ELSEVIER

International Journal of Pharmaceutics 174 (1998) 29–37

international journal of pharmaceutics

The stabilization of parenteral fat emulsion using non-ionic ABA copolymer surfactant

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Received 19 December 1997; received in revised form 24 June 1998; accepted 3 July 1998

Abstract

The effect of autoclaving on the stability of emulsions with different oil phases and different non-ionic surfactants was evaluated in order to develop a stable formulation. The effect of heating on the physicochemical properties during the autoclaving was determined by the changes in the emulsion droplet size. It was found that a combination of non-ionic copolymer surfactant (F68) with an oil phase mixture consisting of castor oil with either soybean oil or middle-chain triglycerides (MCT) 1:1 w/w yielded fine emulsions with particle sizes ranging from 120 to 140 nm. These emulsions did not show significant changes in their droplet sizes upon autoclaving and showed a good stability both in the presence of Ca^{2+} ions and at different pH values (5–9). In contrast to F68, emulsions prepared using other non-ionic emulsifiers as PEG-sorbitan monooleate (Tween 80), polyoxyethylene-660-hydroxystearate (Solutol H15) and polyoxyethylene-35-ricinoleate (Cremophor EL) showed an increase in droplet size upon autoclaving. The results could be explained on the basis of high cloud point of F68 resulting in more resistance against dehydration during autoclaving and subsequently no emulsifier damage. Due to the influence of castor oil on the interfacial tension it can act additionally as a co-surfactant. These factors avoid the flocculation of the emulsifier and can hinder the coalescence of the oil droplets during the autoclaving process.

Keywords: Fat parenteral emulsions; Non-ionic surfactants; Oil phase mixture; Interfacial tension; Emulsion stability; Cloud point.

1. Introduction

Up to the present, parenteral fat emulsions have usually been prepared using either egg yolk or soya lecithin as a surfactant (Meyer et al., 1957; Benita et al., 1989a,b) due to their metabolizing properties and a wide safety margin (Darby et al., 1979). Although emulsions stabilized with phospholipids could be autoclaved without a significant change in their physicochemical properties (Hansrani et al., 1983; Yamaguchi et al.,

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00222-1

1995a) the stability of these emulsions is somewhat critical. One factor to be considered is that the presence of ions results in a surface charge reduction with a decrease in the collision energy barrier and subsequently in a droplet coalescence, which reduces the stability (Singleton et al., 1965; Müller et al., 1994). Another factor is that the emulsion stability is pH dependent, whereas the acidic emulsion showed increased droplet sizes upon autoclaving due to the influence on the film thickness and a reduction in the dissociation of the free fatty acids (Chaturvedi et al., 1992; Bock et al., 1994).

Taking these factors into account, non-ionic surfactants were usually combined with phospholipids to improve the stability of these emulsions utilizing the steric stability of the surfactant layer (Benita et al., 1989a; Yamaguchi et al., 1995b). A thermo resistant close-packed mixed film was obtained by combination of F68 with phospholipids, which confers steric stability to the dispersed droplets (Weingarten et al., 1991). Moreover, the fractional removal rate of the parenteral emulsions from the blood stream decreased greatly by using only the non-ionic surfactants (Jeppsson et al., 1975; Müller et al., 1992). However, medium chain trigylcerides (MCT) or soybean emulsions stabilized using only F68 undergo changes in particle size upon autoclaving process (Lucks et al., 1993; Bock et al., 1994).

Therefore the effort in this study was to develop an emulsion formulation stabilized only with a non-ionic surfactants by the modification of the oil phase, which has a great influence on the emulsion stability (Benita et al., 1991; Jumaa et al., 1998). These oils chosen were soybean oil, MCT, tributyrate and castor oil alone or in mixtures, which are proved for parenteral application (Riffkin et al., 1964; Bach et al., 1989). The surfactants used were F68, Solutol H15, Tween 80 and Cremophor EL which are known for the parenteral administration (Kellner et al., 1951; Reihart et al., 1995).

2. Materials and methods

2.1. Materials

Purified castor oil and soybean oil were pur-

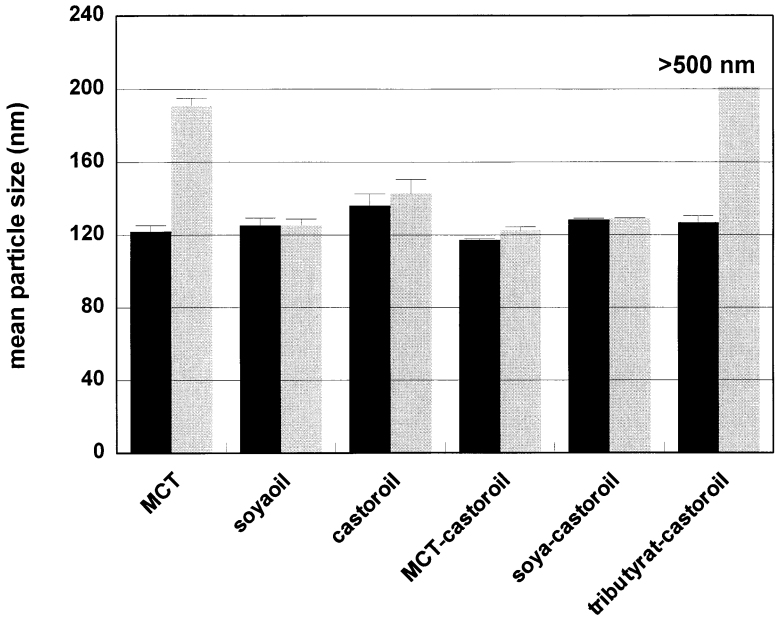

Fig. 1. Influence of the oil phase on mean particle size of the emulsions upon autoclaving (before autoclaving (closed), and after autoclaving (open)).

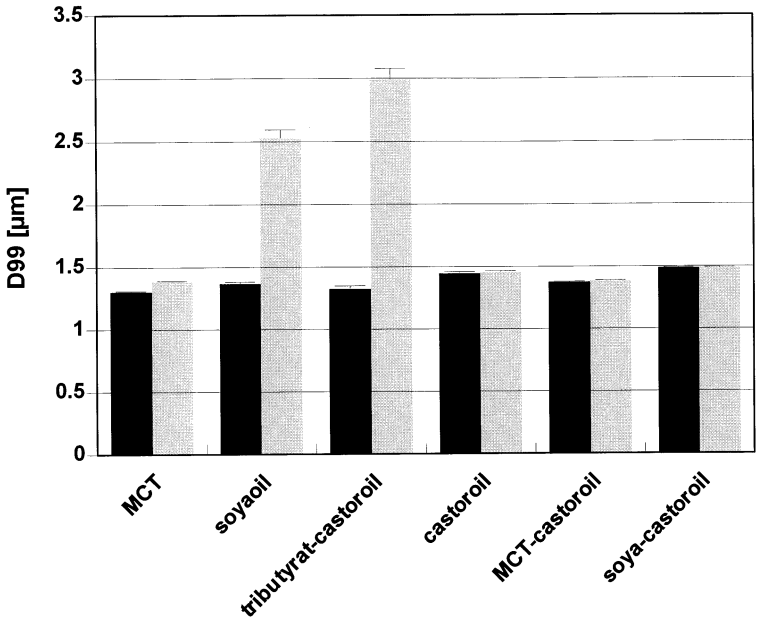

Fig. 2. Influence of the oil phase on D99 of the emulsions upon autoclaving (before autoclaving (closed), after autoclaving (open)).

chased from Henry Lamotte (Bremen, Germany) and medium chain triglyceride (Miglyol 812) was obtained from Hüls (Witten/Ruhr, Germany). Non-ionic ABA copolymer surfactant (Synperonic F68) and PEG-sorbitan monooleate (Tween 80) were supplied by ICI (Cleveland, UK) polyoxyethylene-660-hydroxystearate (Solutol H15) and polyoxyethylene-35-ricinoleate (Cremophor EL) were obtained from BASF. Tributyrate was purchased from ICN (Cleveland, UK.) and glycerol was purchased from Merck (Darmstadt, Germany). Doubled distilled water was used. All other chemicals were of reagent grade.

2.2. Preparation of emulsions

The emulsions were prepared as follows: The non-ionic surfactants were dissolved in 2.5% aqueous solutions of glycerol (for adjustment of isotonicity). The oil phase and the aqueous solution were heated separately to about 50–55°C. The oil phase was added to the aqueous solution and this mixture was pre-emulsified using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany) at 8000 rpm for 3 min.

Final emulsification was carried out by passing 40 ml of the coarse emulsion through a high pressure homogenizer (Micron Lab 40, APV Gaulin, Luebeck, Germany) eight times at a pressure of 20 MPa. The homogenization was performed at a temperature of 40°C.

The pH of the resulting emulsions was adjusted before autoclaving to different values (from 5 to 9) using 0.1 N sodium hydroxide solution. The pH was measured directly in the emulsion using Microprocessor pH/ion Meter pMX 2000 (WTW, Weilhein, Germany). The emulsions were filled into 50 ml vials and sterilized using steam autoclave (KI5T, Keller, Weinhein, Germany)at 121°C for 20 min.

2.3. Measurements

The mean diameter of the bulk population was determined by photon correlation spectroscopy (PCS) covering the size range 5 nm to approximately 3 μm (Malvern spectrometer RR 102, Malvern, U.K, with Helium-Neon laser $\lambda = 632.8$ nm, Siemens, Germany). The width of the size was expressed as polydispersity index (PI) (Becher

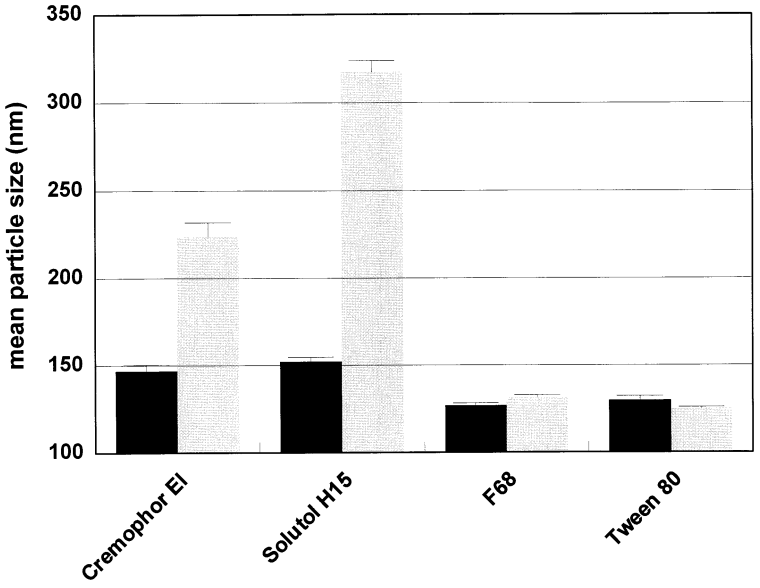

Fig. 3. Changes in mean particle size of lipid emulsions containing various cosurfactants induced by autoclaving (before autoclaving (closed), and after autoclaving (open)).

et al., 1988; Müller et al., 1991). The PI of the parenteral fat emulsions are typically in the range 0.1–0.2. For size analysis approximately 1 μl fat emulsion was added to 1 ml distilled water in order to obtain the optimum scattering intensity.

Larger particles (range 0.1–35 μm) were detected much better by laser diffractometry (Helos, Sympatec. Clausthal-Zellerfeld, Germany)

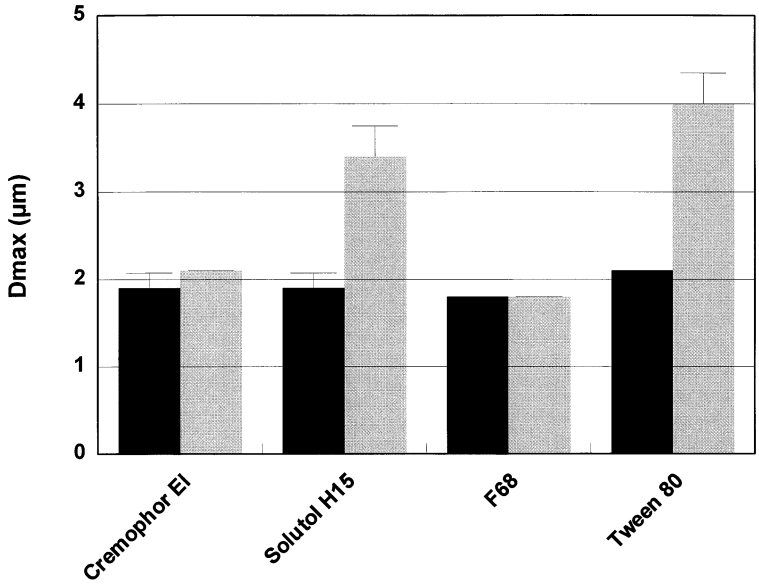

Fig. 4. Influence of the emulsifiers on Dmax of the emulsions upon autoclaving (before autoclaving (closed), after autoclaving (open)).

which yielded a particle size distribution. The emulsions were characterized by their volume diameters D50, D99 and Dmax, that means 50, 99% or all of the particles are below the given size. An additional parameter is the specific surface area calculated from the volume data (m^2/ml).

Interfacial tensions were determined by an electronic tensiometer (K122, Krüss, Hamburg-Germany) employing the plate detachment method. The interfacial tension measurements were carried out at a temperature of 40°C corresponding to the homogenization temperature.

2.4. Determination of cloud point and phase inversion temperature

The cloud point and the phase inversion of the emulsifier micellar solutions (1% w/w) were determined by heating the solutions at a rate of 1°C per min and noting the onset of turbidity. The temperature at which the solution started to become hazy was recorded. After further heating to 3°C, the solution was gradually cooled and the temperature at which the solution become clear was noted. The average temperature between these two values was the cloud point of the micellar solution. The final results were recorded as the mean of three determinations.

3. Results and discussion

3.1. Influence of different oil phases and different non-ionic surfactants on the emulsion physicochemical properties

As can be seen from the literature the nature of the oil phase has a great influence on the emulsion stability (Benita et al., 1991; Jumaa et al., 1998). Therefore different oils were suggested for the use in fat emulsion production using only non-ionic surfactants.

Table 1
The cloud points of the non-ionic surfactants (n = 3, ± Standard deviation)

Surfactant	Cloud point (°C)
F68	>100
Solutol H15	75.5 ± 1.23
Tween 80	72.5 ± 1.21
Cremophor EL	71.5 ± 1.52

In the first stage F68 was used as an emulsifier with MCT, tributyrate, soybean oil and castor oil alone or in their mixtures 1:1 (w/w). The emulsion prepared with tributyrate as a single oil phase showed at once phase separation after the autoclaving, therefore, the data are not listed. Fig. 1 shows the changes in the mean particle sizes of the emulsions 24 h after autoclaving prepared using F68 in combination with different lipid phases. The mean particle sizes of the tributyrate mixed with castor oil (1:1) or MCT alone as a single oil phase displayed the largest changes, whereas the emulsions prepared using soybean oil and castor oil as a single phase or a mixture of castor oil with either soybean oil or MCT 1:1 (w/w) showed a negligible increase in mean particle sizes. Analysis of the larger droplets by laser diffractometry revealed the same results with exception of soybean oil, which undergoes a great increase in the D99 upon autoclaving (Fig. 2). Thus it could be deduced from PCS and LD data that only either castor oil or its mixtures with MCT or soybean oil (1:1 w/w) gave an appropriate stability. Interestingly from a previous study it clearly can be seen (Jumaa et al., 1998) that emulsions prepared with castor oil alone need a higher shear rate to reduce the particle size distribution due to the high viscosity of castor oil and this leads to the formation of larger oil droplets resulting in an emulsion instability.

In the second stage these caster oil mixtures were used with different non-ionic surfactants (2% concentration w/w). Fig. 3 showed the changes in mean particle size using F68, Tween 80, Cremophor EL and Solutol H15, as emulsifiers with a mixture of castor oil/MCT 1:1 w/w. Autoclaving has a strong negative influence on the mean particle sizes of Cremophor El and Solutol H15 preparation, while the mean particle size of F68 and Tween 80 remains unchanged. Moreover Dmax from LD revealed, however, a coalescence of the Tween 80, whereas the F68 showed no changes after autoclaving (Fig. 4). Yamaguchi and his group reported that emulsions stabilized with

Table 2
Effect of F68 emulsifier concentration on emulsion droplet diameters and the emulsion stability upon autoclaving

F68 concentration w/w (%)	mean particle size (nm)		D50 (μm)		D99(μm)		Sv (m^2/ml)	
	b	a	b	a	b	a	b	a
1%	178 ± 6.5	202 ± 5	0.70 ± 0.02	0.74 ± 0.03	1.60 ± 0.03	2.50 ± 0.1	9.70 ± 0.06	9.3 ± 0.06
1.5%	148 ± 3.1	165 ± 2.5	0.61 ± 0.02	0.65 ± 0.03	1.44 ± 0.02	1.47 ± 0.04	10.1 ± 0.05	9.8 ± 0.04
2%	127 ± 3.5	132 ± 4.7	0.59 ± 0.01	0.60 ± 0.02	1.41 ± 0.02	1.42 ± 0.03	11.3 ± 0.3	11.1 ± 0.1
2.5%	118 ± 4.5	121 ± 5.3	0.56 ± 0.02	0.56 ± 0.02	1.38 ± 0.01	1.38 ± 0.03	12.1 ± 0.1	12.0 ± 0.1
3%	115 ± 4.3	120 ± 4.9	0.54 ± 0.02	0.55 ± 0.02	1.31 ± 0.02	1.32 ± 0.03	12.0 ± 0.2	11.8 ± 0.1
4%	114 ± 5.6	121 ± 5.9	0.53 ± 0.02	0.53 ± 0.03	1.28 ± 0.01	1.3 ± 0.03	12.9 ± 0.2	12.7 ± 0.2

b, before sterilization
a, after sterilization
$\pm$, standard deviation

Tween 80, undergo a great change in the particle size after autoclaving (Yamaguchi et al., 1995a,b) which is in agreement with these results.

These results could be explained on the basis of the interfacial tension properties of castor oil together with the high cloud point of F68. As shown in Fig. 5, the presence of castor oil results in a remarkable decrease in interfacial tension due to its high free fatty acid fraction, which acts as a cosurfactant (Patent, 1992; Yamaguchi et al., 1995a,b). Moreover, F68 showed the highest cloud point (as shown in Table 1) compared to other non-ionic surfactants (Bahadur et al., 1991). This means that F68 showed more resistance to undergoing dehydration at high temperature during autoclaving resulting in more stable film, which can prevent the coalescence of the oil droplets upon autoclaving (Clint et al., 1988). Therefore no changes of particle size would be observed, while Solutol H15, Tween 80 and Cremophor EL are precipitated at the sterilization temperature and this leads to breakdown of the film around the oil droplets. The partial coalescence resulted in a sudden increase in the particle size. In addition, the interfacial tension properties of castor oil itself also help to prevent coalescence of particles.

It could be deduced from the above results that emulsions consisting of F68 and a mixture of castor oil with either soybean oil or MCT showed good stability upon autoclaving. Therefore these emulsions were subjected to further studies in order to determine the appropriate emulsifier concentration to stabilize the emulsion. The data summarized in Table 2 demonstrate that increasing the emulsifier concentration up to 2–2.5% resulted in a remarkable decrease in mean particle size as well as in particle size distribution (D50, D99). This particle size distribution then levelled off at concentration higher than 2.5% and was accompanied by a maximum stability, whereas lower emulsifier concentrations showed unstable

Table 3
Effect of Ca^{2+} ions incubation (5 mmol) on stability of F68 and phospholipids emulsions

Emulsion diameters (μm)	Lipofundin S 20%			F68 emulsion			
	0	1 h	1 day	0	1 h	1 day	1 month
D50	0.45	0.76	Unstable	0.58	0.58	0.59	0.59
D99	1.15	4.95	Unstable	1.4	1.41	1.4	1.4
Dmax	1.50	10.2	Unstable	1.8	1.8	1.8	1.8
Macroscopic aspect	Creamed top layer			No change			

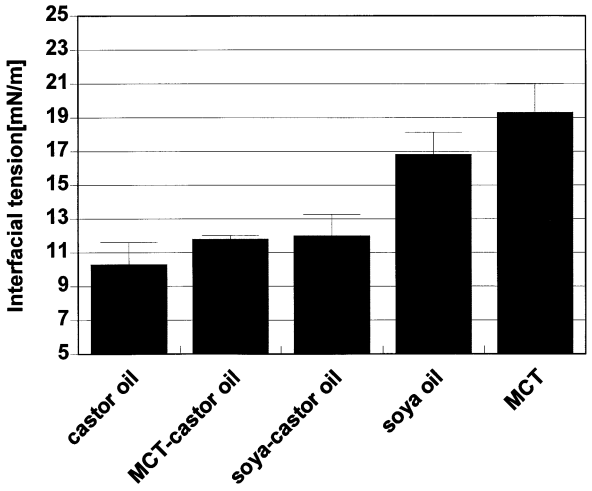

Fig. 5. Effect of the addition of castor oil on interfacial tension.

preparations upon autoclaving. This was also reflected by the specific area (S_v) obtained from LD, which increases with increasing emulsifier concentrations due to the decrease in particle sizes and thus leads to increase in the specific surface area. The insufficient stability of the emulsions with emulsifier concentration lower than 2% could be attributed to an insufficient amount of F68 to properly coat oil droplets, thus enabling them to resist sterilization. Consequently an emulsion formulation with 2–2.5% F68 was chosen because of its excellent stability (Table 2) and to avoid a high emulsifier concentration, which usually leads to toxicity side effects, an increase in emulsion viscosity (Ishii et al., 1990), and instability problems (Krafft et al., 1991; Bock et al., 1994).

3.2. Long term stability

The long term stability of the best emulsion formulations (2–2.5% F68) was further investigated in different pH values and different Ca^{2+} ion concentrations.

Fig. 6 demonstrates the storage stability of the F68 emulsions under different pH values. The data show that no significant changes in the droplet sizes of the emulsions were observed upon storage, while phospholipid emulsions are stable only in the alkaline region (Crommelin et al., 1989; Bock et al., 1994).

The effect of Ca^{2+} ion concentrations (3, 5 and 12 mmol Ca^{2+}) on the stability of F68 and phospholipid emulsions was studied upon storage. Table 3 shows that the droplet sizes of the F68 emulsions remain fairly constant for 30 days, while the opposite held true for the commercial emulsion (Lipofundin 20% MCT) stabilized with phospholipids. They rose dramatically in droplet size within the first 1 h and showed large oil droplets in the next days. In Table 3 only the 5

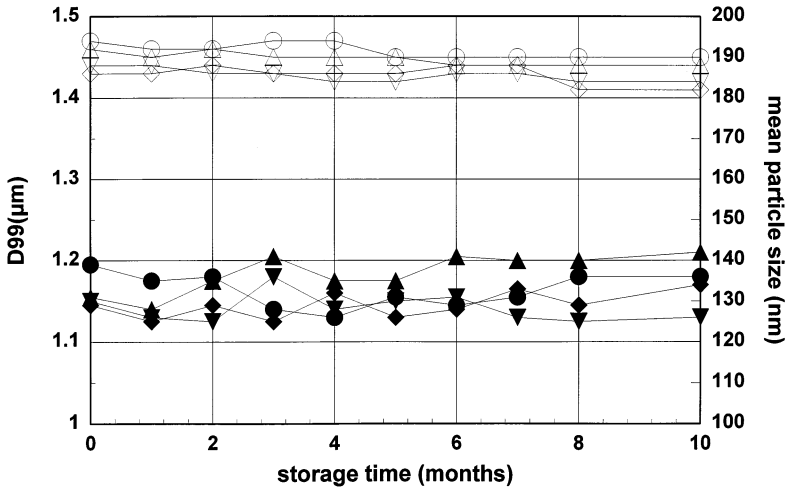

Fig. 6. Stability of F68 emulsions stored under different pH values, [mean particle size (closed), D99 (open), pH 6(△), pH 7 (○) pH 8 (◇) and pH 9 (▽)].

mmol Ca^{2+} concentration is shown because the same trend was noted in the other concentrations.

It was evident from the literature that 3–5 mmol Ca^{2+} ions led to uncharged emulsions stabilized with phospholipids. Higher concentrations lead to positive charged emulsions, but the positive zeta potential is normally not high enough for sufficient stabilization (Washington et al., 1987; Müller et al., 1994). The instability of phospholipids emulsions could be explained by the ability of divalent cations to reverse the surface charge of the phospholipids layer and reduced electrostatic repulsion by low zeta potential value (Singleton et al., 1965; Müller et al., 1994).

4. Conclusion

A parenteral fat emulsion stabilized only with F68 was developed. Utilizing the dehydration resistance of F68 during the autoclaving process according to its high cloud point and supported by the low interfacial tension of castor oil, which acts through its free fatty acid fraction as a co-surfactant, a stable emulsion formulations could be achieved. These emulsions showed an excellent stability at either different pH-values or in the presence of Ca^{2+} ions in comparison with emulsions which were stabilized with phospholipids.

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