.2±6.7*	162.6±17.6*

Each value represents the mean ± S.D. (n=4).
*, $p<0.01$ versus A-1; **, $p<0.05$ versus A-1.

accelerated by the coprecipitate formulation with or without core compound (Fig. 2). Further, the concentration of griseofulvin outside of tube-1 at later times was higher than the equilibrium solubility of griseofulvin in the buffered-saline. This was also observed in the dissolution study, when coprecipitate of Code C-2, LC-2 or LC-0.5 were examined (Fig. 2).

In terms of the transport of griseofulvin through the cellulose membrane of tube-1 with the large pore size, it was observed that the transport with formulations of Code C-2, LC-2 and LC-0.5 was greater than that in Code L-7.5 (Fig. 2). However, in terms of the transport of griseofulvin through the membrane of tube-2 with the smaller pore size, significant

differences among the above four formulations were not observed (Fig. 2).

The transport of griseofulvin across the intestine was investigated briefly (Table 3), using an in vitro at small intestinal sac method. The coprecipitate in formulations accelerated the transport of griseofulvin markedly in comparison to griseofulvin alone. The physical mixture seemed to also accelerate the transport of griseofulvin, but not significantly.

DISCUSSION

An increase in surface area of drug particle is of importance in the acceleration of the dissolution of a drug (2). In the present study, it was observed that the reduction of particle size of pure griseofulvin (Code A-1 compared to A-2) and of the formulation of griseofulvin which included lactose as a bulking substance (Code L-7.5), which were prepared to increase the exposed surface area of griseofulvin, accelerated both the griseofulvin dissolution and griseofulvin transport through a cellulose membrane. The bulky substance may also act as an inert spacer to prevent aggregation.

It is known that phospholipids readily produce a lipid film after being dissolved in chloroform followed by evaporation (17), and that this film can

also be used as an artificial membrane (18). Lipid films of phospholipids easily incorporate lipophilic compounds such as cholesterol. Therefore, in the present study, coprecipitates of griseofulvin with phospholipid were prepared according to the film method described in experimental section. To diminish the crystallinity of griseofulvin in the coprecipitate (i.e., one in which the crystallinity as accessed briefly by X-ray analysis was diminished), the amount of phospholipid required was more than 2 times the amount of griseofluvin (Fig. 1).

It has been reported that the solubilities of phospholipids such as phosphatidylcholine and phosphatydiylethanolamine are low but that they can hydrate (16), and phospholipids are known to be surface active agents.

Since phospholipid is a surface active agent, it is possible that the phospholipid-coprecipitate in the test solution probably aggregate to form micelles which include griseofulvin as a lipoidal compound. In the present study, the observed supersaturation of griseofulvin (Table II and Fig. 2) may be due to griseofulvin being included in such aggregates, which may often have a small size with an apparent molecular weight of less than 14,000, which can penetrate the cellulose membrane of tube-1 (Fig. 2). The apparent

griseofluvin transport through the cellulose membrane of tube-1 in terms of the coprecipitate forms was observed in following order: LC-2>C-2>LC-0.5>L-7.5=L-0.5 (Fig. 2). However, significant differences were not observed among the above five formulations in the transport of griseofulvin through the cellulose membrane of tube-2 with the smaller pore size (Fig. 2). These results indicate that the apparent molecular weight of the proposed aggregates formed from the coprecipitate (C-2, LC-2 and LC-0.5) in causing the apparent supersaturation of griseofulvin is larger than 3,500, but less than 14,000. Thus, the apparent supersaturation of griseofulvin from coprecipitates seems to be due to the formation of aggregates in aqueous solution, but these aggregates may bind together with time so that they do not pass through a Millipore filter during sampling (Table 1).

In terms of the in vitro transport of griseofulvin across rat small intestine, the use of coprecipitate accelerated the transport significantly (Table 3). Although it is not clear that the possible aggregation is involved in the increase in griseofulvin transport across the intestine, the coprecipitate is expected to accelerate the griseofulvin absorption from intestine after oral administration.

The coprecipitates of griseofulvin prepared with phospholipid increased the griseofulvin dissolution

rate. It should be also noticed that the use of lactose or calcium phosphate as a bulking (core) substance facilitated the dissolution of griseofluvin from the formulation. Thus, combinations of a coprecipitate and a suitable bulking (core) substance may be helpful in accelerating the dissolution of of such lipoophilic compounds.

REFERENCES

(1) Fincher, J. H., J. Pharm. Sci., 57, 1825 (1968).

(2) Duncan, W. A. H., Macdonald, G., and Thortor, M. J., J. Pharm. Pharmacol., 14, 217 (1962).

(3) Varia, S. A., Chuller, S., and Stella, V. J., J. Pharm. Sci., 73, 1074 (1984).

(4) Varia, S. A., and Stella, V. J., J. Pharm. Sci., 73, 1080 (1984).

(5) Sekiguchi, K., and Obi, N., Chem. Pharm. Bull., 9, 1080 (1961).

(6) Sekikawa, H., Nakano, M., and Arita, T., Chem. Pharm. Bull., 26, 118 (1978).

(7) Gibaldi, M., and Weintraub, H., J. Pharm. Sci., 54, 832 (1978).

(8) Simonelli, A. P., Mehta, S. G., and Higuchi, W. I., J. Pharm. Sci., 58, 538 (1969).

(9) Corrigan, O. I. and Timony, R. F., J. Pharm. Pharmacol., 27, 759 (1975).

(10) Crommelin, D. J. A. and Van Bloois, L., Int. J. Pharm., 17, 135 (1983).

(11) Ford, J. L., Stewart, A. F., and Dubois, J-L., Int J. Pharm., 28, 11 (1986).

(12) Jousma, H., Talsma, H., Spies, F., Joosten, J. G. H., Junginge, H. E. and Crommelin, D. J. A., Int. J. Pharm., 35, 263 (1987).

(13) Jachowig, R., Int. J. Pharm., 35, 1 (1987).

(14) Sugimoto, I., Kuchiki, A., Nakagawa, H., Tohgo, K., Kondo, S., Iwane, I., and Takahashi, K., Drug Dev. Ind. Pharm., 6, 137 (1980).

(15) Tachibana, T., and Nakamura, A., Kolloidal-Z. Polym., 203, 130 (1965).

(16) Lumberg, B., Sevens, E., and Ekman, S., Chem. Phys. Lipids, 22, 285 (1978).

(17) Olson, F., Hunt, C. A., Szoha, F. C., Vail, W. J., and Paraphadjopoulos, D., Biochim. Biophys. Acta., 557, 9 (1979).

(18) Nishihata, T. and Higuchi, T., Biochim. Biophys. Acta., 775, 269 (1984).