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Research paper

Parenteral emulsions stabilized with a mixture of phospholipids and PEG-660-12-hydroxy-stearate: evaluation of accelerated and long-term stability

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Abstract

Different emulsion formulations were prepared using phospholipids (Lipoid S57) and PEG-660-12-hydroxy-stearate (Solutol HS15) as single emulsifiers or in mixtures. The accelerated stability after autoclaving, freezing and centrifugation was investigated. The long-term stability was also studied at different temperatures (4, 20, and 37 °C) for 8 months. Emulsion stabilized with phospholipids displayed a stable behavior after the autoclaving and centrifugation, but it broke down after the freezing process. In mixture with Solutol HS15, however, the emulsion showed appropriate shelf stability at different temperatures for 8 months. A change in the particle size of the emulsion prepared only with Solutol HS15 was observed after centrifugation (slight) and after autoclaving (marked). In contrast to phospholipid emulsion, this emulsion (with only Solutol HS15) was less prone to breaking down after the freezing, as no complete phase separation was observed. The results obtained using an emulsifier mixture revealed that a combination of an anionic surfactant (phospholipids) and non-ionic surfactant (PEG-660-12-hydroxy-stearate) improves the emulsion's stability, compared to the emulsion's stability prepared using only a single emulsifier. However, no direct correlation could be found between the accelerated and the long-term stability data. © 2002 Published by Elsevier Science B.V.

Keywords: Lipid emulsion; Accelerated stability; Long-term stability; Phospholipids; PEG-660-12-hydroxy-stearate; Emulsifier mixture

1. Introduction

Lipid emulsions have received increasing importance as a targeting system for poorly water-soluble drugs [1,2]. Therefore, many studies aim to formulate various drugs in lipid emulsion form [3–5]. The decrease in toxicity and the concomitant increase in the therapeutic window are potential advantages of incorporating drugs into lipid emulsions [6,7]. Also, lipid emulsions have been shown to enhance the activity and the bioavailability of the incorporated drugs [8,9].

Since emulsions are intrinsically unstable, rapid changes in their particle size and particle size distribution are considered to reflect poor physical stability [10]. Usually, the lipid emulsions were stabilized using phospholipids as an emulsifier. However, the stability of these emulsions is very sensitive to pH changes, the presence of electrolytes, and the presence of cationic molecules [11]. For example, [12] have shown that higher pH resulted in higher zeta potential value, and consequently, better stability. The presence of the electrolytes and cationic molecules disturbs the phospholipid monolayers and reduces the zeta potential, which will consequently destabilize the emulsion [13,14]. In addition, incorporating drugs into lipid emulsions can result in an interaction with the emulsifier film leading to break down of the emulsion [15].

Therefore, the enhancement of the emulsion stability has evoked great attention to achieve an emulsion formulation with an improved stability [16,17]. For example, a combination of phospholipids and a non-ionic copolymer (Pluronic F68) surfactant leads to the formation of close-packed mixed film, which confers improved stability, which is attributed to the steric stabilization of the non-ionic surfactant [18–20]. More recently, we studied the surface properties of phospholipid monolayers using Langmuir film balance after the addition of PEG-660-12-hydroxy-stearate (Solutol HS15). It was shown that this non-ionic surfactant could also form a close-packed mixed film when it was mixed with phospholipids [21]. PEG-660-12-hydroxy-stearate is a non-ionic non-toxic surfactant that could be used as

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substitute for PEG-35-ricinoleate (Cremophor EL), which was previously used in parenteral formulations [22].

Based on this background, the objective of this study was to elucidate the stability of different emulsion formulations using phospholipids, Solutol HS15 and different mixtures of Solutol HS15 with phospholipids after autoclaving and after 8 months storage time (at different temperatures). The accelerated stability after freezing and centrifugation was also studied with the intent to try to find out the best stable formulation.

2. Materials and methods

2.1. Materials

Phospholipids (Lipoid S75 (S75)) were isolated from soybean oil and contained according to the manufacturer (Lipoid GmbH, Ludwigshafen, Germany) a minimum of 70% phosphatidylcholine, 10% phosphatidylethanolamine and 1.7% lysophosphatidylcholine. Medium-chain triglycerides (MCT) were obtained from Hüls (Miglyol 812, Witten/Ruhr, Germany). Purified castor oil was purchased from Henry Lamotte (Bremen, Germany). Polyoxyethylene-660-12-hydroxystearat (Solutol HS15®) was supplied by BASF (Ludwigshafen, Germany). Sorbitol for parenteral application was purchased from Merck (Darmstadt, Germany). Double distilled water was used for all preparations. All other chemicals were of reagent grade or higher.

2.2. Experimental methods

2.2.1. Preparation of the emulsions

Typical oil-in-water (o/w) emulsions were prepared, as previously described [23], using a 20% oil phase containing a mixture of castor oil with MCT (1:1). Different emulsion systems were prepared using phospholipids S75 (1.5%), Solutol HS15 (2%), and mixtures of phospholipids with Solutol HS15. The aqueous phase contained the non-ionic surfactants and 5% aqueous solutions of sorbitol to enable adjustment to isotonicity. The oil phase, in which the phospholipids were dissolved, and the water phase were heated separately to about 50–55 °C. The oil phase was added to the aqueous solution and the mixture was pre-emulsified using an Ultra-Turrax T25 (Jahnke & Kunkel, Staufen, Germany) at 8000 rev./min for 3 min. A submicron emulsion was obtained by passing the coarse emulsion through a high pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany) eight times at a pressure of 20 MPa. Subsequently, the pH of the resulting emulsions was adjusted to 8 ± 0.05 using 0.1 N sodium hydroxide solution (because lipid emulsions are only stable in a pH value higher than 7.5). The homogenizer capacity is 40 ml, therefore, 40 ml of each emulsion formulation was prepared and the emulsions were filled into 15 ml vials. The vials were sealed and the emulsions were sterilized by autoclaving (K15T, Keller, Weinheim, Germany) at 121 °C for 15 min. All emulsion formulations were carried out in triplicate.

2.2.2. Emulsion characterization

The mean droplet size of the emulsions was determined by photon correlation spectroscopy (PCS) covering the size range from 5 nm to approximately 3 μm (Malvern spectrometer RR 102, Malvern, UK, with Helium-Neon laser $\lambda = 632.8$ nm, Siemens, Germany). For size analysis approximately 1 μl emulsion was added to 1 ml distilled water in order to obtain the optimum scattering intensity. Larger particles (range 0.18–35 μm) were detected by laser diffraction analysis (LDA) (Helos, Sympatec, Claushal-Zellerfeld, Germany), which yielded a particle size distribution using a lens with a 20 mm focal length. The emulsions were characterized by their volume diameters D_{50}, D_{99} and D_{max}, which means 50%, 99% or all of the particles are below the given size. The surface charge (zeta potential) was measured using a ZetaSizer 3 (Malvern Instruments, Malvern, UK). The electrolyte solution used for dilution consisted of double distilled water with a conductivity of 50 μS/cm adjusted by NaCl (0.5 mmol/l). A total of 500 μl of each emulsion formulation was diluted with 20 ml electrolyte solution.

2.2.3. Accelerated stability tests (centrifugation and freezing)

The freezing and centrifugation tests were performed as reported in the literature [18].

The centrifugation test was performed at ambient temperature ($2000 \times g$/60 min). In the freezing/thaw test, the emulsion vials were stored at −18 °C for 24 h. These emulsions were stored for another 24 h at 25 °C.

All emulsion formulations were characterized before and after centrifugation and freezing cycle using Helos and PCS. Also, the macroscopical changes were observed and reported. All experiments were performed in triplicate.

3. Results and discussion

3.1. Stability of emulsions with a single emulsifier

Emulsions stabilized with Solutol HS15 or with phospholipids showed sufficient stability after the centrifugation process and no phase separation as well as no marked change in their particle size were observed (Table 1). In contrast to this, both emulsions underwent great changes in their physicochemical properties after the freezing process. Emulsion stabilized with phospholipids showed a visual phase separation, whereas the Solutol emulsions were less prone to breaking down after the freezing process (Table 1).

Because their particles are larger than the filter's pores the filtration method is not an appropriate technique to sterilize lipid emulsions, and thus the application of heat sterilization

Table 1
Accelerated stability of the emulsions stabilized with phospholipids or with Solutol H15 (as a single emulsifier)[a]

Stress test	D_{50} (μm)		D_{99} (μm)		D_{max} (μm)		PCS (nm)	
	Before	After	Before	After	Before	After	Before	After
Phospholipids								
Centrifuging	0.65	0.62	1.47	1.44	1.8	1.8	156.8	135.9
Freezing	Complete phase separation							
Autoclaving	0.65	0.65	1.47	1.48	1.8	1.8	157.5	164.1
Solutol H15								
Centrifuging	0.6	0.51	1.42	1.23	1.8	1.5	153.9	118.9
Freezing	Thin oily film on the surface (no complete phase separation)							
Autoclaving	0.6	0.66	1.42	1.96	1.8	3.6	153.5	322.7

[a] The variation coefficient for the LDA measurements was lower than 2% and for PCS measurements lower than 3% (calculated from three measurements).

is required. Since lipid emulsions are thermodynamically unstable systems, the autoclaving process often accelerates the degradation of the emulsions. At the same time, this process could also be considered as an additional accelerated stability test. Emulsions stabilized with phospholipids displayed a high stability during the autoclaving process, whereas Solutol emulsion underwent a great change in its particle size after autoclaving (Table 1). Both emulsion systems, in the long-term stability, showed no phase separation after 8 months at 4, 20, and 37 °C. The particle size measurements of phospholipid emulsions also showed the same pattern and no significant change in their particles were observed, whereas Solutol emulsion displayed a slight change in its particle size at 37 °C (Table 2). The stability results of phospholipid emulsion correspond well with findings in the literature [24].

From the above results, it can be seen that both emulsions underwent significant changes in their physicochemical properties after the freezing process but negligible changes after centrifugation. Moreover, only the phospholipid emulsions displayed a good stability after autoclaving even after 8 months at different temperatures.

The Solutol emulsion was, in contrast, damaged after the autoclaving (a slight change in the particle size was also observed after 8 months storage at 37 °C). Hence, no significant correlation between the accelerated and the long-term stability tests could be drawn. The observed changes in the emulsion systems in the accelerated tests could in most cases be attributed to the direct change of the emulsifier properties after application of such stress treatments, which resulted in a sudden instability of the systems studied [25,26].

Table 2
Long-term stability of the emulsions stabilized with phospholipids or with Solutol H15 (as a single emulsifier) after 8 months at different temperatures[a]

Temperature (°C)	D_{50} (μm)	D_{99} (μm)	D_{max} (μm)	PCS (nm)
Phospholipids				
4	0.64	1.47	1.8	169.4
22	0.64	1.47	1.8	161.8
37	0.66	1.49	1.8	172.8
Solutol H15				
4	0.62	1.44	1.8	159.4
22	0.64	1.47	1.8	168.9
37	0.65	1.49	1.5	171.8

[a] The variation coefficient for all measurements (LDA and PCS) was lower than 3.5% (calculated from three measurements).

3.2. Stability of emulsions prepared with emulsifier mixtures

The above results indicate that Solutol HS15 alone cannot appropriately stabilize parenteral emulsions, since it shows insufficient stability after the autoclaving process. In contrast, phospholipid emulsion showed a stable behavior during the autoclaving process. However, as reported in the literature, this stability is critical. This is because of the high sensitivity of the phospholipids (electrostatic stability) to the changes in the pH value especially in the presence of electrolytes [13,14]. Hence, a combination of the phospholipids offering an electrostatic stabilization and Solutol HS15, which offers a steric stabilization, would result in a more stable formulation. Therefore, a mixture of S75 and Solutol HS15 (in different ratios) was used and the stability of the different formulations was investigated. In this trial, the total concentration of the emulsifier's mixture was kept constant (1.5%) but the composition of the mixture was varied.

Fig. 1 shows the effect of the Solutol HS15 ratio on the particle size and the surface charge (zeta potential) of the resulting emulsions. The addition of Solutol HS15 to phospholipids leads to an initial decrease in the particle size. A further increase of the Solutol ratio resulted again in a slight increase in the emulsion particle size. However, the lowest particle size was observed at the ratio 1:1 (S75/Solutol HS15). This indicates clearly that using a mixture of phospholipids with Solutol HS15 can emulsify the oil phase more effectively than the single emulsifiers (either phospholipids or Solutol HS15 alone). Moreover, as might be

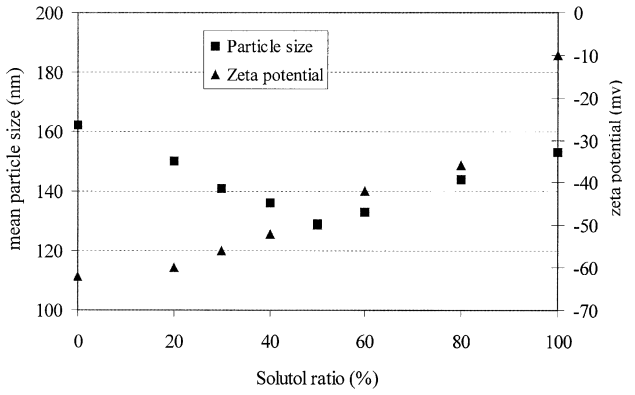

Fig. 1. The effect of the Solutol HS15 ratio on the particle size and the zeta potential of the resulting formulations.

expected, the addition of Solutol to the phospholipids led to a decrease in surface charges of the oil droplets resulting in a decreased ratio of the anionic surfactant (phospholipids).

A comparison of the zeta potential data with the particle size results showed that a decrease in particle sizes of emulsions was accompanied by a decrease in negative surface charge values. This indicates that Solutol participated in the emulsifier film formation. Furthermore, previous work [21] on the surface properties of phospholipid monolayers after the addition of Solutol HS15 has shown that the Solutol HS15 molecules are intercalated between the phospholipid monolayers forming a molecularly mixed film. Hence, based on the surface properties and the physicochemical properties, a mixed interfacial film comprising the Solutol HS15 and phospholipid molecules was formed at the o/w interface. Therefore, the physicochemical properties and the stability (accelerated and long-term stability) of emulsions prepared from a mixture of phospholipids with Solutol HS15 in different ratios were studied. The aim was to find out the more suitable formulation with an appropriate stability after the autoclaving process and with sufficient long-term stability.

Table 3 shows that formulations prepared with Solutol ratios higher than 60% displayed great changes in their particle size after the autoclaving process. Formulations containing less than 60% Solutol showed stable behavior during autoclaving with no changes in their particle sizes. The reason may be two-fold: (i) decreasing the Solutol ratio will result in an increase in the zeta potential value; consequently, this will increase the stability of the emulsion [27]; (ii) increasing phospholipid concentrations will increase the cloud point of the resulting emulsifier mixture, which will also increase the phase inversion temperature (PIT) resulting in more stable behavior during autoclaving. Formulations with a high cloud point are more resistant to dehydration at high temperatures occurring during the autoclaving [25]. As a result, the coalescence of the oil droplets should be prevented to achieve a stable emulsion behavior [10,23]. In order to show this, the long-term stability and the stability after freezing and centrifugation of formulations with Solutol ratios lower than 60% were further investigated. Since formulations with 20 and 50% Solutol HS15 give an overall idea of the stability of the other formulations (Solutol ratios between 10 and 60%), the data of these two formulations were shown. These formulations displayed enhanced stability during the centrifugation as no significant changes in their particle size were detected (Table 4). Additionally, it was noticed that formulations with a higher Solutol ratio (50%) (Table 4) tend to show less phase separation after the freezing process. This different behavior among the formulations (Solutol free and with different ratios of Solutol) could be attributed to the disturbance in the lamellar structure of phospholipids [28,29]. This means that during freezing the lamellar associated water will crystallize, which will damage the lamellar structure of phospholipid monolayers and consequently result in an instabilization of the emulsions. The freezing process will be less effective in the case of Solutol formulations, since it does not show this lamellar structure. Thus, the freezing process will be less effective and the emulsions will show more stable behavior.

The long-term stability carried out with 20% Solutol formulation showed a good stability with no changes in its

Table 3
Effect of PEG-660-12-hydroxy-stearate ratio on the emulsions droplet diameter upon autoclaving[a]

Solutol HS15 ratio (%)	PCS (nm)		D_{99} (μm)		D_{max} (μm)	
	Before	After	Before	After	Before	After
0	176	184	1.59	1.6	1.8	1.8
20	163	168	1.48	1.49	1.8	1.8
30	158	165	1.48	1.48	1.8	1.8
40	156	162	1.47	1.48	1.8	1.8
50	148	153	1.46	1.46	1.8	1.8
60	155	161	1.48	1.49	1.8	1.8
70	159	163	1.49	1.49	1.8	3.6

[a] The variation coefficient for the LDA measurements was lower than 3% and for PCS measurements lower than 1.5% (calculated from three measurements).

Table 4
Accelerated stability of the emulsions stabilized with a mixture of phospholipids with Solutol H15[a]

Stress test	D_{50} (μm)		D_{99} (μm)		D_{max} (μm)	
	Before	After	Before	After	Before	After
20% Solutol HS15 ratio						
Centrifuging	0.6	0.58	1.41	1.38	1.5	1.5
Freezing			Phase separation			
50% Solutol HS15 ratio						
Centrifuging	0.54	0.53	1.35	1.33	1.5	1.5
Freezing	0.55	0.66	1.39	9.8	1.58	12.25

[a] The variation coefficient for the LDA measurements was lower than 3% and for PCS measurements lower than 3% (calculated from three measurements).

Table 5
Long-term stability of the emulsions stabilized with a mixture of phospholipids with Solutol H15 after 8 months[a]

Temperature (°C)	D_{50} (μm)	D_{99} (μm)	D_{max} (μm)
20% Solutol HS15 ratio			
4	0.58	1.44	1.8
22	0.58	1.46	1.8
37	0.60	1.47	1.8
50% Solutol HS15 ratio			
4	0.53	1.36	1.5
22	0.53	1.37	1.5
37	0.54	1.37	1.5

[a] The variation coefficient for all measurements was lower than 3.5% (calculated from three measurements).

physicochemical properties (Table 5). Similarly, good stability at different temperature was observed for formulation at 50% Solutol. In contrast to the formulation prepared only with Solutol as emulsifier, this 50% Solutol formulation did not undergo any changes in its physicochemical properties after 8 months at different temperatures (Table 5).

Moreover, using the non-ionic surfactant Solutol greatly enhanced the stability of the emulsions at different pH values. As shown in Fig. 2, the emulsion stabilized with an emulsifier mixture (1:1 S75 and Solutol) showed no significant changes in its large particle (D_{99}) after 8 weeks. Emulsions stabilized with only phospholipids, as well known from the literature, are only stable in the alkaline region [12]. Furthermore, using the non-ionic surfactant as a part of the emulsifier film will also enhance the stability of the lipid emulsions in the presence of Ca^{2+} ions compared to emulsions stabilized only with phospholipids [23]. This is due to the utilization of the steric stabilization effect offered by the non-ionic surfactant, which is usually pH- and ion-independent. However, this stability depends on the ratio of the non-ion surfactant [30]. Hence, a formulation of 1:1 phospholipids and Solutol HS15 seems to be an attractive alternative formulation, which offers adequate electrostatic and steric stability.

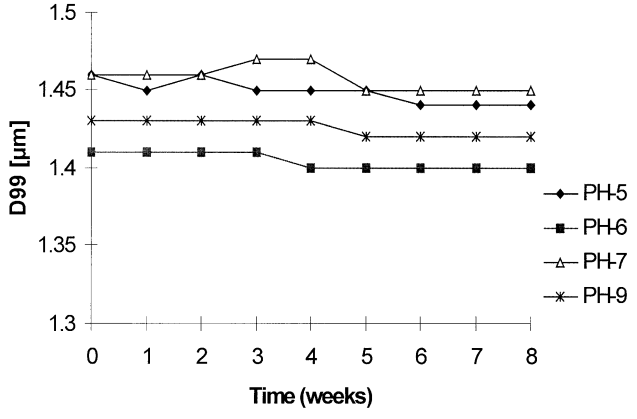

Fig. 2. Stability of emulsions stabilized with emulsifier mixture (1:1 phospholipids and Solutol HS15).

Hence, the presence of a non-ionic emulsifier Solutol offers a steric stabilization, which can enhance the stability at different pH values and in the presence of electrolytes compared to the emulsion stabilized only with phospholipids. Simultaneously, the use of the phospholipids improved the stability of the Solutol emulsion during autoclaving as well as during storage at shelf. A formulation prepared using this mixture appeared to be appealing because it yields much more stable emulsions than those obtained with only single emulsifier.

4. Conclusion

The combination of anionic and non-ionic surfactants, namely phospholipids and Solutol HS15, yields a formulation with an improved stability compared to the formulations stabilized only with one emulsifier. However, monitoring the long-term stability seems to be necessary because the stability under stress conditions is not always the appropriate way to predict the long-term stability.

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