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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. ($n = 3$).

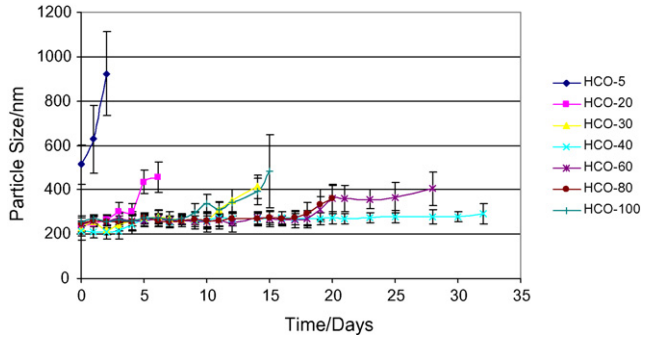

Fig. 1. The particle sizes of 10% (w/w) of seal oil emulsions prepared using 2.5% of HCO-5, HCO-20, HCO-30, HCO-40, HCO-60, HCO-80 and HCO-100, respectively, as the emulsifying agent. Samples were incubated at 55 ± 0.2 °C in a constantly shaking water bath. The particle size of the emulsions was analyzed daily for 32 days.

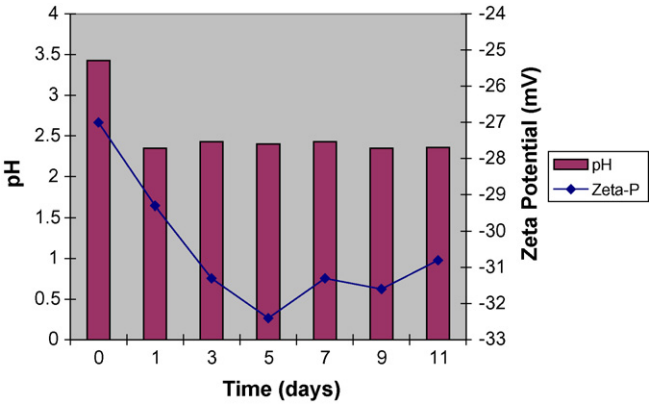

Fig. 2. The pH and zeta-potential changes in the 10% (w/w) seal oil emulsion prepared using 2.5% of HCO-40 as the emulsifying agent. The seal oil emulsion was incubated at 55 ± 0.2 °C in a constantly shaking water bath. The pH and zeta-potential of the emulsion samples were analyzed every other day for 11 days.

25,000 psi. After four passes, the mean diameters of the emulsion particles were found to be in the range of 240–270 nm.

It should be noted that the chain length of the polyoxyethylene in the HCO derivatives increases in the following order: HCO-5, HCO-20, HCO-30, HCO-40, HCO-60, HCO-80 and HCO-100. As a result, their corresponding HLB values are as follows: 6 (HCO-5), 10.5 (HCO-20), 11 (HCO-30), 12.5 (HCO-40), 14 (HCO-60), 15 (HCO-80) and 16.5 (HCO-100).

The particle size of the 10% of seal oil emulsions prepared using different emulsifying agents was monitored for 32 days following their preparation and the results are shown in Fig. 1. Mean particle sizes of 400 nm or higher were considered undesirable and used as an indication for physical instability. It is clear from Fig. 1 that HCO-5 (HLB = 6) was not an effective emulsifying agent as the particle size of the emulsion was found to be larger than 400 nm upon preparation and continued to increase over time. As the chain length of polyoxyethylene in the HCO derivatives increases (from HCO-5 to HCO-40), the stability of the emulsion produced was found to improve. However, as the chain length of polyoxyethylene further increases (HCO-60, HCO-80 and HCO-80), the stability of the emulsion was found to decrease. Consequently, HCO-40 was found to be the most effective emulsifying agent for the 10% of seal oil in water emulsion.

The zeta-potential and pH value of the 10% of seal oil emulsion using 2.5% of HCO-40 as the emulsifying agent were monitored for 11 days following preparation. The results are shown in Fig. 2. It was found that the initial zeta-potential was around −27 mV which decreased to about −31 mV over the course of 11 days. The initial pH value was 3.5 which gradually decreased to about 2.4 over the same time period. These changes are believed to be due to the hydrolysis of the triacylglycerides in the seal oil, which results in the production of negatively charged free fatty acids.

To evaluate the effect of charged surfactants on the stability of the 10% of seal oil emulsion prepared using 2.5% of HCO-40 as the emulsifying agent, various amounts (0.1%, 0.25% or 0.5%) of Crodasinic LS-30 or Incroquat Benhenyl TMS were added. The results are shown in Fig. 3. It was found that the negatively charged Crodasinic LS-30 had very little effect on the particle size of the emulsion. However, the effect of Incroquat Benhenyl TMS is shown to be dependent on its concentration. There was little effect demonstrated when 0.1% of Incroquat Benhenyl TMS was used, while higher concentrations (0.25% and 0.5%) had an adverse effect on the stability of the emulsion.

The zeta-potential and pH value of the 10% of seal oil emulsion using a combination of 2.5% of HCO-40 and various concentrations (0.1%, 0.25% or 0.5%) of Crodasinic LS-30 or Incroquat Benhenyl TMS were monitored for 23 days following preparation. The results are shown in Figs. 4 and 5. It was demonstrated that the negatively charged Crodasinic LS-30 had very little effect on the zeta-potential (∼−30 mV) while the positively charged Incroquat Benhenyl TMS showed significant impact on the zeta-potential of the emulsion produced. Zeta-potentials of the emulsions were found to have changed to about −14, +16 and +30, respectively, when 0.1%, 0.25% and 0.5% of Incroquat Benhenyl TMS was used. It was also found that the respective zeta-potentials decreased over time to

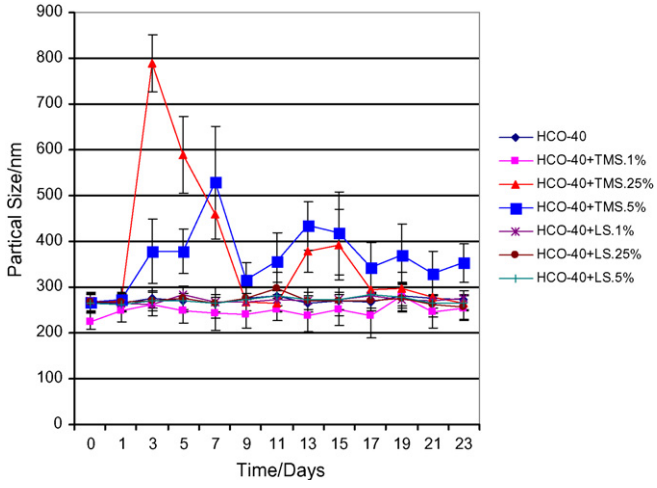

Fig. 3. The particle sizes of 10% (w/w) of seal oil emulsions prepared using 2.5% of HCO-40 with varying amounts of Incroquat Benhenyl TMS or Crodasinic LS-30. The seal oil emulsions were incubated at 55 ± 0.2 °C in a constantly shaking water bath. The pH and zeta-potential of the emulsion samples were analyzed every other day for 23 days.

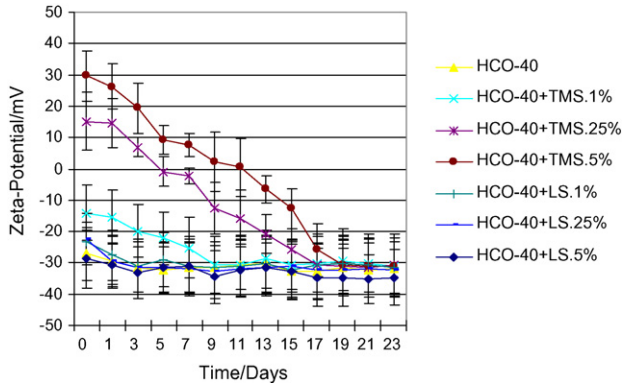

Fig. 4. The zeta-potential changes of the 10% (w/w) seal oil emulsion prepared using 2.5% of HCO-40 in combination with varying amounts (0.1%, 0.25% and 0.5%) of Incroquat Benhenyl TMS or Crodasinic LS-30. The emulsion samples were incubated at 55 ± 0.2 °C in a constantly shaking water bath. The zeta-potential value of each sample was analyzed every other day for 23 days.

about ~30 mV (Fig. 4). The addition of Crodasinic LS-30 was found to have very little effect on the pH value of the emulsion. However, Incroquat Benhenyl TMS was found to affect the pH of the emulsion and the effect was concentration dependent. The pH values of the emulsions were found to be 4.7, 5.4, and 6.2, respectively, when the amounts of Incroquat Benhenyl TMS were 0.1%, 0.25% and 0.5%, respectively. It was noted that the pH values of the respective emulsions gradually decreased over the period monitored (Fig. 5).

To study the possible causes of the decreased pH and zeta-potentials over time observed above with the seal oil emulsions, an emulsion containing 10% light mineral oil (a non-ionizable) emulsion was prepared using 2.5% HCO-40 as the emulsifying agent. The particle size, pH value and zeta-potential of the preparation were monitored for 20 days following preparation. As shown in Fig. 6, the particle size, pH and zeta-potential of the mineral oil emulsion remained unchanged, suggesting that the observed changes with the seal oil emulsions were likely due to the hydrolysis of triacylglycerides in the seal oil to free fatty acids.

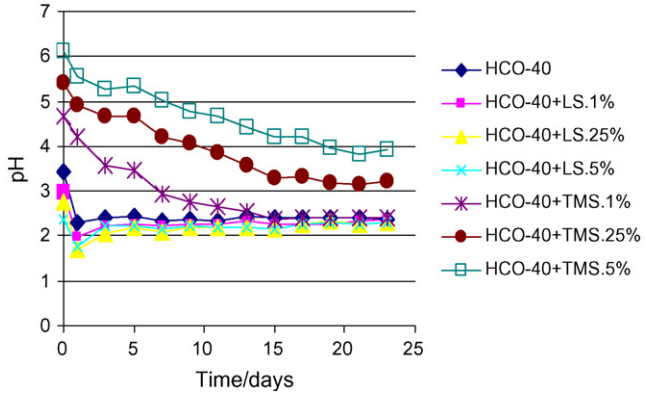

Fig. 5. The pH changes of the 10% (w/w) seal oil emulsion prepared using 2.5% of HCO-40 in combination with varying amounts (0.1%, 0.25% and 0.5%) of Incroquat Benhenyl TMS or Crodasinic LS-30. The emulsion samples were incubated at 55 ± 0.2 °C in a constantly shaking water bath. The pH of the emulsion samples was analyzed every other day for 23 days.

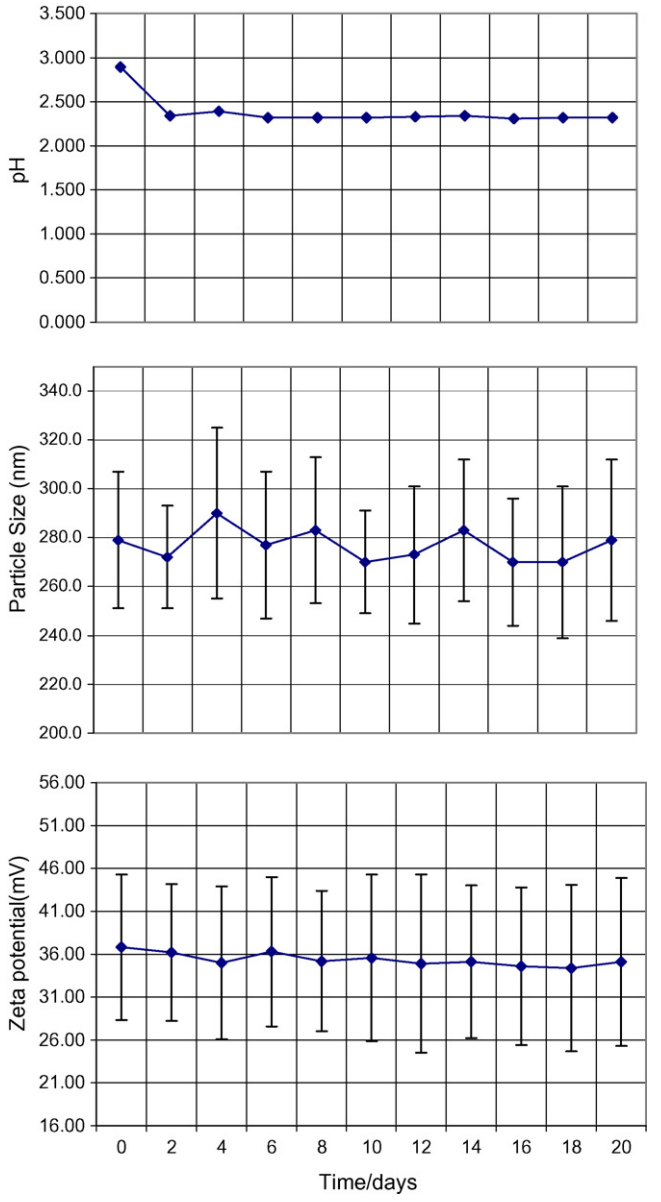

Fig. 6. The particle size, pH and zeta-potential changes of 10% light mineral oil emulsion prepared using 2.5% of HCO-40. The emulsion was incubated at 55 ± 0.2 °C in a constantly shaking water bath. The analysis was performed every other day for 20 days.

4. Discussion

Emulsions are of pharmaceutical importance including their use as drug carriers. Seal oil is believed to possess many health benefits over vegetable oils and fish oils. Therefore, a seal oil emulsion was prepared. It is known that emulsifying agent(s) could affect the characteristics of the emulsions. Seven HCO derivatives, HCO-5, HCO-20, HCO-30, HCO-40, HCO-60, HCO-80 and HCO-100, were studied as emulsifying agents in the preparation of the 10% of seal oil emulsion and the HLB values of the emulsifying agents are 6, 10.5, 11, 12.5, 14, 15 and 16.5, respectively. Hydrogenated castor oil derivatives were chosen because they are found in some important lipid soluble drug formulations such as cyclosporin (Thomas et al., 2005). In

this study it was found that HCO-40 resulted in the most stable seal oil emulsion. The particle sizes of the emulsion were found to remain largely unchanged during the experiment period (32 days) while the particle sizes of the emulsions using other emulsifying agents have increased. The results suggested that the HCO-40 possessed the required HLB for forming a stable seal oil emulsion.

It is generally understood that increased surface charge on the emulsion particles leads to increased repulsion between particles which would subsequently lead to increased physical stability of the emulsion. It was found that the zeta-potential of the emulsion where HCO-40 was used as the emulsifying agent was about −30 mV although HCO-40 is considered as a non-ionizable surfactant. The negative charge shown by the emulsion is believed to be the result of the ionization of the free fatty acids derived from the hydrolysis of triacylglycerides in the seal oil.

The current studies demonstrated that the surface charge or zeta-potential of the seal oil emulsion was affected by the presence of positively charged surfactant, Incroquat Benhenyl TMS, while it was not influenced by negatively charged surfactant, Crodasinic LS-30. Although both Incroquat Benhenyl TMS and Crodainic LS-30 are not commonly used in pharmaceutical industry for making emulsion formulations, they were chosen as example surfactants in this study purely because of their unique characteristics of charge. The zeta-potential of the seal oil emulsion was shown to increase in the presence of Incroquat Benhenyl TMS, which is likely the result of neutralization of the negative charge on the surface by the positive charge of the surfactant. The increase was shown to be dependent upon the concentration of Incroquat Benhenyl TMS. The zeta-potential changed from −14 mV to +30 mV when the concentration of Incroquat Benhenyl TMS changed from 0.1% to 0.5%. As time increased, the zeta-potentials of those emulsions decreased gradually, which is likely due to the gradual hydrolysis of triacylglycerides in the seal oil to free fatty acids which are associated with a negative charge. It was also shown that the pH of the emulsions decreased over time and reached to about 2.2 which was likely due to the production of free fatty acids following the hydrolysis of triacylglycerides. It would be difficult to prove the hydrolysis of triacylglycerides in our systems. The change in pH observed could be due to the hydrolysis of an infinitesimal amount of the triacylglycerides, the major lipid component in the emulsion. This small change in fatty acid composition would not be easily detected by GC. For this reason, we used an indirect approach in which a non-hydrolyzable lipid was used to demonstrate that the drop in pH in the seal oil emulsions was likely due to a slight hydrolysis of the triacylglycerides.

When a large quantity of positively charged surfactant, Incroquat Benhenyl TMS, was used in the seal oil emulsion, the overall system became positively charged, which would promote the hydrolysis of triacylglycerides producing free fatty acids. The fatty acids produced would subsequently be ionized. As more negatively charged $R–COO^-$ accumulated on the surface, the zeta-potential of the system started to drop. To confirm that the change in zeta-potential and pH was a result of the hydrolysis of triacylglycerides in the seal oil, a mineral oil emulsion was prepared. It was found that both zeta-potential and pH of the mineral oil emulsion did not change over time, which confirmed our speculation that the change in zeta-potential and pH in the seal oil emulsion was due to the hydrolysis of triacylglycerides in the seal oil. It should be noted that the emulsion systems studied in this report were not buffered. In addition, readers should be reminded that the fatty acids in the emulsion systems may not behave the same as if they are dissolved in water.

The current study demonstrated that harp seal oil can be formulated as stable oil-in-water emulsions. Because of its unique content of omega-3 fatty acids, especially DHA, DPA and EPA, and its superior chemical and biological characteristics compared with fish oil, it is conceivable that the currently underutilized seal oil may provide an excellent vehicle for dissolving lipid soluble drugs.

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