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Preparation and physical characterization of a novel marine oil emulsion as a potential new formulation vehicle for lipid soluble drugs

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Abstract

Emulsions often contain vegetable oils such as soybean oil. In this study, a 10% (w/w) of marine mammal oil emulsion was prepared. The effect of a group of emulsifying agents on the stability of the 10% of seal oil emulsion was examined. The emulsifying agents studied were hydrogenated castor oil coated with various polyoxyethylene derivatives. It was found that 2.5% of HCO-40 resulted in the most stable seal oil emulsion. The size of the emulsified droplets defined by their diameters was found to be around 240–270 nm. The initial zeta-potential and pH value of the emulsion were found to be around −27 mV and 3.5, respectively, which decreased over time, to about −31 mV and 2.4, respectively. This is believed to be a result of the hydrolysis of triacylglycerides into free fatty acids in the emulsion. The effect of various amounts of Crodasinic LS-30, a negatively charged surfactant, and Incroqal Behenyl TMS, a positively charged surfactant, on the emulsion was investigated. It was shown that Crodasinic LS-30 had very little effect on the particle size, zeta-potential and pH, while the effect of Incroquat Benhenyl TMS was found to be dependent upon the concentration of the surfactant used.

Keywords: Seal oil emulsion; Zeta-potential; Particle size; Emulsifying agents; Hydrogenated castor oil; Crodasinic LS-30; Incroqal Behenyl TMS

1. Introduction

Emulsions are mixtures consisting of one (or more) immiscible liquid phase(s) dispersed in another. The size range for the dispersed droplets can be from 0.1 to 20 μm (Lance et al., 1995; Koh et al., 2000; Collins-Gold et al., 2000). Oil-in-water (O/W) emulsions have been widely used in pharmaceutical formulations such as total parenteral nutrition (TPN). They are also used as carriers for the delivery of water insoluble drugs. In addition, emulsions have the potential to achieve sustained drug release, and for site-specific drug delivery by binding ligands for various cell surface receptors to the particle surface (Prankerd and Stella, 1990; Kang et al., 2003). Oils used in most of the O/W emulsions are derived from plant sources such as soybean oil. In recent years, marine oils have attracted a lot of attention because of the beneficial health effects reported. Marine oils contain large quantities of long chain omega-3 polyunsaturated fatty acids (PUFAs). The interest in marine oils stemmed from the epidemiological studies of the diet of Greenland Eskimos, in which fish and seal meat were the important sources of dietary lipid. The incidence of cardiovascular disease (CVD) in Eskimos was found to be significantly lower than that of the Danish population, despite their high fat intakes (Dyerberg et al., 1978; Nobmann et al., 2005). It has been demonstrated that omega-3 PUFAs can lower serum triacylglycerides (de Lorgeril et al., 2005) and reduce cardiovascular risk factors (Conquer et al., 1999). In addition, omega-3 PUFAs are essential for the normal growth and development of brain and retina. Inadequate amounts of docosahexaenoic acid (DHA), one of the main PUFAs found in marine oils, have been linked to a wide variety of abnormalities ranging from reduced visual acuity and learning irregularities to depression and suicide (Alessandri et al., 2004; Stillwell et al., 2005). PUFAs also play an important role in the prevention and treatment of hypertension, arthritis, and inflammatory and autoimmune diseases (Morlion et al., 1996; Roulet et al., 1997; Mayser et al., 1998; Gadek et al., 1999; Lanza-Jacoby et al., 2001; Calder, 2004).

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Fish oil emulsions have been developed and marketed in some parts of the world and they are believed to be better in some patient populations due to the presence of the omega-3 PUFAs. Although fish oil contains eicosapentaenoic acid (EPA) and DHA, it has a very small quantity of docosapentaenoic acid (DPA).

The harp seal (*Phoca groenlandica*), a marine mammal found abundantly in the waters off the North Atlantic Ocean, is another source of omega-3 PUFAs. Seal oil is believed to be better than fish oil because of: more consistent contents of omega-3 PUFA, especially DPA; lower levels of contaminants; reduced susceptibility to chemical degradation. Furthermore, the omega-3 PUFAs in seal oil are found to be at the sn-1 and sn-3 positions of the triacyglycerides (Ikeda et al., 1998) which are believed to be better substrates for human pancreatic lipase and lipoprotein lipase since the lipases in human preferably hydrolyze triacyglycerides at sn-1 and sn-3 positions. On the other hand, the omega-3 PUFAs in fish oil are primarily at the sn-2 position of triacylglycerides. Therefore, the plasma clearance of fish oil made emulsions was shown to be slower compared to soybean oil made emulsions. Earlier data showed that omega-3 triacylglycerides from fish oil are poorly hydrolyzed in extracellular media and therefore are delivered to tissues as part of the core of emulsion remnants (Treskova et al., 1999). Unfortunately, the majority of currently processed seal oil has been used as lubricants in the auto and aerospace industries. Only a small portion of seal oil (about 10–15%) has been marketed as nutraceutical supplements for omega-3 fatty acids. Our group has investigated the use of seal oil emulsions as potential drug carriers. In previous studies, it was found that seal oil could serve as a vehicle to dissolve certain hydrophobic compounds (Xiao et al., 1999; Kang et al., 2003). In this report, we prepared a 10% seal oil emulsion and studied the effect of a group of hydrogenated castor oil (HCO) derivatives as emulsifying agents on the physical stability of the seal oil emulsion. In addition, the effect of Crodasinic LS-30, an example of negatively charged surfactants, and Incroqal Behenyl TMS, an example of positively charged surfactants, on the physical stability of the seal oil emulsion using HCO-40 as the emulsifying agent was also studied.

2. Materials and methods

Seal oil was provided as a gift from Caboto Sea Food Ltd., Baie Verte, Nfld, Canada. The composition of the fatty acids in the seal oil was analyzed using an HP-5890 capillary gas chromatograph (GC) equipped with a SupelcoWax10 capillary column. The seal oil triglycerides were converted to fatty acid methyl esters by transmethylation in methanol–sulfuric acid (94/6 v/v) in the presence of hydroquinone at 70 °C for 5 h. The methyl esters were extracted in hexane, dried and dissolved in carbon disulfide. Chromatograms were obtained using a helium carrier gas and detected by flame ionization. Identification and quantitation were done using fatty acid methyl ester standards obtained from Sigma–Aldrich Ltd., Burlington, Ont., Canada. HCO derivatives (hydrogenated caster oil coated with polyoxyethylene of various lengths), HCO-5, HCO-20, HCO-30, HCO-40, HCO-60, HCO-80 and HCO-100, were gifts from Nikko Chemicals Co. Ltd., Tokyo, Japan. Incroqal Behenyl TMS and Crodasinic LS-30 were kindly provided by Croda Inc., Cedex, France. All other chemicals were purchased from Sigma–Aldrich Canada Ltd., Burlington, Ont., Canada.

All seal oil emulsions consisted of 10% of seal oil (w/w) and 2.5% of the respective HCO derivatives or a combination of 2.5% of HCO-40 and Crodasinic LS-30 or Incroqal Behenyl TMS (0.1%, 0.25% or 0.5%). To prepare the respective seal oil emulsions, seal oil and the emulsifying agent or a mixture of emulsifying agents were thoroughly mixed with water. The mixture was passed in a high pressured homogenizer (C5-model, Avestin Inc., Ottawa, Ont., Canada) four times at 25,000 psi to form an emulsion. To examine the particle size, zeta-potential and pH value changes, the emulsion prepared was placed in a 55 ± 0.2 °C water bath and samples were removed at different time intervals. Particle size and zeta-potential were analyzed using a Beckman Coulter Delsa 440SX zeta-potential analyzer (Beckman Coulter Co., Fullerton, CA, USA). The pH value of the samples was determined using a pH meter. In addition, a 10% of mineral oil in water emulsion using 2.5% of HCO-40 as the emulsifying agent was prepared. The particle size, pH and zeta-potential changes were monitored as described above.

3. Results

The composition results of the fatty acids in the seal oil analyzed by GC are shown in Table 1. It was found that the amount of omega-3 fatty acids was about 25%.

Ten percent (10%, w/w) of seal oil emulsions containing 2.5% of HCO-5, HCO-20, HCO-30, HCO-40, HCO-60, HCO-80 or HCO-100, or a combination of 2.5% of HCO-40 and Crodasinic LS-30 or Incroqal Behenyl TMS (0.1%, 0.25% or 0.5%), respectively, were prepared. The emulsions were prepared using a high pressure homogenizer (also known as a microfluidizer) at

Table 1
Compositional analysis of fatty acids in seal oil

Fatty acids	Common names	Percentage
14:0	Myristatic acid	5.70 ± 2.57
14:1	Myristoleic acid	1.33 ± 0.44
16:0	Palmitic acid	7.89 ± 1.54
16:1 ω7	Palmitoleic acid	16.18 ± 1.67
18:0	Steric acid	1.05 ± 0.20
18:1 ω9	Oleic acid	19.76 ± 2.63
18:1 ω7		3.91 ± 0.79
18:2 ω6	Linoleic acid	1.63 ± 0.33
18:3 ω6	Gamma-linolenic acid	<1.0
18:3 ω3	Alpha-linolenic acid	<1.0
18:4 ω3		<1.0
20:1 ω9		12.65 ± 0.90
20:4 ω6	Arachidonic acid (AA)	<1.0
20:5 ω3	Eicosapentaenoic acid (EPA)	7.54 ± 0.54
22:1 ω11		3.83 ± 1.63
22:1 ω9		<1.0
22:4 ω6		<0.1
22:5 ω3	Docosapentaenoic acid (DPA)	4.62 ± 0.96
22:6 ω3	Docosahexaenoic acid (DHA)	10.29 ± 2.53

Note: values reported are mean ± S.D. ($n = 3$).