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Studies using a non-ionic surfactant-containing drug delivery system designed for hard gelatin capsule compatibility

S.K. Cole [a], M.J. Story [a], D. Attwood [b], T. Laudanski [c], J. Robertson [a,1] and S.G. Barnwell [a]

[a] *Cortecs Research & Development Division, Techbase 1, Newtech Square, Deeside Industrial Park, Deeside CH5 2NT, Clwyd (UK),* [b] *University of Manchester, Pharmacy Dept, Manchester M13 9PL (UK) and* [c] *Institute of Obstetrics and Gynecology, Medical Academy, Bialystok (Poland)*

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Summary

A drug delivery system has been designed which uses non-ionic surfactants to enhance the dissolution and absorption of poorly soluble lipophilic drugs, vitamins and fat-soluble nutrients. Vitamin E absorption using this system, was shown to be independent of food intake in a healthy human volunteer study. Stability studies using formulations containing vitamin E or vitamin E and β-carotene indicated that the incompatibility normally encountered when using hard gelatin capsules with non-ionic surfactants may be overcome by including small amounts of glycerol and water

Introduction

Hard gelatin capsules are being increasingly used for the encapsulation of oily liquid formulations such as garlic oil, fish oil, vitamin E and Evening Primrose Oil. The preferred use of hard gelatin capsules for this purpose rather than the more commonly used soft gelatin capsules, probably results from their reliable and cost-effective manufacture, barrier properties for products with strong odours, shorter disintegration time and reduced risk of microbial contamination (Cadé et al., 1986a, b; Ridgway et al., 1987).

Surfactants are widely used as excipients in pharmaceutical formulations and as food additives and are known to affect absorption from the gastrointestinal tract (Gibaldi and Feldman, 1970; Kaneda et al., 1974; Samaha and Gadalia, 1987; Bermejo et al., 1991; White et al., 1991). In vivo, the surfactant properties of bile acids are required for the absorption of fat soluble vitamins such as vitamin E (MacMahon and Thomson, 1970). In disorders which result from bile acid deficiency such as cystic fibrosis (Harries and Muller, 1971) non-ionic surfactants have been used to compensate for the action of bile acids and reverse the depletion of essential fat soluble nutrients from the body (King et al., 1979).

Correspondence to: S.G. Barnwell, Cortecs Ltd, Research & Development Division, Techbase 1, Newtech Square, Deeside Industrial Park, Deeside, Clwyd CH5 2NT, U K

[1] *Present address* Convatec, Techbase 3, Newtech Square, Deeside Industrial Park, Deeside CH5 2NT, Clwyd, U K

Previous studies (Cole, 1990) have shown that liquid-filled hard gelatin capsules containing formulations with high concentrations of non-ionic surfactants are subject to problems arising from the embrittlement of the capsule shell and subsequent leakage of the contents. This phenomenon is probably caused by the removal of water from the protein structure of the capsule with resulting loss of mechanical strength. A means of overcoming hard gelatin capsule embrittlement has been developed (Story, 1990). The present study describes the application of this technology to a drug delivery system designed to enhance the bioavailability of lipophilic drugs, exemplified by d-α-tocopherol, d-α-tocopheryl acetate (vitamin E) and β-carotene (pro-vitamin A), and includes an assessment of in vitro dissolution characteristics, chemical and physical stability together with clinical performance in healthy human volunteers.

Materials and Methods

Materials

β-Carotene as a 30% micronised fluid suspension in vegetable oil, d-α-tocopherol and d-α-tocopheryl acetate were obtained from Roche or Henkel for use as actives in pharmaceutical formulations and from Sigma as high-purity reference standards for analytical work. Tween 80 (polysorbate 80) was sourced from ICI Speciality Chemicals. Cremophor EL (polyoxyethylene glycerol tririconoleate) and Cremophor RH40 (glycerol polyethylene glycol oxystearate) were obtained from BASF. Glycerol (B.P. Grade) was supplied by William Rawson Ltd. Hard gelatin capsules, Licaps® transparent size 1, were obtained from Capsugel (Pontypool). All materials used in the manufacture of dosage forms for stability studies and human clinical trials were of a suitable pharmaceutical or food grade. All other chemicals used were of an appropriate grade and supplied by either Sigma, BDH or Metlab (Hawarden, U.K.).

Manufacture of dosage forms

Dosage forms containing non-ionic surfactants in hard gelatin capsules were prepared with (i) d-α-tocopheryl acetate, (ii) d-α-tocopherol and (iii) β-carotene and d-α-tocopherol. Pilot scale manufacture of liquid filled hard gelatin capsules was performed by mixing the liquid excipients at 40°C for about 3 h, in the absence of light, using a suitable glass or stainless steel mixing vessel. The formulations were accurately filled into hard gelatin capsules using a positive displacement pipette and sealed using a bench scale Licaps® machine (Capsugel) with 57% (v/v) ethanol:water solution (Cadé et al., 1986a, b). Each batch contained about 500 capsules. Large scale manufacture of formulations used for stability studies and human clinical trials, was performed by Pharma-Kapsel (Germany). The manufacturing procedures were essentially similar to those used for pilot-scale except that the capsules were sealed by the technique of gelatin banding (Ridgway et al., 1987). The batch sizes produced were of approx. 6000 capsules.

Assessment of capsule content by HPLC

Determination of d-α-tocopherol or d-α-tocopheryl acetate content of encapsulated formulations For the purpose of determining uniformity of content, 10 individual capsules were initially assayed. Subsequent stability determinations were carried out using the combined contents of ten capsules. Capsule(s) were weighed, the weight of empty capsule(s) subtracted and the contents transferred to a 200 ml volumetric flask containing 100 ml of warm distilled water at 30°C, sonicated until the gelatin capsules dissolved, and diluted to volume with methanol. A 20 ml aliquot of this solution was further diluted to 100 ml with methanol and filtered through a 0.45 μm cellulose acetate filter before analysis by HPLC. Recovery of d-α-tocopheryl acetate and d-α-tocopherol from the capsules was found to be approx. 100% using this procedure.

A reverse-phase HPLC assay was developed for the determination of d-α-tocopherol and d-α-tocopheryl acetate. The method used was based on that described by Bieri et al. (1979) and was performed using a Varian® 5500 liquid chromatography system. The mobile phase used was methanol, at a flow rate of 2 ml min^{-1}, through a

150 mm × 4.6 mm (i.d.) 5 μm Spherisorb® octyldecylsilane (ODS1) column (Phase Separations Ltd, U.K.). Detection was at 285 nm and the detector range was controlled by a Varian 4270 integrator set at an attenuation of 128. Using a total injection volume of 20 μl the response to both d-α-tocopherol and d-α-tocopheryl acetate was shown to be linear between 0.63 and 1.6 mg ml^{-1} with a correlation coefficient of 0.999 for both compounds. The approximate retention times for d-α-tocopherol and d-α-tocopheryl acetate were 2 and 3 min, respectively. The reproducibility of the system was determined by six consecutive measurements of reference standards which indicated a coefficient of variance of less than 2%.

Determination of the d-α-tocopherol and β-carotene content of encapsulated formulations 10 individual capsules were initially analysed for the purpose of determining uniformity of content. Subsequent stability testing was performed using the contents of 10 capsules. The contents of the capsules were removed by needle puncture and made up to a final volume of 200 ml with chloroform. A 10 ml aliquot of the solution was removed and diluted to 100 ml with chloroform: methanol (1:1) before analysis by HPLC. Using this system the d-α-tocopherol and β-carotene were found to be 95.5 and 95.0% recoverable, respectively, with a coefficient of variance of less than 2%.

A reverse-phase HPLC method was developed based on those described by Bieri et al. (1979), Nierenburg (1985) and Miller and Yang (1985). A Varian® 5500 liquid chromatography system was used incorporating a programmable wavelength ultraviolet/visible detector. The column, mobile phase and flow rate were similar to those of the system described above, however, detection was at 285 nm for 4.5 min followed by 436 nm for a further 4.5 min. The detector sensitivity was controlled by the Varian® 4270 integrator set at an attenuation of 128. The linear response range for β-carotene was between 0.63 and 3.83 μg ml^{-1} with a correlation coefficient of 0.999. The linear response range for d-α-tocopherol in this system was between 51.8 and 207.2 μg ml^{-1} with a correlation coefficient of 0.999. The approximate retention times were 3 and 7 min for d-α-tocopherol and β-carotene respectively. The reproducibility of the system was checked as described above and found to have a coefficient of variance of less than 2%.

Determination of vitamin E in human plasma To determine the d-α-tocopherol content of plasma samples obtained from human subjects the technique described by Bieri et al. (1979) was used.

Dissolution testing

The dissolution apparatus used was a Hanson® 12 dissolution tester incorporating a rotating basket method as described in the US Pharmacopoeia (1985). Dissolution studies were carried out at 37°C in either pH 1.2, pH 5.0 or pH 7.4 buffer and samples taken at 5, 10, 20, 30 and 60 min. Each 20 ml sample was removed and passed through a 0.45 μm cellulose acetate filter. A 5 ml aliquot of this filtrate was then further diluted to 25 ml, with methanol, and analysed by the HPLC technique described above for d-α-tocopherol and d-α-tocopheryl acetate.

For analysis of the samples from the dissolution studies the detector sensitivity, controlled by the Varian® integrator, was set to an attenuation of 16. The linear response range was between 0.62 and 5.0 μg ml^{-1} with a correlation coefficient of 0.999. The standard solutions were compared to a d-α-tocopherol or d-α-tocopheryl acetate standard which was equivalent to the potency of the active component of one capsule dissolved in 750 ml.

Stability studies

To assess the effect of temperature and storage on stability, the encapsulated formulations were packaged into white, high density polyethylene containers. The capsules were stored at 4, 25, 30, 37 and 50°C. They were also stored at 30 and 37°C at a relative humidity of 75%.

At specific intervals of 1, 3, 6 and 12 months, the capsules were assessed for chemical and physical stability. The d-α-tocopheryl acetate, d-α-tocopherol or d-α-tocopherol and β-carotene contents of the capsules were determined by the HPLC methods outlined above.

Capsules were also subjected to dissolution testing and physical assessment. Physical assessment consisted of an inspection of the overall appearance of the capsules and a standard compression test, to check for capsule embrittlement. Following the compression test the capsules were spread on absorbent paper overnight to identify signs of capsule leakage.

Clinical studies

10 healthy males, within $\pm 10\%$ of ideal body weight, participated in the study. Subjects were shown to be in good health by a physical examination and a series of hospital laboratory tests. The protocol for the study was approved by independent researchers at the Academy of Medicine, Bialystok, Poland, and the subjects abstained from taking other medication for at least 2 weeks before the start of the study and until after collection of the last blood sample. Following a 12 h overnight fast, a light breakfast was allowed 3 h post-dose after which time subjects were allowed to follow their normal daily diets.

The study was of a randomised two-way crossover design with the subjects receiving either two size 1 hard gelatin capsules, containing 200 i.u. of a standard oily preparation of vitamin E, or two size '1' hard gelatin capsules containing 200 i.u. of d-α-tocopheryl acetate, the non-ionic surfactant Cremophor EL, glycerol and water. The capsules were taken with 250 ml of boiled tap water. The zero time blood samples were taken within a 5 min period preceding the administration of the capsules. Post-dose samples were taken at 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 h. Blood samples were collected in lithium heparin tubes, mixed and centrifuged at $2.0 \times 10^3 \cdot g$ min within 15 min of collection to remove erythrocytes. The resulting plasma was transferred to clean tubes and stored at -20°C until analysed. After a 1 week interval the subjects received the alternative medication and the same sampling protocol used. Extraction and detection of vitamin E in human plasma was by the method described above. No degradation of vitamin E was observed under the storage conditions used.

TABLE 1

Effect of storage conditions on the chemical and physical stability of capsules containing d-α-tocopheryl acetate

Storage conditions (°C)	Storage time (months)		
	1	3	6
4	100.3 P	94.8 P	92.8 P
25	101 7 P	99 7 P	96.8 P
37	99 1 P	102 0 P	100.3 P
37 (75) [a]	98 5 P	94 8 P	95 3 P
50	88 4 F	119.2 F	ND F

Values are expressed as % initial d-α-tocopheryl acetate content [a] % relative humidity: ND, not determined. Results of the physical assessment P, pass; F, fail

Results

Preformulation studies using d-α-tocopheryl acetate and d-α-tocopherol

Preformulation studies were conducted to select a suitable non-ionic surfactant for solubilising either d-α-tocopheryl acetate or d-α-tocopherol. Formulations were initially prepared with either d-α-tocopherol (1100 units/g) or d-α-tocopheryl acetate (1100 units/g) and the non-ionic surfactants Cremophor RH40 and Cremophor EL. Formulations containing vitamin E:surfactant ratios of 1:1, 1:2 and 1:3 (w/w) were assessed for clarity by examining 2% (w/w) solutions, in pH 7.4 USP buffer. Formulations containing Cremophor EL produced a clear solution at a vitamin E:surfactant ratio of 1:1 for d-α-tocopheryl acetate and 1:2 for d-α-tocopherol. Cremophor RH40 did not result in efficient solubilisation of either vitamin E preparation, the solutions remaining opaque for all formulations.

Hard gelatin capsules were filled with 200 I.U./capsule of d-α-tocopheryl acetate or 100 I.U./capsule d-α-tocopherol, using either 1:1 or 1:2 (w/w) vitamin E:surfactant ratios, respectively, and sealed. Within 7 days of encapsulation, the capsules were found to be brittle and subsequently split, releasing the contents. A series of studies subsequently found the incorporation of up to 5% (w/w) glycerol and 5% (w/w) of purified water into the formulation resulted in hard gelatin capsules resistant to the effects of non-ionic surfactants (Story, 1990).

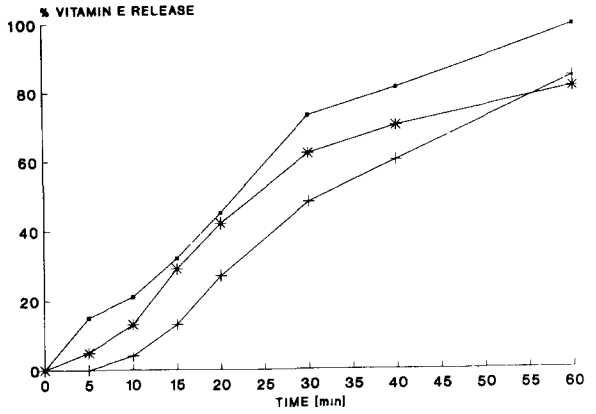

Fig. 1 Effect of pH on the dissolution of capsules containing formulations of d-α-tocopheryl acetate, Cremophor EL, glycerol and water Values correspond to pH 1 2 (■), pH 5 0 (+) and pH 7 4 (*) buffer.

Initial stability studies indicated that the formulations containing either d-α-tocopherol or d-α-tocopheryl acetate together with glycerol and water, prepared using the pilot manufacturing procedure, were stable for at least 3 months. Stability was determined by physical assessment at ambient temperatures only.

Stability and dissolution studies using capsules containing d-α-tocopheryl acetate formulations

The results from a pilot stability study using capsules containing d-α-tocopheryl acetate, Cremophor EL, glycerol and water, stored at a range of temperatures and conditions, are shown in Table 1. The results show that the vitamin E content of the capsules is stable for up to 6 months at all temperatures except 50°C. Similarly, the physical assessment of the capsules showed their condition to be satisfactory, except at 50°C where they became brittle.

Dissolution studies were also carried out on the capsules containing d-α-tocopheryl acetate, Cremophor EL, glycerol and water. The results in Fig. 1 show the effects of pH on the release of d-α-tocopheryl acetate. The rate and extent of solubilisation were found to be greater at pH1.2 than at pH 5.0 or pH 7.4, however, this was possibly due to the incomplete dissolution of the capsules at the higher pH values. These results would suggest that the content of the capsules would be efficiently solubilised in the acid environment of the stomach before reaching the higher pH encountered in the duodenum.

Stability and dissolution studies using capsules containing d-α-tocopherol formulations

For more detailed stability assessment hard gelatin capsules containing d-α-tocopherol, at a potency of 100 I.U. per capsule, Cremophor EL, glycerol and water were filled and sealed, using gelatin banding, by Pharma-Kapsel. The dissolution profile of this formulation showed a slower release rate compared to the capsules containing d-α-tocopheryl acetate (cf. Figs 1 and 2). It is still likely, however, that efficient solubilisation of the d-α-tocopherol would take place in the stomach before the formulation passed into the duodenum. Storage time did not significantly affect dissolution rate during the 12 month study period. Similar dissolution results were obtained from each batch, A and B, of the capsules used.

The stability of d-α-tocopherol in two batches, A and B, of capsules manufactured by Pharma-Kapsel is detailed in Table 2. The initial potency of 10 individual capsules from each batch, as determined by the HPLC method described earlier, indicated a mean d-α-tocopherol content of 236 ± 1.6 mg ml^{-1} for batch A and 237 ± 10.4 mg ml^{-1} (S.D.) for batch B. These initial determinations of capsule d-α-tocopherol content were

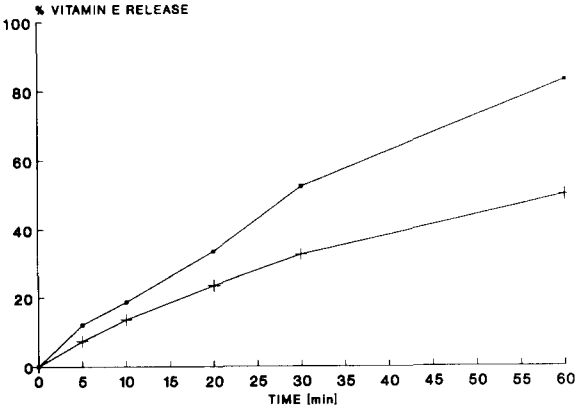

Fig 2 Effect of pH on the dissolution of capsules containing d-α-tocopherol, Cremophor EL, glycerol and water Values correspond to pH 1 2 (■) and pH 7.4 (+)

TABLE 2

Effect of storage conditions on the d-α-tocopherol content and physical stability of encapsulated formulations

Storage conditions	3 months	6 months	12 months
Batch A			
4	101 3 P	97 4 P	107 8 P
25	103 9 P	97 4 P	101 9 P
30	102 0 P	99 3 P	105 1 P
37	ND P	102 0	101 9 P
30 (75)	104.9 P	98 0 P	106.6 P
37 (75)	98.7 P	96 7 P	96 7 P
Batch B			
4	100 0 P	97 5 P	113 3 P
25	101 9 P	93.8 P	88 1 P
30	ND P	95.7 P	100 6 P
37	ND P	90.1 P	97 5 P
30 (75)	96 3 P	106 2 P	95 6 P
37 (75)	98 8 P	96 9 P	98 1 P

Values are expressed as % zero time activity, ND, not determined, P, passed physical assessment, F, failed physical assessment Storage conditions are expressed in °C and (%) relative humidity

taken as the zero time potency of 100%. Subsequent stability points were assessed by comparison to this potency. The values in Table 2 show that formulations containing d-α-tocopherol are generally stable both in terms of d-α-tocopherol content and physical stability, in the presence of non-ionic surfactant, for 12 months when stored at 30°C or below.

Formulation, dissolution and stability studies using formulations containing mixtures of d-α-tocopherol and β-carotene

Two batches of hard gelatin capsules, C and D, containing d-α-tocopherol, β-carotene, Cremophor EL, glycerol and water were filled by Pharma-Kapsel and sealed by gelatin banding. Each capsule contained 100 I.U. of d-α-tocopherol and 10 mg of β-carotene. The initial potency of 10 individual capsules from each batch was assessed by the extraction and HPLC technique described earlier which was developed for the purpose of fully resolving d-α-tocopherol and β-carotene. The initial values for batch C were β-carotene 25 ± 0.4 mg ml^{-1} and d-α-tocopherol 230 ± 1.5 mg ml^{-1} (S.D.). For batch D the initial mean potency of 10 individual capsules was β-carotene 24 ± 0.9 mg ml^{-1} and d-α-tocopherol 230 ± 13.4 mg ml^{-1} (S.D.).

The results in Table 3 show the effects of storage conditions and time on the chemical stability of β-carotene and d-α-tocopherol together

TABLE 3

Effect of storage conditions on chemical and physical stability of hard gelatin capsules containing vitamin E and β-carotene

Storage conditions (°C) (%RH)	3 months		6 months		12 months	
	β-Carotene	d-α-Tocopherol	β-Carotene	d-α-Tocopherol	β-Carotene	d-α-Tocopherol
Batch C						
4	108 0	111.5	108 0	101 3	108.0	107 1
25	96.0	105.8	108 0	98 7	86 4	101 3
30	100 0	100.6	ND	100 6	108 0	120 5
37	ND	ND	108 0	92 9	96.4	105 0
30 (75)	92 0	ND	ND	101 9	74 4	92 3
37 (75)	101 9	101.9	ND	97.4	95 8	101 7
Batch D						
4	98 1	98 1	104 2	96 2	101.3	96 8
25	ND	ND	95 8	97 5	98.8	106 4
30	100 1	104 4	ND	97 5	79.2	85.2
37	ND	ND	104 2	98.7	100.5 [a]	113.4 [a]
30 (75)	101 0	103 8	ND	97 5	77 1 [a]	89 9 [a]
37 (75)	100 0	ND	95 8	94.3	50 0 [a]	95.5 [a]

Values are expressed as % zero time for β-carotene and d-α-tocopherol activity; ND, not determined; [a] Capsules failed physical assessment

with the physical stability of the capsules containing the formulation. The results demonstrate that the formulations are stable for a 12 month period following manufacture and the capsules remained in a satisfactory condition when stored at 30°C or below. Storage at these temperatures did not adversely affect the dissolution characteristics of the capsules.

The dissolution profiles of d-α-tocopherol, at pH 1.2 and pH 7.4, typical of capsules from batches C and D are shown in Fig. 3. Studies of the formulations used for encapsulation showed that d-α-tocopherol was completely solubilised in the Cremophor EL whereas the β-carotene, although fully dispersed, was only partially solubilised. Centrifugation of capsule contents for $2.5 \times 10^3 \cdot g$ min produced a β-carotene pellet with a slightly red coloured supernatant. Dissolution studies using capsules from batches C and D, in pH 1.2 and 7.4 buffers, followed by centrifugation of the samples for $2.5 \times 10^3 \cdot g$ min and HPLC analysis, indicated that only about 2% of the β-carotene was solubilised. The results show that d-α-tocopherol is probably efficiently solubilised in the acid environment of the stomach. The effect of partial solubilisation on β-carotene absorption in vivo is difficult to predict.

Clinical studies

A clinical study was performed in healthy human volunteers comparing a formulation containing d-α-tocopheryl acetate, Cremophor EL, glycerol and water, with a standard oily vitamin E preparation. The potency, stability and dissolution characteristics of the capsules, containing d-α-tocopheryl acetate, Cremophor EL, Glycerol and water, used in this study are shown in Table 1 and Fig. 1. The concentrations of d-α-tocopherol measured in the plasma of the subjects receiving standard vitamin E capsules and the formulation containing the excipients above are shown in Table 4. The initially high levels of d-α-tocopherol present in the plasma of the subjects in the pre-dose period prevented conventional analysis of AUCs resulting from the administered formulations. The conventional formulation of vitamin E resulted in a minimal increase in plasma vitamin E levels until after the subjects received their first meal 3 h post-dose. In contrast, the subjects dosed with the formulation containing d-α-tocopheryl acetate, Cremophor EL, glycerol and water showed a sustained increase in plasma vitamin E levels from the time of dosing until the end of the sampling period.

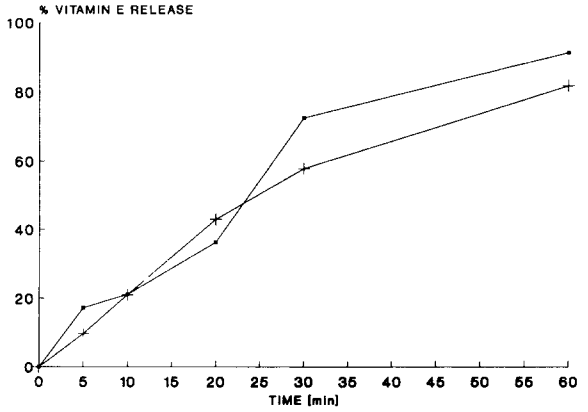

Fig 3 Effect of pH on the dissolution of d-α-tocopherol from capsules also containing β-carotene, Cremophor EL, glycerol and water Values correspond to pH 1.2 (■) and pH 7 4 (+).

TABLE 4

Plasma vitamin E concentrations in subjects receiving a conventional vitamin E formulation and a formulation containing non-ionic surfactant

Time (h)	Conventional vitamin E formulation	Non-ionic surfactant/ vitamin E formulation
0	10 5±2 7	9 9±2 1
1.0	10 6±2 4	9 5±2 6
2.0	10 3±2 5	11 8±2 1
3 0	10.3±1.9	13 6±2.7
4.0	9 5±1 6	14 3±2 9
5.0	13 7±2 1	15 6±2.8
6 0	13.3±2 0	15 5±3 2

Values are means (μg ml^{-1}) ±S E. for 10 subjects receiving each formulation. Each individual plasma concentration was in turn the mean of duplicate or triplicate determinations

Discussion

The present investigation reports pharmaceutical data concerning the physical and chemical

stability of formulations containing lipid soluble vitamins and non-ionic surfactants filled into hard gelatin capsules.

Vitamin E is probably the most well known biological antioxidant. One of the most characteristic reactions of unsaturated fatty acids exposed to oxygen is the formation of peroxides. The free radicals produced during peroxide formation probably leads to the deterioration of tissues to which they are exposed. One of the most important roles of vitamin E in vivo is believed to be the suppression of free radical formation. A number of medical conditions result from vitamin E deficiency, for example, haemorrhage resulting from platelet dysfunction in pre-term infants (Khurshid et al., 1975; Sinha et al., 1987), retrolental fibriplasia (Hittner et al., 1981), bronchopulmonary dysplasia (Ehrenkranz et al., 1978) and tardive dyskinesia (Elkashef et al., 1990). Interestingly, the incidence of tardive dyskinesia, a disabling neurological syndrome, is particularly common among subjects with impaired vitamin E absorption caused by either the deficiency of endogenous bile acids, such as in cystic fibrosis (Howard, 1990), congenital biliary atresia and primary biliary cirrhosis, or impaired lipid transport typically found in a-β-lipoproteinemia (Gassull et al., 1976).

Also of interest is the potential role of biological anti-oxidants as prophylactics, to prevent or reduce the risk of cancer. A number of studies have shown that both vitamin E and β-carotene may have some protective effects upon individuals taking supplements of these vitamins (Kvale et al., 1983; Mathews-Roth, 1983; Alam et al., 1984; Knekt et al., 1988). It may be envisaged that efficient and consistent absorption of these materials would enhance their effectiveness as prophylactic treatments (Wolf, 1984).

The dissolution studies indicate that vitamin E, in the form of d-α-tocopherol or d-α-tocopheryl acetate, is rapidly released and solubilised over a wide range of pH values characteristic of either the acid environment of the stomach or the variable but increasing pH encountered in the duodenum (see Figs 1 and 2). In the potential application of this drug delivery system to other pharmaceutically useful compounds the ability of the non-ionic surfactant to solubilise a lipophilic pharmaceutical active in a pH independent manner would be useful if its sensitivity to acid conditions required the liquid-filled hard gelatin capsule to be enteric-coated. Thus efficient solubilisation would occur whether release from the capsule was designed to take place in the stomach or the duodenum. In the case of β-carotene, only partial solubilisation occurred in the non-ionic surfactant used in the formulation, while complete solubilisation of d-α-tocopherol was maintained. Dissolution studies using this formulation (Fig. 3) indicated that the d-α-tocopherol was rapidly released and solubilised, however, only about 2% of the β-carotene was solubilised when released. The implication of this observation in vivo is difficult to predict, however, previous studies have shown that β-carotene is broken down in the gastrointestinal tract to a form which is more readily solubilised and adsorbed (Goodman et al., 1966).

A possible interpretation of the results of the dissolution studies, using either d-α-tocopherol or d-α-tocopheryl acetate as the source of vitamin E, would be that the non-ionic surfactant contained in the formulations could replace or at least supplement the solubilising properties of bile acids in vivo (Tables 1 and 2). Further evidence that the non-ionic surfactant present in the formulations could replace the function of bile acids in vivo is demonstrated by the results of the human bioavailability study (Table 4). The effect of the drug delivery system was to cause an increase in plasma vitamin E levels soon after dosing whereas the standard preparation did not increase plasma vitamin E levels until after 3 h. A likely explanation for the delayed increase in absorption observed in the case of the standard preparation is that solubilisation, and therefore absorption, would not be able to take place until bile acids were made available in vivo. Interestingly, the first meal was allowed 3 h post-dose, resulting in gall-bladder contraction and therefore the availability of bile acids to promote vitamin E absorption (MacMahon and Thomson, 1970). The effect of gall-bladder contraction upon the absorption and bioavailability of drugs has also been discussed by Cole et al. (1992). These observations

confirm previous studies which suggest that non-ionic surfactants can function as a replacement for bile acids in vivo (Harries and Muller, 1971; King et al., 1979; Bland and Prestbo, 1984). It is believed that similar or better results would be obtained in clinical studies using d-α-tocopherol, since non-ionic surfactant-induced absorption could take place without the preceding requirement for ester hydrolysis of d-α-tocopheryl acetate (Baker et al., 1980).

In summary, the present study shows that the addition of glycerol and water to formulations containing non-ionic surfactants enables them to be filled into hard gelatin capsules without causing embrittlement of the capsule shells. Dissolution and stability studies, with vitamin E, indicate the suitability of this system for the efficient delivery of lipophilic drugs which would otherwise have poor dissolution and absorption characteristics.

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