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The development and characterisation of triglyceride-based 'spontaneous' multiple emulsions

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Abstract

The formation of multiple emulsion droplets from two oil systems (Labrafil M 1944 CS and Labrafac Lipophile WL 1349) via a one-step process involving minimal agitation in aqueous media has been investigated in terms of the multiple character of the droplets, the particle size distribution, the stability and the lipolysis profile in the presence of pancreatic lipase. It was noted that multiple emulsion droplets were formed from both oils in the presence and absence of Tween 80, with the stability and particle size of the droplets being dependent on the composition and choice of media. It was noted that optimum stability of up to several days was obtained using 10% Tween 80 for both oils, with two stages of the breakdown process being apparent, the relative propensities of which being dependent on the oil used. The particle size distribution in distilled water indicated the presence of two distinct size populations corresponding to multiple and single droplets. It was noted that droplet breakdown was greatly accelerated in simulated intestinal fluid and in high pH media, with evidence for liquid crystal structure formation apparent. Lipolysis studies indicated that pegylation and the presence of surfactant slowed the degradation process. The study has indicated that 'spontaneous' multiple emulsion formation is indeed possible, with a reasonable if not necessarily optimal stability profile being observed for these systems.

Keywords: Lipid; Triglyceride; Multiple emulsion; Particle size; Microscopy

1. Introduction

Multiple emulsions have been widely studied as a means of delivering drugs via the oral, topical and parenteral routes, with applications including protein delivery (Cournarie et al., 2004), delivery of antibiotics to the vagina (Tedajo et al., 2005), sustained delivery (Vaziri and Warburton, 1994) and vaccine delivery (Bozkir and Hayta, 2004). Other pharmaceutical applications include taste masking of unpalatable drugs (Vaziri and Warburton, 1994), production of biodegradable microspheres (O'Donnell and

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McGinity, 1996) and in the treatment of drug overdose (Chiang et al., 1978). The production of such emulsions usually involves a series of relatively complex steps in order to allow incorporation of the continuous phase into the disperse phase droplets. Three methods of formation have been cited, namely phase inversion, one-step and two-step emulsification. However, phase inversion and one-step emulsification have not been widely adopted as doubts have been cast on reproducibility, degree of multiplicity and stability (Florence and Whitehill, 1982; Garti and Aserin, 1996). The most reliable and reproducible method of manufacture involves the re-emulsification of a primary emulsion by a two-step technique. The first step involves the production of an ordinary w/o emulsion under conditions of high shear. The drug, solute or marker is added to the aqueous phase together with a primary low HLB surfactant. The primary emulsion is then further emulsified in a second step under low shear to produce the final w/o/w multiple emulsion, with the bulk aqueous phase in the second step containing a hydrophilic surfactant. Despite this technique providing a reproducible method of manufacture, scale-up issues and sensitivity to formation conditions has limited industrial application.

Recently we have been studying the self-emulsifying properties of Labrafil oils mixed with non-ionic surface active agents with a view to delineating the physico-chemical and structural features of these systems in terms of their suitability as self-emulsifying drug delivery systems (Devani et al., 2004). Labrafils are composed of triglycerides which have been partially reacted with polyethylene glycol (PEG) of defined chain length to form mixtures of mono-, di- and tri-glycerides and polyethylene glycol mono and diesters. The chemical diversity of the PEG esterified lipids affords a number of different functionalities to the resulting product, with mono and diesters of PEG having surfactant properties, whereas monoglycerides are believed to exhibit co-surfactant properties; the remaining di- and tri-glycerides constitute the oily phase. To date the primary role of these oils has been as surfactant components due to their resemblance to amphiphilic non-ionic surfactant molecules (Shah et al., 1994). In addition, as the mono- and diglycerides constituents act as co-solvents, the percentage of non-ionic surfactant required for self-emulsification is reduced (Bachynsky et al., 1997) and indeed many of these lipid systems can be used as self-emulsifying systems in their own right (Craig et al., 1995). In performing these studies it was noted that certain oil/surfactant mixes produced multiple emulsions on simple agitation with water (Craig et al., 1995; Devani et al., 2004). Such an observation is of interest as a potential route for simple large scale or indeed in vivo production of multiple emulsions via a one-step process but without necessitating the use of significant shear. In this investigation we examine specifically the possibilities and limitations afforded by this approach to generating multiple emulsions in terms of emulsion type, stability and formation in physiologically relevant media. In addition we have studied the lipolysis profile of these emulsions with a view to investigating the likely biological fate of these systems on oral ingestion.

2. Materials and methods

2.1. Materials and emulsion preparation

Labrafil M 1944 CS (composed largely of triglycerides based on oleic and linoleic acid (C18) and pegylated derivatives, HLB 4) and Labrafac Lipophile WL 1349 (composed largely of non-pegylated caprylic and capric acid triglycerides (C8, C10), HLB 1) were selected due to their ability to form multiple emulsions, as indicated in the previous study (Devani et al., 2004). Both were supplied via generous donation from Gattefosse s.a. (France). For the lipolysis studies, further oils were used from the same supplier (Labrafac CM 10, Labrafil M 2125 CS, Labrasol and Labrafil WL 2609 BS) so as to allow correlation with chemical structure. Polyoxyethylene sorbitan mono-oleate, Tween 80 (HLB 15) was used as obtained (ICI Buckinghamshire, England). All materials used were commercial grade quality. Emulsions were prepared by adding 250 μl of oil/surfactant mix (% v/v) to 300 ml of distilled water and mixed under conditions of gentle agitation (100 rpm for 10 min).

2.2. Characterisation of emulsions

For verification of the nature of the multiple emulsions a range of fluorescent lipid-soluble and water-soluble dyes were incorporated as indicated. For observation of the emulsions, a Differential

Interference Contrast (DIC) microscope, the Olympus BX 50, was employed with a Nikon-F-60 IM 35 mm camera; the principles of this technique have been described in a previous communication (Devani et al., 2004). The rate of development and percentage ratio of multiple to simple droplets were assessed for 100% oil to 70% oil/30% surfactant mixes. To study the rate of multiple droplet formation the droplet structure was examined every 2 h using the microscope. A sedgewick rafter counting chamber of dimensions 50 mm × 20 mm × 1 ml with a base divided into 1 mm squares was used to examine the droplets. Multiple emulsion stability was assessed by filling three 50 ml stoppered measuring cylinders with the emulsion corresponding to each oil/surfactant concentration at room temperature, 18–20 °C. The degree of multiplicity, changes in internal droplet size and number, the ratio of multiple to simple droplets and evidence of rupture of the oil phase and the presence of free oil was assessed microscopically. Observations were made periodically every 2 h over the first 12 h, then daily for a maximum of 3 weeks.

The size distributions of the emulsions were measured using a Malvern Mastersizer S laser diffraction particle analyser. Stability was assessed via observation of changes in the volume median diameter D (v, 0.5). The point at which large multiple structures are broken down to smaller simple o/w droplets was recorded. Hundred percent Labrafac Lipophile WL 1349 emulsions were not sized over time because the very oily nature of the emulsion left residues on the sample cell lens of the Malvern Mastersizer S causing problems in reproducibility and cleaning. Four batches for each oil/surfactant mix were measured five times at each time point. Measurements were taken initially every 4 h for 12 h then every 24 h for 5 days.

0.1 M hydrochloric acid pH 1.4 and pH 6.8 phosphate buffer (BP) were used as a crude simulation of gastric and intestinal conditions. To examine the effect of pH, standard phosphate buffers with a pH range of 6.4–7.7 and an ionic strength $I = 0.01$ were prepared. All salts were of standard laboratory grade and used as obtained from BDH Laboratories (Poole, UK). The emulsion stability in media more closely simulating physiological conditions was assessed using simulated fasted gastric and intestinal fluid as outlined by Dressman et al. (1998). Two hundred and fifty microliters of the 90% oil/10% surfactant mix (% v/v) was added to 300 ml of distilled water (10 min at 100 rpm). After a standing time of two hours (to allow sufficient time for structural development), a proportion of the emulsion was transferred to 300 ml of fasted simulated gastric or intestinal fluid. The mixture was stirred at 100 rpm for a final 2 h. In between each transfer the structure of the multiple droplets were examined using the microscope under optical and cross polar settings. The size of the droplets was assessed using the Malvern Mastersizer S. All salts and egg lecithin were used as obtained from BDH Laboratories (Poole, UK). The bile salt, sodium taurocholate was used as obtained from Sigma-Aldrich (Dorest, UK) and sodium dodeceyl sulfate was used as obtained from Fisher Scientific (Loughborough, UK).

2.3. Lipolysis studies

Pancreatic lipase in the presence of its co-factor colipase catalyses the lipolysis of a triglyceride substrate into 1 mol of 2-monoglyceride and 2 mol of fatty acids. It is possible to assess the rate and extent of lipolysis by continuously titrating the liberated fatty acid with a known concentration of sodium hydroxide. A pH stat method was used as an in vitro technique for assessing the lipolysis of oily formulations. In a low buffered medium, as the triglyceride is hydrolysed, the fatty acid released causes the pH of the medium to fall. In response, sodium hydroxide is dispensed to maintain a constant pH. The number of moles of sodium hydroxide would then equate to the number of moles of fatty acid and, by comparison with the calculated fatty acid content of completely digested lipid, the value may therefore be related to the extent and rate of lipolysis (MacGregor et al., 1997).

A 736 GP Titrino (Metrohm UK) was used as the titrating unit. A constant temperature of 37 °C was maintained by the use of a water-jacketed vessel. The pH electrode was calibrated with standard pH 4 and 7 buffers. All experiments were performed in freshly prepared fasted simulated intestinal fluid as outlined above. This buffer formulation was obtained from a study designed to investigate the likely situation in the human small intestine under both fasted and fed state. The simulated media contains a single bile salt, sodium taurocholate and lecithin to represent a bile mixture in the small intestine. One gram of substrate was added to 100 ml of media and transferred to the

reaction vessel. The stirrer was activated at a suitable level to ensure adequate agitation. 0.1 M sodium hydroxide solution was chosen as the titration concentration. Two milliliters of distilled water was added to 0.5 g of porcine pancreatin (Sigma-Aldrich, Dorest UK) which was pre-incubated at 37 °C for 20 min. The pH of the reaction mixture was adjusted to pH 6.8 and monitored to ensure a constant pH and temperature over a 30 min time interval. After 30 min, the timer was set to 0 and 1.0 ml of pancreatin solution was added to the vessel (2.0 ml pancreatin suspension was centrifuged at 15 000 rpm for 10 min). The titration system was immediately activated with the end-point set at pH 6.8. Titration readings were noted every 3 s. After 3 h and 30 min the total volume of titrant dispensed was noted. In vitro digestion for each formulation was repeated four times.

To compare the extent of lipolysis of different formulations, the percentage of lipolysis over a fixed time of 12 000 s was determined; all formulations had come to effective completion over this time period. Using an estimation of the mean molecular mass of the lipid, it is possible to estimate the potential number of fatty acid molecules produced assuming the reaction went to 100% completion and from this the percentage of total lipolysis. The low buffering capacity of the media could allow the pH to fall by the atmospheric absorption of carbon dioxide and digestion of lecithin and/or surfactant. To monitor any changes in pH caused by means other than digestion of the lipid substrate, two control experiments were performed over the same time period. The first contained only the fasted simulated intestinal fluid (FaSIF: lecithin, bile salts and mineral salts) while the second contained 1.0 g of Tween 80 in FaSIF. The total volume of sodium hydroxide dispensed was less than 0.01 ml for both experiments, indicating negligible changes in pH due to external variables.

3. Results

3.1. Formation and visual stability of multiple droplets

As previously reported (Devani et al., 2004), the two oils produced multiple emulsions both alone and in the presence of Tween 80, with the predominant droplet type being Type B (large droplets with numerous small internal droplets), as defined using the classification proposed by Florence and Whitehill (1981). The multiple nature of the observed emulsions was confirmed using Sudan Black and fluorescein (data not shown). Ninety to 95% of all large to medium droplets were of multiple character for 100% oil to 70% oil/30% surfactant for both oils.

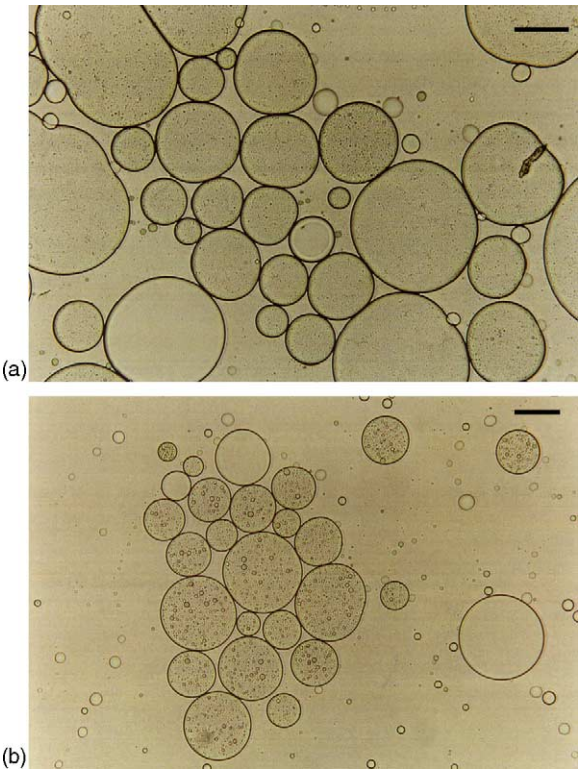

Fig. 1. (a) Eighty percent Labrafac Lipophile WL 1349/20% Tween 80 multiple droplet at 0 h (bar represents 100 μm). (b) Eighty percent Labrafac Lipophile WL 1349/20% Tween 80 multiple droplet at 4 h (bar represents 100 μm).

Fig. 1 shows photomicrographs taken immediately after formation and after 4 h for 80% Labrafil M 1944 CS/20% Tween 80. Labrafac Lipophile WL 1349 formulations developed to a stage whereby clearly discernible multiple emulsion droplets were visible over timescales of up to 2 h, while the presence of up to 30% Tween 80 slowed the process to 2–4 h. The more hydrophilic Labrafil M 1944 CS formed emulsions at a slower rate, with the oil alone and in the presence of 10% Tween 80 showing discernible formation over 2–4 h, slowing to 6–8 h in the presence of 30% Tween 80.

Table 1
Stages of w/o/w multiple emulsion breakdown

Stage	Features observed
I	Some small/medium simple oil droplets
II	Increases in inner water droplet size, reduction in degree of multiplicity
III	Increases in the number of large simple oil droplets. Multiple droplets still exist but inner droplets sizes increase further
IV	Large-scale destruction begins, substantial increase in the numbers of small simple o/w droplets and a reduction in multiple droplets, rupture of the oil membrane, which separates the internal and external aqueous phases
V	Very few multiple emulsion droplets left if any, those remaining are usually small

Several stages of subsequent breakdown were identified and are reported in Table 1, with selected photomicrographs shown in Fig. 2(a)–(c) for 70% Labrafac Lipophile WL 1349/30% Tween 80. Fig. 2(a) shows the multiple emulsion formed after 2 h, with no sign of breakdown being apparent. The early stages of breakdown (stages II and III) include the compromise of multiple character through the reduction in the number and size of internal droplets; this could be attributed to expulsion of internal droplets. In addition, however, an enlargement of internal droplets was also noted in some cases (Fig. 2(b)). Such an increase

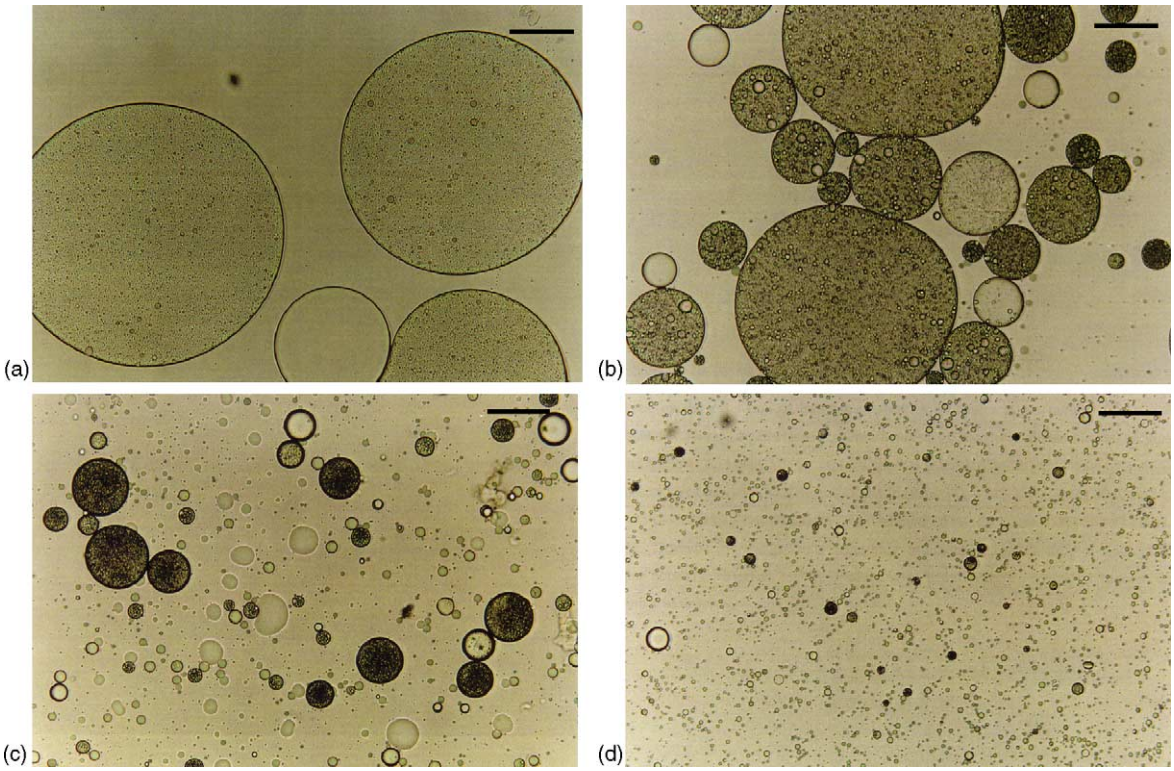

Fig. 2. (a) Seventy percent Labrafac Lipophile WL 1349/30% Tween 80, time 2 h (before breakdown) (bar represents 100 μm). (b) Seventy percent Labrafac Lipophile WL 1349/30% Tween 80, time 8 h, showing an increase in internal droplet size (bar represents 100 μm). (c) Seventy percent Labrafac Lipophile WL 1349/30% Tween 80, time 24 h, showing rupture of the oil membrane, loss of distinct multiple structure, a decrease in the size of multiple droplets and an increase in the number of simple droplets (bar represents 100 μm). (d) Seventy percent Labrafac Lipophile WL 1349/30% Tween 80, time 96 h, showing substantial inner droplet breakdown, rupture of the internal oil membrane (bar represents 100 μm).

Table 2a
Times at which each stage of breakdown began for Labrafil M 1944 CS/Tween 80 emulsions

Stage	% composition/time (h)			
	100% Labrafil M 1944 CS	90% Labrafil M 1944 CS/10% Tween 80	80% Labrafil M 1944 CS/20% Tween 80	70% Labrafil M 1944 CS/30% Tween 80
I	12	12	8	4–6
II	24	24	10–12	10–12
III	48	72	24	24
IV	48	120	48	48
V	48–72	360+	96–120	96

in internal water droplet size could also occur as a result of migration of water from the external to the internal phase through the oil membrane, evidenced by the close proximity of these large droplets to the external surface of the multiple droplet. A decrease in the number and size of multiple droplets continued to occur with a loss of distinct multiple character (Fig. 2(c)). At stage IV and V, large-scale breakdown took place depicted by a decrease in the number of multiple droplets and an increase in the number of simple o/w droplets (Fig. 2(d)).

Tables 2a and 2b display the times at which each stage of breakdown occurred for the oil/surfactant mixes examined. In general, Labrafac Lipophile WL 1349 formulations were more resistant to large-scale breakdown (stage IV) than Labrafil M 1944 CS formulations. However, the initial stage of structural change such as an increase in the internal droplets size and a reduction in the multiple character (stages II and III) occurred at a faster rate for Labrafac Lipophile WL 1349 emulsions. The presence of surfactant appeared to accelerate the early stages of breakdown for both oils. However, at stage V it was observed that for both oils, 90% oil/10% Tween 80 were the most stable formulation and 100% oil the least stable system. A change in the ratio of multiple to simple droplets was also observed over time for both oils. Initially 90–95% of all large to medium size droplets were multiple in nature. However, at the time corresponding to stage III, only 60% of the droplets were multiple in character. A large number of empty oil droplets were observed, possibly caused by the expulsion of internal droplets. For example, a gradual reduction in the percentage of multiple droplets was observed for 80% Labrafac Lipophile WL 1349/20% Tween 80: 0 h, 90–95%; 4 h, 81%; 8 h, 72%; 12 h, 62%; 24 h, 53%; and 48 h, 56% (based on observing circa 250 droplets). Thereafter, it was difficult to continue to assess changes in droplet ratio, as the rafter counting chamber become increasingly 'busy' with a general increase in the number of droplets.

3.2. Particle size analysis

Particle size analysis was used to detect changes in overall size distribution and alterations in the volume median diameter D (v, 0.5). In the case where two distribution peaks were observed (designated A and B), the D (v, 0.5) value was taken for peak B (the larger size peak). This was achieved by 'killing'

Table 2b
Times at which each stage of breakdown began for Labrafac Lipophile WL 1349/Tween 80 emulsions

Stage	% composition/time (h)			
	100% Labrafac Lipophile WL 1349	90% Labrafac Lipophile WL 1349/10% Tween 80	80% Labrafac Lipophile WL 1349/20% Tween 80	70% Labrafac Lipophile WL 1349/30% Tween 80
I	4	4	4	4
II	12	12	8	8
III	10	10	10	8–10
IV	48	96–120	96	48
V	72	336	168–192	168–192

Table 3a
Average volume median diameter $D(v, 0.5)$ in μm as a function of time (h) for Labrafil M 1944 CS emulsions (standard deviations in parenthesis)

Emulsion system (% oil/Tween 80)	0 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h
100	43.4 (7.8)	50.1 (17.1)	48.1 (7.1)	45.8 (9.1)	32.8 (11.2)	34.8 (5.1)	50.1 (8.7)	46.7 (3.7)	34.5 (3.9)
90	41.5 (2.8)	39.5 (3.0)	37.1 (2.8)	39.7 (2.9)	35.9 (2.5)	34.4 (1.6)	46.1 (0.9)	44.4 (1.3)	41.5 (1.6)
80	64.6 (1.6)	62.6 (4.1)	60.5 (5.9)	55.7 (2.8)	46.7 (2.4)	45.7 (5.6)	62.2 (8.9)	51.6 (7.3)	39.1 (6.8)
70	66.5 (2.3)	71.2 (2.7)	53.9 (10.5)	56.8 (3.3)	43.6 (3.3)	41.1 (5.7)	65.6 (10.2)	51.9 (7.0)	39.7 (5.7)

the data channels representing the smaller size distribution, peak A, as this distribution could not be fully evaluated due to the lower detection limits of the instrument. The particle size data over time are displayed in Tables 3a and 3b for Labrafil M 1944 CS and Labrafac Lipophile WL 1349 formulations respectively. Labrafil M 1944 CS formulations displayed two distinct populations before stability studies were performed. In general, a reduction in the percentage frequency of droplets in peak B was observed with a concurrent increase in the percentage of droplets in peak A, indicating a substantial increase in the number of droplets in the submicron size range. From the particle size data it is not possible to establish whether these observations are due to the destruction of multiple structures to simple droplets or simply a reduction in the size of the multiple droplets since this instrument cannot distinguish between multiple and simple droplets. However, microscopic observations imply that droplets in the submicron range were predominantly simple in nature. These results therefore suggest that over time the dramatic change in the percentage of droplets in peaks A and B may represent further conversion to a simple emulsion system.

Labrafac Lipophile WL 1349 formulations initially displayed only a single peak with a large $D(v, 0.5)$ value. Over time droplets in the submicron size range were detected as indicated in Fig. 3. Furthermore, the percentage of droplets now in peak A increased over time as the percentage of droplets in peak B decreased. The $D(v, 0.5)$ size of droplets in peak B also decreased in value and displayed increasing polydispersity with time.

3.3. Effect of different media on size and stability (10% Tween 80 emulsions)

3.3.1. Influence of 0.1 M hydrochloric acid and phosphate buffer pH 6.8

Clear structural differences were observed between emulsions formed using both 0.1 M hydrochloric acid and phosphate buffer pH 6.8 compared to distilled water. Poorly developed multiple structures in the presence of a considerable amount of free oil were observed for 90% Labrafac Lipophile WL 1349/10% Tween 80 under both conditions. In contrast, several multiple structures were identified for 90% Labrafil M 1944 CS/10% Tween 80 formulations. However on closer examination under cross-polarised light these internal structures were birefringent in nature. This indicates that a crystalline phase had formed in the internal droplet phase in both media.

The volume median diameter $D(v, 0.5)$ was not affected by the formulation media for 90% Labrafac Lipophile WL 1349/10% Tween 80 (a *t*-test found no significant difference at the 5% level for this group of

Table 3b
Average volume median diameter $D(v, 0.5)$ in μm as a function of time (h) for Lipophile WL 1349 emulsions (standard deviations in parenthesis)

Emulsion system (% oil/Tween 80)	0 h	4 h	8 h	12 h	24 h	48 h	72 h	96 h	120 h
100	183.5 (7.2)	158.4 (8.3)	164.3 (1.5)	162.9 (1.5)	148.2 (1.7)	141.3 (8.5)	166.8 (7.2)	154.2 (7.3)	137.3 (8.1)
90	170.2 (5.5)	140.6 (9.1)	139.3 (5.5)	142.1 (2.2)	137.5 (2.9)	134.9 (8.8)	141.8 (1.7)	126.5 (1.8)	120.8 (6.8)
80	126.9 (7.4)	106.8 (8.6)	108.9 (3.4)	112.1 (4.1)	108.8 (5.1)	94.4 (9.4)	81.2 (17.6)	60.9 (15.8)	61.2 (13.9)
70	126.9 (7.4)	106.8 (8.6)	108.9 (3.4)	112.1 (4.1)	108.8 (5.1)	94.4 (9.4)	81.2 (17.6)	60.9 (15.8)	61.2 (13.9)

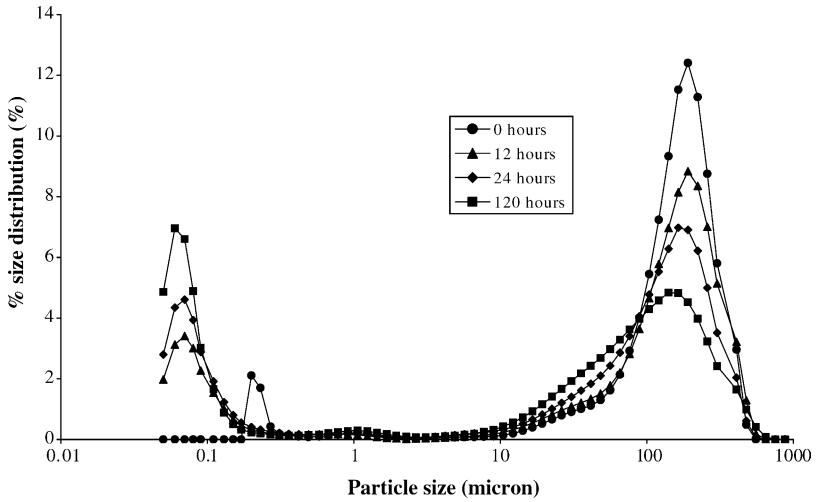

Fig. 3. Size distribution over time for 90% Labrafac Lipophile WL 1349/10% Tween 80 emulsions.

data). In contrast, notable changes were observed for 90% Labrafil M 1944 CS/10% Tween 80 formulations. A reduction in D (v, 0.5) and a change in the overall size distribution was detected. The lower end size distribution, denoted by peak A, completely disappeared in both media. This was replaced by a bi-modal distribution of larger droplets in peak B which covered a broad size range (Fig. 4).

3.3.2. The effect of pH at constant low ionic strength

As the pH was increased the defined internal arrangement for 90% Labrafil M 1944 CS/10% Tween 80 formulations disappeared and was replaced by what appeared to be sparse, aggregated structures. Birefringent material was again observed instead of internal water droplets in Labrafil M 1944 CS emulsions. At

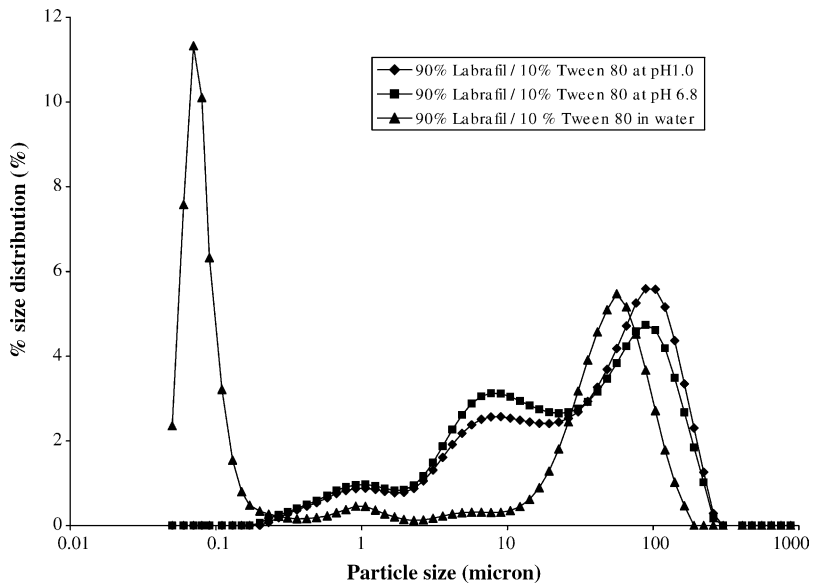

Fig. 4. The effect of 0.1 M HCl and phosphate buffer pH 6.8 on the size distribution for 90% Labrafil M 1944 CS/10% Tween 80.

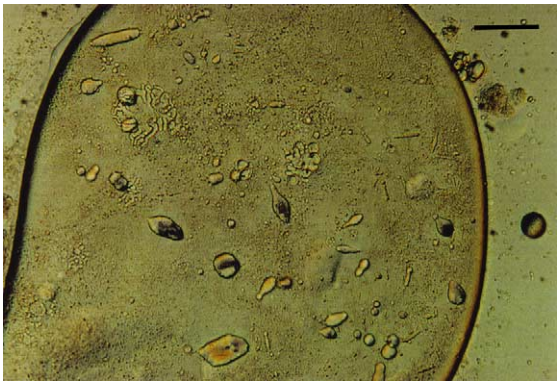

Fig. 5. 90% Labrafil M 1944 CS/10% Tween 80 in phosphate buffer pH 7.0 (image width represents 100 μm).

pH 6.4 these structures were almost spherical in nature but became irregular as the pH was increased (Fig. 5).

3.3.3. Stability of multiple droplets in fasted simulated gastric and intestinal media

The median D (v, 0.5) particle size values for Labrafac Lipophile WL 1349 (118.2 μm, S.D. 8.3) and Labrafil M 1944 CS (35.5 μm, S.D. 3.2) in gastric fluid were lower than those obtained in water. From microscopic observations in gastric media, Labrafil M 1944 CS produced smaller multiple droplets with liquid crystalline material in the internal structure. Labrafac Lipophile WL 1349 produced well-defined multiple droplets though distinctly smaller than that produced in water. However, when the multiple droplets were transferred to simulated intestinal fluid, complete destruction of the multiple structure occurred. Labrafac Lipophile WL 1349 emulsions 'cracked' releasing free oil, which floated on the surface. Labrafil M 1944 CS emulsions underwent complete phase inversion to a simple o/w emulsion.

3.4. Lipolysis of oils and multiple emulsions

The lipolysis profiles over 12 000 s for 100% oil formulations are presented in Fig. 6; we include a fuller range of Labrafil oils as used in the previous study (Devani et al., 2004) so as to allow analysis of the relationship between lipolysis and composition. The rank order starting from the most digestible lipid was as follows: Labrafac Lipophile WL 1349, Labrafac CM 10, Labrafil M 2125 CS, Labrafil M 1944 CS, Labrasol and Labrafil WL 2609 BS. It is evident that the hydrolysis of 100% Labrafac Lipophile WL 1349 (unesterified medium chain triglyceride (MCT)) emulsion proceeds almost to completion (98.6%). In contrast, the other oils, all of which are PEG esterified, were not digested rapidly irrespective of the fatty acid chain length. In addition, medium chain triglycerides systems (Labrasol, Labrafac CM 10) underwent lipolysis to a greater

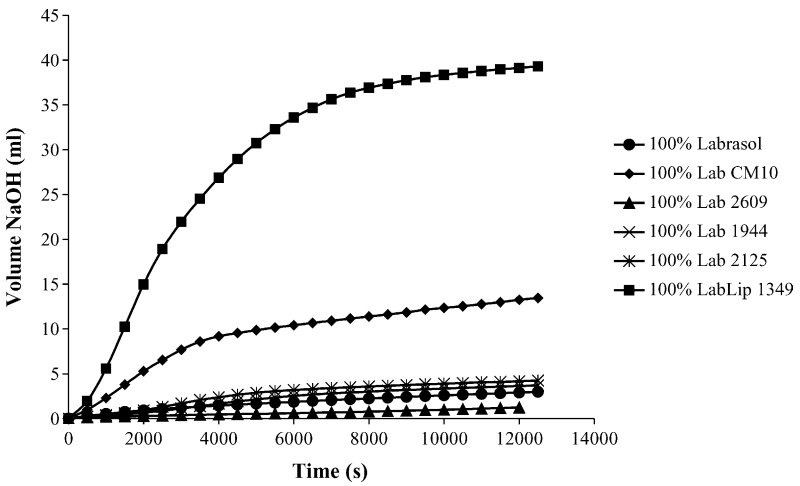

Fig. 6. The lipolysis of 100% Labrafil oil emulsions (the error incurred was within the size of the symbols).

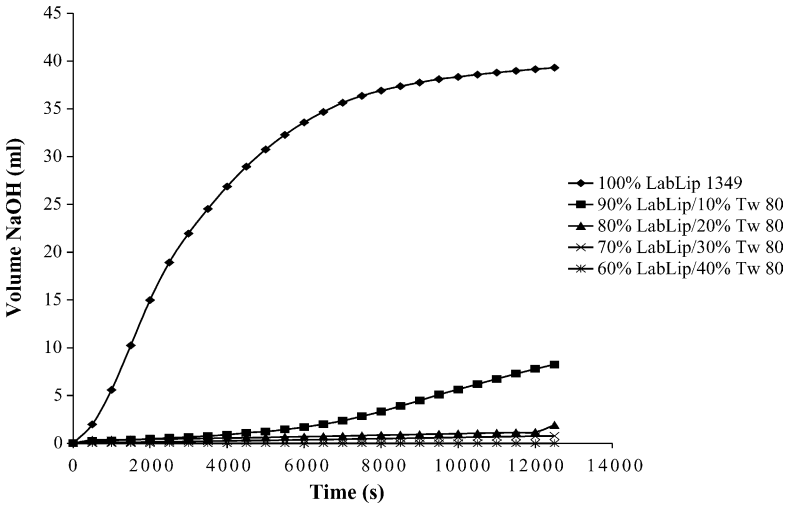

Fig. 7. The effect of Tween 80 surfactant concentration on Labrafac Lipophile WL 1349 formulations (the error incurred was within the size of the symbols).

extent than long chain triglyceride lipids (Labrafil WL 2609 BS, Labrafil M 1944 CS, Labrafil M 2125 CS). However, the effect of fatty acid chain length was difficult to determine as the size of the PEG molecule also varied for different lipid formulations. Nevertheless, a three-fold increase in the extent of lipolysis was noted for Labrafac CM 10 at 28.3% (MCT, PEG 200) compared to Labrafil M 1944 CS at 9.5% (long chain triglyceride (LCT), 8 PEG chain) despite the larger PEG group of the former. Labrafac Lipophile WL 1349 (unesterified MCT) essentially underwent complete lipolysis compared to Labrafil M 2125 CS (LCT, 6 PEG chain). However, when comparing the extent of lipolysis for Labrasol (MCT, PEG 400) and Labrafil WL 2609 BS (LCT, PEG 400), the medium chain triglyceride shows only a marginally greater percent lipolysis than the long chain triglyceride preparation. This suggests that at high PEG esterification differences in fatty acid chain length are superseded by the size of the PEG group. These results clearly indicate that in these cases the effect of PEG esterification appeared to play a more significant role in the overall extent of lipolysis than the fatty acid chain length. The effect of Tween 80 surfactant concentration on the extent and rate of lipolysis of Labrafac Lipophile WL 1349 are presented in Fig. 7. It is evident that there is a substantial reduction in the extent of lipolysis even at 10% surfactant levels.

4. Discussion

Visual and microscopic investigations confirmed that both oils studied formed multiple emulsions on gentle agitation with water, as previously reported (Devani et al., 2004). The tendency of these oils to form such emulsions with a minimum of agitation and without the need for a complex manufacturing protocol is of interest, both for preparing multiple emulsions for subsequent use using a simple and inexpensive protocol and also for the possibility of the formation of multiple emulsions on ingestion. Indeed, the systems used are more usually associated with SEDDS, hence it is entirely reasonable to suggest that the propensity to ‘spontaneously’ self-emulsify is strongly associated with the rapid emulsification behaviour shown by this family of oils. The observation that it is the more hydrophobic oils in the series (HLB 4 and 1 for Labrafil M 1944 CS and Labrafac Lipophile WL 1349 respectively) that are inclined to form multiple emulsions is perhaps surprising, given that it has been previously reported that hydrocarbon oils with greater water-solubility exhibit a greater tendency for water to pass from the external bulk medium to the internal phase and thus enhance the growth of internal droplets (Omotosho et al., 1986). The lipophilic surfactant nature of the oil phase may reduce the w/o interfacial tensions; Labrafil oils contain mono- and di-glycerides

of fatty acids and sometimes PEG esters, which act as lipophilic and hydrophilic surfactant molecules respectively. Omotosho et al. (1986) noted a correlation between the water/oil interfacial tension and the size of the internal droplets, with lower interfacial tension systems correlated with smaller internal droplets.

The rate of multiple droplet formation was found to be dependent on the nature of the oil phase and the oil/surfactant ratio, with the more hydrophobic oil (Labrafac Lipophile WL 1349) and lower surfactant concentrations resulting in more rapid maturation of the droplets. The implication from these observations is again, perhaps surprisingly, that hydrophobicity seems to be the dominant feature of rapidly forming systems rather than surface activity per se. The migration of water from the external to the internal phase has been proposed to occur by two main methods; direct diffusion and inverse micellar transport via surfactant micelles (Kita et al., 1977; Matsumoto and Kohda, 1980). Given the above correlation with hydrophobicity, the data suggests that micellar solubilisation of water by hydrophobic surface active molecules may be the dominant mechanism. Alternatively, other workers have reported a decrease in interphase transport as the viscosity of the oil phase increases (Whitehill, 1980). Labrafac Lipophile WL 1349 displays a lower viscosity of 25–35 mPa s than Labrafil M 1944 CS which has a viscosity of 75–95 mPa s at 20 °C. In addition, since Tween 80 has a much greater viscosity of 425 mPa s at 25 °C, higher concentrations of this surfactant may result in a significant increase in viscosity and hence possibly a reduction in the rate of development.

Irrespective of the mechanism of formation, a key feature associated with any applications for these systems is the stability of the formed emulsions. Instability may manifest in three principle ways; phase separation and the appearance of free oil, changes in the overall size of the particles and the loss of integrity of the multiple nature of the droplets. Free oil was observed for Labrafac Lipophile WL 1349 emulsions even at the start of the experiment, therefore it is likely that this fraction is a result of incomplete emulsification as opposed to cracking of the emulsion. The main pathways for the breakdown of multiple droplets have been described by Florence and Whitehill (1981) as being expulsion, coalescence of oil droplets, coalescence of internal water droplets and shrinkage or swelling of the internal droplets due to osmotic pressure gradients. Microscopic observations in this study indicated that early stages of alteration were noted as increases in internal droplet size (as a result of internal coalescence or external migration of water into the internal phase) and expulsion of internal droplets resulting in sparsely populated or simple oil droplets, with later stages reflecting partial phase inversion to a simple o/w emulsions. The stability of the two oils studied was dependent on the nature of the oil phase and the oil/surfactant ratio. Labrafac Lipophile WL 1349 emulsions were more susceptible to the initial stages of breakdown compared to Labrafil M 1944 CS. This may be because Labrafil M 1944 CS emulsions take longer for complete multiple development to occur and thus the features of breakdown are not evident until this stages is reached, with Labrafac Lipophile WL 1349 having a lower viscosity and hence greater tendency for internal droplet coalescence. Labrafac Lipophile WL 1349 emulsions were, however, more resistant to the latter stages of breakdown. Labrafil M 1944 CS contains PEG esterified fatty acids, which possess hydrophilic surfactant properties. Given that Adeyeye and Price (1991) have suggested that hydrophilic surfactants result in less stable multiple emulsion systems, susceptibility to phase inversion may be increased due to the presence of a greater amount of hydrophilic surfactant material (PEG ester plus Tween 80).

90% oil/10% surfactant was the most stable formulation for both oils. Several workers have noted that the ratio between the hydrophilic and lipophilic surfactant is central to the stability of the multiple emulsion (Magdassi et al., 1984; Adeyeye and Price, 1991). Although the ideal percentage of lipophilic to hydrophilic surfactant varies according to the study, the general consensus was a larger concentration of lipophilic surfactant is required to stabilise the larger interfacial area (Laugel et al., 1996). If the percentage of hydrophilic surfactant increased beyond the ideal, destruction of the multiple droplet occurs, the most cited reason being the solubilisation of the lipophilic surfactant in the micellar core of the hydrophilic surfactant (Laugel et al., 1996; Florence et al., 1989). In the present case, whereby we suggest two processes occurring over different time periods (coalescence of internal droplets, droplet expulsion/phase inversion) our hypothesis would suggest that the presence of Tween 80 would slow the coalescence of the internal

droplets but accelerate the phase inversion and droplet expulsion processes; the existence of an optimal concentration may be a reflection of these two competing considerations.

Although poorly developed multiple droplets were produced for both oils in the higher pH media investigated, Labrafil M 1944 CS produced internal liquid crystalline phases opposed to water droplets. Other workers have reported the presence of liquid crystals at the interfaces in the presence of electrolytes (Ohwaki et al., 1992; Vaziri and Warburton, 1995). Ohwaki et al. (1992) postulated that in the presence of certain additives, the interfacial tension would decrease, leading to the orientation of hydrophilic surfactants at the w/o interface to form liquid crystals.

Several studies have reported that the extent and rate of lipolysis may vary according to the fatty acid chain length, polarity and the degree of unsaturation of the lipid phase (Lowe, 1994; MacGregor et al., 1997). In this study, medium chain triglycerides were hydrolysed to a greater extent than long chain triglycerides. Other workers have also reported greater digestibility of medium chain triglycerides compared to long chain triglycerides (Yamahira et al., 1979; MacGregor et al., 1997). The presence of PEG esterified fatty acids and the non-ionic surfactant showed significant inhibition or hindrance to the lipolysis process. Several other workers have reported an inhibition of lipid digestion by hydrophilic surfactant molecules (e.g. Solomon et al., 1996). A number of theories have been proposed to explain the inhibition of lipase activity by surfactant molecules. For example, the presence of surfactant molecules at the o/w interface may occlude the attachment of lipase and/or its co-factor colipase to the interface by stearic or electrostatic inhibition (Borgstrom and Erlanson, 1973). It has been suggested that competition occurs for hydrophobic interfacial interactions between the surfactant and the enzyme/co-factor (Borgstrom, 1977). Others have suggested direct binding of the surfactant to the enzyme or co-factor altering its confirmation and thus preventing it from binding to the interface (Borgstrom and Erlanson-Albertsson, 1984; Embleton and Pouton, 1997). A study by Nano and Sarvary (1976) concluded that a reduction in the interfacial tension below a critical value may inhibit the adsorption of lipase to the interface. This would be dependent on the effectiveness of the surfactant to adsorb to the interface.

Overall, therefore, the study has indicated that multiple emulsions may indeed be formed ‘spontaneously’ via a one-step process with minimal agitation using these two oils and that, in distilled water at least, the stability of these systems may range from tens to hundreds of hours. Clearly, there is a need to optimise these systems so as to promote their stability further, particularly in a biological environment (although there may well be advantages to systems that break down on reaching specified regions of the GI tract). Nevertheless, there is undoubtedly potential for the development of these systems as a novel approach to multiple emulsion formation.

5. Conclusions

The study has demonstrated that Labrafil M 1944 CS and Labrafac Lipophile WL 1349 form multiple emulsions on gentle agitation in water, the stability of the resulting droplets being highly dependent on composition and choice of media. In general, Labrafil M 1944 CS showed a greater stability with respect to internal droplet coalescence while Labrafac Lipophile WL 1349 showed greater stability to gross breakdown, with a low concentration of Tween 80 (10%) enhancing stability in both cases. The breakdown of the droplets is considerably accelerated in simulated intestinal fluid and at high pH media, indicating that a combination of pH and ionic strength may lead to droplet breakdown. Similarly, lipolysis by pancreatic lipase was greatest for systems with lower pegylation and medium triglyceride chain length. Overall, the study has indicated that it is indeed possible to produce reasonably stable multiple emulsions via a simple one-step process, although further investigations are clearly required in order to optimise these systems as viable dosage forms.

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