[Chem. Pharm. Bull. 34(5)2196—2201(1986)]

A New Method for Dissolution Testing of Vitamin E Preparations in Test Medium Containing Sodium Glycochenodeoxycholate[1)]

YUKI TSUSHIMA,*[,a] SATOSHI YAMAGIWA,[a] KIMIO TATSUISHI,[a] MASANORI KAYANO,[a] TADAKAZU TOKUMURA,[a] YOSHIHARU MACHIDA[b] and TSUNEJI NAGAI[b]

Research Center of Technological Development, Eisai Co., Ltd.,[a] Minami 2-3-14, Honjo-shi, Saitama 367, Japan and Faculty of Pharmaceutical Sciences, Hoshi University,[b] Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan

(Received January 6, 1986)

A method for dissolution testing of oily and water-insoluble drug preparations was developed. It was found that dissolution testing of a preparation containing *d*-α-tocopherol as a model drug could be done by using the paddle method in JP X and the 2nd fluid in JP X containing 20 mM sodium glycochenodeoxycholate. The data obtained by the method were useful for predicting *in vivo* absorption of the drug. However, in the case of a capsule containing *d*-α-tocopherol dissolved in cottonseed oil, the drug and vehicle floated on the surface of the dissolution test medium, and good dissolution was not observed.

Keywords——dissolution test method; paddle method; oily drug; *d*-α-tocopherol; bile salts; sodium glycochenodeoxycholate

A dissolution test for peroral solid preparations is widely required for quality control. Further, the dissolution rate is known to be the rate-determining step of *in vivo* absorption for slightly water-soluble drugs. A dissolution test, from this point of view, could also be an important method for predicting the bioavailability of drugs. Generally, water, diluted HCl or various buffer solutions have been used as the media in dissolution tests. However, the ordinary dissolution test method is not applicable to a pharmaceutical preparation containing a water-insoluble drug, because they are not soluble in such dissolution media. In order to solve this problem, several dissolution test methods have been developed, for example, a method employing a 20 l vessel and a large amount of dissolution test medium,[2)] the two-phase method,[3)] a method involving addition of synthetic surfactant[4-7)] and the usage of mixed solvents[8-12)] such as an alcohol and water. However, the use of special apparatus which differs completely from the JP apparatus seems undesirable, and the addition of synthetic surfactant or alcohol to the dissolution medium would make it difficult to predict the bioavailability. A method using a synthetic surfactant or alcohol may be useful only for a few drugs or preparations, because of the differences in interaction between the synthetic surfactant and drugs and/or additives. Moreover, the solubility of drugs or additives in an organic solvent which is added to a dissolution medium seem to differ from case to case. Consequently, these methods do not offer reliable prediction of bioavailability.

On the other hand, if a dissolution medium containing a biological surfactant is used, the problem mentioned above might be overcome, because the biological surfactant (represented by bile salts) affects all drugs given by oral administration. These biological surfactants are known to increase the dissolution rates of slightly water-soluble drugs.[13-20)] In addition, it is known that fat-soluble vitamins are absorbed after being dissolved in digestive fluids owing to the influence of bile salts.[21-23)] Thus, bile salts seem to play an important role in the dissolution and absorption processes of slightly water-soluble and/or water-insoluble drugs in

the gastro-intestinal tract. By choosing suitable bile salts for the dissolution test medium, as well as concentration and stirring conditions, it may be possible to model effectively the conditions of the gastro-intestinal tract. In a study on the application of biological surfactants in dissolution testing, the mechanism of the solubilizing effect was reported,[13-20] but its relation to the bioavailability has not been reported. In addition, a method of dissolution testing using bile salts has not yet been developed.

Bile salts in the gastro-intestinal tract may act on drugs in the following two ways: (1) acting as a surfactant to increase the effective surface area of the drug for dissolution, (2) solubilization by forming micelles with the drug. Many of the water-insoluble and fat-soluble drugs are oily substances, and the second factor could be important. Vitamins A, D and E seem to be representatives of the oily drugs.

The aim of the present investigation was to develop a new method for dissolution testing of oily drug preparations. *d*-α-Tocopherol was used as a model drug, and 6 kind of bile salts were used.

Experimental

Materials——*d*-α-Tocopherol was obtained from Eisai Co., Ltd. Bile salts, *i.e.*, sodium glycocholate (GC-Na), sodium taurocholate (TC-Na), sodium taurodeoxycholate (TDC-Na), sodium glycodeoxycholate (GDC-Na), sodium taurochenodeoxycholate (TCDC-Na) and sodium glycochenodeoxycholate (GCDC-Na), were synthesized according to the method described by Norman.[24] All cholates were checked for purity by high performance liquid chromatography (HPLC)[25] and all of them were confirmed to be more than 98% pure. Other chemicals used were of reagent grade. Distilled water was used in all experiments.

Solubility Studies——Each bile salt was dissolved in the 2nd fluid of JP X at three different concentrations, 20, 40, and 80 mM. An 8 ml aliquot of each solution and *d*-α-tocopherol was added to a 10 ml centrifuge tube; 0.2, 0.4 and 0.8 g of *d*-α-tocopherol was added to 20, 40 and 80 mM solutions of bile salts, respectively. After stirring of each solution for 5 d at 37 °C, 1 ml was taken, and filtered through a 0.22 μm Millipore® filter. The concentration of *d*-α-tocopherol in the filtrate was determined by HPLC using a Shimadzu LC-5A HPLC. The chromatograph was operated at a flow rate of 1.5 ml/min and the eluate was monitored at 292 nm by using a Shimadzu SPD-2A UV monitor. The column was Nucleosil C_{18} (5 μm in 4 mm × 15 cm). *dl*-α-Tocopherol acetate and a mixture of methanol and water (97 : 3) were used as the internal standard and mobile phase, respectively.

Model Preparations of *d*-α-Tocopherol for Dissolution Test——Each tablet contained 50 mg of *d*-α-tocopherol. Preparation A: each capsule contained 100 mg of *d*-α-tocopherol dissolved in HCO-60 and PEG-400. Preparation B: each capsule contained 100 mg of *d*-α-tocopherol dissolved in cottonseed oil and decaglycerin monolaurate. Preparation C: each tablet contained 50 mg of *d*-α-tocopherol. Preparation D: each capsule contained 100 mg of *d*-α-tocopherol dissolved in cottonseed oil. The lymphatic absorption of preparations A, B, C and D in rats has been reported.[26]

Procedure for Dissolution Study——The paddle method and rotatory basket method in JP X were used. The dissolution test media were the 2nd fluid and the 2nd fluid containing 20—127 mM bile salts. Each preparation (corresponding to 100 mg of *d*-α-tocopherol) was placed in a beaker containing the dissolution test medium. The dissolution test was carried out at 100 or 200 rpm using 1000 ml or 250 ml of dissolution test medium equilibrated to 37 °C. A 5 ml aliquot of the sample solution was taken and passed through a Millipore® filter (0.22 μm) at appropriate intervals.

Results and Discussion

Solubility of *d*-α-Tocopherol in Bile Salts Solutions

Table I shows the effect of bile salts on the solubility of *d*-α-tocopherol. The solubility was above 1.0 mg/ml in 20 mM GCDC-Na, GDC-Na, TCDC-Na and TDC-Na, while in 80 mM GCDC-Na, GDC-Na and TDC-Na, solubility above 10 mg/ml was observed. These results indicate that the addition of bile salts to the 2nd fluid dose increase the solubility of *d*-α-tocopherol, and that GCDC-Na, GDC-Na or TDC-Na would be suitable as the solubilizer for the dissolution test medium. From the above results, and because the concentration of bile salts in the gastro-intestinal tract was reported to be about 20 mM,[27] the 20 mM bile salt

TABLE I. Solubility of *d*-α-Tocopherol in Bile Salts Solutions

Bile salt	Concentration (mM)	Solubility (mg/ml)
GCDC-Na	20	1.33
	40	5.76
	80	14.31
GDC-Na	20	3.43
	40	9.59
	80	19.64
TCDC-Na	20	1.95
	40	3.89
	80	9.91
TC-Na	20	0.14
	40	0.84
	80	4.12
GC-Na	20	0.57
	40	2.98
	80	3.92
TDC-Na	20	2.57
	40	5.88
	80	14.57

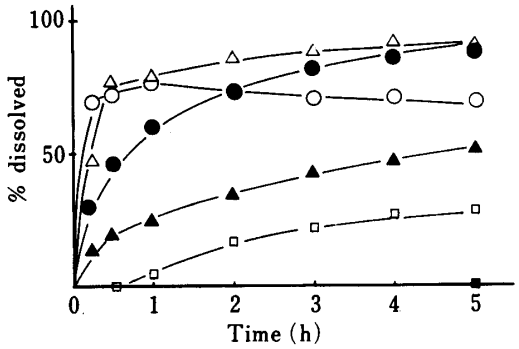

Fig. 1. Dissolution Behavior of *d*-α-Tocopherol from Tablets in the 2nd Fluid with or without 20 mM Bile Salts

The dissolution was measured in 1000 ml of each dissolution medium at 100 rpm.
■, the 2nd fluid; □, TC-Na; ▲, TCDC-Na; ○, GDC-Na; ●, GCDC-Na; △, TDC-Na.

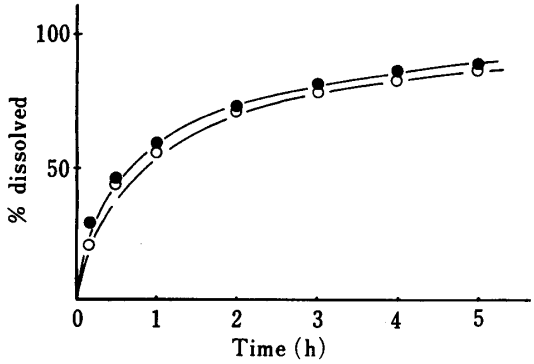

Fig. 2. Dissolution Behavior of *d*-α-Tocopherol from Tablets in the Paddle Method or Rotatory Basket Method at 100 rpm in 1000 ml of the 2nd Fluid with 20 mM GCDC-Na

●, paddle method; ○, rotatory basket method.

solution was selected as the dissolution test medium.

Effect of Bile Salts on the Dissolution of *d*-α-Tocopherol from Tablets

Figure 2 shows the results of dissolution tests using the 2nd fluid of JP X and 20 mM solutions of 5 different bile salts, TDC-Na, GCDC-Na, GDC-Na, TCDC-Na and TC-Na, as dissolution test media. No dissolution of *d*-α-tocopherol was observed in the 2nd fluid. Especially good dissolution was observed with TDC-Na, GCDC-Na and GDC-Na, and this is consistent with the results of the solubility study.

Bile salts are usually expensive and difficult to obtain in high purity. However, GCDC-Na can be synthesized relatively easily and obtained in high purity. In addition, glycochenodeoxycholic acid has been reported to be one of the major bile acids in human.[28] Therefore, GCDC-Na was selected as a solubilizer to be added to the dissolution test medium.

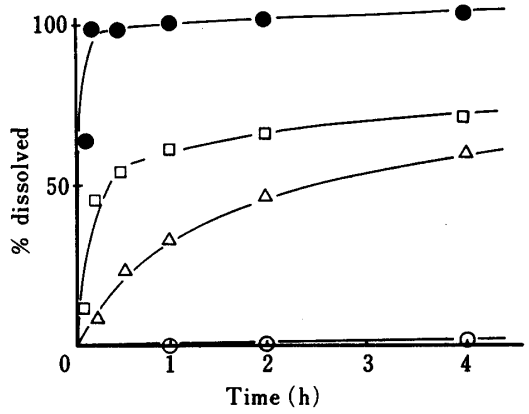

Fig. 3. Dissolution Behavior of *d*-α-Tocopherol from Four Different Preparations, A, B, C and D, in 1000 ml of the 2nd Fluid with 20 mM GCDC-Na

The dissolution was measured by the paddle method at 100 rpm.
●, preparation A; □, preparation B; △, preparation C; ○, preparation D.

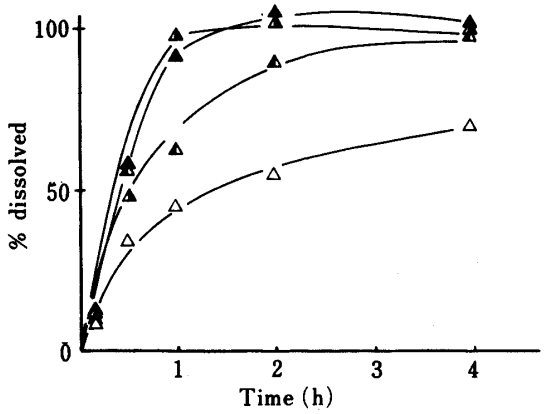

Fig. 4. Effect of GCDC-Na Concentration on the Dissolution Behavior of *d*-α-Tocopherol from Preparation C

The dissolution was measured by the paddle method at 100 rpm in 250 ml of dissolution medium.
△, 20 mM; ▲, 40 mM; ▲, 80 mM; ▲, 127 mM.

Comparison of the Paddle Method and Rotatory Basket Method

Figure 3 shows the results of dissolution tests using the paddle method and rotatory basket method described in JP X. No marked difference of dissolution patterns between the two methods was observed, and the reproducibility was good. In the following experiments, the paddle method was used because it seemed more convenient in terms of handling and observation of the sample preparations.

Comparative Study *in Vitro* and *in Vivo*

The conditions of the new dissolution test method were determined as described above, and this dissolution test was applied to four preparations whose lymphatic absorption properties were known.[26] The results are shown in Fig. 3. The values of percent transferred in lymphatic absorption of *d*-α-tocopherol after oral administration of preparations A, B, C and D in rat were reported to be 52.0, 44.6, 33.7 and 34.2%, respectively.[26] It was found from a comparison of the limphatic absorption and dissolution test data that the order of the dissolution rates of preparations A, B and C agreed with the order of the percent transferred. This indicates that the new method using GCDC-Na solution can be utilized for the dissolution testing of oily drug preparations.

However, in preparation D, the percent transferred in lymphatic absorption was comparable to that of preparation C in spite of the very low dissolution rate compared with preparation C. The reason for this difference appeared to be that the drug and the plant oil used as the vehicle of preparation D floated on the surface of the dissolution test medium after the disintegration of the capsule.

For the dissolution of *d*-α-tocopherol from preparation D, the effects of GCDC-Na and the paddle rotation speed were examined.

Figures 4 and 5 show the effect of the concentration of GCDC-Na on the dissolution from preparations C and D, which gave the same *in vivo* absorption. The increase of GCDC-Na from 20 mM to 127 mM enhanced the dissolution rate from preparation D. However, the same phenomenon was observed with preparation C, and the difference in dissolution rate between preparations C and D was not reduced.

Figure 6 shows the effect of the paddle rotation speed on the dissolution rate from

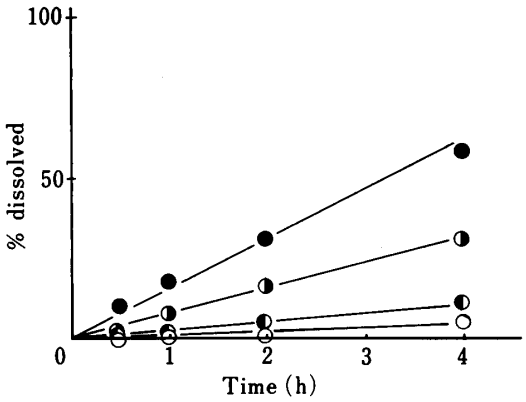

Fig. 5. Effect of GCDC-Na Concentration on the Dissolution Behavior of *d*-α-Tocopherol from Preparation D

The dissolution was measured by the paddle method at 100 rpm in 250 ml of the dissolution medium. ○, 20 mM; ◐, 40 mM; ◑, 80 mM; ●, 127 mM.

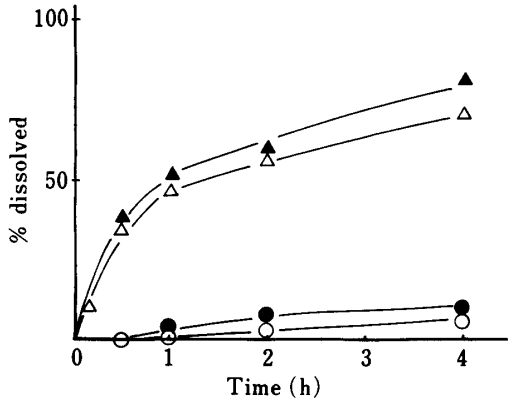

Fig. 6. Dissolution Behavior of *d*-α-Tocopherol from Preparations C and D at 100 or 200 rpm

The dissolution was measured by the paddle method in 250 ml of the 2nd fluid with 20 mM GCDC-Na. △, preparation C at 100 rpm; ▲, preparation C at 200 rpm; ○, preparation D at 100 rpm; ●, preparation D at 200 rpm.

preparations C and D. It was found that the paddle rotation speed did not greatly affect the dissolution of *d*-α-tocopherol.

The results suggest that this new method of dissolution testing for oily drug preparations, using GCDC-Na solution, is useful. Further improvement (*e.g.*, of the test apparatus) will be required for application to capsules of oily drugs.

Acknowledgement The authors are very grateful to Mr. Masayoshi Onozaki and Miss Yuriko Ushiyama for their assistance in the experimental work.

References and Notes

1) A part of this work was presented at the 105th Annual Meeting of the Pharmaceutical Society of Japan, Kanazawa, April 1985.
2) B. Katchen and S. Symchowicz, *J. Pharm. Sci.*, **56**, 1108 (1967).
3) M. Gibaldi and S. Feldman, *J. Pharm. Sci.*, **56**, 1238 (1967).
4) H. Weintraub and M. Gibaldi, *J. Pharm. Sci.*, **58**, 1368 (1969).
5) A. T. Florence, *J. Pharm. Pharmacol.*, **22**, 256 (1970).
6) H. Schott, L. C. Kwan and S. Feldman, *J. Pharm. Sci.*, **71**, 1038 (1982).
7) M. Nagata, K. Matsuba, S. Hasegawa, T. Okuda, M. Harada, K. Kubo, M. Ishijima and J. Watanabe, *Yakugaku Zasshi*, **99**, 965 (1979).
8) M. Takasugi, F. Shono, S. Kyoya, K. Mizuguchi and K. Miyata, *Yakubutsu Ryoho*, **12**, 1433 (1979).
9) S. Nishii, K. Nishikawa, H. Kishi, N. Onoshi and E. Hiraoka, *Byoin Yakugaku*, **7**, 350 (1982).
10) T. Nishiyama, K. Iwashige, H. Ina, T. Maegawa, S. Takada, T. Ishimasa and H. Shinzaki, *JJHPA*, **18**, 25 (1982).
11) S. Nishii, M. Wada, E. Maruta, H. Kishi, E. Hiraoka and T. Kishi, *Byoin Yakugaku*, **9**, 14 (1983).
12) T. Okamoto, K. Takahashi, T. Kishi, S. Nishii, E. Maruta, M. Wada, H. Kishi and E. Hiraoka, *Byoin Yakugaku*, **9**, 19 (1983).
13) T. R. Bates, S. Lin and M. Gibaldi, *J. Pharm. Sci.*, **56**, 1492 (1967).
14) S. Lin, J. Menig and L. Lachman, *J. Pharm. Sci.*, **57**, 2143 (1968).
15) T. R. Bates, M. Gibaldi and J. L. Kanig, *J. Pharm. Sci.*, **55**, 191 (1966).
16) S. Miyazaki, H. Inoue, T. Yamahira and T. Nadai, *Chem. Pharm. Bull.*, **27**, 2468 (1979).
17) S. Miyazaki, T. Yamahira, Y. Morimoto and T. Nadai, *Int. J. Pharm.*, **8**, 303 (1981).
18) M. A. Kassem, A. G. Mattha, A. E. El-Nimr and S. M. Omar, *Int. J. Pharm.*, **12**, 1 (1982).
19) A. G. Mattha, S. M. Omar and M. A. Kassem, *Int. J. Pharm.*, **11**, 27 (1982).
20) T. R. Bates, M. Gibaldi and J. L. Kanig, *Nature* (London), **210**, 1331 (1966).
21) K. Bernhard, G. Ritzel and E. Scheitlin, *Helv. Physiol. Pharmacol. Acta*, **10**, c47 (1952).

22) J. D. Greaves, *Amer. J. Physiol.*, **125**, 423 (1939).
23) J. D. Greaves and C. L. A. Schmidt, *J. Biol. Chem.*, **102**, 101 (1933).
24) A. Norman, *Arkiv För Kemi.*, **8**, 331 (1955).
25) *a*) F. Nakayama and M. Nakagaki, *J. Chromatogr.*, **183**, 287 (1980); *b*) O. W. Cass, A. E. Cowen, A. F. Hofmann and S. B. Coffin, *J. Lipid Res.*, **16**, 159 (1975).
26) Y. Tsushima, T. Tokumura, S. Yamagiwa, K. Tatsuishi, M. Kayano, Y. Machida and T. Nagai, Abstracts of Papers, the 6th Symposium on Development and Evaluation of Pharmaceutical Preparations, Tokyo, October 1985, pp. 105—108.
27) *a*) T. Tebagchali, J. Hatzioannou and C. C. Booth, *Lancet*, **II**, 12 (1968); *b*) J. Sjovall, *Acta Physiol. Scand.*, **46**, 339 (1959).
28) J. Sjovall, *Clin. Chim. Acta*, **5**, 33 (1960).