PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :
A61K 31/12, 31/59, 31/175
A61K 31/225, 31/355, 31/595

A1

(11) International Publication Number: **WO 87/ 01035**

(43) International Publication Date: 26 February 1987 (26.02.87)

(21) International Application Number: PCT/US86/01671

(22) International Filing Date: 13 August 1986 (13.08.86)

(31) Priority Application Number: 765,359

(32) Priority Date: 13 August 1985 (13.08.85)

(33) Priority Country: US

(60) Parent Application or Grant
(63) Related by Continuation
US 765,359 (CIP)
Filed on 13 August 1985 (13.08.85)

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(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: PHARMACEUTICAL MICROEMULSIONS

(57) Abstract

A method for parenteral administration of fat-soluble pharmaceuticals and vitamins using microemulsions. The microemulsions are comprised of a naturally occurring amphipatic substance and a hydrophobic lipid along with the active ingredient, are size selected for 1,000 ± 300 Å pseudomicelles, and permit safe intravenous injection of the active ingredient. Levels of the active ingredient in the various lipoprotein fractions of serum appear to mimic the natural distribution of the administered drug if taken orally.

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PHARMACEUTICAL MICROEMULSIONS

Technical Field

The invention relates to the administration of compositions containing effective amounts of fat soluble vitamins or drugs. In particular, the invention relates to microemulsions suitable for parenteral administration of vitamins E, A, D, and K and hydrophobic pharmaceuticals.

Background Art

The administration of fat soluble vitamins and pharmaceuticals becomes a medical problem when deficiencies in or needs for these substances are displayed by subjects not capable of normal intestinal absorption of these vitamins into the bloodstream. While normal children and adults can employ commercially available oral compositions, such as old-fashioned cod liver oil, to obtain needed supplements of fat soluble vitamins, individuals with malabsorption syndromes and premature infants require parenteral administration in order to make dosage effective. Similarly, while the majority of people can take oral formulations of fat-soluble drugs, often those most in need of them - i.e., seriously ill patients, are often not capable of swallowing or otherwise employing the normal gastrointestinal process.

In particular, for example, attention has been focused on the administration of α-tocopherol (vitamin E), because administration by non-oral routes has led to serious problems. It is rare for simple nutritional deprivation of this vitamin to cause a deficiency in humans. Adults with normal metabolism apparently store sufficient vitamin E to weather long periods of

diminished intake. The function of the vitamin is largely unknown, but it appears to be localized in the membranes in association with highly unsaturated fatty acids and there is, at present, a consensus that the vitamin stabilizes these membranes, at least in part by virtue of its antioxidant properties and by virtue of its formation of complexes with free fatty acids (Erin, A.N., et al, Biochim et Biophys Acta (1984) 774:96-102; Whitin, J.C., et al, J Lipid Res (1982) 23:276-282). The most readily demonstrated effect of vitamin E deficiency is an in vitro erythrocyte hemolysis in the presence of hydrogen peroxide; the clinical symptoms are associated with loss of integrity of the membranes of the various physiological systems both in humans and in animals.

Vitamin E deficiency in humans is most often encountered in premature infants and in adults or children with abnormal rates of absorption of fats from the intestine, thus necessitating parenteral administration. The importance of vitamin E administration in premature infants has increased measurably over the past several decades as the number of such infants maintained viable has greatly increased. Administration of vitamin E is particularly important to counterbalance the negative effects of the administration of oxygen. Oxygen is directly beneficial in treating infants with hyaline membrane disease, but has a side effect of severe damage to the retina. It is believed by some investigators that adequate levels of vitamin E can mitigate this. However, oral administration of vitamin E to these infants results in gastrointestinal problems and intravenous administration has been implicated in several neonatal deaths (Bodenstein, C.J., Pediatrics (1984) 73:733). Current

products for such parenteral administration are aqueous emulsions which contain detergents as the emulsifying agent.

In addition, with respect to vitamin E per se, a small group of individuals with a genetic hereditary disorder, abetalipoproteinemia, i.e., who lack plasma ß-lipoprotein, also exhibit the need for parenteral vitamin E. Finally, severe malnutrition, especially in infants and children, results in a deficiency which warrants parenteral administration of this vitamin, and patients subjected to trauma requiring administration of high levels of oxygen may benefit from its antioxidant properties.

Individuals with malabsorption syndromes in general exhibit a need for parenteral administration of fat soluble vitamins and drugs. These individuals include patients suffering from cystic fibrosis, chronic pancreatitis, pancreatic carcinoma, cirrhosis of the liver, glutin enteropathy, tropical sprue, regional enteritis, ulcerative colitis, and persons who have been subjected to gastrointestinal surgery. All of these individuals are inadequately capable or incapable of proper transport of orally administered fat soluble nutrients and pharmaceuticals to the bloodstream for subsequent metabolic utilization.

While the foregoing discussion has focused on deficiencies of vitamins or the need for therapy using fat soluble substances in humans, it is recognized that other mammals may also benefit from suitable parenteral administration of dietary supplements containing fat soluble vitamins and may require similar therapy.

Presently known routes of administration are less than perfect. There have been reports of intramuscular administration of vitamin E in the

veterinary field (Caravaggi, C., et al, N.Z.J. Agric Res (1968) 11:313-318), however the effectiveness of this route in transporting the vitamin to the bloodstream appears to vary with the nature of the subject. Intramuscular administration has also been used in humans (Bauernfeind, J.C., et al, Am J Clin Nutr (1974) 27:234-253). However, most emphasis for parenteral dosing has been placed on intravenous administration. Two commercially available preparations, Intralipid (soybean oil based), disclosed in U.S. 3,164,094, and Liposyn (safflower oil based) have been used, but they have been shown to be relatively poor sources of vitamin E (Gutcher, G.R., et al, J Parent & Entreal Nutr (1984) 8:269-273). U.S. 3,384,545 discloses an aqueous emulsion utilizing the polyoxyethylene ether of castor oil as an emulsifying agent. Other compositions have used detergents such as polysorbates. None of these compositions is satisfactory and their performance record in trouble-free administration of active ingredients has been poor.

The composition of the present invention has the unique property of delivering fat soluble substances to the plasma in a form and distribution which mimics that naturally occurring in normal subjects. It is therefore free of the problems encountered in the compositions presently known in the art. In addition, in certain embodiments, the carrier in the composition serves as a source of essential nutrients.

Disclosure of the Invention

The compositions of the invention provide safe and effective delivery of fat soluble substances, for example, of vitamin E, to the circulatory system in a form which is readily recognized by the host.

Accordingly, the vitamin or other substance is treated as in the normal course of metabolism, and no destructive side effects are found. This is accomplished by supplying the desired fat soluble substance in a microemulsion, i.e., an aqueous suspension of pseudomicelles, wherein the pseudomicelles are of such size and density as to mimic the chylomicron particles normally formed by fats transported across the intestinal wall. These small chylomicron pseudomicelles are on the order of 1,000 Å in diameter and are essentially fat globules surrounded by a hydrophilic surface which is generated from a monolayer of amphipatic molecules. The particles are sufficiently small to enter the liver, which appears to play a significant role in the distribution and utilization of a number of vitamins and drug metabolites. They are sufficiently large to prevent the side effect of hypercholesterolemia, a reaction often associated with use of small unilamellar liposomes (50-300 Å) to administer drugs. Microemulsions have in fact been used to model the lipoprotein components of plasma (Chen, G.C., et al, Biochemistry (1984) 23:6530-6538).

Thus, in one aspect, the invention relates to a composition comprising an effective amount of a fat soluble active ingredient harbored in a microemulsion of pseudomicelles 500-1,500 Å, preferably 700-1,300 Å, in diameter. Microemulsions containing pseudomicelles of this diameter generally have densities of approximately 1.005-1.1 g/ml, but the density range depends on the nature of the composition components. The final percentage composition of the microemulsion depends on particle size and the nature of the components. To prepare the microemulsion, initial ranges of about 40%-70% by weight of hydrophobic lipid,

and 30%-60% by weight of a naturally occurring amphipatic substance are practical.

In other aspects, the invention relates to methods of administering fat soluble substances using the composition of the invention and to methods of preparing this composition.

Brief Description of the Drawings

Figure 1 shows the results of an in vitro assay whereby tocopherol contained in microemulsions of the invention distributes itself among the lipoprotein components of serum.

Figure 2 shows the levels of tocopherol and tocopherol acetate in liver extracts of the same rats administered the composition of the invention.

Modes of Carrying Out the Invention

A. Definitions

As used herein "microemulsion" refers to an aqueous suspension of pseudomicelles which contain relatively hydrophobic lipid centers surrounded by a monolayer of amphipatic (sometimes spelled amphipatic) molecules bearing hydrophilic moieties. The concentration of pseudomicelles in the aqueous suspension is typically about 0.1 mg/ml-10 mg/ml. In the microemulsions of the invention, the diameter of the pseudomicelles is approximately 500-1,500 Å, preferably 700-1,300 Å. The nature of the pseudomicellular particle is different from that of liposomes, which are unilamellar or multilamellar particles of bilayers, wherein the bilayers have hydrophilic surfaces on either side. Thus, liposomes have hydrophilic interiors as well as exteriors and are often of smaller dimension -- i.e., of the order of

50-300 Å. Liposomes are appropriate for delivery of hydrophilic, rather than hydrophobic, active ingredients.

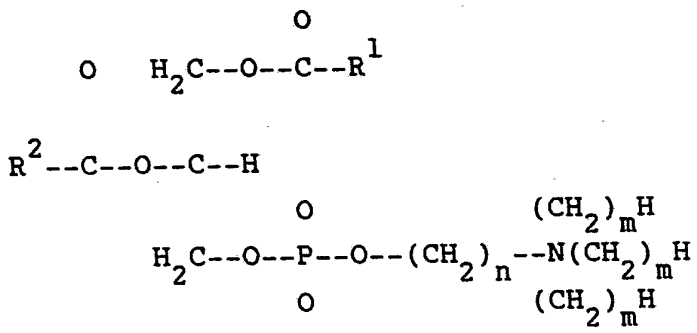
"Pseudomicelles" by definition, also differ from "micelles". Micelles are aggregations of amphipatic molecules--i.e., molecules which have both hydrophobic and hydrophilic portions such that the outer layer of the micelles is compatible with the surrounding medium. In a typical case, micelles in aqueous medium contain hydrophilic surfaces and hydrophobic interiors. Pseudomicelles have these characteristics, but differ in that the hydrophobic interiors are supplemented with additional hydrophobic material.

Micelles are generally very small in diameter (about 50-100 Å, similar to small unilamellar liposomes). Pseudomicelles can be substantially larger, depending upon the quantity of hydrophobic "filler" used in their centers. As the particles become larger, they become, in general, less dense as the low density hydrophobic fillers become a greater and greater proportion of the composition. Since the quantity of the hydrophobic center increases as the cube of the radius while that of the more dense hydrophilic surface layer increases only as the square, the ratio of the less dense hydrophobic material to the more dense amphipatic molecules making up the surface becomes larger.

In summary, pseudomicelles are comprised of amphipatic molecules on their surfaces, oriented so that the hydrophilic portions of the amphipatic surface molecules face the medium, and a hydrophobic core. In terms of definition, as used herein, "amphipatic" substances are those which have hydrophobic and hydrophilic portions, which are thereby capable of forming micelles when in aqueous suspension, or of

supporting the formation of pseudomicelles when mixed with water and a hydrophobic lipid. The amphipatic substances used in the compositions of the present invention are those which are found in the biosystem. Naturally occurring substances are used because, as explained below, many synthetic amphipatic materials -- i.e., detergents, are toxic. The most commonly occurring amphipatic substances are the phospholipids, among which the lecithins are preferred.

"Lecithin" refers to a phospholipid derived from glycerol. Two of the glycerol hydroxyls are esterified to two long chain (8-24C) fatty acids. The third hydroxyl is one of the two alcohols of a phosphodiester; a tertiary amino alcohol is the other alcohol component. Thus, lecithins generally have the formula



wherein R^1 and R^2 are independently hydrocarbyls of 7-23 carbons; n is an integer from 2-4 and each m is independently an integer of 1-4. Naturally occurring lecithins generally are comprised of choline esterified to the phosphate -- i.e., n is 2 and all m are 1. In addition, in naturally occurring lecithins, R^2 is unsaturated and the glycerol carbon to which it is attached has the R configuration, as shown. In the lecithins employed in the invention, racemic mixtures as well as resolved enantiomers may of course be employed.

Chirality of the center carbon of the glycerol is not relevant to the properties exhibited by the lecithins used in the invention.

Common biological sources for lecithins include soybean and egg yolk. Also functional, though less effective, are the ethanolamine analogs of the lecithins (cephalins) and more complex bipolar lipids such as sphingomyelins.

Of importance in determining the effectiveness of the amphipatic molecule is the degree of polarity provided by the hydrophilic portion. It is recognized that polarity is, in general, a sliding scale where no sharp boundaries are found. However, there is a clear difference in class when a readily ionizable hydrogen is present. Thus, phospholipids, which contain an ionized phosphate diester at neutral pH, contain clearly polar portions along with the long chain fatty acid hydrophobic portions. However, even the monoglycerides (which contain two free hydroxyl groups) are amphipatic, although to a lesser extent. Strictly speaking, even the triglycerides which are included in the definition below as "hydrophobic" lipids contain a moderately polar region in the glycerol region. However, the polarity of the triester is not sufficient to render the whole amphipatic.

"Hydrophobic lipid" refers to a lipid which is not sufficiently amphipatic to stabilize an emulsion. Triglycerides are classic examples. When mixed with water, the momentary suspension of lipid particles in the aqueous medium immediately separates into two homogeneous oil and water layers. Of particular relevance to the invention is the observation that triglycerides, vitamin A acetate and tocopherol acetate

(the acetylated form of vitamin E) behave as hydrophobic lipids, and can form the pseudomicelle core.

Between the extremes of the hydrophobic lipids which form the cores of the pseudomicelles of the invention and the amphipatic molecules which are responsible for supporting the emulsion and comprise the outer layer are a class of molecules which are not sufficiently amphipatic to provide, themselves, the desired outer layer, but are not sufficiently hydrophobic to comprise a separate core. These compounds, when included in the compositions of the invention, distribute themselves so that the majority of the molecules reside in the outer layer. In this category, for example, is cholesterol. Mixtures of cholesterol with amphipatic materials fail to result in pseudomicelles, but rather in the formation of liposomes. On the other hand, cholesterol mixed solely with a hydrophobic lipid fails to support an emulsion of any kind. Cholesterol has been found useful in the constructure of liposomes, as it is believed to close up the "pores" in the bilayer by occupying spaces between the lipid side chains at the interior. Similarly, in the pseudomicelles of the present invention, the cholesterol, if contained, resides mostly in the outer layer. On the other hand, cholesterol esters are hydrophobic lipids and can be used as the core material.

As set forth above, the pseudomicelles of the invention are thus envisioned as containing amphipatic outer layers wherein the amphipatic substances are oriented with their hydrophilic portions facing outward, and are filled with hydrophobic substances in the core. The active ingredient included in the pseudomicelles will distribute itself within the pseudomicelle according its own polarity characteristics. Fat soluble

substances which are substantially entirely hydrophobic are found mostly in the core. Moderately polar fats such as vitamin E itself, vitamin K and vitamin A, and steroid compounds containing hydroxyl groups such as vitamin D and cholesterol distribute themselves more densely in the boundary between the surface molecules and the core or in the surface monolayer. Any classification of the active ingredient into "hydrophobic" lipid or "amphipatic" is to a large extent arbitrary. There will be a continuum of distributions between the boundary layer and the core. In some cases, the active ingredient can serve as the boundary layer or the core.

B. General Method

The compositions of the invention are capable of effecting the distribution of a fat soluble active ingredient in plasma in a manner which mimics that encountered in the normal physiology of the subject. With respect to vitamin E specifically, there has been considerable study of the normal transport of this vitamin in plasma.

A summary of the distribution of α-tocopherol in the serum lipoproteins and various cellular components in blood was published by Kayden, H.J., Tocopherol, Oxygen, and Biomembranes (1978) Elsevier/North Holland Biomedical Press, pp 131-142. The total tocopherol in plasma, as opposed to that of the cellular components, seems correlated to the total lipid content of the plasma. However, the variation found in erythrocytes, platelets, lymphocytes, and granulocytes parallels the variation of tocopherol in plasma as altered by increased oral intake of this vitamin. Tocopherol was found to be associated with all

the lipoprotein components of plasma, but had a higher correlation with the low density lipoproteins (LDL) in humans than with the other two major lipoprotein constituents, high density lipoproteins (HDL) and very low density lipoproteins (VLDL). In other species the distribution may be different. (The lipoprotein components of the blood are classified according to their densities (Havel, R.J., et al, J Clin Invest (1955) 34:1345-1353). While there are no sharp boundaries, there appear to be clusters of lipoprotein components in the gradient. The VLDL has a density range of approximately 0.95-1.006 g/ml; LDL of approximately 1.006-1.063 g/ml; and HDL of approximately 1.063-1.21 g/ml.)

As tocopherol appears to be functional in the context of membranes, studies have also been made concerning the transfer of this vitamin into the membrane structure. For example, Murphy, D.J., J Biol Chem (1981) 256:10464-10468 showed that components of the rat liver cytosol mediate the transfer of tocopherol between membranes including transfer from an in vitro liposome preparation to liver microsomes. It would appear that each fat soluble vitamin or drug has its own transport system in the plasma as exemplified, for example, by the differences shown for α-tocopherol and ß-carotene in human blood by Bjornson, L.K., et al, J Lipid Res (1976) 17:343-352. A further paper shows the transfer of d-α-tocopherol from the plasma to a receptor protein in rat liver nuclei (Nair, P.P., et al Tocopherol, Oxygen & Biomembranes, (1978, supra) pp 121-130).

Plasma transport of the active ingredient of an orally administered composition is a precursor to the manifestation of biological activity at the intended

sites. This transport is normally initiated by transfer of chylomicron particles comprising the active ingredient in question across the intestinal walls. However, there are a number of instances, as outlined above in the case of vitamin E, and applicable as well to other fat soluble pharmaceuticals, wherein oral administration is undesirable and the vitamins or drugs must be administered parenterally, preferably intravenously. In these cases, the natural assembly of the chylomicron particles is bypassed and whatever form is injected must substitute for them as a vehicle.

In general, in order to inject liquid compositions containing fat soluble materials, measures must be taken to assure a uniform suspension of the desired substance in water. A number of approaches have been taken toward this result. One recently popular general technique involves the formation of liposomes to behave as carriers of the active ingredient. Two characteristics make such particles unsuitable in this case. First, since the multi- or unilamellar liposomes are composed of bilayers with hydrophilic boundaries, they are more hospitable to polar substances than to lipophilic materials. Second, the liposome particles have a high concentration of amphipatic substance at their surfaces. These substances are capable of solubilizing cholesterol from the cell membranes and inducing hepatic cholesterol synthesis. Liposomes can thus cause hypercholesterolemia (Byers, S.O., et al, J Biol Chem (1962) 237:3375-3380; Jakoi, L., et al, J Biol Chem (1974) 269:5840-5844).

Another approach has been to use synthetic detergents to solubilize lipid soluble substances.

Detergents mediate the interaction between lipophiles and aqueous medium at a molecular level and are thus able to provide a uniform distribution. However, many detergents are toxic when used in effective amounts. For example, materials such as sodium lauryl sulfate (SDS) and Triton X-100 are not usable in solutions to be administered to mammalian subjects. Less toxic detergents such as polysorbate 80 and its derivatives are more appropriate, but nevertheless appear to constitute a health hazard.

In the approach of the present invention, a microemulsion is used as a carrier for the active ingredient. To obtain the microemulsion, a naturally occurring amphipatic substance, specifically a phospholipid, such as a lecithin or ethanolamine analog or sphingomyelin, is used as an emulsifying agent to form the pseudomicelles in an aqueous suspension of sufficient hydrophobic lipid to form the cores. The amphipatic substance forms the boundary layer of the pseudomicelles. Selection is made to obtain pseudomicelles approximately 500-1,500 Å, preferably 700-1,300 Å, in diameter. This size particle is sufficiently small to undergo uptake by the liver, as is the normal course of fatty substance metabolism, but is large enough to be capable of containing effective amounts of active ingredients and to minimize the hypercholesterolemia which occurs at high concentrations of amphipatic substances.

The pseudomicelles for transport of active ingredient are formed by mixing the active ingredient, hydrophobic lipid material, and naturally occurring amphipatic substance in a total amount in aqueous suspension to that the total of the components amounts to 0.1-10 mg/ml. These components are supplied in a

ratio suitable to generate a large proportion of pseudomicelles of the proper size. (An approximate exemplary ratio is 5:40:55 by weight for vitamin E, tocopherol acetate, and lecithin, for example.) The workable starting ratio has no precise limits and can vary widely, but yields are increased if the initial ratio approximates that in the final pseudomicelle composition.

In one approach, the suspension containing about 10-50 mg/ml components in water is then sonicated to form a mixture of various size pseudomicelles. The sonicated suspension is then subjected to gradient density segregation and those pseudomicelles having the approximate density of 1.005-1.1 g/ml depending on the nature of the components are selected from the gradient to obtain particles of 500-1,500 Å, preferably 700-1,300 Å, diameter. These pseudomicelles contain the very approximate original composition of the mixture, if the above exemplified ratio is used for the illustrated components, and are used directly for administration of the active ingredient.

Another approach to the formation of pseudomicelles in the suspension utilizes a newer technology called "microfluidization". Microfluidization is effected by the interaction of two fluid streams in defined microchannel under high pressure, as high as 10,000 psi. The process is carried out in the Microfluidizer™, a product of Microfluidics Corporation, Newton, MA, which is described in Pharmaceutical Manufacturing (August 1985), pp. 18-22, and in Drug and Cosmetic Industry (November 1984). These reports describe the use of the Microfluidizer to form liposome compositions using aqueous suspensions of an amphipatic material, optionally containing an

intermediate substance, such as cholesterol. Since no hydrophobic lipid is present in these compositions, pseudomicelles cannot be formed.

For preparation of the pseudomicelles of the invention using microfluidization, a suspension containing 0.1-10 mg lipids/ml is supplied. The initial relative amounts of the various materials in the composition appear to be more significant when this technique is used than it is when sonication is applied. The size of particles formed appears to influenced by a number of factors besides the initial composition, and smaller particles appear to be obtained at lower total lipid concentrations, as well as after repeated passes through the Microfluidizer. In general, it is more critical to start at the proper composition, and if this is done, no size separation is necessary. By appropriately choosing the starting position and concentration of total lipids, as described in the illustration below, more than 90% of the resulting pseudomicelles are in the desired size range of 1,000 Å $\pm$ 300 Å. The particle size, it should be noted, also varies with the pressure, higher pressures resulting in smaller particles.

In typical preparations, a ratio of hydrophobic lipid:amphipatic lipid:active ingredient of the order 55:45:5 is used. There is, of course, some flexibility in this ratio. A convenient concentration of lipids in aqueous suspension is in the range of 0.5-5 mg/ml, and the pressure range is of the order of 10,000 psi. The fluidization can be conducted at room temperature, and 60 cycles are run.

Exemplary embodiments of the hydrophobic lipid which is used to form the core portion include vitamin A acetate, tocopherol acetate, triglyceride preparations,

or combinations thereof. Preferred embodiments of the naturally occurring amphipatic substance include lecithin, e.g., phosphatidylcholine which can be obtained from egg yolk, other phospholipids and partial glycerides. Active ingredients (which distribute according to their own character between the boundary and core) include tocopherol, tocopherol acetate, vitamin A, vitamin E, vitamin D, phenesterine, daunorubicin, doxorubicin, mitotane, visadine, halonitrosoureas, anthrocyclines, hydrophobic proteins, ellipticine or combinations thereof, and diazepam. Relatively hydrophilic compounds among these, such as doxorubicin or daunorubicin, can be converted to more hydrophobic forms by methods known in the art per se, such as esterification. Of course, mixtures of these active ingredients can be used. The active ingredient will distribute in the pseudomicelle according to its character on the amphipatic/hydrophobic scale. Some active ingredients, such as tocopherol acetate are sufficiently hydrophobic to be used as the hydrophobic lipid in forming the core. Others, such as vitamin A, are sufficiently amphipatic to form the boundary. In these instances, the active ingredient may be used simultaneously as a part of the carrier, as well as for its activity. In addition, if triglycerides are used as the hydrophobic lipid, they are hydrolyzed and metabolized as nutrients. Similarly, if vitamin A acetate or tocopherol acetate are used as core, they are hydrolyzed to the active vitamins.

C. Administration and Use

For administration, the compositions of the invention are injected into a human or animal subject intravenously in an amount suitable for the condition

being treated and the subject. As a general proposition, the amount of active ingredient will often be on the order of approximately 1%-10% of the total content of microemulsion in the suspension depending on the amount desired to be administered. However, if the active ingredient is a "hydrophobic lipid" or sufficiently amphipatic to form the boundary, the percentage can be increased -- i.e., the active ingredient can itself form the lipid core or the boundary layer.

When injected intravenously, the active ingredient contained in the microemulsion is transferred to the blood lipoproteins in a manner entirely analogous to that observed for orally administered dosages of the same material. Therefore, the pseudomicelles of the invention are capable of effectively placing the active substance into its normal progress of physiological activity.

Regardless of the mode of administration, it appears that the pseudomicellular composition is attracted to the liver, and approximately half of the administered dose homes to the liver and is metabolized there. Thus, the compositions of the invention are particularly advantageous in treating conditions in which the liver is a target organ. Such diseases include Type 2 Crigler-Najjar Disease, which can thus be treated with phenobarbitol suitably derivatized; bacterial liver disease, which can be treated with antibiotics; and amoebic liver abcesses, which are treated with metronidazole or chloroquine.

D. Examples

The following examples are intended to illustrate but not to limit the invention.

The compositions of Examples 1-10 were prepared using the following general procedure: The active ingredient, if necessary dissolved in alcohol, is mixed with a suitable hydrophobic lipid such as soybean oil, triglyceride, or tocopherol acetate along with the amphipatic substance. The by weight percentages in the starting material for the active ingredients are about 1%-10% of the active ingredient (if it is used as active ingredient per se) up to about 60%-70% if the active ingredient can also serve as part of the boundary layer or core. The starting composition overall includes about 40%-70% of a hydrophobic lipid and about 30%-60% of an amphipatic substance. Thus, if the active ingredient is itself, for example, a hydrophobic lipid, this core-forming component of the mixture can be included as the active ingredient and thus enhance the dosage level by nearly a factor of 10, if this is desired.

The ingredients were pipetted into a pyrogen-free glass tube and the solvent was evaporated using a rotary evaporator. The dried lipids (about 100 mg) are resuspended in 5 ml of saline pH 7.4, buffered with 20 mM sodium phosphate, and the cloudy suspension is sonicated for 1 hr at 55°C under a stream of nitrogen. (Sonication may be performed with a W-225 R sonicator (Ultrasonics Inc.) for example.)

The sonicated emulsion is purified by ultracentrifugal floatation in a three-layered discontinuous sucrose gradient in the tube of a Beckman SW41 rotor. Sucrose is added to 2.5 ml of the sonicated suspension to a final density of 1.060 g/ml in a polyalymer tube (Beckman, Spinco Division, Palo Alto, CA) and overlaid with a density 1.020 g/ml sucrose-NaCl up to 1.5 cm from the top of the tube and then saline to

0.5 cm from the top. The tube is centrifuged at 20,000 rpm at 10°C for 60 min and the gradient is collected into 12 fractions labeled 1-12 from bottom to top of the tube. In all cases, fractions of approximately 6-10 as noted specifically in the examples were collected and contained microemulsions having a density which averaged 1.010-1.1 g/ml. In all cases the purified fractions were free from liposomes.

Example 1

The original mixture contained:

5 mg α-tocopherol (dl) in ethanol;

40 mg α-tocopherol (dl) acetate in ethanol; and

55 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered were 7-19 and had an average density of 1.02 g/ml and an average particle size of 750 Å.

The composition of the pseudomicellular compartments was 6.5% tocopherol; 45.5% tocopherol acetate; and 48% lecithin. In this and subsequent examples the tocopherol and tocopherol acetate quantitations were determined by reverse phase HPLC (Perkin-Elmer C_{18}) using a mobile phase of methanol:water on 97:3 v/v and phospholipids were determined by the method of Takayama, et al, Clin Chim Acta (1977) 79:93-98.

Table 1 below shows the composition of each fraction. Fractions 7-9, the fractions collected, are shown to have approximately the same distribution of components. Whereas fractions 3-5 had particle sizes in the range of 300 Å diameter, the particles of fractions 10-12 had diameters of roughly 2,000 Å.

Example 2

The original mixture contained:

5 mg α-tocopherol in ethanol;

40 mg soybean oil; and

55 mg phosphatidylcholine (egg yolk type III) in ethanol.

Fractions 8-11 were collected and microemulsion had an average density of 1.010 g/ml and an average diameter of 840 Å.

The resulting particles had the composition of 5.3% α-tocopherol; 17.4% lecithin; and 77.3% triglyceride.

Example 3

The original mixture contained:

10 mg α-tocopherol (dl) in ethanol;

35 mg α-tocopherol (dl) acetate in ethanol; and

55 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered were 7-10 and had an average density of 1.020 g/ml and an average particle size of 710 Å.

Example 4

The original mixture contained:

45 mg tocopherol acetate in ethanol; and

55 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered were 7-10 and had an average density of 1.025 g/ml and an average particle size of 700 Å.

Example 5

The original mixture contained:

2 mg α-tocopherol in ethanol;

40 mg vitamin A acetate in ethanol; and

58 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered were 7-10 and had an average density of 1.020 g/ml and an average particle size of 750 Å.

Example 6

The original mixture contains:

10 mg α-tocopherol in ethanol;

25 mg vitamin A acetate in ethanol; and

65 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered are 7-10 and had an average density of 1.025 g/ml and an average particle size of 700 Å.

Example 7

The original mixture contains:

2.5 mg α-tocopherol in ethanol;

2.5 mg vitamin A in ethanol;

65 mg soybean oil; and

30 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered are 8-11 and had an average density of 1.01 g/ml and an average particle size of 800 Å.

Example 8

The original mixture contains:

5 mg α-tocopherol in ethanol;

2 mg vitamin D_2 in ethanol;

2 mg vitamin K_1 complex in ethanol;

61 mg vitamin A acetate in ethanol; and

30 mg of egg phosphatidylcholine type III in ethanol.

The gradient fractions recovered are 6-10 and had an average density of 1.015 g/ml and an average particle size of 750 Å.

Example 9

The original mixture contains:

10 mg of BCNU (a nitrosourea, in particular Carmustine™, Merck Index) in 50% ethanol;

40 mg egg phosphatidylcholine type III in ethanol; and

50 mg soybean oil;

The gradient fractions recovered are 8-11 and had an average density of 1.010 g/ml and an average particle size of 900 Å.

Example 10

The original mixture contains:

5 mg visadine in ethanol;

50 mg egg phosphatidylcholine type III in ethanol;

40 mg soybean oil; and

5 mg of cephalin in ethanol.

The gradient fractions recovered are 8-11 and had an average density of 1.010 g/ml and an average particle size of 850 Å.

Example 11

Vitamin E Transfer from Microemulsion to Plasma

The microemulsion prepared in Example 1 containing pseudomicelles at a concentration of 5 mg/ml in water was treated with human and rabbit serum and the resulting mixtures were underlayered in a sodium chloride gradient. The diffusion of tocopherol in the mixtures was compared with the distribution of lipoproteins in the same mixtures and with the distribution of tocopherol from the untreated emulsion.

In more detail, 900 µl of human serum was incubated with 100 µl of the microemulsion of Example 1 for 1 hr at 37° in a shaker bath. For a control, 100 µl of the microemulsion were incubated with 900 µl of saline solution under the same conditions. Each mixture was underlayered in a sodium chloride gradient and separated by ultracentrifugation according to Foreman, et al, J Lipid Research (1977) 18:759-767. The fractions of various densities were collected and each fraction was assayed for the content of tocopherol, tocopherol acetate, and cholesterol. The results are shown in Figure 1. The microemulsion alone mixed with saline gives a single peak for tocopherol at a density of approximately 1.018 g/ml. The tocopherol from the microemulsion mixed with serum, however, distributes itself in a pattern which is similar to that of cholesterol, thus indicating its distribution among the HDL, LDL, and VLDL fractions. The treatment of microemulsion with serum effects the transfer of the tocopherol to serum in conjunction with the lipoprotein content thereof. Similar results are obtained with rabbit serum.

Example 12

Stability of Tocopherol Acetate

Nine hundred µl of normal human serum and 100 µl of the microemulsion of Example 1 were incubated at 37°C in a shaker bath and samples were withdrawn every 30 min up to 2.5 hr and assayed for α-tocopherol and tocopherol acetate. A similar assay was performed using heparinized serum. During this period of time, the ratio of α-tocopherol to tocopherol acetate was unchanged indicating that hydrolysis of the acetate to the free vitamin was extremely slow. The results were the same when heparin treated human serum was used. This is in contrast to the results in vivo as set forth below

Example 13

Apolipoprotein Transfer to the Microemulsion

The transfer of ApoA4, ApoA1, ApoE, and ApoCS, all apolipoproteins found in serum, to the microemulsion was studied by mixing various volumes of the microemulsion with a serum fraction having a density of 1.006 g/ml obtained from humans who had fasted for 12 hr. The serum fraction was prepared by ultracentrifugation from serum, spun at its own density (1.006 g/ml) overnight, and the samples taken from the bottom of the tubes.

The serum samples were incubated with approximately equal volumes of the microemulsions for 30 min at 37°C in a shaker bath and the microemulsion phase separated using ultracentrifugation over a sucrose gradient, as described in connection with the original preparation of the microemulsion. Briefly, sucrose was added to 2.5 ml of the mixtures to a final density of

1.060 g/ml, overlayered with 1.020 g/ml sucrose NaCl and then with saline. The tubes were centrifuged for 2 hr at 20,000 rpm, as described above, and the top fraction, containing the microemulsion, was separated using a tube-slicing technique. This fraction was dialyzed, delipidated with ethanol:ether 3:1 at -20°C overnight, then with ether alone. The precipitated proteins were solubilized in sample buffer containing SDS and 2-mercaptoethanol and subjected to SDS-PAGE. The resulting gels, stained with Coomassie blue, showed the presence in the microemulsion fraction of significant amounts of ApoA4, ApoAl, ApoE, and ApoCS.

Example 14

Transfer of Vitamin E from the Microemulsion to Plasma in Vivo

A. Short Term Results

Fifteen ml of the preparation of Example 1 were injected intravenously to New Zealand male rabbits and the levels of α-tocopherol and tocopherol acetate in the bloodstream were determined periodically over a period of 4 hr. The rabbits were then sacrificed and the tissues analyzed for their content of tocopherol and tocopherol acetate.

The results of the serum analysis showed that after 5 min, the levels of tocopherol in the blood had risen to approximately 40 µg/ml or approximately 10 times normal. The concentration then slowly decreased linearly to about 20 µg/ml over a period of 4 hr. The α-tocopherol acetate after 5 min was at a level of 280 µg/ml or about 70 times the normal α-tocopherol level, and then decreased slowly, finally disappearing from the circulation by the end of the 4 hr period.

The serum samples were also layered under a sodium chloride gradient and centrifuged. The distribution of the injected α-tocopherol and tocopherol acetate among the lipoprotein fractions was assessed. While the tocopherol acetate remained mainly at the density of the microemulsion alone, vitamin E was transferred, apparently to the LDL fraction, in exchange for cholesterol.

Analysis of the tissues showed accumulation of α-tocopherol in the liver, and to a lesser extent in the spleen. After 4 hr no α-tocopherol acetate could be detected in liver, spleen, and other tissues.

B. Long Term Results

New Zealand male rabbits were injected daily with 1 ml doses of the preparation of Example 1 for 30 days. Serum samples taken at 1 week intervals showed elevated levels of α-tocopherol and no change in cholesterol concentration. No tocopherol acetate was detectable in any of the samples.

In other experiments, rats were injected IV with 1 ml of the preparation of Example 1. The animals were sacrificed at 15 minute intervals for the first hour, after two hours, and then after 1, 3, 5, and 10 days. The levels of α-tocopherol and tocopherol acetate were assayed in the sera and liver extracts.

The serum levels of tocopherol acetate rose abruptly but declined almost immediately to normal. The α-tocopherol levels in serum rose to a lesser extent and then remained slightly above the control level over a period of about ten days.

Figure 3 shows the levels of tocopherol and tocopherol acetate in liver extracts. The concentration of tocopherol acetate in liver rose dramatically but

declined almost to normal levels before the end of a two hour period. Lagging, and corresponding to this decrease, was a peak in the tocopherol level in the liver indicating that the tocopherol acetate was completely hydrolyzed after approximately two hours. The tocopherol then disappeared from the liver after about one day, presumably causing the rise in the plasma levels at that time.

These results show that tocopherol acetate can be used as a source of tocopherol in vivo. Hydrolysis is apparently carried out efficiently by the liver. Therefore microemulsions using tocopherol acetate as active ingredient can be used as a source of vitamin E. The dosage level can thus be elevated by a factor of approximately 10 over that which would have been possible had it been necessary to use tocopherol per se.

Example 15

Distribution of Tocopherol in Tissues

The microemulsion preparation of the invention was used to administer vitamin E to rats for the purpose of studying the tissue distribution of vitamin E. The composition of Example 1 was administered to male Simonsen albino rats (Simonsen Laboratories, Gilroy, CA) in both experimental (vitamin E deficient) and control diets. The concentrations of α-tocopherol, α-tocopherol acetate, triglycerides, and cholesterol were determined in serum, and the concentrations of vitamin E were determined in liver, spleen, and adipose tissue.

In vitamin E-deficient rats, the α-tocopherol levels rose in serum immediately after injection and then decreased to normal after the first day. Thereafter, the level decreased to lower than normal,

but stayed above the original deficient level. In the controls, the α-tocopherol level rises but then drops after 2-4 hr to remain at a normal level. Cholesterol and triglycerides do not change significantly in either groups of rats over the experimental period.

The concentration of vitamin E acetate in the livers of both control and experimental rats rose immediately from zero µg/g of liver to approximately 66 µg/g 15 min after injection. However, vitamin E acetate was cleared from the liver in both groups after 2 hr. The concentration of vitamin E in the liver of deficient rats arose from approximately 1.7 µg/g at the time of injection to a peak of 122 µg/g after 2 hr. Thereafter, the concentration decreased to a level of 4.6 µg/g after 96 hr. In control rats the vitamin E level rose from approximately 7 µg/g at the time of injection to a peak of 105 µg/g after 2 hr and then decreased again to normal levels after 96 hr.

The concentration of vitamin E in spleen of rats fed normal diets rose from approximately 9 µg/g at the time of injection to a peak level of 23 µg/g after 1/2 hr, which then diminished rapidly to approximately 12-13 µg/g, a level which was maintained over the experimental period of 96 hr. In vitamin E-deficient rats, the original level of 0.7 µg/g rose to a peak of 12 µg/g after 24 hr, and then rapidly diminished to 5 µg/g after 96 hr.

In fat, the vitamin E deficient rats showed no increase in vitamin E in their fat tissues until approximately 24 hr; the levels rose from an unmeasurable quantity at the time of injection to 1.3 µg/g after 24 hr, and increased to 4.2 µg/g after 96 hr. In normal rats, the level at injection of 13.7

µg/g slowly increased to reach 30 µg/g after 24 hr and 37 µg/g after 96 hr.

Thus, vitamin E acetate is hydrolyzed rapidly and disappears from the plasma and the liver only a few hours after injection. The liver is a site for temporary storage, whereas the adipose tissue appears to accumulate the vitamin.

Example 16

Stability

The microemulsions were assessed for particle size and density as well as composition over a 3 month period. No significant changes in these parameters were noted.

Example 17

Microfluidization

Compositions containing tocopherol and tocopherol acetate as a microemulsion were also prepared using microfluidization rather than sonication to create the pseudomicelles. To prepare the composition, 22.5 mg of tocopherol, 116 mg of tocopherol acetate, and 94 mg of egg yolk phosphatidyl choline were dissolved in 25 ml ethanol, and the solution subjected to rotoevaporation. The dried solids were resuspended into 25 ml water by mixing immediately before pouring the suspension into the Microfluidizer.

The pump of the Microfluidizer was powered by compressed air at 80 psi, translating into a pressure at the outlet for the microemulsion of 11,000 psi. This is based on a multiplier coefficient of 140 for the SC Hydraulic Pump, Model No. 10-500-8.

The suspension was circulated through the Microfluidizer for 8 min at room temperature, and the

resulting microemulsion was collected and stored at 4°C. Determination of the composition of the microemulsion by HPLC showed it to contain 432 µg/ml tocopherol, 2800 µg/ml tocopherol acetate, and 2190 µg/ml egg phosphatidyl choline, representing a weight ratio of 8:52:40 of tocopherol:hydrophobic lipid:amphipatic substance.

The particle size was determined by laser light-scattering analysis using a Submicron Particle Analyzer Model N-4MD from Coulter Electronics Incorporated. Ninety percent of the particles had a mean size value of 1,000 ± 300 Å.

Example 18

Effectiveness of the Microfluidized Preparation

Rats made vitamin E deficient by a vitamin E-free diet over a three-month period were injected by the preparation of Example 17 by injection into the femoral vein. The composition was injected in amounts of 1 ml, 0.75 ml, 0.5 ml, 0.25 ml, and 0.125 ml.

The levels of tocopherol in serum were determined by HPLC at various time periods after injection. The serum levels rose above the 6 µg/ml normal level for all dosages except for 0.125 ml. Dosage levels 0.5 ml or greater were able to maintain the tocopherol level in the serum at above or normal level over a two-day period. The levels were reduced below that level in subsequent days of the experiment, but did not return to zero over an 11-day period.

When multiple injections of dosage containing 1 ml, 0.5 ml, and 0.25 ml were used over 5 successive days, the levels of tocopherol increased linearly during injection and then decreased during the 5th day.

Example 19

Administration of Vitamin A

Vitamin A was administered using the microemulsion of the invention containing vitamin E as an enhancer of absorption. The microemulsion was prepared according to the method of Examples 1-10 using sonication. The original mixture contained 5 mg α-tocopherol, 40 mg vitamin A acetate, and 55 mg egg lecithin, which were dissolved in ethanol, dried in a rotary evaporator, and taken up in 5 ml phosphate-buffered saline. The resulting cloudy suspension was sonicated for 30 min at 55°C. The resulting suspension was centrifuged over a sucrose gradient, as described above, except that 16 fractions were collected and fractions 7-10 used as the microemulsion composition. These fractions had an average particle diameter of 700 Å and a composition of 5% α-tocopherol, 38% vitamin A acetate, and 57% phospholipid. The concentration of pseudomicelles in the aqueous suspension was 5 mg/ml.

Example 20

Administration of Vitamin A with a Vitamin E Enhancer

In Vitro

Fifty µl of the vitamin A microemulsion was incubated with 4,950 µl or serum at 37°C in a shaker bath. Five hundred µl samples were removed and frozen at various times, and assayed for the presence of vitamin A and vitamin A acetate.

Unlike tocopherol, vitamin A acetate is apparently readily hydrolyzed in serum. The initial levels of more than 14 µg/ml of vitamin A acetate is reduced to zero after 24 hr with a concomitant rise in

vitamin A to 3 µg/ml. Since vitamin A is relatively unstable in serum, the final concentration does not equate to the original concentration of vitamin A acetate.

In Vivo

Normal and vitamin A deficient rats were injected intravenously with 1 ml of the vitamin A microemulsion prepared in Example 19. Determination was made of the amount of vitamin A, vitamin A acetate, and vitamin A palmitate in serum and tissue. It is believed that vitamin A is converted to vitamin A palmitate in the liver.

For both control and vitamin A deficient rats, the vitamin A acetate disappeared within 1 hr from plasma, and vitamin A increased to reach a peak at 0.5 hr after infusion, then decreased slowly to return to the normal level after 1 day. Vitamin A palmitate reached a peak in serum 1 hr after injection and then disappeared from circulation after 4 days. Liver extracts showed an immediate increase in vitamin A palmitate, increasing to a maximum after about 48 hr, followed by a return to normal levels in the control rats. Similar increases occurred in the liver of vitamin A deficient rats.

The adipose tissue of both control and experimental rats was also assayed for vitamin A and vitamin A palmitate. After an initial peak, the vitamin A content in both control and experimental animals decreased to levels of less than 1.5 µg/ml. The vitamin A palmitate content of the fat, however, was maintained over a period of 6 days at a level of 2.5 µg/g in the normal rat, and 5.5 µg/g in the vitamin A deficient rat.

Claims

1. A composition for the administration of fat-soluble active ingredient to an animal subject, which composition comprises:

a microemulsion comprised of pseudomicelles wherein 90% of said pseudomicelles have diameters in the range of 1000 ± 300 Å in diameter.

2. The composition of claim 1 wherein the pseudomicelles are comprised of a naturally occurring amphipatic substance and a hydrophobic lipid and include said active ingredient.

3. The composition of claim 2 wherein the amphipatic substance comprises a phospholipid.

4. The composition of claim 2 wherein the hydrophobic lipid is selected from tocopherol acetate, vitamin A acetate, and triglycerides.

5. The composition of claim 1 wherein the active ingredient is selected from tocopherol, tocopherol acetate, vitamin A, vitamin A acetate, vitamin D, and vitamin K.

6. The composition of claim 1 wherein the pseudomicelles were present in the microemulsion at 0.1-10 mg/ml.

7. A method to prepare the composition of claim 1 which comprises selecting microemulsions of 500-1,500 Å diameter pseudomicelles from an sonicated mixture of a naturally occurring amphipatic substance

and a hydrophobic lipid which includes an active ingredient.

8. A method to prepare the composition of claim 1 which comprises subjecting a mixture of hydrophobic lipid and an amphipatic substance, including said active ingredient, to microfluidization under conditions wherein pseudomicelles having the required diameters result.

9. A method of administering fat-soluble pharmaceuticals which comprises injecting a subject in need of such treatment with an effective amount of the composition of claim 1.

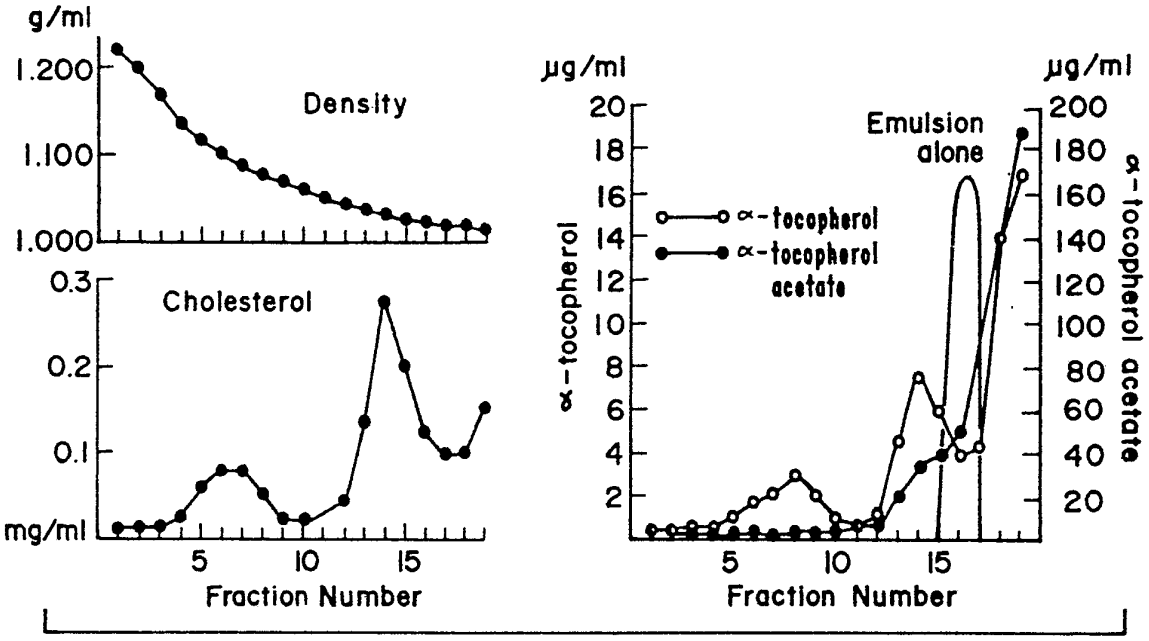

g/ml
1.200
1.100
1.000
Density
0.3
0.2
0.1
mg/ml
Cholesterol
5
10
15
Fraction Number
µg/ml
20
18
16
14
12
10
8
6
4
2
α-tocopherol
α-tocopherol
α-tocopherol acetate
Emulsion alone
µg/ml
200
180
160
140
120
100
80
60
40
20
α-tocopherol acetate
5
10
15
Fraction Number

FIG. 1

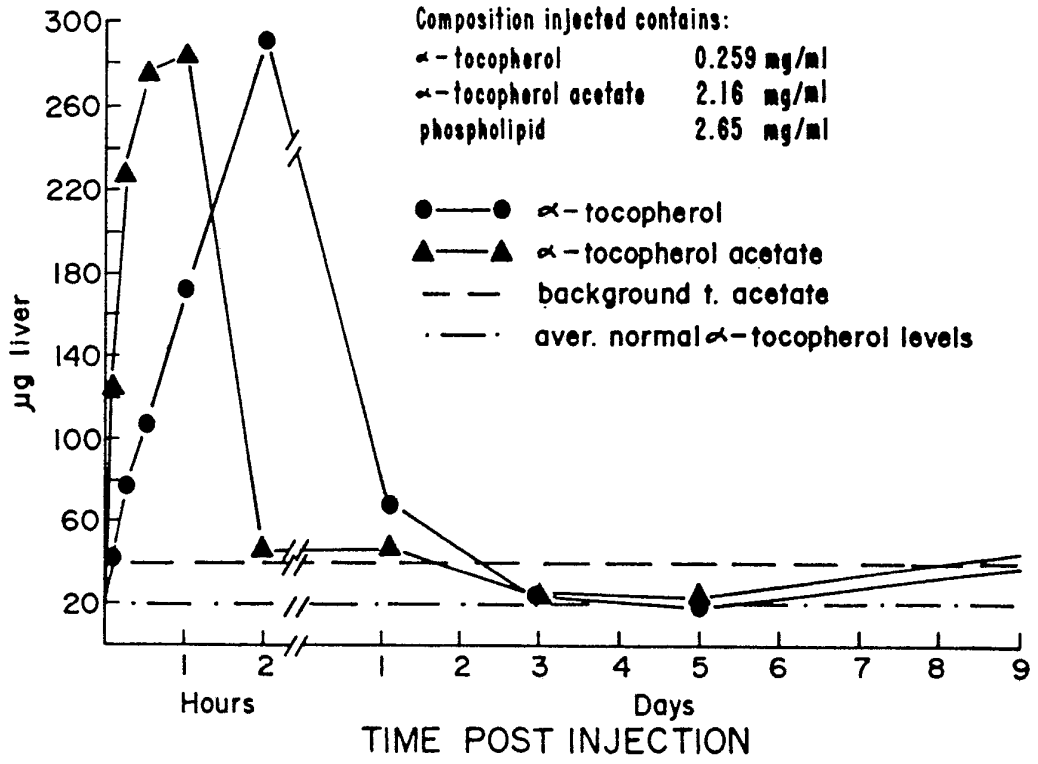

Composition injected contains:
α-tocopherol 0.259 mg/ml
α-tocopherol acetate 2.16 mg/ml
phospholipid 2.65 mg/ml
α-tocopherol
α-tocopherol acetate
background t. acetate
aver. normal α-tocopherol levels
µg liver
300
260
220
180
140
100
60
20
1
2
1
2
3
4
5
6
7
8
9
Hours
Days
TIME POST INJECTION

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US86/01671

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): A61K 31/12,31/59,31/175,31/225,31/355,31/595

U.S. CL: 514/167,514/168,514/458,514/548,514/589,514/681

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	514/167;514/168;514/458;514/548;514/589;514/681

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Cas-On-Line: tocopherol, vitamin A, vitamin D, vitamin K, pseudomicelles,micelles, soybean, lipid, lecithin

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	US,A 4,158,707 (STEFFEN) 19 June 1979, See column 2, lines 50-56 and claims 1 and 6.	1-9
X	US,A, 4,271,196 (SCHMIDT) 02 June 1981, See column 2, lines 29-35 and column 3, lines 18-20.	1-9
X	US,A, 4,328,222 (SCHMIDT) 04 May 1982, See column 2, lines 29-35 and column 3, lines 18-20.	1-9
X	JP,B, 57-163313 (NIPPON SHINYAKU KK) 07 October 1982, See attached abstract of entire document.	1-9

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21 October 1986	29 OCT 1986
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	D.W. Robinson

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
X	Chemical Abstracts, Volume 98, No. 12, published 21 March 1983 (Columbus, Ohio, USA) Daigo Nutritive Chemicals, Ltd. "Fat-soluble vitamin compound intravenous injections" See page 375, column 2, the abstract No. 95647b, Jpn Kokai Tokkyo Koho JP 57,209,216, 22 December 1982.	1-9
X	Chemical Abstracts, Volume 100, No. 18, published 30 April 1984 (Columbus, Ohio, USA) Eisai Co., Ltd. "Stable pharmaceutical and cosmetic emulsion preparations" See page 367, column 1, abstract No. 145017d, Jpn. Kokai Tokkyo JP 59 10,511, 20 January 1984.	1-9
A	US,A, 4,377,567 (GEHO) 22 March 1983, See entire document.	1-9
A	US,A, 3,384,545 (AIELLO) 21 May 1968, See entire document.	1-9
A	The American Journal of Clinical Nutrition, Volume 27, published March 1974, (USA), J.C. Bauernfeind, "Vitamins A and E nutrition via intramuscular or oral route", See pages 234-253.	1-9
A	Pediatrics, Volume 73, published 1984, C.J. Bodenstein, "Intravenous Vitamin E and Deaths in the Intensive Care Unit", See page 733.	1-9
A	N.Z.J. Agric. Res., Volume 11, published 1968, C. Caravaggi, "The Appearance of Tocopherol in the Blood of Sheep After Intramuscular injection of a-Tocopheryl Acetate", See pages 313-318	1-9

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate. of the relevant passages [17]	Relevant to Claim No [18]
A	Biochemica et Biophysica Acta. Volume 774, published 1984, A.N. Erin. "Formation of a-Tocopherol complexes with fatty acids -A Hypothetical Mechanism of Stabilization of Biomembrances by Vitamin E", See pages 96-102.	1-9
A	Journal of Parenteral and Enteral Nutrition, Volume 8, No. 3, published 1984, (USA), G.R. Gutcher, "Tocopherol Isomers in Intravenous Lipid Emulsion and Resultant Plasma Concentrations", See pages 269-273.	1-9
A	Journal of Lipid Research, Volume 23, published 1982, John C. Whitin, "The effect of vitamin E deficiency on some platelet membrane properties", See pages 276-282.	1-9
A	Journal of Lipid Research, Volume 17, published 1976, Loring K. Bjornson, "The transport of a-tocopherol and B-carotene in human blood", See pages 343-352	1-9
A	The American Journal of Clinical Nutrition, Volume 30, published May 1977, (USA), J.G. Bieri, "Factors affecting the exchange of tocopherol between red blood cells and plasma", See pages 686-690.	1-9
A	Clinica Chimica Acta, Volume 24, published 1969, T. Davies, "Interrelation of Serum Lipoprotein and Tocopherol Levels", See pages 431-436	1-9
A	The Journal of Biological Chemistry, Volume 256, No. 20, published 25 October 1981, (USA) Dennis J. Murphy, "Membrane Transfer of a-Tocopherol", See pages 10464-10468.	1-9

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
A	American Society of Clinical Pathologists, Volume 79, No. 6, published June 1983, Meir J. Stampfer, "Effect of Vitamin E on Lipids", See pages 714-716.	1-9
A	Pediat. Res., Volume 6, published 1972, Barbara A. Underwood, "Blood and Liver Concentration of Vitamins A and E in Children with Cystic Fibrosis of the Pancreas", See pages 26-31.	1-9
A	Nutrition Reports International, Volume 22, No. 3, published September 1980, David Kritchevsky, "Influence of Vitamin E Supplementation on Cholesterol Metabolish in Rats", See pages 339-342.	1-9
A	International Journal for Vitamin and Nutrition Research, Volume 41, No. 3, "Tissue Uptake and Metabolism of d,l-3,4-H_2-a-Tocopheryl Nicotinate and d,l-a-Tocopheryl-1', 2'-H_2-Acetate Following Intravenous Administration", See pages 339-354.	1-9
A	Biochemistry, Volume 23, No. 26, published 1984, G. Chi Chen, "Circular Dichroic Spectra of Apolipoprotein E in Model Complexes and Cholesterol-Rich Lipoproteins: Lipid Contribution", See pages 6530-6538	1-9

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Chemical Abstracts, Volume 95, No.16, published 19 October 1981 (Columbus, Ohio, USA) Teikiku Chemical Industry Co., Ltd., "Vitamin K injection solutions". See page 384, column 2, the abstract no. 138633b, Jpn. Kokai Tokkyo Koho 81 65,817, 03 June 1981.	1-9

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.