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(54) Title: SEAL OIL BASED LIPID EMULSIONS AND USES THEREOF

(57) Abstract: The present invention provides seal oil based lipid emulsions that are rich in long chain Ω-3 fatty acids. The present invention further provides methods of preparing the emulsions and for the use of the emulsions in clinical settings for a variety of applications, including total parental nutrition.

# SEAL OIL BASED LIPID EMULSIONS AND USES THEREOF

## FIELD OF THE INVENTION

The present invention pertains to the field of lipid emulsion systems and in particular to seal oil based lipid emulsions for parenteral administration.

## BACKGROUND

Lipid emulsions have been widely used as the basis of total parenteral nutrition (TPN) infusion solutions, providing a source of both calories and essential fatty acids for those patients unable to accept orally administered nutrition. For example, Intralipid® intravenous lipid emulsion was developed in Europe during the 1960's, and by 1975 was approved for use in the United States. The lipid emulsions currently available commercially for parenteral use are composed of a vegetable oil, such as soybean oil or safflower oil, an emulsifying agent such as egg phospholipids, glycerol, and water. Such commercially available emulsions include Liposyn® and Liposyn II® (Abbott Laboratories); Travemusion® (Baxter Healthcare); and Soyacal® (Alpha Therapeutics).

Progress in lipid biochemistry has established the biological essentiality of polyunsaturated Omega-3 (ω-3) and Omega-6 (ω-6) fatty acids. Fatty acids are characterised by the number of C atoms and double bonds they possess and by the position of the first double bond relative to the methyl end of the molecule. Thus, the designation C18:2 ω-6 indicates a fatty acid with 18 C atoms and 2 double bonds, the first double bond being at the sixth C atom from the methyl end. Polyunsaturated fatty acids are important as membrane building blocks and modulators of biochemical processes. The most biologically important ω-6 fatty acids are arachidonic acid (AA; C20:4) and its precursor, linoleic acid (C18:2). α-Linolenic acid is the parent substance of the long-chain ω-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), which are essential

fatty acids in humans. As precursors in the synthesis of the highly biologically active eiconaoids, AA and EPA influence inflammatory reactions (Burton JL. Lancet, 1989, 433-5) and immunological resistance (Endrers S, *et al.,* Eur J Clin Invest, 1995, 25:629-38), as well as cardiovascular diseases (Burr ML, *et al.,* Lancet, 1989, 2:757-61; Hu FB, *et al.,* JAMA, 2002, 287(14):1815-21), lipid metabolism disorders, thrombotic processes and neoplastic diseases (Jeeski LI, *et al.,* Pro Soc Exp Biol Med 1995, 210:227-33; Tevar R, *et al.,* J Parenter Enteral Nutr, 2002, 26(5):285-9; Barber MD, *et al.,* Nutr Cancer, 2001, 40(2):118-24; Senzaki H, *et al.,* World Rev Nutr Diet, 2001, 88:117-25). Clinical symptoms, such as lesions of the skin, cessation of growth, renal degeneration and increased metabolic rate have been associated with the deficiency of ω-6 fatty acids (Caldwell M.D., Human essential fatty acids deficiency: A review in fat emulsion in parenteral nutrition. Meng H.C. and Wilmore D.W. (eds.) Chicago, IL: *Amer. Med. Assoc.* P. 24, 1978).

Epidemiological studies, which show a correlation between the fat content of food and the occurrence of cardiovascular disease, led to the recognition that polyunsaturated fatty acids were prophylactically effective. In this context, the ω-3 fatty acids found in marine oils have proved to be particularly beneficial. Recently, the need to maintain a balance between ω-3 and ω-6 fatty acid consumption has been recognised to be necessary for the protective pharmacological effects of these fatty acids to be achieved. Ratios of ω-3 to ω-6 fatty acids of 1:2 to 1:4 have been recommended (Helmut G., *et al., JPEN*, 1994, 18:417-421).

As indicated above, the majority of commercially available lipid emulsions are derived from vegetable oils. The drawbacks associated with vegetable oil derived emulsions are two-fold. Firstly, vegetable oils lack ω-3 unsaturated fatty acids and secondly, vegetable oils contain significant amounts of vitamin K (for example, the vitamin K1 concentrations in 10% emulsions of Intralipid® and Liposyn II® were found to be 30.8 and 13.2 micrograms/dL, respectively). Vitamin K is one of the key factors in the blood coagulation process and thus may complicate surgical procedures, particularly for patients receiving anticoagulant therapy.

In response to current research indicating that supplementation of parenteral nutrition with ω-3 fatty acids elicits a variety of protective effects in certain clinical conditions,[10, 11] a fish oil based lipid emulsion, Omegaven® (Fresenius Kabi), has been developed and approved for use in European market. Omegaven® is a 10% fish oil emulsion with a high percentage of the ω-3 fatty acids, EPA and DHA. A number of biological benefits have been shown to be associated with the use of Omegaven® including increased $LTC_5$ production, reduced platelet aggregation, improved immunological functions and slowed oedema formation. Simultaneous intravenous administration of Omegaven® with a basic lipid emulsion such as Intralipid® also provides an optimal ratio of ω-3 to ω-6 fatty acids.

Fish oil, however, contains very low levels of the ω-3 fatty acid DPA. Like the polyunsaturated fatty acids EPA and DHA, DPA is important nutritionally, with about one-third of the long chain ω-3 fatty acids circulating in human blood being attributable to DPA. Recent research has demonstrated that EPA may actually be converted to DPA in the blood vessel walls and that DPA itself may be the effective agent under these circumstances. Although it is generally believed that EPA is the key factor in producing prostaglandin, which keeps the artery wall soft and free of plaque, this study indicated that DPA may be 10-20 times more powerful than EPA in this respect. DPA can thus act as a powerful anti-atherogenic factor (Schiefermeier M. & Yavin E., J Lipid Res, 2002, 43(1):124-31; Hansen JB, *et al.*, J Lipid Res, 2000, 41(9):1376-83).

Another potential drawback to the use of fish oil in lipid emulsions is that methods of extracting fish oil currently rely on steam or heat, which oxidises ω-3 fatty acids. High temperatures also have the potential to accelerate the isomerisation of ω-3 fatty acids from their natural *cis*-geometry to a *trans*-geometry. Both oxidised and *trans*-fatty acids are detrimental to human health.

A need remains, therefore, for a lipid emulsion containing a high percentage of the ω-3 fatty acids, EPA, DPA and DHA, a low amount of vitamin K and low proportions of oxidised and *trans*-fatty acids.

This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention.

## SUMMARY OF THE INVENTION

An object of the present invention is to provide seal oil based lipid emulsions and uses thereof. In accordance with an aspect of the present invention, there is provided a lipid emulsion comprising:

(a) 5–40% by weight of a purified seal oil;

(b) 0.5–5% by weight of an emulsifying agent, and

(c) water,

wherein the emulsion has a final pH of between 6.0 and 9.0.

In accordance with another aspect of the invention, there is provided a nutritive composition comprising:

(a) a lipid emulsion comprising 5–40% by weight of a purified seal oil, 0.5–5% by weight of an emulsifying agent and water, and

(b) one or more nutritional compounds,

wherein the composition has a final pH of between 6.0 and 9.0.

In accordance with another aspect of the invention, there is provided a nutritive composition comprising a lipid emulsion comprising 5–40% by weight of a purified seal oil, 0.5–5% by weight of an emulsifying agent and water, and one or more therapeutic agents.

In accordance with another aspect of the invention, there is provided a nutritive composition comprising a lipid emulsion comprising 5–40% by weight of a purified seal oil, 0.5–5% by weight of an emulsifying agent and water, and one or more diagnostic agents.

In accordance with another aspect of the invention, there is provided a use of a lipid emulsion comprising 5–40% by weight of a purified seal oil, 0.5–5% by weight of an emulsifying agent and water, for total parenteral nutrition in a subject in need thereof.

In accordance with another aspect of the invention, there is provided a use of a lipid emulsion comprising 5–40% by weight of a purified seal oil, 0.5–5% by weight of an emulsifying agent and water, as a delivery vehicle for therapeutic or diagnostic agents.

## BRIEF DESCRIPTION OF THE FIGURES

**Figure 1** depicts the effect of the lecithin to seal oil ratio on the globule size of a 10% seal oil emulsion.

**Figure 2** depicts the effect of pH on the zeta potential of a 10% seal oil emulsion.

**Figure 3** depicts the effect of pH on peroxidation of a 10% seal oil emulsion.

**Figure 4** depicts the effect of EDTA and/or Vitamin E on peroxidation of a 10% seal oil emulsion.

**Figure 5** depicts the effect of EDTA concentration on peroxidation of a 10% seal oil emulsion.

**Figure 6** depicts the peroxidation values for emulsions containing different oils. Diamond: Intralipid® made from soybean oil; Square: Omegaven® made from fish oil; Triangle: 10% seal oil emulsion.

**Figure 7** depicts the effect of autoclave time on peroxidation of a 10% seal oil emulsion.

**Figure 8** depicts the effect of autoclave time on pH of a 10% seal oil emulsion.

**Figure 9** depicts the effect of autoclave time on zeta potential of a 10% seal oil emulsion.

**Figure 10** depicts the effect of autoclave time on particle size of a 10% seal oil emulsion.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a lipid emulsion comprising seal oil as the core lipid for use in therapeutic applications, including total parenteral nutrition.

*Definitions*

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

As used herein, the term "about" refers to a +/-5% variation from the nominal value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to.

**Composition of the Lipid Emulsions**

In accordance with the present invention, the lipid emulsion comprises seal oil and an emulsifying agent dispersed in water. The emulsion may further comprise additional components such as, antioxidants, chelating agents, buffers, co-emulsifiers, osmolality modifiers, neutralisation agents and the like that improve the stability, uniformity and/or other properties of the emulsion.

The seal oil based lipid emulsion of the present invention are more stable and more resistant to oxidation than currently available fish oil based emulsions. In addition, the seal oil based emulsions contain the essential fatty acid docosapentaenoic acid (DPA), which is present only at low levels in fish oil based emulsions.

### *1. Seal Oil*

The lipid emulsions of the present invention comprise seal oil as the core lipid. A variety of seal species are suitable as sources of oil for the emulsions. In one embodiment of the present invention, the oil is derived from harp seals.

As mentioned above, currently available lipid emulsions for therapeutic applications contain either a vegetable-derived oil or a fish oil as the core lipid. Seal oil provides a number of advantages over fish or vegetable oils. For example, whereas most vegetable-derived oils have a low content of beneficial ω-3 fatty acids and a relatively high content of vitamin K, seal oil contains high levels of ω-3 fatty acids and low levels of Vitamin K. In addition, the ω-3 fatty acid content of seal oil is higher than that found in fish oils, for example, the ω-3 fatty acid content of harp seal oil is typically about 20–35%. Furthermore, most ω-3 fatty acids in seal oil occupy the 1- and 3- positions of the substituted triglyceride molecule, the same positions as in humans, whereas the ω-3 fatty acids in fish oil mainly occupy the 2-position. The substitution pattern of seal oil, therefore, can provide for better absorption of the administered lipids within the emulsion by the recipients, as the seal oil lipids more closely resemble endogenous lipids.

In terms of the ω-3 fatty acid content, seal oil contains a higher ratio of docosahexaenoic acid (DHA) to eicosapentaenoic acid (EPA) than fish oil, as well as lower levels of undesirable monounsaturated and saturated fatty acids. Moreover, seal oil contains significant amounts of the essential fatty acid docosapentaenoic acid (DPA), which is present at very low levels in fish oils. Together with making seal oil well-suited for therapeutic purposes, the above properties confer on seal oil superior fluidic characteristics, making it less likely to form semi-solids that could clog blood vessels.

In accordance with the present invention, the seal oil for use as the core lipid of the emulsion is highly purified and contains a high concentration of polyunsaturated fatty acids, especially ω-3 fatty acids. The oil further contains a higher proportion of ω-3 fatty acids than ω-6 fatty acids.

The concentration of ω-3 fatty acids in seal oil is typically about 20–35% by total weight and the concentration of ω-6 fatty acids is typically about 2–10% by total weight. However, one skilled in the art will appreciate that the composition of the fatty acids of the oil will vary depending on the method of extraction and refinery.

In accordance with the present invention, the seal oil for use in the emulsions comprises greater than about 20% by total weight of ω-3 fatty acids. In one embodiment, the seal oil comprises greater than about 25% by total weight of ω-3 fatty acids. In another embodiment, the seal oil comprises greater than about 27% by total weight of ω-3 fatty acids.

In seal oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) comprise a high proportion of the ω-3 fatty acid content of the oil. For example, the concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) typically account for about 45–70% of total weight of ω-3 fatty acids in harp seal oil, whereas the linolenic acids account for about 0.5–1.5% of total weight of ω-3 fatty acids.

In accordance with the present invention, the ratio of DHA to EPA in the seal oil for use in the emulsions of the present invention is between about 1:1 and about 3.5:1.

The fatty acid content of the seal oil may also be in the form of esters of fatty acids such as esters of linoleic acid (18:2n6), arachidonic acid (20;4n6), linolenic acid (18:3n3), eicosapentaenoic acid or EPA (20:5n3) and docosahexaenoic acid or DHA (22:6n3), as well as those of other saturated fatty acids such as myristic acid (14:00), palmitic acid (16:00) and stearic acid (18:00). Seal oils typically have a low content of free fatty acid esters (between about 0.2 and 0.9 ml/g) in terms of the acid value (according to United States Pharmacopoeia 1993). One skilled in the art will appreciate that the amount of fatty acid esters present in the oil is dependent on the process employed in the refining of the oil.

In accordance with the present invention, the free fatty acid content of the seal oil used in the emulsions is less than about 0.5 ml/g. In one embodiment, the free fatty acid content is less than about 0.4 ml/g. In another embodiment, the free fatty acid content is less than about 0.3 ml/g (all values measured according to United States Pharmacopoeia 1993).

The quality of the oil can be assessed by parameters known in the art, for example, peroxide value, iodine value or fatty acid composition. Such parameters can be measured by a number of techniques known to one skilled in the art, for example, the

parameters can be measured by gas chromatography (see also analytical procedures described in the United States Pharmacopoeia XXI and XXII, and the British Pharmacopoeia 1993 and 1998).

In accordance with the present invention, the seal oil for use in the emulsions has a peroxide value between about 3 mEq/kg and about 35 mEq/kg and an average iodine value of above 60 mEq/kg. In one embodiment, the seal oil has an iodine value of between about 85 mEq/kg and about 100 mEq/kg.

Methods of extracting and refining oils are well-known in the art. For example, International Patent Application No. PCT/CA00/00028 describes a method of refining animal and vegetable oils using low heat. The use of low temperature methods minimises the amount of detrimental oxidised and trans-fatty acids that are present in the purified oil. Seal oil additionally tends to be more resistant to natural oxidative processes than fish oils, with preliminary experiments demonstrating that that the extent of oxidisation of ω-3 polyunsaturated fatty acids in seal oil *in vitro* was less than half that observed in fish oils. In accordance with the present invention, the seal oil has a low content of oxidised and *trans*-fatty acids.

In one embodiment of the present invention, the seal oil is extracted and refined by a low temperature process to minimise oxidation. In another embodiment, the temperature of the process is maintained at or below 22°C.

The lipid emulsions of the present invention typically comprise between about 5% and about 40% (w/v) of seal oil. In one embodiment, the emulsion comprises between about 5% and about 30% (w/v) of seal oil. In another embodiment, the emulsion comprises between about 5% and about 20% (w/v) of seal oil. In another embodiment, the emulsion comprises between about 5% and about 15% (w/v) of seal oil. In another embodiment, the emulsion comprises about 10% (w/v) of seal oil.

### *2. Emulsifying Agent*

To prepare the lipid emulsions in accordance with the present invention, one or more emulsifying agents are mixed with the seal oil component. Emulsifying agents for this purpose are generally phospholipids of natural, synthetic or semi-synthetic origin. A

variety of suitable emulsifying agents are known in the art. Examples of suitable emulsifying agents include, but are not limited to, egg phosphatidylcholine, egg lecithin, L-α-dipalmitoyl phosphatidylcholine (DPPC), DL-α-dipalmitoyl phosphatidylethanolamine (DPPE), dioleoyl phosphatidylcholine (DOPC) and soy lecithin. In accordance with the present invention, the total concentration of triglycerides as well as free fatty acids in the emulsifier should be low in order to minimise the contribution to the total oil concentration of the emulsion. In one embodiment of the present invention, the total concentration of triglycerides as well as free fatty acids in the emulsifier is less than about 3.5%.

In one embodiment of the present invention, lecithin is used as the emulsifying agent in the lipid emulsions. In another embodiment, egg lecithin is used as the emulsifying agent. In another embodiment, egg lecithin containing 80-85% phosphatidyl choline and less than about 3.5% of fat is used as an emulsifying agent. One skilled in the art will appreciate that other components may be present in the egg lecithin without adversely affecting the emulsifying properties. For example, the egg lecithin may contain one or more of phosphatidyl ethanolamine, lysophosphatidyl choline, lysophosphatidyl ethanolamine, sphingomeylin and other natural components.

The lipid emulsions according to the present invention typically contain between about 0.5% and about 5% (w/v) emulsifying agent. In one embodiment of the present invention, the emulsion contains between about 0.6% and about 2% (w/v) emulsifying agent. In another embodiment, the emulsion contains between about 0.8% and about 1.8% (w/v) emulsifying agent. In another embodiment, the emulsion contains between about 1.0% and about 1.5% (w/v) emulsifying agent. In another embodiment, the emulsion contains between about 1.2% (w/v) emulsifying agent.

The ratio of lecithin to seal oil in the emulsion is important in determining the size of the oil globules formed within the emulsion. In accordance with the present invention, the globule size in the emulsion is below 1 μm. The ratio of lecithin to seal oil is therefore between about 1:4 and about 1:20. In one embodiment of the present invention, the ratio is between about 1:4 and about 1:18. In another embodiment, the ratio is between about 1:4 and about 1:15. In another embodiment, the ratio is between about 1:4 and about 1:10.

### 3. *Other Additives*

The lipid emulsion in accordance with the present invention can further comprise additional components such as, antioxidants, chelating agents, osmolality modifiers, buffers, neutralisation agents and the like that improve the stability, uniformity and/or other properties of the emulsion.

The present invention contemplates addition of one or more antioxidants to the lipid emulsion in order to help prevent the formation of undesirable oxidised fatty acids. Suitable antioxidants that can be added to the lipid emulsions include, but are not limited to, alpha-tocopherol (vitamin E) and tocotrienols. As is known in the art, tocotrienols are a natural blend of tocotrienols and vitamin E extract concentrated from rice bran oil distillate, which have an antioxidant activity similar to that of alpha-tocopherol (vitamin E). Tocotrienols have a similar structure to vitamin E and contain three double bonds in the carbon side chain of the molecule.

When used, the concentration of antioxidant added to the emulsion is typically between about 0.002 and about 1.0% (w/v). In one embodiment, the concentration of antioxidant used in the emulsion is between about 0.02% and about 0.5% (w/v).

In one embodiment of the present invention, tocotrienols are added to the emulsion as an antioxidant. In another embodiment, about 0.5% (w/v) tocotrienols are added to the emulsion. In still another embodiment, vitamin E is added to the emulsion as an antioxidant. In another embodiment, about 0.02% (w/v) vitamin E is added to the emulsion.

The emulsion can further comprise a chelating agent to improve the stability of the emulsion and reduce the formation of oxidised fatty acids. Suitable chelating agents are known in the art and are those that are generally recognised as safe (GRAS) compounds. Examples include, but are not limited to, EDTA. In one embodiment of the present invention, the emulsion comprises EDTA. In another embodiment, the emulsion comprises concentrations of EDTA between about $1 \times 10^{-6}$ M and $5 \times 10^{-5}$ M.

An osmolality modifier can also be incorporated into the emulsion to adjust the osmolality of the emulsion to a value suitable for parenteral administration. Amounts and types of osmolality modifiers for use in parenteral emulsions are well-known in the art. An example of a suitable osmolality modifier is glycerol. The concentration of osmolality modifier typically ranges from about 2% to about 5% (w/v). In one embodiment of the present invention, the amount of osmolality modifier added to the emulsion is between about 2% and about 4%. In another embodiment, the amount of osmolality modifier added to the emulsion is between about 2% and about 3%. In another embodiment, about 2.25% (w/v) glycerol is added to the emulsion as an osmolality modifier.

One skilled in the art will understand that the pH of the emulsion can be adjusted through the use of buffers or neutralisation agents. Emulsions with pH values close to physiological pH or above have been shown to be less prone to fatty acid peroxidation. One skilled in the art will appreciate that the pH of the emulsions can be adjusted through the use of an appropriate base that neutralises the negative charge on the fatty acids, through the use of an appropriate buffer, or a combination thereof. A variety of bases and buffers are suitable for use with the emulsions of the present invention. One skilled in the art will appreciate that the addition of buffer to the emulsion will affect not only on the final pH, but also the ionic strength of the emulsion. High ionic strengths may negatively impact the zeta potential of the emulsion (*i.e.* the surface charge of the oil globules) and are, therefore, not desirable. Selection of an appropriate buffer strength to provide a suitable pH and zeta potential as defined herein is considered to be within the ordinary skills of a worker in the art.

In one embodiment of the present invention, the pH of the emulsion is adjusted using sodium hydroxide. In another embodiment, the pH is adjusted with a buffer. In another embodiment, the buffer is a phosphate buffer. In another embodiment, both sodium hydroxide and a phosphate buffer are added to the emulsion.

The final pH of the emulsion is typically between about 6.0 and about 9.0. In one embodiment of the present invention, the pH of the emulsion is between about 7.0 and about 8.5. In another embodiment, the pH of emulsion is between about 7.0 and about 8.0.

The lipid emulsion can further comprise components for adjusting the stability of the emulsion, for example, amino acids or carbohydrates, such as fructose or glucose. The lipid emulsion can also be formulated to include nutrients such as glucose, amino acids, vitamins, or other parenteral nutritional supplements. The formulation of the lipid emulsion to incorporate a therapeutic agent is also considered to be within the scope of the present invention. A "therapeutic agent" as used herein refers to a physiologically or pharmacologically active substance that produces a localised or systemic effect or effects in animals and refers generally to drugs, nutritional supplements, vitamins, minerals, enzymes, hormones, proteins, polypeptides, antigens and other therapeutically or diagnostically useful compounds.

**Preparation of the Lipid Emulsions**

The lipid emulsions in accordance with the present invention can be prepared by a number of conventional techniques known to those skilled in the art. In general, the core lipid is first mixed with the emulsifier and the antioxidant, if one is being used. The emulsion is then prepared by slowly adding this oil phase into water with constant agitation. If an osmolality modifier is being used, it is added to the water prior to mixture with the oil phase. The pH can be adjusted at this stage, if necessary, and the final volume adjusted with water, if required.

The size of the oil globules of the emulsion (*i.e.* the particle size) is an important parameter with respect to therapeutic effects and the quality of the emulsion. Since lipid particles are removed from the systemic circulation in a manner similar to chylomicrons, the size of lipid particles in the emulsion need to remain within or below the size range of the naturally occurring chylomicron, which is 0.4-1.0 μm. If the particle size is larger than this, the lipid particles may be deposited in the liver, spleen and lungs resulting in significant fat load following infusion (Rahui C.M., *et al., Am. J. Hosp. Pharm.* 1992, 49:2749-2755). Lipids with small particle sizes disperse better in the emulsion and tend to produce safer and more stable emulsions. The lipid emulsions in accordance with the present invention typically have a particle size that is smaller and more uniform than commercially available lipid emulsions. Appropriate particle sizes are those with an average diameter below 1 μm, typically

between about 0.05 μm and about 0.5 μm. In one embodiment of the present invention, the average particle size is between about 100 nm and about 500 nm. In another embodiment, the average particle size is between about 200 nm and about 400 nm. In other embodiments, the average particle size is between about 250 nm and about 350 nm and between about 300 nm and about 350 nm. One skilled in the art will understand that the particle sizes obtainable will be dependent on the composition of the formulation as well as the preparation procedure.

Lipid emulsions with an appropriate average particle size (*i.e.* less than 1 μm in diameter) can be prepared by a number of techniques known in the art. For example, the dispersion prepared from addition of the oil phase into water, as described above, can be submitted to homogenisation, sonication or microfluidization. A variety of suitable mechanical devices to achieve the preparation of emulsions are commercially available. In one embodiment of the present invention, the dispersion is passed one or more times through a homogenizer. In another embodiment, a pressure of 20,000-27,000 Psi is used to create the emulsion using a homogenizer. In another embodiment, a pressure of 180,000 kPa is used to create the emulsion using a homogenizer. One skilled in the art will appreciate that the particle size of an emulsion prepared in this manner is associated with the oil content, concentration of the emulsifiers, as well as the pressure of, and the number of passages through, the homogenizer. Selection of the appropriate conditions foe the preparation of the emulsions according to the present invention is considered to be within the ordinary skills of a worker in the art.

At this stage, the resultant emulsion can be filtered through an appropriate filter, for example, a 0.45 μm membrane, if required. Alternatively, the emulsion can be sterilised by autoclaving. The emulsions comprising seal oil according to the present invention have been found to be stable to autoclaving for up to 60 minutes at 121°C.

The final emulsion is generally maintained in sterile conditions and can be packaged in a hermetically sealed container for short or long-term storage.

The preparation of the lipid emulsion under an inert atmosphere to minimise the occurrence of lipid oxidation during the process is also contemplated by the present invention.

In one embodiment of the present invention, the lipid emulsion comprises 5–20 % seal oil, 0.5–5% egg lecithin containing 80–85% phosphatide, 0.002–0.5% tocotrienols or alpha-tocopherol, 2–5% glycerol and water. In another embodiment, the seal oil contains 20 – 35% ω-3 fatty acids. In another embodiment, the seal oil has been refined using a process that maintains the temperature at or below 22°C. In another embodiment, the average particle size of the emulsion is in the range from about 250nm to about 350nm. In still another embodiment, the emulsion has a final pH between about 7 and about 8.

**Stability of the Lipid Emulsion**

Minimisation of lipid oxidation is particularly important in lipid emulsions as oxidation leads to the development of rancidity and the generation of potentially toxic molecules. As is known in the art, the size of the oil globules, the concentration of the oil, the nature of the emulsifiers, and the presence of antioxidants are important determining factors in the amount of lipid oxidation that takes place in emulsions.

The stability of the lipid emulsions can be determined by a number of tests known in the art and described herein and elsewhere including, but not limited to, visual examination of the emulsion for creaming and / or separation, accelerated temperature testing, high speed centrifugation, measurement of particle size, and distribution and alteration of zeta-potential. The oxidative status of seal oil can be pre-evaluated by measuring the peroxide value and thus determining the quality of the emulsion. Methods of determining the peroxide content of oils are known in the art (for example see the analytical procedures described in the United States Pharmacopoeia XXI, or the British Pharmacopoeia 1993). Seal oil typically has low peroxide values of about 4 mEq/kg to about 32 mEq/kg depending on the manufacturing process. Thus, in one embodiment of the present invention the seal oil has a peroxide value less than 35

mEq/kg. In another embodiment, the seal oil has a peroxide value in the range from about 5 mEq/kg to about 30 mEq/kg.

The oxidation level of the lipid emulsion can also be determined by measuring the peroxide value, by monitoring the oxygen consumption or by detecting lipid peroxides.

**Toxicity Testing**

The toxicity of the lipid emulsions can be tested *in vitro* and *in vivo* using standard techniques known in the art (see, for example, *Current Protocols in Toxicology,* Maines, M., *et al.* (ed.), J. Wiley & Sons, Inc., New York, NY).

For example, the emulsions can be tested *in vitro* for the presence of endotoxin using the Limulus Amebocyte Lysate (LAL) blood clot test (Marilyn J. Gould, Nephrology News & Issues, November 1988, pp26). Alternatively, or in addition, the emulsions can be tested *in vivo* using standard acute toxicity tests in which the emulsions are administered to a suitable test animal (typically a mouse or a rat). After an appropriate amount of time, the animals are sacrificed and tissue from the major organs is collected and analysed. The results are compared to a control group of animals to which a placebo or a standard product was administered.

**Testing the Lipid Emulsions**

A number of standard clinical tests known to one skilled in the art can be used to determine the efficacy of the lipid emulsions according to the present invention in the context of total parenteral nutrition, or for therapeutic applications. For example, the ability of the lipid emulsions to be taken up by the body can be determined by standard bioequivalency studies (see, for example, *Current Protocols in Toxicology,* Maines, M., *et al.* (ed.), J. Wiley & Sons, Inc., New York, NY). In these studies the disappearance of the fatty acids from the bloodstream of an appropriate animal model subsequent to infusion with the lipid emulsion of the present invention, or a control emulsion (typically a commercially available vegetable or fish oil based emulsion) is monitored. The fatty acid profile of the liver lipids can also be measured by standard

procedures and the distribution of the fatty acids determined by examining the histopathology of, for example, the liver, kidneys, heart, lungs, spleen, or thymus.

The therapeutic effects of the lipid emulsions according to the present invention can be measured in an appropriate animal disease model. For example, the burnt mouse model is frequently used to test the therapeutic benefit of lipid emulsions [see, Hayashi, *Nutrition,* **17**:211 (2001)]. In this case the lipid emulsion is administered to the mice and various physiological parameters are monitored, for example, the blood and urine levels of nitrogen, or the regeneration of certain proteins, such as blood albumin.

Other bioavailability studies and toxicity tests known in the art can also be carried out following short- or long-term schedules.

**Uses of the Lipid Emulsions**

The lipid emulsions in accordance with the present invention can be used as *cis*-ω-3 fatty acid enriched total parenteral nutrition (TPN) products. For this purpose, the lipid emulsions can be combined with ω-6 rich lipid emulsions, such as vegetable oil based emulsions, to provide optimal ω-3:ω-6 fatty acid ratios, if desired. In addition, the lipid emulsion in accordance with the present invention can be formulated as a nutritive composition to include one or more other nutrients such as glucose, amino acids, vitamins and/or other parenteral nutritional supplements by standard techniques.

As mentioned previously, ω-3 fatty acids are known to influence inflammatory reactions and immunological resistance, as well as cardiovascular diseases, fat metabolism disorders, thrombotic processes and neoplastic diseases. Therefore, the lipid emulsions in accordance with the present invention can also be used to benefit postsurgical and posttraumatic patients (particularly those who are under treatment with anti-coagulants), patients experiencing early stages of sepsis/systemic inflammatory response syndrome (SIRS), patients at risk of hyperinflammatory processes, patients whose immune functions need support, patients with inflammatory bowl diseases (Crohn's disease, ulcerative colitis), patients with inflammatory skin diseases (psoriasis, atopic eczema), patients with arthritis, patients with cardiovascular

diseases, patients with cancer, patients with pulmonary disease such as asthma and diabetic patients.

The use of the lipid emulsions to formulate compositions that incorporate one or more therapeutic or diagnostic compounds is also contemplated by the present invention. In this embodiment, the lipid emulsion acts as a delivery vehicle for the therapeutic or diagnostic compound(s) as well as providing beneficial effects as indicated above. Formulation of the lipid emulsions to contain one or more therapeutic or diagnostic compound can be readily achieved by a worker skilled in the art using standard techniques.

Methods of administering lipid emulsions to patients for TPN applications or therapeutic benefit are known in the art. Typically the emulsions are administered by infusion over a suitable period of time. Appropriate dosages and administration regimens can readily be determined by one skilled in the clinical arts.

**KITS**

The present invention additionally provides for kits containing the lipid emulsions for administration to a subject. The kit would provide an appropriate dosing regimen for a prescribed period.

The kits of the invention comprise one or more packages or containers containing the lipid emulsion in combination with a set of instructions, generally written instructions, relating to the use and dosage of the lipid emulsion. The kits may further comprise additional containers containing one or more nutrients or therapeutic or diagnostic compounds that may be added to the emulsion prior to administration. The packages containing the lipid emulsion may be in the form of unit doses or bulk packages (for example, multi-dose packages). The doses may be packaged in a format such that each dose is associated, for example, with a day of the week. There may also be associated with the kit a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of biological products, which notice reflects approval by the agency of manufacture, use or sale for human or animal administration.

To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

## EXAMPLES

### *EXAMPLE 1: Preparation of Lipid Emulsions containing Harp Seal Oil.*

Phospholipids, seal oil, glycerol, tocotrienols or alpha-tocopherol and cholesterol were mixed well by stirring for about 30 minutes at room temperature. Sterile water was added to the mixture and it was stirred for another 2-3 minutes. The entire mixture was then poured into the chamber of a homogenizer (EmulsiFelex®-C5, Avestin Inc., Ottawa, Canada) and passaged through the homogenizer at high pressure (20,000-27,000 psi) several times.

In one combination, seal oil 10%, egg lecithin 2.4%, tocotrienols 0.5%, glycerol 2.25%, and cholesterol 5%, the average size of lipid droplet was 452nm after 4 passages of the emulsion through the homogenizer under a pressure of 20,000 Psi. The particle size dropped to 340 nm after a single passage through the homogenizer under an increased pressure of 27,000 Psi. However, the particle sizes of 280nm could be obtained under a pressure of 20,000 Psi when the cholesterol was omitted from the combination.

### *EXAMPLE 2: Optimisation of the Emulsion Composition*

Since lipid emulsions are thermodynamically unstable systems, a number of factors need to be considered in order to prepare a stable and effective pharmaceutical product. These factors include the nature of the oil and emulsifier, the concentration of each component, and the methodology of formulation. However, testing all factors at various levels is impractical and, therefore, factorial or fractional factorial designs are often used. Factorial or fractional factorial designs provide an effective way to screen various factors and optimise the formulation of the emulsion, see Phan-Tan-Luu, R., and Didier, M. Experimental design in emulsion and suspension formulations:

theoretical aspects. P.465-535; and Neilloud, F., Marti-Mestres, G., Maillols, H. Application of experimental methodology to emulsions and suspensions. P. 535-557, Pharmaceutical Emulsions and Suspensions. Edited by Neilloud, F. and Marti-Mestres, G., Marcel Dekker Inc., 2000).

Depicted below is a sample of a full factorial design used to assess the consequence of varying certain factors of the lipid emulsion on the stability properties of a 10% seal oil emulsion.

The domain of variation of the three factors is shown in Table 1 below.

**Table 1.**

| *Number* | *Factors* | *Level 1 (w/v)* | *Level 2 (w/v)* |
|---|---|---|---|
| 1 | Seal Oil | 10.00% | 20.00% |
| 2 | Egg Lecithin | 1.20% | 2.40% |
| 3 | Cholesterol | 0.00% | 0.50% |

The response taken into account is the particle size of the emulsion in this sample. Other parameters of lipid oxidation as well as bioavailability data can also be assessed by this method.

Full factorial design ($2^3$) and response are shown in Table 2.

**Table 2.**

| *No.* | *Seal Oil %* | *Egg Lecithin %* | *Cholesterol %* | *Size nm* |
|---|---|---|---|---|
| 1 | 10 | 1.2 | 0 | 287±45 |
| 2 | 20 | 1.2 | 0 | 324±48 |
| 3 | 10 | 2.4 | 0 | 280±30 |
| 4 | 20 | 2.4 | 0 | 290±46 |

| *No.* | *Seal Oil %* | *Egg Lecithin %* | *Cholesterol %* | *Size nm* |
|---|---|---|---|---|
| 5 | 10 | 1.2 | 0.5 | 383±79 |
| 6 | 20 | 1.2 | 0.5 | 373±57 |
| 7 | 10 | 2.4 | 0.5 | 452±110 |
| 8 | 20 | 2.4 | 0.5 | 363±62 |
| Effect Matrix | -13 | 4.5 | 97.5 | |

Calculation of the matrix effect in this sample indicates that the presence and concentration of cholesterol is an important factor in determining the particle size of this type of emulsion.

***EXAMPLE 3: Adjustment of the Particle Size in the Lipid Emulsions***

Particle size of the emulsion is related to several factors as mentioned above. Control of particle size and maintenance of this size within a certain range have great influence on the stability of the emulsion and further determine the effectiveness and toxicity of the emulsion. An emulsion composed of the following combination: seal oil 10%, egg lecithin 2.4%, tocotrienols 0.5%, glycerol 2.25%, and cholesterol 5%, had an average lipid droplet size of 452 nm after 4 passages of the emulsion through a homogenizer under a pressure of 20,000 Psi. This lipid droplet size dropped to 340 nm after only one passage when the pressure was increased to 27,000 Psi. However, the particle size of the emulsion can reach 280 nm under a 20,000 Psi pressure if the cholesterol is omitted from this combination.

***EXAMPLE 4: Preparation of a 10% Seal Oil Emulsion***

Lecithin (12 g, Lipoid E80®, Lipoid GmbH, Ludwigshafen, Germany), glycerol (22.5 g, Sigma-Aldrich, Burlington, Ontario, Canada), seal oil (100 g, either from Caboto Seafood Ltd., Baie Verte, Newfoundland and Labrador, Canada or Seafreez Seafood

Ltd, Catalina, Newfoundland and Labrador, Canada), vitamin E (0.2 g, Sigma-Aldrich, Burlington, Ontario, Canada), 0.1 M NaOH solution (2.0 g, Sigma-Aldrich, Burlington, Ontario, Canada), $K_2HPO_4$ (0.38 g, Sigma-Aldrich, Burlington, Ontario, Canada), EDTA (0.75 mg, Sigma-Aldrich, Burlington, Ontario, Canada) and water for injection, USP (Baxter Canada Ltd., Mississauga, Ontario, Canada) to 1000 g, were stirred for 30 min. The emulsion was prepared by passing the mixture through a high pressured homogenizer 4-8 times (EmulsiFlex-C5, Avestin, Ottawa, Ontario, Canada). The pressure was maintained at 180,000 kPa. The product was sterilised by autoclave at 121 °C for 20 min and packaged in bottles or plastic bags under aseptic conditions.

***EXAMPLE 5: Compositional Analysis of a 10% Seal Oil Emulsion***

Gas chromatography (GC) and infrared spectrophotometry were used to analyse the composition of fatty acids in three different preparations of harp seal oils obtained from various suppliers. Briefly, the seal oil was hydrolysed into free fatty acids using NaOH and then transmethylated. The esterified fatty acid methyl esters were analysed on a HP-5964 capillary gas chromatographometer. Fatty acids standards purchased from Sigma were esterified using the same procedures. The fatty acid compositions of seal oil were quantified. (see Fidler N, *et al.,* J Lipid Res, 2000, 41(9):1376-83). The results are summarised in Table 3 below.

**Table 3**: Composition of Seal Oil Emulsion

| Fatty Acids | Common names | % | | |
|---|---|---|---|---|
| | | Sample 1 | Sample 2 | Sample 3 |
| 14:0 | Myristic acid | 4.063 | 4.367 | 8.664 |
| 14:1 | | 1.111 | 1.034 | 1.838 |
| 16:0 | Palmitic acid | 6.406 | 7.795 | 9.478 |
| 16:1 ω7 | | 17.581 | 14.341 | 16.632 |

| Fatty Acids | Common names | % | | |
|---|---|---|---|---|
| | | Sample 1 | Sample 2 | Sample 3 |
| 18:0 | Steric acid | 0.851 | 1.249 | 1.043 |
| 18:1 ω9 | | 22.029 | 20.377 | 16.88 |
| 18:1 ω7 | | 4.555 | 4.136 | 3.035 |
| 18:2 ω6 | Linoleic acid | 1.938 | 1.661 | 1.277 |
| 18:3 ω6 | gamma-linolenic acid | 0.226 | 0.739 | n/a |
| 18:3 ω3 | alpha-linolenic acid | 0.722 | 1.513 | 0.569 |
| 18:4 ω3 | | 1.509 | ? | 2.336 |
| 20:1 ω9 | | 11.902 | 12.403 | 13.642 |
| 20:4 ω6 | Arachidonic acid (AA) | 0.544 | 0.531 | n/a |
| 20:5 ω3 | Eicosapentaenoic acid (EPA) | 8.062 | 7.564 | 6.985 |
| 22:1 ω11 | | 2.292 | 3.563 | 5.629 |
| 22:1 ω9 | | 0.558 | 0.699 | 0.896 |
| 22:4 ω6 | | 0.0903 | n/a | n/a |
| 22:5 ω3 | Docosapentaenoic acid (DPA) | 4.829 | 5.452 | 3.573 |
| 22:6 ω3 | Docosahexaenoic acid (DHA) | 10.731 | 12.575 | 7.573 |

From Table 3, it can be seen that the compositions of long chain ω-3 polyunsaturated fatty acids in fish and seal oil are very different. Although fish oil contains EPA and

DHA, it has very small quantity of DPA. Fish oil also has a higher level of EPA than that of DHA.

***EXAMPLE 6: Acid Values of Seal Oil***

Acid values were determined according to the U.S. Pharmacopeia and are expressed as the number of mL of 0.1 N alkali required to neutralise the free fatty acids in 10.0 g of harp seal oil.

| | |
|---|---|
| Oil from Supplier #1: | 2.356 |
| Old harp oil from Supplier #2: | 2.262 |
| Beater (old) oil from Supplier #2: | 1.695 |
| Beater (young) oil from Supplier #2: | 0.986 |
| Bedlamer oil from Supplier #2: | 2.300 |

Beater: a young harp or grey seal in its first year of life which has completed its first moult to a soft spotted grey coat at an age of three to four weeks.

Bedlamer: a juvenile harp or grey seal from about one to five years of age which has a spotted coat. Harp seals in the early years of sexual maturity gradually develop the distinctive saddle or harp shaped markings.

***EXAMPLE 7: Analysis of Heavy Metal Content and PCBs***

ICP-Mass spectrophotometric analysis of trace metals (USEPA method #3050B), mercury (USEPA method #245.5) and polychlorinated biphenyls (PCBs) were done by the PSC Analytical Services, Bedford, NS, Canada.

Five oil samples and three lipid emulsion samples made of seal oil, fish oil (Omegaven®) and soybean oil (Intralipid®) were tested. All samples showed non-detectable levels of heavy metals and PCBs.

***EXAMPLE 8: Various Factors affecting the Quality of Seal Oil Emulsions***

Physical stability was evaluated by monitoring the size of oil globules in the emulsion. The test performed was under accelerated conditions, high speed centrifugation or at high temperatures. Emulsion samples (10 mL) were subjected to high speed centrifugation at 41,600 g at 15 °C for one week. Aliquots (50 μL) of the emulsion were collected each day for particle size analysis. In a separate set of experiments, emulsion samples (10 mL) were kept in a water bath at temperatures of 37, 45 and 60°C for 5 days. At each 24 hour interval, aliquots (50 μL) of samples were removed for particle size analysis.

The chemical stability was defined by the peroxidation value. The emulsion samples were kept in test tubes at 37 °C. Samples were collected on days 0, 1, 2, 3 and 4 and analysed for peroxidation value.

Emulsion, 0.2 mL, was added to 1.5 mL of isooctane/isopropanol (3:2, v/v). The mixture was vortexed for about 30 seconds and centrifuged at 2000 x g for 2 min. The top layer (20 μL) was collected, and mixed with 2.8 mL of methanol/1-butanol (2:1, v/v) and 15 μL of thiocyanate/$Fe^{2+}$ solution*. The mixture was then vortexed. Following incubation at room temperature for 20 min, absorbance at 510 nm was measured. The concentration of peroxide was determined from a standard curve obtained from a series of known concentrations of cumene hydroperoxide.

* The thiocyanate/$Fe^{2+}$ solution was prepared by mixing 3.94 M thiocyanate solution with 0.072 M $Fe^{2+}$ solution at 1:1 ratio.

*8.1. The effect of lecithin to seal oil ratio on globule size*

As stated earlier, globule size is one of the characteristics of emulsions. We found that the surfactant used (lecithin) affected the size of oil globules formed. As shown in Figure 1, as the ratio of lecithin to oil decreased, the globule size increased.

*8.2. The effect of pH on the surface charges*

The charge on the surface of globules is known to have an impact on the stability of the emulsion. A high charge keeps the globules repelled from each other. As a result, the globules will stay in the emulsion longer. Zeta potential is used to define the charge on the surface of globules. The zeta potentials of the seal oil emulsion at different pH conditions were measured and the results are shown in Figure 2. The results demonstrate that as the pH of the emulsion increases the zeta potential increases.

*8.3. The effect of pH on peroxidation*

Seal oil contains polyunsaturated fatty acids which are prone to peroxidation. Oxidized fatty acids have been implicated in cancer, cardiovascular diseases and inflammatory process. Peroxidation of fatty acids is a major concerns for oils containing unsaturated fatty acids. It is known that the extent and/or rate of peroxidation reactions can be influenced by many factors including pH. The effect of pH on peroxidation of the seal oil emulsion was evaluated and the results are shown in Figure 3. The results demonstrate that peroxidation was slower when the pH of the emulsion was ~7 or pH <3. More peroxidation reactions occurred in the pH range between 4 to 6.

Based on the results above, phosphate buffer was selected to control the pH of the emulsions at 7. The concentration of $K_2HPO_4$ used was 2.5 mM. At this pH, the zeta potential was found to be in the range of 35-42 mV. Although higher concentrations of phosphate buffer would increase the buffer capacity, it would also increase the ionic strength of solution, which could result in the rapid decrease of zeta potential which is not desirable.

*8.4 The effect of anti-oxidant and chelating agent on peroxidation*

Figure 4 shows the results of peroxidation of the seal oil emulsion in the absence and presence of vitamin E (as an antioxidant), EDTA, and Vitamin E/EDTA together. The results demonstrate that both EDTA and vitamin E decreased the peroxidation reactions significantly. Combination of EDTA and vitamin E was very effective. It has been reported that trace amounts of transition metal ions such as $Cu^{2+}$ and $Fe^{3+}$ are present in lecithin (from egg or soya). Those metal ions are known to be the excellent

catalysts for oxidation reactions. Chelating agents such as EDTA are believed to be able to form complexes with metal ions. As a result, reactions catalysed by the ions are inhibited. However, the presence of a large quantity of EDTA in a product to be infused to plasma of patients is not desirable. To find out the lowest effective concentration of EDTA in seal oil lipid emulsion, different amounts of EDTA were tested. Peroxidation reactions were found to be eliminated when the concentration of EDTA was higher than $2X10^{-6}$ M (Figure 5). This concentration was, therefore, chosen for all seal oil emulsion preparations. Concentration of vitamin E was 0.02% which is the same value as fish oil emulsion (Omegaven®).

***EXAMPLE 9: Characterisation of a 10% Seal Oil Emulsion***

*9.1. Particle size and zeta potential*

Uniform particle size is a desired characteristic of emulsions. Zeta potential is known to affect the stability of emulsions. Therefore, both particle size and zeta potential of the seal oil emulsions prepared using different compositions and under different conditions were evaluated.

The particle size and zeta potential of the 10% seal oil emulsion prepared according to Example 4 were analysed by a particle analyser, Delsa 440SX manufactured by Beckman Coulter, Fullerton, California, USA, and were found to be 320 ± 40 nm and 44.7 ± 15.3 mV, respectively. The pH of the emulsion determined by a pH meter was found to be 7.5 ± 0.5.

*9.2. Sterility Test*

Sterility was checked by the Medical Microbiology Laboratory of the Health Care Corporation of St. John's, Newfoundland and Labrador, Canada. Sterility tests confirmed that the product is free of bacterial contamination. Table 4 summarises the sterility check results of seal oil of different batches and 10% seal oil emulsions prepared according to Example 4 using those seal oil products.

**Table 4:** Sterility Test

| Description of seal oil | Sample tested | Autoclave time (minutes) | Sterility results (+ indicates not sterile) |
|---|---|---|---|
| extracted at 70 °C from seals of unknown age without chromatographic deordoration from Supplier #1 | seal oil as obtained | 0 | - |
| | 10% seal oil emulsion | 0 | - |
| | 10% seal oil emulsion | 10 | - |
| | 10% seal oil emulsion | 20 | - |
| Extracted at room temperature from very old harp seals with chromatographic deordoration from Supplier #2 | seal oil as obtained | 0 | + |
| | 10% seal oil emulsion | 0 | - |
| | 10% seal oil emulsion | 10 | - |
| | 10% seal oil emulsion | 20 | - |
| Extracted at room temperature from one year old harp seals with chromatographic deordoration from Supplier #2 | seal oil as obtained | 0 | + |
| | 10% seal oil emulsion | 0 | - |
| | 10% seal oil emulsion | 10 | - |
| | 10% seal oil emulsion | 20 | - |
| Extracted at room temperature from beaters with chromatographic deordoration from Supplier #2 | seal oil as obtained | 0 | + |
| | 10% seal oil emulsion | 0 | - |
| | 10% seal oil emulsion | 10 | - |

| Description of seal oil | Sample tested | Autoclave time (minutes) | Sterility results (+ indicates not sterile) |
|---|---|---|---|
| | 10% seal oil emulsion | 20 | - |
| Extracted at room temperature from Bedlamers with chromatographic deordoration from Supplier #2 | seal oil as obtained | 0 | + |
| | 10% seal oil emulsion | 0 | - |
| | 10% seal oil emulsion | 10 | - |
| | 10% seal oil emulsion | 20 | - |

*9.3. Endotoxin test: Limulus Amoebocyte Lysate test*

Endotoxin standard (0.2 ng/mL) and LAL kit were purchased from Associates of Cape Cod Incorporated, Cape Cod, Massachusetts, USA. Sterile water for injection, USP (10 mL/vial, Baxter Canada, Mississauga, Ontario, Canada) was used as negative control. The endotoxin standard (0.2 ng/mL) was diluted to 0.1, 0.05, 0.025 and 0.0125 ng/mL. The 10% seal oil emulsion of example 4 was also diluted to 2, 4, 8, 16 and 32 times. To conduct the test, 0.1 mL of negative control, endotoxin standards (0.2, 0.1, 0.05, 0.025 and 0.0125 ng/mL) or seal oil emulsion and its diluted samples were added to 0.1 mL of LAL kits. The samples were mixed well by shaking. The samples were incubated at 37±1 °C for 1 hour and examined. Presence of endotoxin is indicated by the formation of a gel.

*In vitro* endotoxin test (LAL assay) indicated that the seal oil emulsion is non-pyrogenic (<0.006 ng/mL or 10 EU/ng).

***EXAMPLE 10: Comparison of Chemical Stability of Seal Oil, Vegetable Oil and Fish Oil Emulsions***

The chemical stability (defined by extent of peroxidation) of a 10% seal oil emulsion (prepared as described in Example 4), commercially available vegetable oil emulsion

(Intralipid®) and a commercially available fish oil emulsion (Omegaven®) were tested according to the procedures described in Example 8 and compared. The results are shown in Figure 6 and demonstrate that the seal oil emulsion is much more stable than either the vegetable oil or fish oil emulsions. Both Intralipid® and Omegaven® are the trademarks of Fresenius-Kabi.

***EXAMPLE 11: Effect of Autoclaving on the Physical and Chemical Stability of Seal Oil Emulsions***

As seal oil emulsion formulations are intended for intravenous infusion, autoclave is required to inactivate bacteria and other pathogens. Since polyunsaturated fatty acids of seal oil are labile to high temperature and pressure, it is essential to re-examine the chemical and physical stability of autoclaved samples of the emulsion. Five seal oil emulsion preparations (20 mL each, seal oil harvested from different ages of seals and processed by different technologies and the emulsions prepared as described in Example 4) were subjected to autoclave conditions for 0, 10, 20, 30, 60 minutes at 121°C. Particle sizes, zeta potentials and the rates of peroxidation reactions of samples were examined (Figures 7, 8, 9 and 10). The results indicated that the measured parameters in all samples were not changed significantly. According to US Pharmacopeia, 30 minutes are required to autoclave an injectable preparation with volume of 500 mL. The autoclave conditions used in these experiments clearly demonstrated the stability of seal oil emulsion formulations.

***EXAMPLE 12: Comparison of Seal Oil and Fish Oil Emulsions***

The properties of a 10% seal oil emulsion prepared as described in Example 4 and a commercially available fish oil emulsion (Omegaven®) were compared.

**Table 5:** Comparison of Formulations

| Ingredients (per 100 grams) | Omegaven® (10% fish oil emulsion) | 10% seal oil emulsion |
|---|---|---|
| Total oil | 10 g | 10 g |
| EPA (C20:5 ω-3) | 1.25-2.82g | 0.70-0.81g |
| DPA (C22:5 ω-3) | N/A | 0.34-0.55g |
| DHA (C22:6 ω-3) | 1.44-3.09g | 0.75-1.3g |
| Lecithin | 1.2g | 1.2g |
| Glycerol | 2.5g | 2.5g |
| Vitamin E | 0.015-0.0295g | 0.02g |
| EDTA-$Na_2$ | 0 | 0.007g |

**Table 6:** Comparison of characteristics of Omegaven® and a 10% seal oil emulsion

| Characteristic | Omegaven® (10% fish oil emulsion) | 10% seal oil emulsion |
|---|---|---|
| Particle size (nm) | 330±24 | 320±40 |
| Zeta Potential (mV) | 48.3±17.2 | 44.7±15.3 |
| pH | 7.5 to 8.7 | 7.0 to 8.0 |
| Endotoxin Test (LAL) | - | - |
| Sterility | - | - |
| Osmolality (mosm/kg) | 308-376 | 320±20 (calculated) |
| Peroxidation values | 0.52 | 0.14 |

| Characteristic | Omegaven® (10% fish oil emulsion) | 10% seal oil emulsion |
|---|---|---|
| Expiry | One year after being manufactured | Two years after being manufactured |

***EXAMPLE 13: Acute Toxicity Testing***

SD rats were used and the rats were divided into two groups of three animals each. For a period of ten days the animals were infused with either seal oil or fish oil emulsion for one hour each day via the tail vein. The dose of seal oil or fish oil emulsion was 10 times higher (1 g/kg body weight per day) than the recommended daily dose of Omegaven® (0.1g/kg/day). A control group was treated with saline solution. After the ten-day infusion each animal was sacrificed and tissues including kidney, heart and liver were collected. These tissues were fixed and were submitted to pathological examination by the Pathology Lab of the Health Care Corporation of St. John's. All tissues (control, Omegaven® and seal oil emulsion fed) were similar in kidney, liver, GI tract and heart with no liver deposition of lipids. No acute toxicity was observed in the rat model.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

**THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1. A lipid emulsion comprising:
   (d) 5–40% by weight of a purified seal oil;
   (e) 0.5–5% by weight of an emulsifying agent, and
   (f) water,
   wherein said emulsion has a final pH of between 6.0 and 9.0.

2. The lipid emulsion according to claim 1 further comprising 2–5% by weight of an osmolarity modifier.

3. The lipid emulsion according to claim 2, wherein said osmolarity modifier is glycerol.

4. The lipid emulsion according to any one of claims 1, 2 or 3 further comprising 0.002–1.0% of an antioxidant.

5. The lipid emulsion according to any one of claims 1, 2, 3 or 4 further comprising between $1 \times 10^{-6}$ M and $5 \times 10^{-5}$ M EDTA.

6. The lipid emulsion according to any one of claims 1, 2, 3, 4 or 5, wherein said emulsifying agent is lecithin.

7. A nutritive composition comprising:
   (c) a lipid emulsion comprising 5–40% by weight of a purified seal oil, 0.5–5% by weight of an emulsifying agent and water, and
   (d) one or more nutritional compounds,
   wherein said composition has a final pH of between 6.0 and 9.0.

8. The nutritive composition according to claim 7 further comprising 2–5% by weight of an osmolarity modifier.

9. The nutritive composition according to claim 8, wherein said osmolarity modifier is glycerol.

10. The nutritive composition according to any one of claims 7, 8 or 9 further comprising 0.002–1.0% of an antioxidant.

11. The nutritive composition according to any one of claims 7, 8, 9 or 10 further comprising between 1 x $10^{-6}$ M and 5 x $10^{-5}$ M EDTA.

12. The nutritive composition according to any one of claims 7, 8, 9, 10 or 11, wherein said emulsifying agent is lecithin.

13. A composition comprising the lipid emulsion according to any one of claims 1, 2, 3, 4, 5 or 6 and one or more therapeutic compounds.

14. A composition comprising the lipid emulsion according to any one of claims 1, 2, 3, 4, 5 or 6 and one or more diagnostic compounds.

15. Use of the lipid emulsion according to any one of claims 1, 2, 3, 4, 5 or 6 to prepare a nutritive composition.

16. Use of the lipid emulsion according to any one of claims 1, 2, 3, 4, 5 or 6 to prepare a therapeutic composition for delivery of one or more therapeutic compounds to a subject in need thereof.

17. Use of the lipid emulsion according to any one of claims 1, 2, 3, 4, 5 or 6 to prepare a diagnostic composition for delivery of one or more diagnostic compounds to a subject in need thereof.

18. Use of the lipid emulsion according to any one of claims 1, 2, 3, 4, 5 or 6 for total parenteral nutrition in a subject in need thereof.

19. The use according to claim 18, wherein said emulsion is formulated for administration in combination with a second emulsion comprising vegetable oil.

20. Use of the nutritive composition according to any one of claims 7, 8, 9, 10, 11 or 12 for total parenteral nutrition in a subject in need thereof.

21. The use according to claim 20, wherein said composition is formulated for administration in combination with a second emulsion which comprises vegetable oil.

22. Use of the composition according to claim 13 for parenteral delivery of the one or more therapeutic compound to a subject in need thereof.

23. Use of the composition according to claim 14 for parenteral delivery of the one or more diagnostic compound to a subject in need thereof.

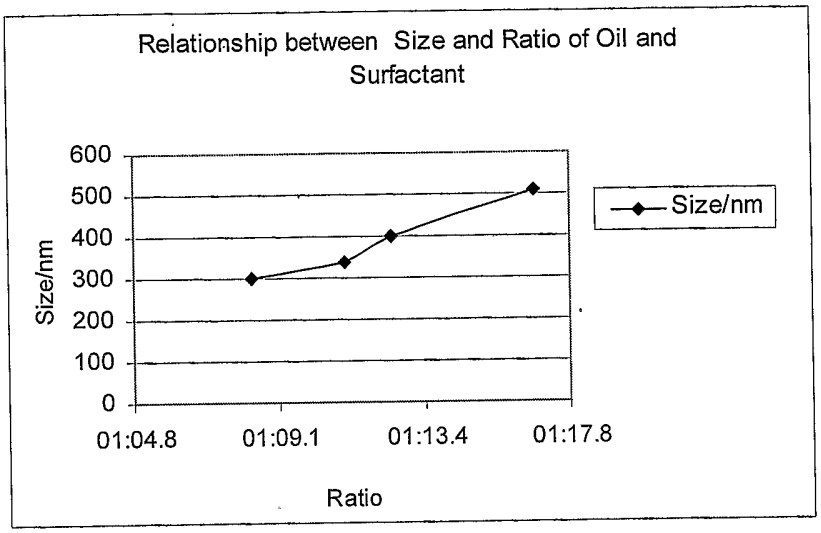
Relationship between Size and Ratio of Oil and Surfactant
600
500
400
300
200
100
0
Size/nm
01:04.8
01:09.1
01:13.4
01:17.8
Ratio
Size/nm

*Figure 1*

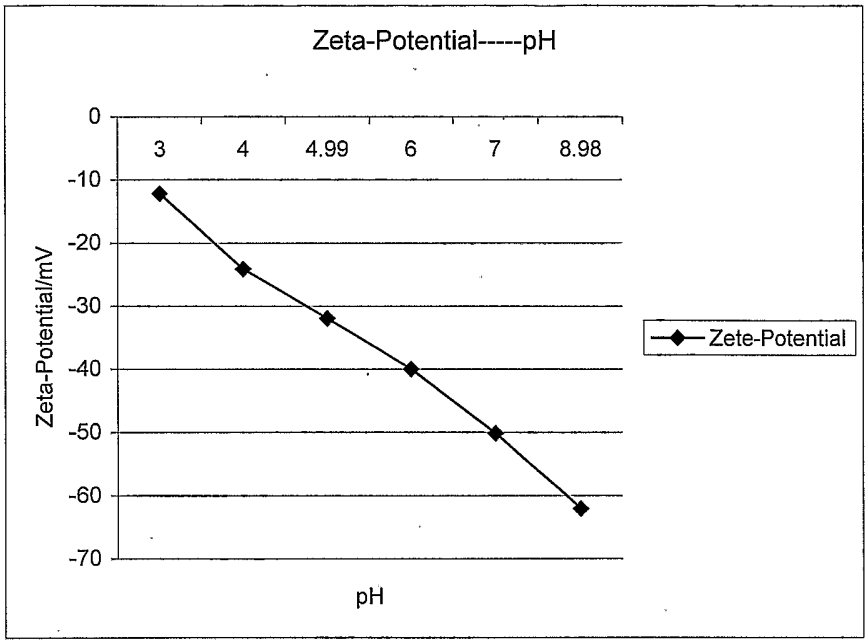
Zeta-Potential-----pH
0
-10
-20
-30
-40
-50
-60
-70
3
4
4.99
6
7
8.98
Zeta-Potential/mV
pH
Zete-Potential

*Figure 2*

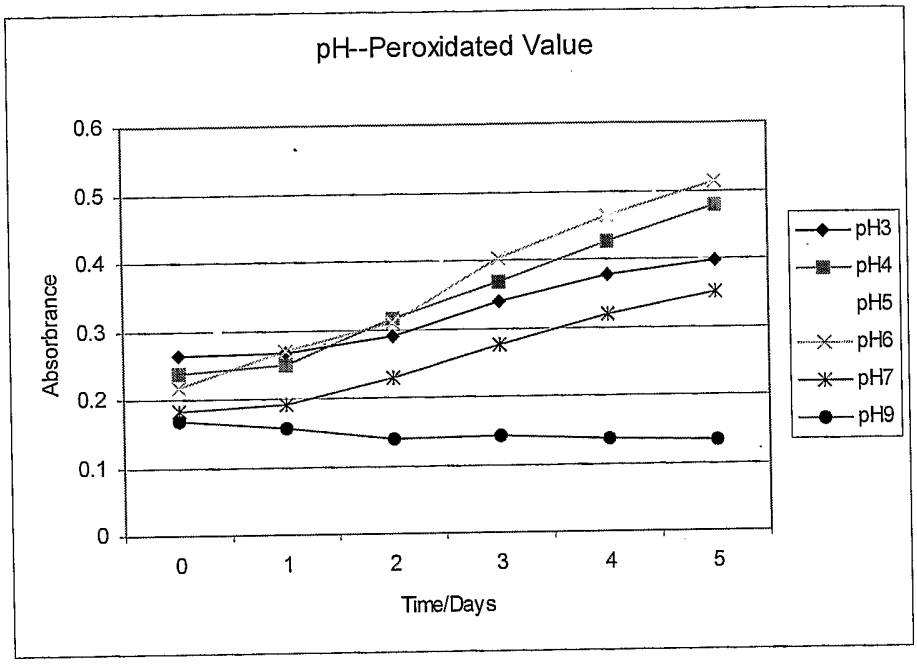
pH--Peroxidated Value
Absorbrance
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
Time/Days
pH3
pH4
pH5
pH6
pH7
pH9

*Figure 3*

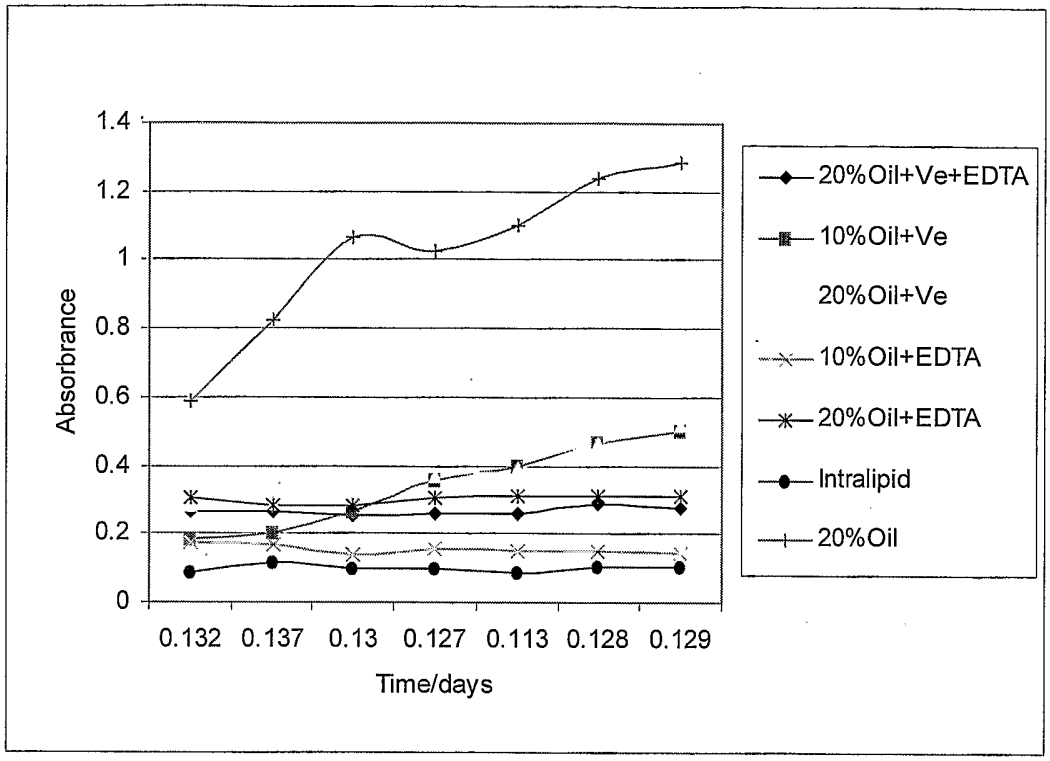
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbrance
0.132
0.137
0.13
0.127
0.113
0.128
0.129
Time/days
20%Oil+Ve+EDTA
10%Oil+Ve
20%Oil+Ve
10%Oil+EDTA
20%Oil+EDTA
Intralipid
20%Oil

*Figure 4*

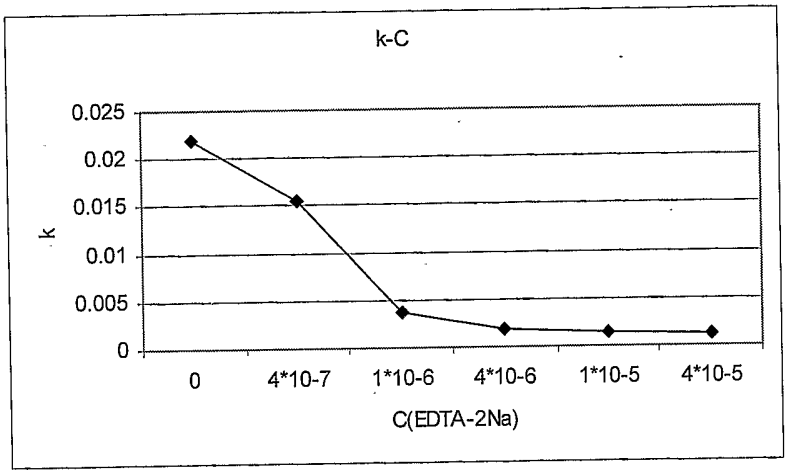
k-C
0.025
0.02
0.015
0.01
0.005
0
k
0
4*10-7
1*10-6
4*10-6
1*10-5
4*10-5
C(EDTA-2Na)

*Figure 5*

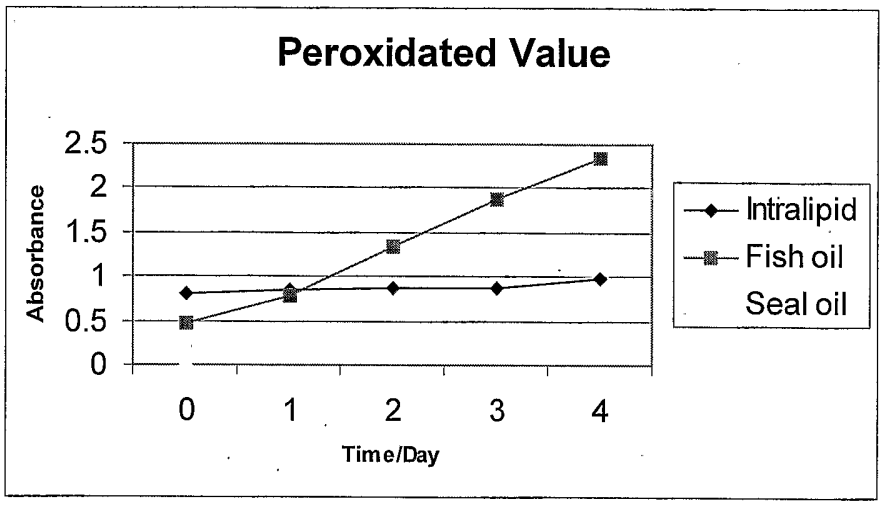
Peroxidated Value
Absorbance
2.5
2
1.5
1
0.5
0
0
1
2
3
4
Time/Day
Intralipid
Fish oil
Seal oil

*Figure 6*

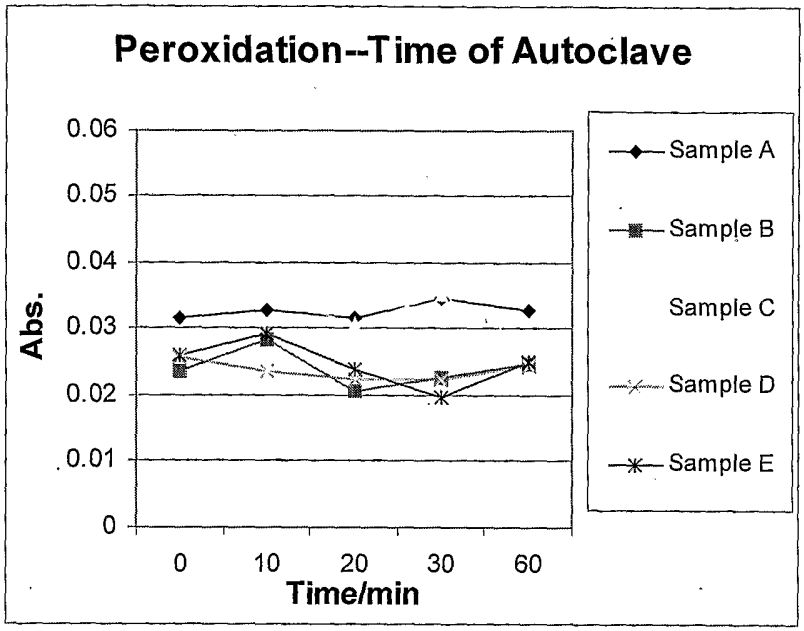
Peroxidation--Time of Autoclave
0.06
0.05
0.04
0.03
0.02
0.01
0
Abs.
0
10
20
30
60
Time/min
Sample A
Sample B
Sample C
Sample D
Sample E

*Figure 7*

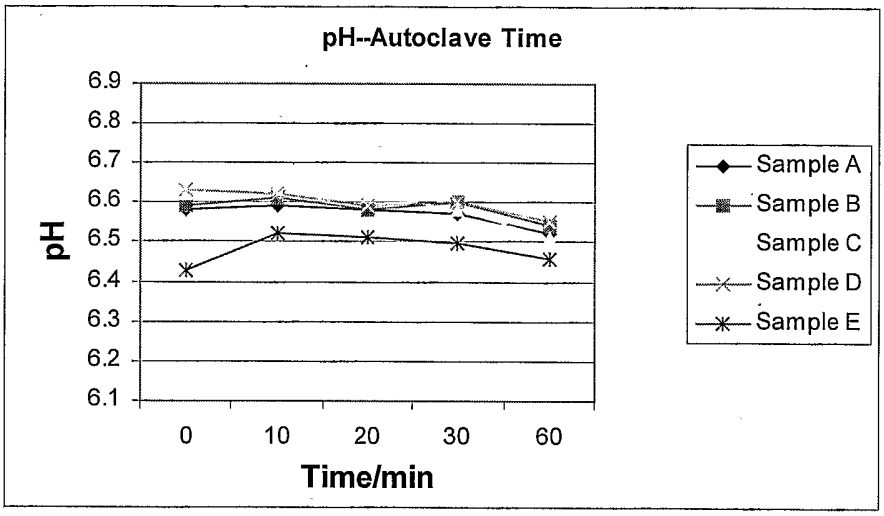
pH--Autoclave Time
6.9
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
pH
0
10
20
30
60
Time/min
Sample A
Sample B
Sample C
Sample D
Sample E

*Figure 8*

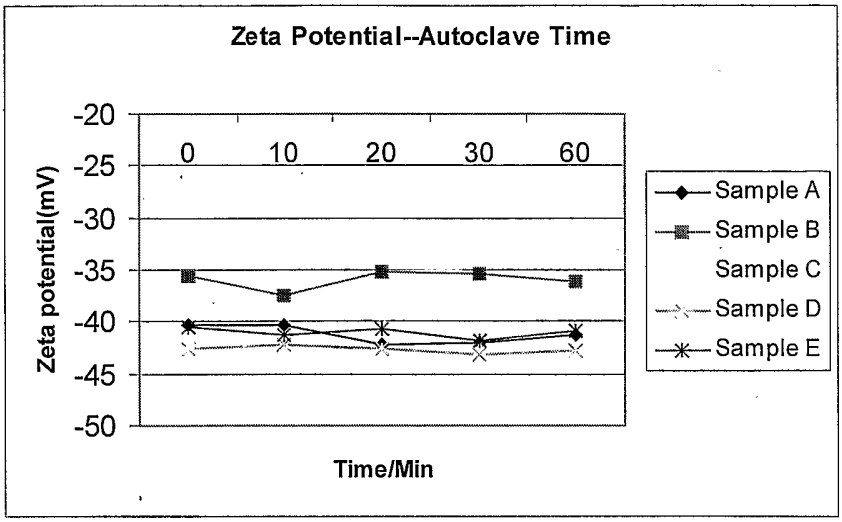
Zeta Potential--Autoclave Time
-20
-25
-30
-35
-40
-45
-50
0
10
20
30
60
Zeta potential(mV)
Time/Min
Sample A
Sample B
Sample C
Sample D
Sample E

*Figure 9*

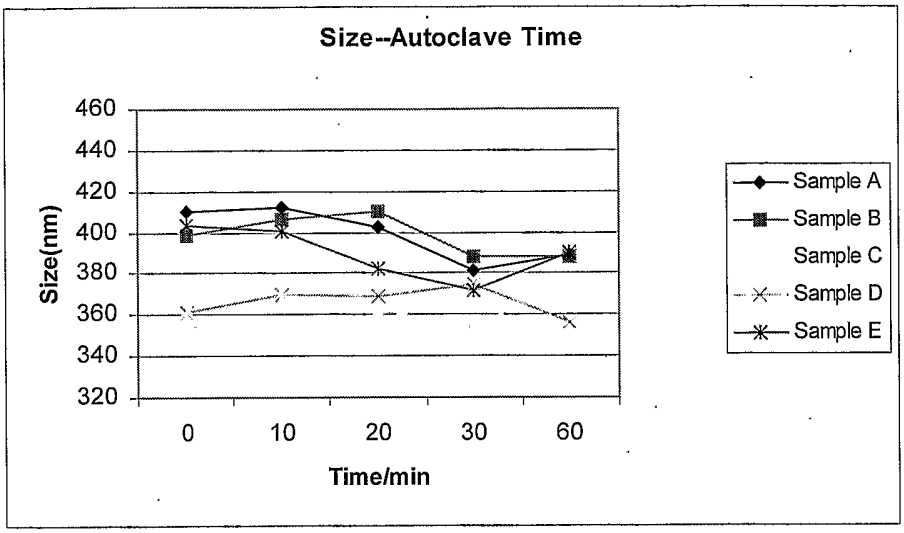
Size--Autoclave Time
460
440
420
400
380
360
340
320
Size(nm)
0
10
20
30
60
Time/min
Sample A
Sample B
Sample C
Sample D
Sample E

*Figure 10*

# INTERNATIONAL SEARCH REPORT

Ir :ional Application No
PCT/CA 02/01981

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23D7/00 A23L1/30 A61K35/12 A61K9/107

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23D A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 14 April 2003 | 06/05/2003 |

| Name and mailing address of the ISA | Authorized officer |
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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Groh, B |

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In tional Application No
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