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Mutual effect of egg albumin and fatty acids on bioavailability of dl-α-tocopherol

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Abstract

We studied the dissolution behavior and oral absorption of dl-α-tocopherol (VE) from solid dispersions of egg albumin in the presence or absence of a series of saturated fatty acids. The solubility diagram of VE–egg albumin was of the Bs-type. The solubility of VE was increased by 300-fold in the presence of egg albumin. The egg albumin complex of the drug was obtained in a molar ratio of 20:1 (VE:egg albumin) from the aqueous solubility diagram. The apparent dissolution rate of VE from solid dispersion with egg albumin was markedly enhanced in comparison with VE alone. Addition of various fatty acids to VE–egg albumin solid dispersions had different effects on the dissolution of VE due to mutual effect of fatty acid and egg albumin. Myristic acid was found to improve the dissolution of VE maximally, while capric acid decreased the solubility of VE obtained from egg albumin solid dispersion. The mean serum levels of VE following oral administration of egg albumin solid dispersions, especially egg albumin solid dispersion containing myristic acid, were significantly higher than those of the drug alone. No significant differences were found between the mean residence times of drug and its solid dispersions. In addition, degradation of VE was inhibited by dispersion in egg albumin. © 1997 Elsevier Science B.V.

Keywords: dl-α-Tocopherol; Egg albumin; Fatty acid; Dissolution; Bioavailability; Stability

1. Introduction

dl-α-Tocopherol (VE) is a poorly soluble antioxidant vitamin. The drug is oxidized on exposure to air and light, darkening in color. It has been previously reported that VE following oral administration is poorly absorbed into the body and fails to reach sufficient concentrations at the site of action (Gallo-Torres, 1980; Bjørneboe et al., 1986; Knight and Roberts, 1985). There have been numerous studies of solubilization of VE using bile salts, surfactants and other chemicals (Imai et al., 1983; Tsushima et al., 1986). However, results from other studies showed that VE

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formulations are toxic to the hepatic, renal and hematopoietic systems because of additives such as emulsifiers, detergents and organic solvents used to solubilize the vitamin for administration (Phelps, 1984; Mintz-Hitter, 1989).

Various strategies have been used to formulate drugs with limited aqueous solubility/dissolution rates; e.g. particle size reduction, use of solubilizing or wetting agents, use of alternative crystal forms, solid dispersion and cosolvate formation. We have been investigating the possible use of natural polymers such as water-soluble gelatin, casein hydrolysate, chitosan, alginates and egg albumin as solubilizing/wetting agents to improve the absorption of poorly water-soluble drugs (Kimura et al., 1991; Imai et al., 1991a,b). Recently, increasing attention has been focused on these agents because of their inert nature and relative safety for clinical use. Some of these natural carriers in addition to improving the bioavailability of drugs, provide other advantages such as masking the taste and lowering toxicity of drugs (Kimura et al., 1992; Shiraishi et al., 1991). Use of these carriers in combination with other absorption promoters such as fatty acid has the potential to improve the absorption of poorly soluble drugs further by mechanisms different from only improving solubility and may also contribute to other advantages of the formulation.

In this investigation, VE was formulated with albumin in the presence or absence of fatty acids as solid dispersions and their effects on the oral bioavailability and stability of the drug were studied.

2. Materials and methods

2.1. Materials

The VE and egg albumin (MW 45 000) were purchased from Nacalai Chemical (Kyoto, Japan). Fatty acids were supplied by Tokyo Kasei Kogyo (Tokyo, Japan). All other reagent were of analytical grade. Deionized double distilled water was used throughout the study.

2.2. Methods

2.2.1. Solubility studies

Aliquots of 2 mg of VE, in its ether solution (40 mg/ml), were added to a series of tubes and dried under nitrogen. Egg albumin (0–1.0, w/w %) in aqueous solution was added to the tubes containing VE and vigorously shaken at 20°C for 3 h to prevent decomposition. The suspensions were centrifuged at 20°C, and 0.5 ml of the supernatant (clear solution) after filtering through a 0.45 μm membrane filter was added to 0.5 ml of methanol and extracted with 5 ml of hexane. The fluorescence intensity of the organic phase was analyzed spectrophotometrically with excitation at 295 nm and emission at 330 nm. For the determination of intrinsic solubility of VE 4 ml supernatant was evaporated and reconstituted in 100 μl of hexane from which aliquot of 20 μl was injected into HPLC. The HPLC system consisted of: Hitachi 655-11 pump; Hitachi F1000 fluorescence detector; column; LiChrosorb Si60 (7 μm, 250X4i.d. mm); eluant, hexane:isopropanol (99.6:0.4 v/v %); flow rate,1.5 ml/min, detection, excitation 290 nm, emission 330 nm. The detection limit of VE was 5×10^{-8} M and the coefficient of variation was 5.1% at 1×10^{-7} M of VE.

The stability constants of complexes were calculated by the Langmuir Eq. (1).

$$\frac{n(n-1)\cdots(n-i+1)K_c^i}{i!} = \frac{[\text{egg albumin} - \text{VE}_i]}{[\text{egg albumin}][\text{VE}]^i} = \frac{([\text{VE}]_t - [\text{VE}]_0/i)}{\{[\text{egg albumin}]_t - ([\text{VE}]_t - [\text{VE}]_0)/i\}\{[\text{VE}]_t - ([\text{VE}]_t - [\text{VE}]_0)\}^i} = \frac{\text{slope}}{(i - \text{slope})[\text{VE}]_0^i} \quad (1)$$

where, K_c is the stability constant, $[\text{egg albumin}]_t$ and $[\text{VE}]_t$ are total concentrations of egg albumin and VE, respectively, $[\text{VE}]_0$ is aqueous solubility of VE, and i is the number of molecules adsorbed to egg albumin. $([\text{VE}]_t - [\text{VE}]_0)/[\text{egg albumin}]_t$ is a slope of initial rising portion.

2.2.2. Preparation of samples

VE–egg albumin (1:3–1:10, w/w) solid dispersions were prepared by the kneading method. The required amounts of drug and egg albumin were weighed and placed in a mortar, then the mixture was kneaded with 1.2 volumes of water for 45 min by hand. Samples were dried under reduced pressure for 3 days at room temperature and screened through a 100 mesh sieve. VE–egg albumin: fatty acid (1:5:0.5, w/w/w) solid dispersions were also prepared by the same method.

2.2.3. Dissolution studies

The dissolution rates of the drug from solid dispersion were determined according to the dispersed amount method (Nogami et al., 1969). A 20 mg equivalent amount of VE sample (<100 mesh) was placed into 250 ml of water kept at 37°C and stirred at 240 rpm. At appropriate intervals, 0.5 ml of solution was withdrawn and filtered through a 0.45 μm membrane filter followed by VE assay as described for the solubility studies. The release of albumin in the medium was measured by Lowry method (Lowry et al., 1951), and fatty acid was determined by GLC. For VE, 0.5 ml of the dissolved oil in ether (40 mg/ml) was placed in a jacket beaker and after drying ether dissolution medium was added.

2.2.4. GLC analysis of fatty acid

Samples of 0.5 ml after mixing with equal volume of 1N HCl and internal standard was extracted with 5 ml of hexane. The organic phase was evaporated and the fatty acid residue was methylated with phenyl trimethyl ammonium hydroxide (PTAH) prior to gas chromatography. The internal standards used were: lauric acid (2×10^{-4} M) for capric acid, palmitic acid (5×10^{-5} M) for myristic acid, and palmitic acid (1×10^{-5} M) for stearic acid. The GLC conditions were as follows: column; silicone OV-17 supported by 3% Chromosorb WAW OMCS 60/80, i.d. 3.4 mm × 2 m; carrier gas, nitrogen at a flow rate of 50 ml/min; injection temperature, 135°C for capric acid, 200°C for myristic acid and 220°C for stearic acid; detection temperature, 220°C (FID).

2.2.5. In vivo absorption studies

Four beagle dogs (10 to 12 kg, 1 to 2 years old) were used at intervals of more than 10 days. The animals were on a liquid diet (Besvion; Snow Brand Milk Product, Tokyo, Japan) for 48 h followed by fasting for 24 h prior to drug administration. One hundred mg of VE or solid dispersion (<100 mesh) containing equivalent amount of VE in hard gelatin capsules (size 00) was administered to each dog in cross-over manner. Blood was sampled from the front leg until 36 h including a liquid diet break just after 24 h. The serum was obtained from blood by centrifugation at 3000 rpm for 20 min.

2.2.6. HPLC analysis of VE

To aliquots of 0.5 ml of serum, 1 ml of d-β-tocopherol in methanol (5 μg/ml) was added as an internal standard followed by vortexing. The mixture was then centrifuged at 3000 rpm for 5 min to separate the deproteinized sample. One ml of clear solution was then extracted with 5 ml of hexane for 10 min followed by evaporation of 4 ml supernatant. The residue was reconstituted in 500 μl of hexane from which aliquots of 5–20 μl were injected into HPLC. The HPLC condition was described in solubility studies. The detection limit of VE was 200 ng/ml in plasma and the coefficient of variation was 3.5% at 1 μg/ml of VE.

All the above experiments were performed in a dark room.

2.2.7. Heat stability analysis

To clarify the effects of egg albumin on the stability of VE in the presence of light and air, VE and its solid dispersions with albumin (1:5, w/w), and with albumin and fatty acid (1:5:0.5, w/w/w), each containing the same amount of drug, were stored at 90°C. At appropriate time intervals, samples were dispersed in methanol by ultrasonication and measured by HPLC after extraction in hexane following the same procedure described above.

2.3. Statistical analysis

All data are presented as means ± SE. The statistical analysis was carried out according to a

one-way analysis of variance (ANOVA), followed by the multiple range test (Duncan's method). Differences were considered to be statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Solubility study

The phase solubility diagram obtained for VE with egg albumin in water at 37°C is shown in Fig. 1. The solubility plot shows a Bs-type curve. The initial rising portion was followed by a plateau region and finally a decrease in total concentration of VE with precipitation at high albumin concentration. The solubility of VE in the plateau was increased by about 300-fold as compared to the intrinsic solubility of VE (9.2×10^{-8} M). The stoichiometry of solid complex was analyzed by the plateau region of solubility diagrams and the chemical analysis of VE for precipitation of complex at the higher concentration of egg albumin. From these results, VE formed a complex with egg albumin at a molar ratio of 20:1. The stability constant (K_c) calculated from the initial linear portion according to the Langmuir equation (Eq. (1)) was 8.7×10^6 M^{-1} based on a 20:1 molar ratio. Our previous study showed that albumin interacts more strongly with acidic than basic drugs through hydrophobic and electrostatic interactions. Acidic drugs are dispersed monomolecularly in the matrix of albumin, while basic drugs are dispersed as separated crystals (Imai et al., 1989). However, the mechanism of interaction of VE, a oily compound, with egg albumin was not clarified in detail here. VE–egg albumin solid dispersion at 1:5 weight ratio corresponding to the same molar ratio (20:1) at which solid complex was formed was prepared by the kneading method.

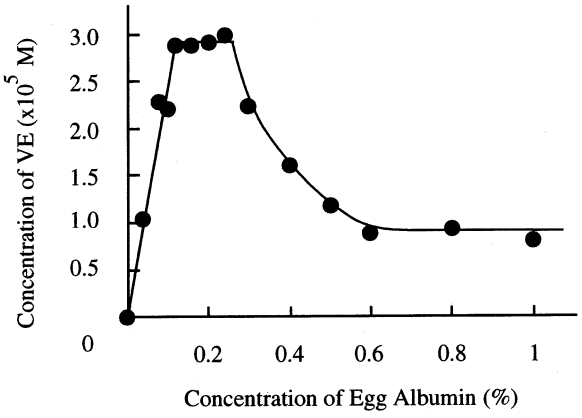

Fig. 1. Phase solubility diagram of VE–egg albumin system in water at 20°C.

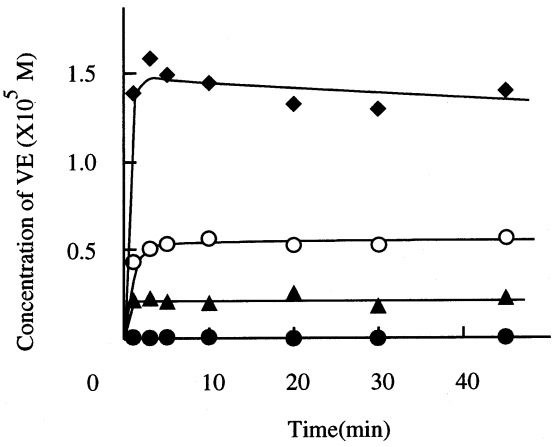

Fig. 2. Dissolution profiles of VE from VE–egg albumin solid dispersions in water at 37°C: ●, VE alone; ▲, VE–egg albumin (1:3); ○, VE–egg albumin (1:5); ◆, VE–egg albumin (1:10).

3.2. Dissolution behavior

Fig. 2 shows the dissolution behavior of VE and its solid dispersion with egg albumin at different stoichiometry in water at 37°C. The dissolution of VE was increased with increases in albumin content compared to the drug alone. The enhanced dissolution rate of VE may have been due to the increased solubility and wide dispersion of VE through complexation with egg albumin. Improvement of wettability of the drug could also be responsible for the observed enhanced dissolution. Previously, the dissolution rates of other slightly soluble drugs were shown to be improved by similar mechanisms from solid dispersions with egg albumin (Imai et al., 1989, 1991b).

The dissolution of VE was further studied by adding saturated fatty acids, capric (C_{10}), lauric

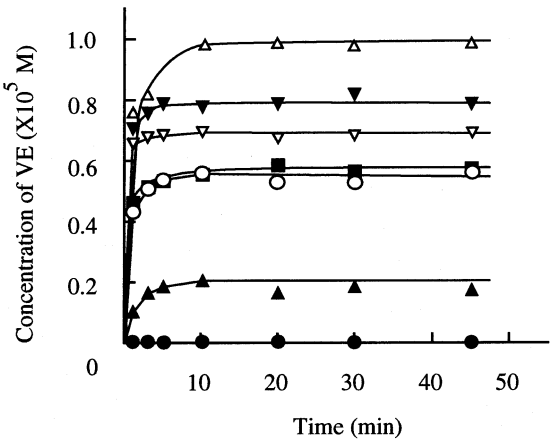

Fig. 3. Dissolution profiles of VE from VE–egg albumin–fatty acid (1:5:0.5) solid dispersions in water at 37°C: ●, VE alone; ○, without fatty acid; ▲, C_{10}; ▽, C_{12}; △, C_{14}; ▼, C_{16}; ■, C_{18}.

(C_{12}), myristic (C_{14}), palmitic (C_{16}), and stearic (C_{18}) acids, to VE–egg albumin solid dispersions (1:5, w/w) to increase absorption of VE. With the exception of C_{10} and C_{18}, the fatty acids further improved the dissolution of VE in the VE–egg albumin solid dispersion relative to that obtained from the VE–egg albumin solid dispersion (Fig. 3), although VE–fatty acid kneaded mixtures showed no enhancement of dissolution of VE (data not shown). The effects of fatty acids on the dissolution of VE were also found to vary depending on the length of the carbon chain. The relationship between carbon chain and enhancing effect of fatty acid was parabolic with maximum effect at C_{14}.

To clarify the roles of individual fatty acids on dissolution, each component of solid dispersions with C_{10}, C_{14} and C_{18} was measured in the dissolution medium, and the results are presented in Table 1. Fatty acids were dissolved in the order of their own solubility ($C_{10} > C_{14} > C_{18}$). C_{10} was easily dissolved in the medium because of its high solubility in water, and the dissolved concentration was comparable to 100% dissolution of C_{10} from solid dispersion. However, C_{18} which has very low solubility compared to the other fatty acids examined was dissolved only slightly in the medium. On the other hand, the dissolution of egg albumin which showed 100% dissolution in the VE–egg albumin solid dispersions decreased to 69% by addition of C_{10}. Although the effects of fatty acids on the dissolution of albumin could not be explained, C_{10}, a feature of short chain fatty acids, might have changed the conformation of albumin during the preparation of solid dispersions, to make it less dispersable/soluble. Thus, the dissolutions of VE, fatty acids and egg albumin from the solid dispersion were independent from one another. Long chain fatty acids such as C_{14} form micelles in solution and thereby entrap drugs to form mixed micelles which facilitate dissolution. The surface tensions of dissolution medium were 47.3, 58.0, 37.9 and 49.0 dynes/cm for VE–egg albumin solid dispersion without fatty acid and with C_{10}, C_{14} and C_{18}, respectively. Thus, the addition of C_{14} to egg albumin solid dispersion improved the dissolution of VE by the virtue of enhancing effects of both fatty acid and egg albumin. However, C_{10} had undesirable effects on egg albumin, which resulted in an in-

Table 1
Concentration of each component in dissolution medium after 20 min of dissolution of VE–egg albumin–fatty acid (1:5:0.5) solid dispersion

Egg albumin dispersion	Concentration of each component (mg/ml)		
	VE ($\times 10^3$)	Egg albumin ($\times 10$)	Fatty acid ($\times 10^2$)
Without fatty acid	2.4	3.9	—
With fatty acid			
C_{10}	0.9	2.7	3.9
C_{14}	4.1	3.5	1.7
C_{18}	2.5	3.8	0.14

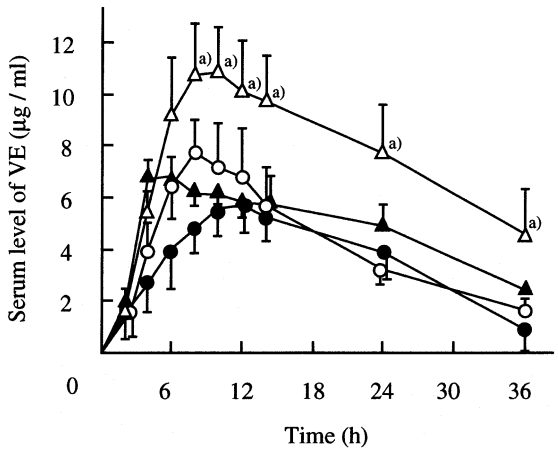

Fig. 4. Mean serum levels (± SE, $n = 4$) of VE following oral administration of VE and solid dispersions (equivalent to 100 mg VE) to beagle dogs: ●, VE alone; ○, VE–egg albumin (1:5); △, VE–egg albumin–C_{14} (1:5:0.5); ▲, VE–C_{14} (2:1).

crease in surface tension and decrease in VE release compared with VE–egg albumin solid dispersion.

3.3. In vivo absorption study

VE is generally present in blood. Before starting in vivo experiments, therefore, the physiological concentration of VE in fasted dog serum was determined as around 10.8 ± 0.2 μg/ml without any significant intra- or interday variation. This endogeneous concentration of VE was agree with the value that Tokumura et al. (1987) reported to be from 10 to 12 μg/ml in plasma of beagle dog. The serum level of VE after administration of VE preparations was subtracted from the physiological VE level. The mean serum levels of VE following oral administration of VE and solid dispersions are shown in Fig. 4, and pharmacokinetic parameters are given in Table 2. VE itself shows limited absorption and delayed t_{max} because of its lymphatic absorption characteristics as an oil. The maximum serum levels (C_{max}) attained with the different preparations were in the order VE < VE–C_{14} mixture = VE–egg albumin < VE–egg albumin–C_{14} solid dispersion. The area under the curve (AUC) of the VE–egg albumin–C_{14} solid dispersion was significantly more than double that obtained with VE alone. The bioavailability of VE from the other preparations was increased but not significant for VE alone. The mean residence time (MRT) values were similar for all the preparations.

Despite the lack of effect of in vitro dissolution of VE from the VE–C_{14} kneaded mixture (1:0.5, w/w), the serum concentration of VE from VE–C_{14} kneaded mixture was increased during 4 to 8 h post dosing in comparison with those of VE alone. These findings suggested the absorption-promoting effect of the fatty acid in vivo. Fatty acid is known to increase the membrane permeability on the intestinal epithelium. The permeation-enhancing effects of fatty acids on intestinal absorption are maximal with C_{10}, which effect can be explained by opening of tight junctions on epithelial membranes caused by the increase of intracellular calcium concentration (Tomita et al., 1995; Lindmark et al., 1995) and enhancing permeability on lipid layer. Our findings indicated that C_{14} has an absorption enhancing effect which might be due not to promotion of epithelial membrane permeability but to enhancement of the solubility of VE. Generally, VE is markdely absorbed after meal in comparison with fasted condition, reflecting the mixed micell formation of VE with various substance in the GI tract such as fat and bile acids. The enhanced bioavailability of VE may be explained by the dissolution of VE and the ability to form mixed micell of VE by combining effects of egg albumin and C_{14}. It was clear that VE, fatty acid and egg albumin were separately dissolved from the solid dispersion in the in vitro dissolution study, although VE strongly interacted with egg albumin (stability constant: 8.7×10^6 M^{-1}). Furthermore, it was reported by Erin et al. (1984) that VE forms complexes with saturated fatty acids from C_{10} to C_{24} with stability constants of around 45 M^{-1}. In GI tract, the dissolved VE in the solid dispersion might be absorbed after formation of mixed micell which might be promoted by C_{14}.

3.4. Degradation behavior

We also evaluated other aspects of VE formulations in solid dispersions. Fig. 5 shows that egg albumin protected VE against oxidation with almost no degradation within 12 h at 90°C, whereas

Table 2
Bioavailability parameters following oral administration of VE and egg albumin dispersions to beagle dogs

System	C_{max} (μg/ml)	t_{max} (h)	$AUC_{0-36\,h}$ (h μg/ml)	MRT (h)
VE alone	6.3 ± 1.7	9.5 ± 2.2	126.5 ± 23.0	15.8 ± 0.7
VE–egg albumin (1:5)	8.2 ± 2.4	8.5 ± 1.9	145.8 ± 24.8	15.3 ± 0.8
VE–egg albumin:C_{14} (1:5:0.5)	11.2 ± 3.1[a]	8.5 ± 1.6	266.8 ± 40.6[a]	17.4 ± 0.2
VE–C_{14} (2:1) mixture	7.7 ± 0.6	4.7 ± 0.9[a]	172.1 ± 14.9	16.5 ± 0.6

[a] $P < 0.05$ versus VE alone.

50% of the drug without albumin was lost by that time under normal storage conditions. Egg albumin probably shielded VE from air and light and thus had a stabilizing effect. Incorporation of fatty acids in the formulation of egg albumin did not have any affect on VE stability.

In conclusion, combinations of two or more natural components with different dissolution/absorption enhancing effects may help in formulating otherwise poorly bioavailable compounds such as VE to improve delivery to the body.

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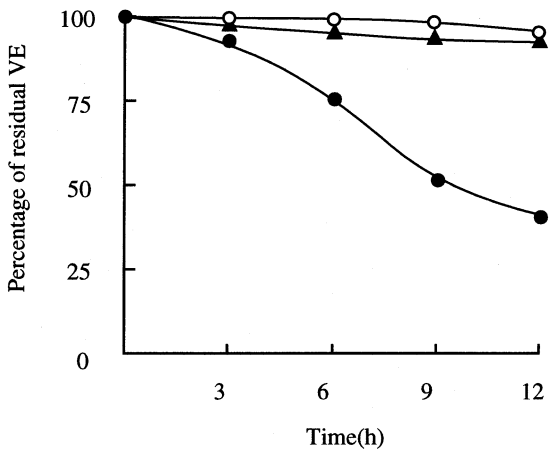

Fig. 5. Time courses of degradation of VE and solid dispersions at 90°C: ●, VE alone; ○, VE–egg albumin (1:5); ▲, VE–egg albumin–C_{14} (1:5:0.5).

References

Bjørneboe, A., Bjørneboe, G.E., Bodd, E., Hagen, B.F., Kveseth, N., Drevon, C.A., 1986. Transport and distribution of alpha-tocopherol in lymph, serum and liver cells in rats. Biochim. Biophys. Acta 889, 310–315.

Erin, A.N., Spirin, M.M., Tabidze, L.V., Kagan, V.E., 1984. Formation of α-tocopherol complexes with fatty acids: A hypothetical mechanism of stabilization of biomembranes by vitamin E. Biochim. Biophys. Acta 774, 96–102.

Gallo-Torres, H.E., 1980. Transport and metabolism. In: Machlin, L.J. (Ed.) Vitamine E: A Comprehensive Treatise. Marcel Dekker, New York, pp. 193-267.

Imai, J., Hayashi, M., Awazu, S., Hanano, M., 1983. Solubility of dl-α-tocopherol by bile salts, polysorbate 80 and egg lecitin. Chem. Pharm. Bull. 31, 4077–4082.

Imai, T., Saito, Y., Matsumoto, H., Satoh, T., Otagiri, M., 1989. Influence of egg albumin on dissolution of several drugs. Int. J. Pharm. 53, 7–12.

Imai, T., Shiraishi, S., Saito, H., Otagiri, M., 1991a. Interaction of indomethacin with low molecular weight chitosan, and improvements of some pharmaceutical properties. Int. J. Pharm. 67, 11–20.

Imai, T., Nodomi, K., Acarturk, F., Otagiri, M., 1991b. Enhancement of dissolution and absorption of mefenamic acid by agg albumin. J. Pharm. Sci. 80, 484–487.

Kimura, K., Imai, T., 1991. Otagiri, M. Effect of low molecular casein and gelatin on absorption of ibuprofen after oral and rectal administration. Pharm. Acta Helv. 66, 120–124.

Kimura, S., Imai, T., Ueno, M., Otagiri, M., 1992. Pharmaceutical evaluation of ibuprofen fast-absorbed syrup containing low molecular weight gelatin. J. Pharm. Sci. 81, 141–144.

Knight, M.E., Roberts, R.J., 1985. Tissue vitamine E levels in newborn rabbits after pharmacologic dosing: influence of dose, dosage form and route of administration. Dev. Pharmacol. Ther. 8, 96–106.

Lindmark, T., Nikkila, T., Artersson, P., 1995. Mechanism of absorption enhancement by medium chain fatty acids in intestinal epithelial Caco-2 cell monolayers. J. Pharmacol. Exp. Ther. 275, 958–964.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mintz-Hitter, H., Vitamine, E., 1989. Biochemistry and health implications. Ann. NY Acad. Sci. 570, 205–207.

Nogami, H., Nagai, T., Yotsuyanagi, T., 1969. Dissolution phenomena of organic medicines involving simultaneous phase changes. Chem. Pharm. Bull. 17, 499–509.

Phelps, D.L., 1984. E-ferol: what happened and what now?. Pediatrics 74, 1114–1116.

Shiraishi, S., Imai, T., Iwaoka, D., Otagiri, M., 1991. Improvement of absorption rate of indomethacin and reduction of stomach irritation by alginate dispersions. J. Pharm. Pharmacol. 43, 615–620.

Tsushima, M., Yamagiwa, S., Tatsuishi, K., Kayano, M., Tokumura, T., Machida, Y., Nagai, T., 1986. A new method for dissolution testing of vitamin E preparations in test medium containing sodium glycochenodeoxycholate. Chem. Pharm. Bull. 34, 2196–2201.

Tokumura, T., Machida, Y., Tsushima, Y., Kayano, M., Nagai, T., 1987. Intestinal absorption of tocopherol in beagle dog and effect of dosage form. Chem. Pharm. Bull. 35, 4592–4596.

Tomita, M., Hayashi, M., Awazu, S., 1995. Absorption-enhancing mechanism of sodium caprate and decanoylcarnitine in Caco-2 cells. J. Pharmacol. Exp. Ther. 272, 739–743.