
PHARMACEUTICAL TECHNOLOGY

**POLYSACCHARIDE FORMULATION FOR IMPROVEMENT
OF RACEMIC VITAMIN E BIOAVAILABILITY**

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Abstract: The relative bioavailability of polysaccharide – racemic vitamin E preparation in comparison with a commercial product was assessed in a kinetic study of plasma α -tocopherol in rabbits. Six male rabbits were used in a cross-over design. α -Tocopherol levels were determined by the fluorometric method. A secondary peak in α -Tocopherol plasma concentration – time profiles was observed in connection with enterohepatic circulation of vitamin E. A simple one-compartment pharmacokinetic model was proposed to explain enterohepatic circulation of α -tocopherol in rabbits. On the basis of the results obtained, it is apparent that polysaccharide – vitamin E preparation achieved a higher rate and extent of absorption. The total AUC of experimental preparation was 1.78-fold greater than that of a commercial capsule preparation, suggesting an increase of bioavailability by 78%.

Keywords: bioavailability, vitamin E, new formulation, pharmacokinetic, enterohepatic circulation, polysaccharide, rabbits

α Tocopherol, known as vitamin E, is a major lipophilic antioxidant (1). It plays an important role in preventing cardiovascular diseases and cancer (2). Due to interest in these beneficial effects, many vitamin E preparations have become widely available. Synthetic vitamin E consists of a mixture of (+)-form and (-)-form which are optical isomers. Natural vitamin E consists of only the (+)-form. The biopotency ratio in IU of natural (+)- α -tocopherol acetate vs. synthetic (\pm)- α -tocopherol acetate ester is 1.36. The bioavailability of tocopherol in human serum or plasma indicates that natural tocopherol is about twice as biologically active as synthetic tocopherol (3-4).

The vitamin E dose requirement for human adult is about 15 mg per day α -tocopherol equivalents (5). Commercial preparations are usually gelatin capsules which contain oily (\pm)- α -tocopherol acetate.

Vitamin E and fat soluble vitamins are better absorbed in the presence of surfactants or from emulsified vehicles than from oily preparations. There is thus an increase of interest in self-emulsifying systems, where lipophilic drugs such as fat soluble vitamins may be stored in concentrated oil – surfactant solution. In addition, it is important to formulate vitamin preparation that is efficiently absorbed from the intestine (6). Physical form of the

supplement can influence the amount of α -tocopherol absorption and hence its bioavailability. To enhance vitamin E bioavailability, it was administered as a self-emulsifying preparation, adsorbed on silica carrier, microcapsulated in lipid matrix or dissolved in oil (6-9). There were no formulations in which polysaccharides were used. Those substances, present naturally in the diet, have the advantages of being safe, nontoxic and easily available. Polysaccharides offer an alternative substrate for the bacterial enzymes present in the colon. Many of them are hydrophilic and swell under exposure to upper gastrointestinal conditions, which results in premature drug release. To overcome this problem natural polysaccharides are chemically modified or mixed with hydrophobic polymers (10-13).

The objective of this study was to assess the bioavailability and pharmacokinetic parameters of vitamin E from commercial oily preparation compared to capsulated granules with polysaccharide-25, when given orally to rabbits.

The polysaccharide was obtained through bacterial starch hydrolysis and tested as an excipient for preparing capsulated granules with vitamin E. The obtained polysaccharide was a short chain amylose which consisted of 25 D-glucopyranose residues linked by α -(1-4) bonds, so the polysaccharide is named “polysaccharide – 25”(14).

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EXPERIMENTAL

Materials and methods

Vitamin E experimental preparation

Granules containing "polysaccharide-25" were prepared by dissolving (\pm)- α -tocopherol acetate in chloroform, then mixed with silica carrier. As the solvent evaporated, the "polysaccharide-25" was added and mixed with 10% ammonium hydroxide to form a wet mass which was granulated. Formulated 1.0 mm granules were put in 1% ascorbic acid in 60% ethanol. Granules were dried at room temperature. Gelatin capsules had been filled with granules in dose 50 mg/kg of rabbit body weight (15).

The other commercially available product was in the form of soft gelatin capsules, each capsule contained 100 mg of oily (\pm)- α -tocopherol acetate (Vitaminum E 0,1, GlaxoWellcome, Poznań)

Animals, drug administration and sampling

The experiment was performed on 6 male New Zealand white rabbits, body weight approximately 3.0 kg, 3 months, old purchased from an animal farm in Chorzelów. The Ethics Committee of the Medical Academy of Lublin approved all the procedures employed in these studies, and the principles of laboratory animal care were followed in accordance with the international guidelines. The animals were housed individually in stainless steel metabolic cages and received the complete diet for rabbits-maintenance and water *ad libitum*. The rabbits were kept in their cages for at least five days before any experimental work was done, and then were fasted for 12 h before study.

Six rabbits were used in a randomized two-period crossover design. A week of wash-out was allowed between the two dosing periods. Each rabbit received p.o. a single dose of test or reference preparation. Both preparations were administered in the morning. The reference preparation was a commercial soft gelatine capsule containing 100 mg of oily (\pm)- α -tocopherol acetate. The test preparation was an experimental capsule filled with granules containing an equivalent amount of vitamin E. Both the test and reference preparations were compared in each subject so that intersubject variables were balanced. The experiment was carried out at room temperature. Serial venous blood samples (1.0 mL) were collected into vacutainers (containing sodium heparin as anticoagulant) by venipuncture from right marginal ear vein at 0 h (before dosing), 1, 2, 3, 4, 5, 6, 9, 10, 12, 24, 36, 48, 52, 60, 72 and 96 h after p.o. dosing. The blood samples were centrifuged for 10 min at 2500 \times g and the plasma was kept frozen at -20°C until assay.

Determination of plasma α -tocopherol

Quantification of α -tocopherol in plasma of rabbit was performed using a fluorometric method

described by Taylor et al. (16). Plasma level of α -tocopherol was analyzed on Shimadzu RF-5301 PC spectrofluorometer. Wavelength settings for detection were 280 nm for excitation and 310 nm for emission.

Pharmacokinetic analysis

Plasma level profiles of the rabbit revealed that absorption of vitamin E was biphasic. Due to enterohepatic circulation of vitamin E, a secondary peak in plasma concentration versus time was observed. The first peak was observed after 3 h and second 24 h after the drugs were administered. This type of kinetics suggests the existence of dual mechanism of absorption.

Data were analyzed according to one compartment model with first order absorption, reabsorption and absorption lag time in two phases. Semilogarithmic functions of concentrations versus time were linear in both phases.

After p.o. administration, α -tocopherol concentration profiles were fitted using the following equations:

$$\text{Phase I: } C^I = B^I \cdot e^{-K^I(t-T_0^I)} - A^I \cdot e^{-K_a^I(t-T_0^I)}$$

$$\text{Phase II: } C^{II} = B^{II} \cdot e^{-K^{II}(t-T_0^{II})} - A^{II} \cdot e^{-K_a^{II}(t-T_0^{II})}$$

The pharmacokinetic parameters were estimated, and the averaged concentration profile was obtained according to equations described above where:

C^I and C^{II} are the serum concentration at time t ,
 A^I , B^I are plasma concentration values at time $t=0$ for fast α -tocopherol disposition,

A^{II} , B^{II} are plasma concentration values at time $t=0$ for slow α -tocopherol disposition,

K^I and K_a^I are the elimination and absorption rate constants following vitamin administration,

K^{II} and K_a^{II} are the elimination and reabsorption rate constants following vitamin circulation,

T_0^I and T_0^{II} are the absorption lag time and reabsorption beginning time,

C_0^I and C_0^{II} are α -tocopherol concentrations at T_0^I or T_0^{II} time.

The values of T_0 and C_0 were calculated separately for phase I (absorption - T_0^I , C_0^I) and phase II (reabsorption - T_0^{II} , C_0^{II}) by solving equations:

$$T_0 = \frac{\ln\left(\frac{A}{B}\right)}{K_{aRe} - K}$$

$$C_0 = A e^{-K_a T_0} \text{ or } C_0 = B e^{-K T_0}$$

C_{max} and T_{max} are maximum plasma concentration and time to reach peak plasma concentration. The values of C_{max} and T_{max} were calculated for phase I (absorption - C_{max}^I , T_{max}^I) and phase II (reabsorption - C_{max}^{II} , T_{max}^{II}) by:

$$T_{max} = \frac{1}{K_a^I - K^I} \ln\left(\frac{K_a^I}{K^I}\right)$$

$$C_{\max} = C_{T_0}(e^{-K_1 T_{\max}} - e^{-K_2 T_{\max}})$$

The areas of plasma concentrations of α -tocopherol as a function of time curve ($AUC_{0-\infty}$) were estimated for both phases by means of the trapezoidal rule with extrapolation to infinity using the last measurable plasma drug concentration divided by the elimination rate constant (K) for phase I and phase II. The K was calculated from the terminal slope of the individual plasma concentration – time curves after logarithmic transformation of the plasma concentration values and application of linear regression. Total AUC was a sum of partial AUC values for phase I and phase II. The relative bioavailability (F) was calculated by dividing the AUC after vitamin E experimental preparation p.o. administration by the AUC after p.o. commercial capsule administration (17).

Statistical analyses

Plasma concentration values are given as arithmetic mean \pm S.D.. Pharmacokinetic parameters were calculated using concentration values obtained from equations for phase I or phase II. Calculated values were compared with concentration values measured in rabbits plasma. The relationship ratio was presented as Pearson correlation coefficients and simple regression equations: $r = 0.95099$, $y = 1.0453x - 0.2800$ for commercial preparation concentration values, $r = 0.72605$, $y = 1.0797x - 0.9859$ for experimental preparation concentration values. Differences with $p < 0.05$ were considered to be significant.

RESULTS

Figure 1 shows the mean plasma α -tocopherol concentration versus time profiles following oral administration of two racemic α -tocopherol preparations to rabbits. The plasma levels of α -tocopherol after administration of vitamin E experimental preparation containing “polysaccharide-25” were distinctly higher compared to those for commercial capsules.

The effect of vitamin E enterohepatic circulation appeared significant in plasma concentration profiles of both preparations. The first peak was observed after 2-3 h after preparations were administered, then α -tocopherol level decreased and next increased after 9 h. The second peak was observed 24 h after administration of both preparations and α -tocopherol level slowly decreased. After 72 h, the plasma level of α -tocopherol was a little higher than initial.

Table 1 shows pharmacokinetic parameters obtained after p.o. administration of two vitamin E preparations. Figures 2 and 3 show the semilogarithmic plot of change of α -tocopherol plasma concentration versus time following p.o. administration of racemic vitamin E in the form of experimental preparation and commercial capsule.

The absorption rate constant (K_a^I) in the first phase was significantly higher following treatment with vitamin E experimental preparation. α -Tocopherol concentration increased more rapidly and time to reach peak plasma levels (T_{\max}^I) was short-

Table 1. Pharmacokinetic parameters of α -tocopherol after p.o. administration of racemic vitamin E experimental preparation and commercial capsule to rabbits.

| | Vitamin E experimental preparation | Commercial capsule |
|-----------------------------|--|---|
| Phase I | $B^I = 12.38 \mu\text{g/mL}$ $K^I = 0.18552 \text{ h}^{-1}$ $A^I = 881.9 \mu\text{g/mL}$ $K_{re}^I = 2.7506 \text{ h}^{-1}$ $T_0^I = 1.66 \text{ h}$ $C_{\max}^I = 7.02 \mu\text{g/mL}$ $T_{\max}^I = 1.05 \text{ h}$ $AUC^I = 55.6 \mu\text{gh/mL}$ | $B^I = 19.59 \mu\text{g/mL}$ $K^I = 0.3725 \text{ h}^{-1}$ $A^I = 34.17 \mu\text{g/mL}$ $K_{re}^I = 1.281 \text{ h}^{-1}$ $T_0^I = 0.61 \text{ h}$ $C_{\max}^I = 6.68 \mu\text{g/mL}$ $T_{\max}^I = 1.36 \text{ h}$ $AUC^I = 28.78 \mu\text{gh/mL}$ |
| Phase II | $B^{II} = 144.57 \mu\text{g/mL}$ $K^{II} = 0.05602 \text{ h}^{-1}$ $A^{II} = 446.35$ $K_{re}^{II} = 0.12135 \text{ h}^{-1}$ $T_0^{II} = 17.25 \text{ h}$ $C_{\max}^{II} = 15.09 \mu\text{g/mL}$ $T_{\max}^{II} = 13.80 \text{ h}$ $AUC^{II} = 628.33 \mu\text{gh/mL}$ | $B^{II} = 59.67 \mu\text{g/mL}$ $K^{II} = 0.05177 \text{ h}^{-1}$ $A^{II} = 100.85$ $K_{re}^{II} = 0.10994 \text{ h}^{-1}$ $T_0^{II} = 9.0 \text{ h}$ $C_{\max}^{II} = 10.13 \mu\text{g/mL}$ $T_{\max}^{II} = 12.9 \text{ h}$ $AUC^{II} = 353.5 \mu\text{gh/mL}$ |
| Total AUC $\mu\text{gh/mL}$ | 683.99 | 383.28 |
| F | 1.78 = 683.99/383.28 | 1 |

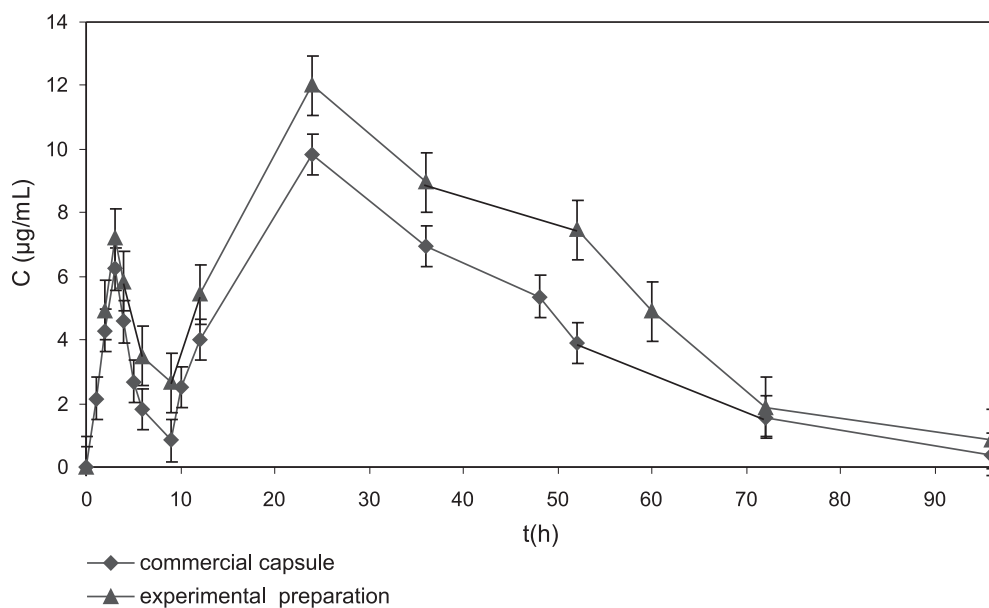


Figure 1. Mean plasma concentration (\pm S.D., $n=6$) versus time profiles of α -tocopherol following oral administration of two racemic vitamin E preparations to rabbits.

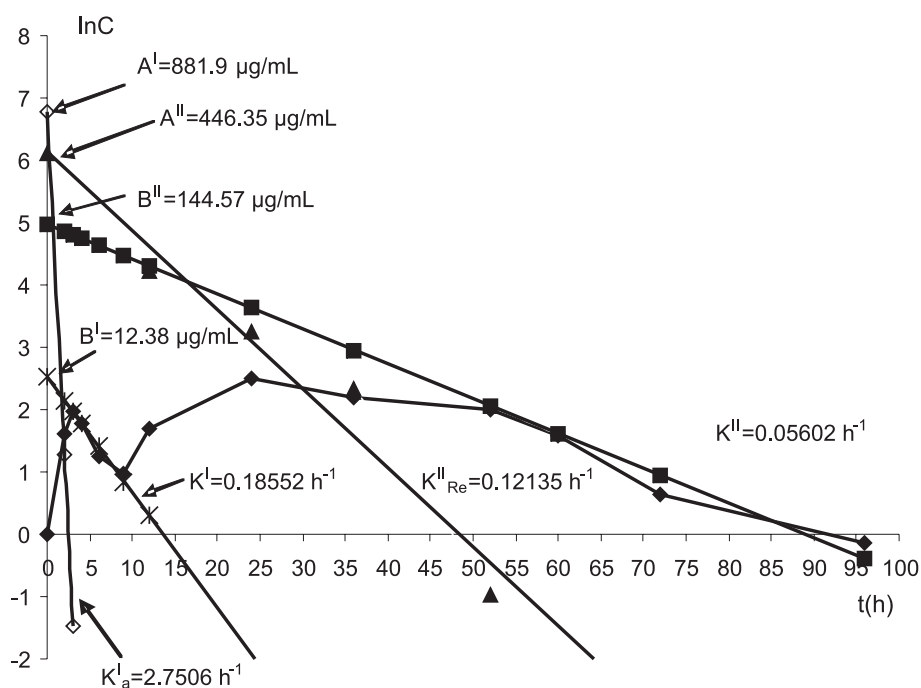


Figure 2. Semilogarithmic plot of change of α -tocopherol plasma concentration as a function of time following p.o. administration of racemic vitamin E in the form of experimental preparation.

er. The maximum plasma concentration (C^I_{max}) value was higher and $AUC^I_{0-\infty}$ value was markedly higher. The elimination rate constant (K^I) was significantly lower, so elimination in the first phase was slower than that of commercial capsule preparation.

The reabsorption rate constant (K^{II}_{Re}) in the second phase was higher but not significantly after administration of experimental vitamin E preparation. The time to reach peak plasma levels after reabsorption process (T^II_{max}) was a little longer but

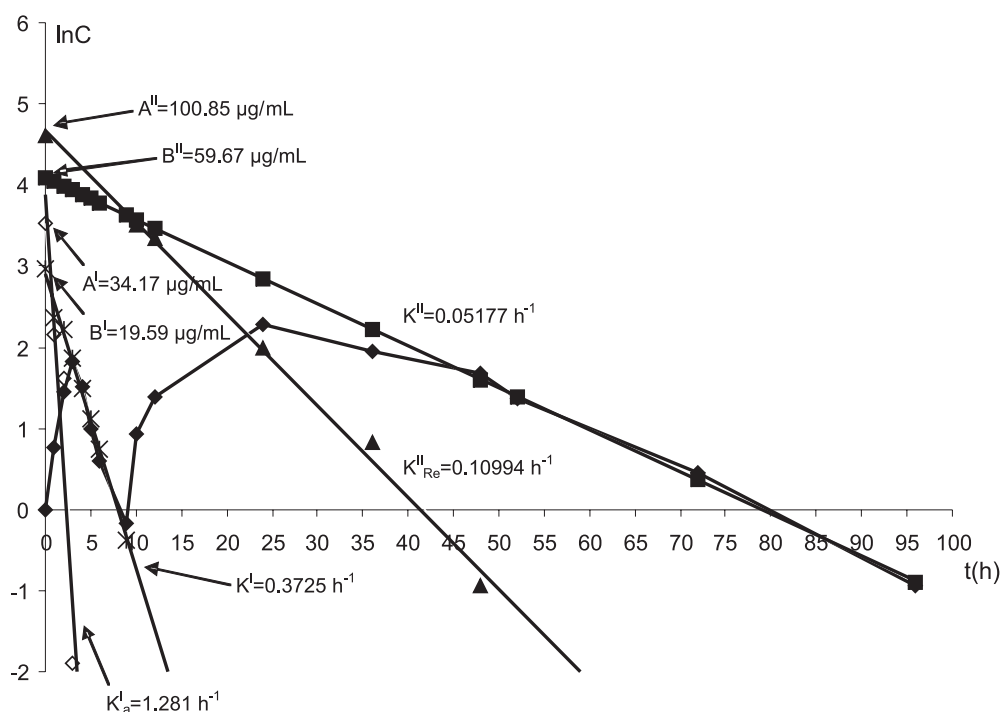


Figure 3. Semilogarithmic plot of change of α -tocopherol plasma concentration as a function of time following p.o. administration of commercial capsule contained racemic α -tocopherol.

the maximum plasma concentration (C_{max}^{II}) value was higher. The elimination rate constant (K^{II}) was slow and did not differ between preparations. The $AUC_{0-\infty}^{II}$ value of the experimental vitamin E preparation was markedly higher than that of commercial capsule preparation. In the vitamin E experimental preparation, the vitamin was adsorbed on the surface of silica which appeared as faster absorption of α -tocopherol. "Polysaccharide-25" contributed to the transfer of absorption process to intestine which appeared in longer absorption lag time or reabsorption time (T_0).

The experimental vitamin E preparation had a greater total AUC , and its relative bioavailability (F) value – 1.78 was significantly higher than that of commercial capsule preparation, suggesting an increase of bioavailability by 78%.

DISCUSSION AND CONCLUSION

Most drugs are absorbed in the intestinal tract and pass into blood in the portal vein and then are introduced directly into the system circulation. During the process of enterohepatic circulation, drugs are taken up from the blood by hepatocytes, secreted into the bile, and then deposited back into the intestinal lumen, where a fraction is reabsorbed by enterocytes and recirculated through the liver (18). Enterohepatic circulation of vitamin E was observed by the authors in human and animals. To explain

enterohepatic circulation of vitamin E, a simple pharmacokinetic model was developed in this study.

The first peak in plasma α -tocopherol concentration versus time profiles following oral administration of both preparations to rabbits was the effect of absorption of a little amount of vitamin E from the proximal part of the intestine which passed through chylomicrons to plasma and liver, while the second peak was the effect of absorption from the distal part of the intestine of the remaining – not absorbed amount of vitamin E and its amount secreted into the bile after the first phase of absorption.

Reabsorption of drug by enterohepatic circulation increases the area under the plasma concentration time curve (AUC) and prolongs the elimination half-life. Compounds excreted in bile and subjected to enterohepatic cycling are for example: imipramin, vitamin A, digoxin, indomethacin and ibuprofen (19). Biphasic absorption of radiolabeled emulsion of vitamin E administered by stomach intubation was observed in rats by Gallo-Torres (20). Two peaks of radioactivity have appeared and suggested biphasic type of kinetics. According to Lee-Kim et al. (21) the magnitude of enterohepatic circulation of α -tocopherol was very small in rats. Hidiroglou et al. (22) administered, radioactive (+)- α -tocopherol orally to sheep. Less than 3% of the total recovered radioactivity was present in bile. The authors described disposition kinetics by the use of a two-compartment model.

A two-compartment model with lag time was proposed to describe the pharmacokinetics of drugs subject to enterohepatic circulation by Steimer et al. and Chen et al. (23, 24). Four-compartment model based on recirculation loops was constructed by Shou et al. (18) to fully evaluate the enterohepatic recirculation. A conventional compartment model that can explain enterohepatic circulation profiles for both single and repeated dose was proposed by Wajima et al. (25).

A tocopherol concentration after single oral dose of a novel self-emulsifying vitamin E preparation and commercial product – Natopherol, available as soft gelatin capsules was investigated by Julianto et al. (8). A self-emulsifying preparation contained vitamin E mixed with Tween 80, Span 80 and dissolved in palm oil. Plasma level of α -tocopherol was evaluated in 8 volunteers. In several volunteers, a secondary peak in plasma concentration versus time profile was observed due to enterohepatic circulation of vitamin E. The pharmacokinetic parameters: C_{max} , T_{max} , and AUC were estimated. The self-emulsifying preparation achieved a faster rate and higher extent of absorption.

Baldi et al. (6) assessed the relative bioavailability of three formulations of (\pm)- α -tocopherol acetate in dairy cows following intraruminal administration of gelatin capsules containing vitamin E adsorbed on silica, microencapsulated and in oil form. Each preparation contained 5000 IU of (\pm)- α -tocopherol acetate. The same dose was also administered intraperitoneally. Vitamin E adsorbed on the surface of silica or microencapsulated in a lipid matrix resulted in different pharmacokinetic profiles and greater relative bioavailability than oil-based preparation.

The kinetic behavior of 3 preparations of α -tocopherol (vitamin E) after oral administration to heifers was assessed by Bontempo et al. (7). A single oral bolus of 5,000 U of α -tocopherol in oil or encapsulated in liposomes or cyclodextrin was administered to each cow. The disappearance rate constant (K) was lower after administration of α -tocopherol encapsulated in liposomes, compared with the other 2 preparations. This formulation may result in longer persistence of the vitamin in plasma than the other 2 preparations.

In summary, we assessed self designed vitamin E preparation and commercial capsule with racemic vitamin E by kinetic methods in New Zealand rabbits. On the basis of the results obtained, it is apparent that self designed vitamin E preparation achieved a higher rate and extent of absorption compared to vitamin E commercial capsules. The relative bioavailability of α -tocopherol was significantly higher after administration of experimental self designed vitamin E preparation. A simple one compartment pharmacokinetic model was proposed to explain enterohepatic circulation of α -tocopherol in rabbits.

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