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Research paper

Insulin-loaded W/O/W multiple emulsions: comparison of the performances of systems prepared with medium-chain-triglycerides and fish oil

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Abstract

Insulin-loaded W/O/W multiple emulsions (ME) composed of medium-chain triglycerides have been shown to decrease the blood glucose level after oral administration to diabetic rats. Fish oil (very long-chain triglycerides) could be an alternative to medium-chain triglycerides because its chronic consumption has beneficial therapeutic effects. The aim of this work was twofold: to obtain stable fish oil containing ME, based on a formulation optimized in a previous work with low medium-chain triglycerides content, and to compare their characteristics to those of ME composed of medium-chain triglycerides. Due to the higher viscosity and surface tension of fish oil compared to medium-chain triglycerides, preparation of ME appeared difficult to achieve. However, a stable unloaded-ME with low fish oil content was formed, by adapting the emulsification process. The characteristics of unloaded fish oil ME were almost similar to those of medium-chain triglycerides ME. In contrast to medium-chain triglycerides ME, the introduction of insulin did not improve the elasticity and consequently the characteristics and stability of fish oil ME. Nevertheless, the insulin-loaded fish oil containing ME was shown to be stable for 6 weeks at 4 °C.

Keywords: Insulin; Multiple emulsion; Medium-chain triglycerides; Very long-chain triglycerides; Fish oil; Elasticity

1. Introduction

W/O/W multiple emulsions (ME) are systems of increasing interest for the oral delivery of hydrophilic drugs which are unstable in the gastrointestinal tract. Medium-chain triglycerides containing multiple emulsion (MCT-ME) and loaded with insulin, have been shown to decrease glycemia in diabetic rats after oral administration [1,2]. However, side effects such as diarrhea, weight loss and steatosis have been observed after repeated administration of those ME containing 35% of oil. To optimize the oral administration of insulin, ME with a lower content of MCT (20%) have been developed, stabilized by a cetyl dimethicone copolyol, as the low HLB surfactant, and by a copolymer of ethylene oxide and propylene oxide, as the high HLB surfactant [3].

Due to the beneficial therapeutic effects of its chronic consumption, fish oil represents a valuable alternative to MCT. Indeed, its high concentration in omega-3 fatty acids is accounted for enhanced insulin action [4], reduction of inflammatory phenomena [5] and incidence of cardiovascular diseases [6]. This would be of importance for diabetics who tend to show cardiovascular disorders [7,8]. Moreover, ME incorporating 2% of omega-3 fatty acids and administered in the rectum produce a rapid absorption of insulin and a strong reduction of glycemia [9].

The aim of this work was twofold: to obtain a stable fish oil containing multiple emulsion (FO-ME) according to the formula previously optimized using MCT [3] and to compare

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the characteristics of the obtained ME, either unloaded or loaded with insulin to those of ME prepared from MCT.

2. Materials and methods

2.1. Materials

Biosynthetic Human Insulin, Umulin®, was a gift from Eli Lilly Company (Indianapolis, USA). The oil phase was constituted of either Miglyol® 810N (MCT, Condea Chemie Company, Germany) or fish oil (FO, Winterisation Europe, France). Abil® EM-90 (Goldschmidt, France) and Arlatone® F127G (ICI Surfactants, Belgium) were used as the low and high HLB surfactants, respectively. Sodium chloride and glucose (VWR International, France) were used as a conductimetric tracer, and for the preparation of iso-osmotic solutions to ME inner aqueous phases, respectively. Desionized water (MilliQ®, Millipore, France) was used in all experiments.

2.2. Methods

2.2.1. Preparation of the multiple emulsions

The ME were prepared using a two-step process. In the first step, a primary emulsion (PE) was formed and composed of 25% of oil (MCT or FO), 4% of Abil® EM-90 (Goldschmidt, France) and 71% of an aqueous solution of insulin (2.8 mg/ml) containing 0.18% of NaCl. In the second step, the ME was obtained by dispersing 80% of the PE in an aqueous solution of Arlatone® F127G (1%). In our reference protocol, both steps were performed at 15 ± 1 °C (to preserve insulin from degradation), as previously described [3]. Three batches of each ME were independently prepared and characterized.

2.2.2. Characterization of the multiple emulsions

Analyses performed on ME consisted of:

- *Macroscopic and microscopic observations*: Microscopic analysis of ME was performed to check the multiple characters of the emulsion droplets using an Optical immersion Laboval 4 microscope, Bioblock, France.
- *Granulometric measurements*: The mean size and size distribution of the multiple globules were measured after dilution of the ME with an iso-osmotic solution, using a laser diffraction Granulometer Coulter LS 230® (Coultronics, France). The data were analysed with the Fraunhofer model. The parameter taken into account was the d_{43} diameter moment/volume.
- *Conductometric measurements*: Conductivity of ME diluted to 1/20 with iso-osmotic solutions was measured at room temperature by means of a CDM 230 Tassel Conductimeter (Radiometer Copenhagen, France). Using this method, the weight fraction of electrolyte released into the outer aqueous phase was measured, and consequently, the encapsulation efficiency [10]. Standardization was performed for each type of ME depending on the nature of its inner aqueous phase.
- *Rheological determination of G' (elastic modulus), G'' (viscous modulus)*: Oscillatory experiments were performed at 20 ± 0.2 °C on a Haake® RS 100 controlled stress, Rhéo, France) at a frequency $N = 1$ Hz with a cone/plate geometry (diameter = 20 mm, angle = 4°).
- *Turbidimetric measurements*: Using a Turbiscan®MA 2000 (Formulaction, France), these measurements allowed foreseeing ME stability [11,12]. A flat bottomed cylindrical glass cell (15 cm in height, 1.2 cm of internal diameter) is filled with the ME and scanned from the bottom to the top by a light source (pulsed near infra-red, 850 nm). A synchronized detector monitors the optical properties of the dispersion along the 5 cm height of the sample. Each scan provides a curve considered as a macroscopic 'fingerprint' of the sample at a given time. ME were scanned just after their preparation, and then repeatedly for six weeks.

All the studies mentioned above were performed at various time intervals, during ME storage at 4 ± 1 °C.

2.2.3. Oil properties

- *Viscosity*: Viscosity of both oils was determined using the Haake® RS 100 controlled stress at 15 °C ± 0.2 °C and 25 ± 0.2 °C, with a cone plate geometry (diameter 60 mm, angle 1°).
- *Interfacial tension measurements*: The interfacial tension of the studied oils with water was determined at 22 ± 1 °C using a Krüss K10 tensiometer (Germany) [13]. Prior to the addition of water, the surface tension of oil was measured. Then, water was added to the oil and the two phases were allowed to stabilize for 20 h. At the end of this period of time, the oil and water phases were separated and their surface tensions measured. The accuracy of the measurements was estimated to be 0.2 mN/m. The interfacial tension ($\gamma_{oil/water}$) was deduced from the relationship (Eq. 1):

$$\gamma_{oil/water} = \gamma_{water(sat)} - \gamma_{oil(sat)} \quad (1)$$

in which, $\gamma_{water(sat)}$ is the surface tension of the water saturated with oil and $\gamma_{oil(sat)}$ is the surface tension of the oil saturated with water. The results are mean of three independent experiments.

All the results relative to the characterization of the ME and oils were obtained from three measurements on each sample.

3. Results and discussion

The strict application to fish oil of the reference formulation and preparation process as described for MCT produced a very thick PE that did not allow formation of any

Table 1
Viscosity of Miglyol 810N® and fish oil measured at 15 ± 0.2 °C and 25 ± 0.2 °C. Results are expressed as mean ± SD

Temperature (°C)	Viscosity (mPa.s)	
	Miglyol 810N®	Fish oil
15	34 ± 2	67 ± 3
25	24 ± 2	46 ± 2

ME. The much higher viscosity of fish oil compared to MCT (Table 1) and its higher interfacial tension might be in a large part responsible for the unfavourable effect of fish oil on subsequent ME formation. Indeed, the interfacial tensions determined at equilibrium were 23.1 mN/m and 16.8 mN/m for fish oil and MCT, respectively. It is important to note that equilibrium surface tension was reached faster for the MCT-water system (4 h) than for fish oil in contact with water (6 h) (Fig. 1). As already shown by Driscoll et al. [14], the longer the hydrocarbon chain length of the oil, the greater the interfacial tension against the aqueous phase. The higher viscosity of the fish oil containing PE made the preparation of the ME more difficult, partly due to the uneasy dispersion of PE into the external aqueous phase that increased the risk of oily globules breaking. Therefore, it appeared necessary to reduce the viscosity of the PE. Since a further change in the composition of the emulsion was unwanted, the emulsification process was modified.

In the aim of reducing the viscosity of the PE, the emulsification temperature was increased from 15 up to 25 °C and the mode of stirring of the PE was reconsidered. The increase in temperature reduced the oil viscosity

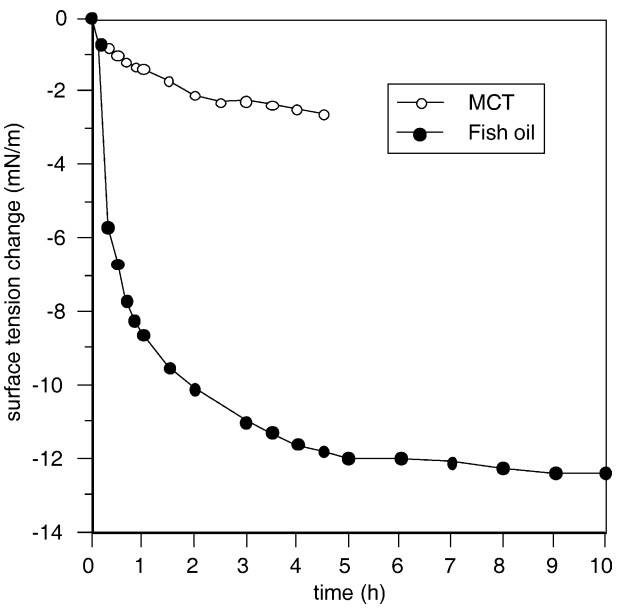

Fig. 1. Surface tension change after 20 h contact of MCT and FO with water.

(Table 1). It was assumed that this increase in temperature also had a lowering effect on the interfacial tension. Indeed, it is well established that an increase in temperature provokes a decrease in the interfacial tension and favours the miscibility of one phase into another. The emulsification processes reported in the literature are generally performed at high temperatures [15]. Although we had also noted, in our previous work [3], that ME formulated at 15 °C had larger sized globules (diameter, 28 ± 11 μm) than ME prepared at 25 °C (diameter, 9 ± 6 μm), we had preferred the low temperature to prevent a possible alteration of the encapsulated insulin. It has been shown that temperature has a profound influence on the rate of hydrolysis and on the formation of higher molecular weight transformation products [16]. In the present work, the preparation of FO-ME was carried out at 25 °C, by replacing the Rayneri® centripetal by a Bioblock® deflocculating stirrer in the first step of emulsification (PE formation), and by reducing the rate and duration of PE stirring (Table 2). All the MCT-ME were also prepared at 25 °C but the emulsification process cited in reference was not modified.

The characteristics of the ME obtained using the new conditions of emulsification are summarized in Tables 3 and 4. The encapsulation efficiencies (Table 3) after preparation appeared to depend more on the presence or absence of encapsulated insulin than on the nature of the chosen oil. They were all higher than 90% except for insulin FO-ME. Insulin MCT-ME showed the best encapsulation efficiency so far, whereas insulin FO-ME showed the lowest one. It was also observed that, for all studied ME, the encapsulation efficiencies slightly decreased during their storage for 6 months at 4 °C. Insulin FO-ME exhibited the more pronounced decrease.

Fig. 2 shows the granulometric distribution of the multiple globules for MCT-ME and FO-ME loaded or not with insulin. The mean size of the multiple globules (Table 3) was larger for FO-ME compared to MCT-ME. Moreover, introduction of insulin into FO-ME led to

Table 2
Characteristics of the fabrication process of multiple emulsions from MCT (Miglyol 810N®) or fish oil, at 25 ° ± 1 °C

		MCT		FO	
		Without insulin	With insulin	Without insulin	With insulin
PE (step 1)	Stirrer type	Rayneri® centripetal		Bioblock® deflocculating	
	Stirring rate (rpm)	3000		1800	1800
	Stirring time (min)	30		9	8
ME (step 2)	Stirrer type	Rayneri® centripetal			
	Stirring rate (rpm)	600	600	600	600
	Stirring time (min)	35	15	16	20

Table 3
Evolution of the globules diameter and encapsulation efficiencies during six months storage at 4 ± 1°C of ME prepared with MCT (Miglyol 810N®) and fish oil. Results are expressed as mean ± SD

		MCT-ME		FO-ME	
		Without insulin	With insulin	Without insulin	With insulin
Encapsulation efficiency (%)	0	91.6 ± 0.5	94.6 ± 0.4	93.3 ± 0.6	88.4 ± 0.3
	2 weeks	91.0 ± 0.5	90.4 ± 0.4	89.1 ± 0.4	85.7 ± 0.4
	6 weeks	90.1 ± 0.5	84.8 ± 0.3	85.7 ± 0.3	82.7 ± 0.4
	6 months	88.7 ± 0.4	84.6 ± 0.3	83 ± 0.3	70 ± 0.3
Globule diameter (μm)	0	11 ± 4	12 ± 9	13 ± 9	21 ± 17
	2 weeks	12 ± 5	12 ± 9	14 ± 9	22 ± 18
	6 weeks	12 ± 5	12 ± 9	15 ± 9	23 ± 18
	6 months	17 ± 7	12 ± 6	19 ± 10	28 ± 22

a dramatic increase in their mean globule size, whereas it did not affect much that of MCT-ME. Pictures of insulin-loaded ME (MCT and FO) are shown on Fig. 3. For all the studied ME no significant increase in size was observed during the first 6 weeks of storage at 4 °C, in agreement with the turbidity studies. Indeed, the retro-diffusion profiles of all studied ME overlaid during this period. The non-evolution of the retro-diffusion profiles accounts for the non-evolution of sizes since the corresponding transmission remained to 0 and unchanged over the period of the experiment (data not shown). This attests for a relatively good stability of the ME over this period of time. Except for insulin-loaded MCT-ME, a significant increase in size was observed for all ME after 6 months of storage. The size is a criterion of importance in the perspective of oral administration of the insulin-loaded ME. As a matter of fact, it is preferred to prepare ME with a globule diameter lower than 20 μm to enhance the intestinal resorption, even if it is expected that larger globules can adapt their shape to become more absorbable. Therefore, insulin-loaded FO-ME have reached the highest limit in term of globule size.

Table 4
Evolution of the rheological properties of the multiple emulsions during 6 weeks storage at 4 ± 1 °C, as determined by oscillatory experiments. Results are expressed as mean ± SD

		Without insulin		With insulin	
		$G' \times 10^3$ (Pa)	$G'' \times 10^2$ (Pa)	$G' \times 10^3$ (Pa)	$G'' \times 10^2$ (Pa)
MCT-ME	0	1.2 ± 0.10	1.5 ± 0.08	1.3 ± 0.07	1.3 ± 0.08
	1 week	1.1 ± 0.09	1.2 ± 0.07	1.0 ± 0.05	1.0 ± 0.05
	2 weeks	0.9 ± 0.85	1.0 ± 0.07	1.0 ± 0.06	1.1 ± 0.07
	3 weeks	0.8 ± 0.65	0.9 ± 0.85	1.0 ± 0.07	1.1 ± 0.08
	6 weeks	0.8 ± 0.70	0.9 ± 0.70	1.0 ± 0.07	1.0 ± 0.06
FO-ME	0	0.8 ± 0.60	1.6 ± 0.10	0.7 ± 0.60	1.2 ± 0.09
	1 week	0.6 ± 0.50	1.2 ± 0.08	0.6 ± 0.55	1.2 ± 0.10
	2 weeks	0.6 ± 0.55	1.3 ± 0.10	0.5 ± 0.45	0.9 ± 0.90
	3 weeks	0.6 ± 0.60	1.3 ± 0.11	0.4 ± 0.40	0.8 ± 0.80
	6 weeks	0.5 ± 0.55	0.9 ± 0.95	0.4 ± 0.35	0.7 ± 0.60

The results of the ME rheological properties are presented in Table 4 and Fig. 4. The elastic modulus G' of all ME was higher than the viscous modulus G'' at low shear, attesting for their predominant elastic behaviour. However, the elastic modulus G' of MCT-ME was higher than that of FO-ME. Consequently, the rheological behaviour of MCT-ME was considered as more elastic than that of FO-ME. It is worth to note that the introduction of insulin in the formulation of ME improved the elasticity of MCT-ME whereas it decreased that of FO-ME.

All these results led to the conclusion that MCT-ME have higher elasticity and subsequently higher stability than FO-ME. They also showed that insulin had opposite effects on both the formation and stability of ME depending on the nature of the oil.

Proteins have been reported in many works to adsorb at the oil/water interface and to be involved in the stabilization of emulsions [17–21]. They interact with the surfactant at the oil/water interface and affect the elasticity and resistance of the droplets. According to Kanouni et al. [22], a high degree of elasticity of the film formed at the first interface would prevent globule breaking, which is highlighted by the present results with MCT. The properties of the fish oil tend to mask the favourable effect of insulin on ME formation

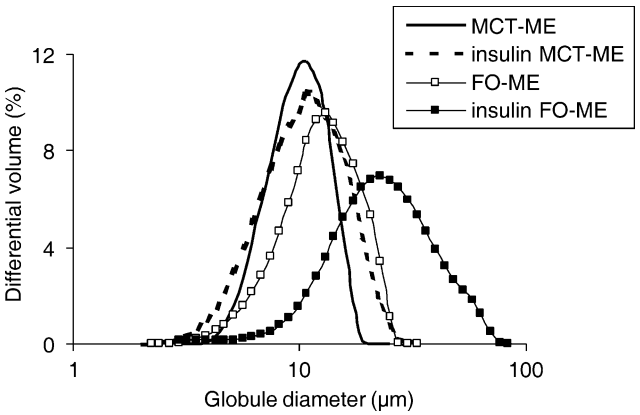

Fig. 2. Granulometric distribution profiles of the multiple globules after preparation.

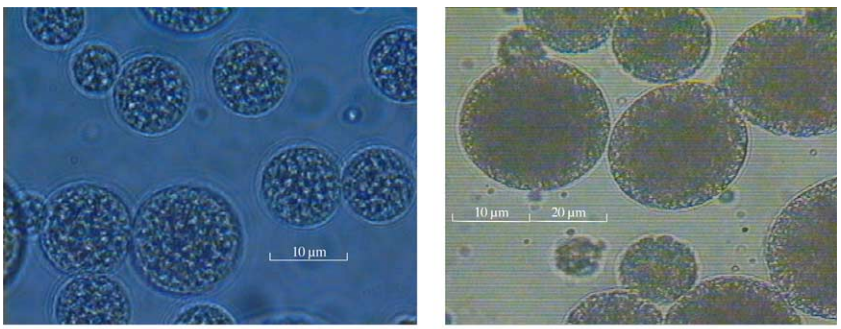

Fig. 3. Microscopic pictures of A insulin MCT-ME, B insulin FO-ME, after preparation. Observations were made at × 40 magnitude.

and elasticity, and subsequently, on its stability. In our previous work [3], we showed that the formation of an insulin–low HLB surfactant complex enhances the formation and stability of MCT-ME. According to our results, this is obviously not the case when MCT is replaced by fish oil. In FO-ME, the oil/water interfacial tension being higher, its lowering requires the adsorption of more surfactant or protein molecules. The extent of the adsorption of one or the other species might be influenced by the nature of the oil. Indeed, the diffusion of surfactant molecules in the oil phase may differ from an oil to another depending on both the affinity of the surfactant for the oil and on oil viscosity. In such conditions, the nature and properties of the interfacial film in FO-ME would be altered compared to MCT-ME. The stability of the whole emulsion would in turn be affected. It has been previously shown that the stability of an emulsion can greatly vary with the composition of its oil phase [14]. The partitioning of a triglyceride from the oil phase to the surfactant at the interface may differ between oils. MCT can displace VLCT (very long chain triglycerides) at the lipid droplet surfaces of MCT-VLCT single emulsions, which consequence is a favourable interfacial location of MCT that improves emulsion stability [14]. This reflects the higher affinity of MCT for a surfactant at the interface compared to VLCT. This phenomenon would account for the lower stability of FO-ME (composed of VLCT), compared to that of MCT-ME. In insulin-loaded ME, it is possible that, in the presence of fish oil, due to a lower interaction between VLCT from the fish oil and the surfactant at the interface, the nature of the interfacial film is significantly altered with an increased interfacial content of insulin, rigidifying the interface and increasing the globule size. If proteins such as bovine serum albumin have been shown to delay the release of electrolyte from ME, there was an optimal concentration above which a higher electrolyte release was observed [18]. In our work, the results suggest that the maximum concentration for insulin for stabilizing FO-ME may have been over passed.

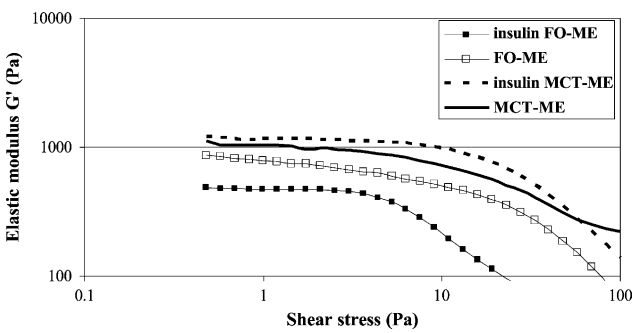

Fig. 4. Rheological profiles of ME after preparation: Evolution of the elastic modulus versus shear stress.

4. Conclusion

A stable unloaded FO-ME with low oil content could be obtained after reducing the viscosity of the PE and presented almost similar characteristics to those of ME prepared from MCT. However, incorporation of insulin had opposite effects on the formation and stability of ME, which can be explained by differences in the interaction of insulin at the oil/surfactant/water interface, depending on the nature of the oil. Obviously, MCT allows formation of more stable insulin ME than fish oil. However, to combine the advantages of both oils i.e. small globules formation for MCT and beneficial therapeutic effects for FO, it might be envision to mix the two oils in the preparation of ME.

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