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Research paper

The influence of alkali fatty acids on the properties and the stability of parenteral O/W emulsions modified with Solutol HS 15®

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Abstract

Arachis oil based parenteral O/W emulsions were prepared using soya bean phosphatidylcholine (SPC) and different combinations of co-emulsifiers containing polyethylene glycol fatty acid esters (Solutol HS 15®) and alkali fatty acids (sodium laurate, sodium stearate). The parameters measured were droplet size (both by photon correlation spectroscopy and laser diffractometry), pH and zeta potential. All emulsions were subjected to autoclaving. The addition of polyethylene glycol 12-hydroxy stearate (Solutol HS 15®) led to a significant decrease of mean oil droplet size. For long-term stability the amount added turned out to be the most important factor. With increased amounts of Solutol HS 15® the packing density of the emulsifier layer and the zeta potential decreased leading to instability. The optimum load of Solutol HS 15® was found to be 15 μmol/ml. Alkali fatty acids markedly improved the physical stability of the emulsions. Improved stability properties conferred to emulsions by alkali fatty acids could be attributed to the zeta potential increase even in the presence of Solutol HS 15®. Consequently a mixed emulsifier film was established in which the ionized fatty acids determined the interface charge. In addition to this a strengthening of the molecular interactions occurring between phospholipid and Solutol HS 15® emulsifier in the presence of ionized fatty acids at the O/W interface can be assumed (L. Rydhag, The importance of the phase behaviour of phospholipids for emulsion stability, Fette Seifen Anstrichm. 81 (1979) 168–173). Different co-emulsifier mixtures were shown to have a pronounced impact on the plasma protein adsorption onto emulsion droplets.

Keywords: O/W emulsion; Solutol HS 15®; Alkali fatty acid; Plasma protein adsorption

1. Introduction

O/W emulsions which are intended to be used as targeted drug delivery systems should be tailor-made to be suitable for different biological environments. A certain specificity in biodistribution can be achieved *passively* by control of the physicochemical properties of the injected carrier systems, such as particle size and dose, together with surface charge and surface characteristics [1]. Emulsion systems which are qualified for a drug targeting need to exhibit a sufficient half-life in the blood to assure the possibility that the drug-carrier-system-conjugate may reach the desired target in intact form [2]. Generally emulsion droplets are retained by the phagocytotic mononuclear cells of the reticuloendothelial system (RES) in liver, spleen and bone marrow [3] after having been opsonized by plasma proteins prior to phagocytosis. Small differences in the composition of the oil-water interface which affect emulsion particle hydrophobicity/hydrophilicity can alter opsonization and RES-uptake significantly. Another difficulty with emulsion particles as drug carriers is to reach any site of action in the extravascular space, especially through intact endothelium (cut-off < 100 nm) due to the relatively larger mean particle size of about 500–700 nm. The relations between opsonization, phagocytosis, RES-uptake, and surface charge are far from simple and concomitant changes in particle size may override any effects produced by variations in surface properties. For an *active* targeting strategy so-called homing devices need to be attached to the particle surface. Those are structures which bind specific to determinants at the target side [4].

The suitability of emulsion droplets for a drug targeting can be considerably improved by adding co-emulsifiers which alter the biodistribution of the carrier systems by their impact on particle size, charge, and surface. On the

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other hand, the incorporation of co-emulsifying excipients improves the stability of the emulsion droplets by enhancing the mechanical strength of the interfacial film formed around the oil droplets, by steric stabilization effects, and/or by electrostatic interactions which create an electrostatic barrier. The effect is much more pronounced with the use of mixed emulsifying agents [5].

It has been reported that non-ionic PEG-derivatives provide stability in the surrounding medium by a repulsion effect [6] leading to an increased circulation half-life as well. The non-ionic solubilizer Solutol HS 15® (polyoxyethylene-660-12-hydroxy stearate) was the co-surfactant chosen due to its physiological compatibility on intravenous application and its potential to provide binding sites for homing devices. The addition of alkali fatty acid molecules to the phospholipid was thought to yield mixed films with a higher packing of interfacial film forming components [7].

The objective of the present paper was to optimize parenteral O/W emulsions by varying formulation parameters to obtain the smallest droplet size of emulsions that can remain stable for a long period of time. Furthermore it was of interest to investigate the impact of different co-emulsifier mixtures on the plasma protein adsorption onto emulsion droplets as an indicator for their in-vivo biodistribution behaviour [8].

2. Experimental section

2.1. Materials

The soya bean lecithin (SPC), Phospholipon 80®, which consists of $76 \pm 3\%$ phosphatidylcholine, was kindly donated by Nattermann GmbH (Köln, Germany). The non-ionic co-emulsifier Solutol HS 15® was supplied by BASF (Ludwigshafen, Germany). The alkali fatty acids, sodium laurate and sodium stearate, were purchased from Fluka Chemicals (Neu-Ulm, Germany). The arachis oil was furnished by Lamotte (Bremen, Germany).

2.2. Emulsion preparation

The phospholipid Phospholipon 80® (1.5% w/w) was dispersed in arachis oil (20%w/w) at 50°C. The non-ionic co-emulsifier Solutol HS 15® and the alkali fatty acid were dissolved in bidistilled water. Both phases were heated separately to 40°C and dispersed by an ultra-turrax (T 25, Janke and Kunkel, Staufen, Germany) for 3 min at 8000 rev./min. This coarse emulsion was passed seven times through a high pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany) with 30 MPa at 40°C. The emulsions were sterilized by steam autoclaving at 121°C for 20 min.

2.3. Particle size

The volume distribution of the particle size was analyzed using laser diffractometry (Helos, Sympatec, Clausthal-Zellerfeld, Germany). All results were calculated according to the Fraunhofer theory. Photon correlation spectroscopy (PCS, Malvern PCS RR 102, Malvern Instruments, Great Malvern, UK) was employed for particle size determination after emulsion preparation and autoclaving respectively. The scattering angle was 90°, the wavelength of the coherent helium-neon laser used was 632.8 nm. The mean diffusion coefficient and the polydispersity were determined by means of cumulants-fit analysis of the intensity.

2.4. Particle charge

The particle charge was determined by electrophoresis measurements using a Malvern Zetasizer 3 (Malvern Instruments, UK) and expressed as zeta potential. All samples were diluted in 0.5 mmol/l NaCl prior to measurement.

2.5. pH measurements

The pH-values of the O/W emulsions were measured by use of a pH-Meter (Microprocessor pH/Ion Meter PMX 2000, WTW Weilheim, Germany) with a Micro-Glasselectrode (Type N 6000 A, Schott, Germany). The equipment was calibrated with standard buffer solutions (Buffer WTW: PL 7: pH 6.865; PL 9: pH 9.18, and PL 4: pH 4.006). Each sample was measured after 5 min of equlibration.

2.6. Two-dimensional polyacrylamide gel electrophoresis analysis

Two dimensional polyacrylamide gel electrophoresis (PAGE) was applied for the determination of protein adsorption onto emulsion droplet surfaces. Two millilitres of emulsion were incubated with 6 ml human plasma at 37°C for 5 min. The dispersed oil phase was separated from excess plasma by four centrifugation steps performed at $15\,000 \times g$ for 60 min alternating with three washing steps with phosphate buffer (pH 7.4). After desorbing the proteins by means of a 10% (w/w) sodium dodecylsulfate (SDS) solution an aliquot was transferred to the first dimension of the 2-D-PAGE analysis for isoelectric focussing by immobilized non-linear pH gradients (3.5–10) (Amersham Pharmacia, Uppsala, Sweden). The samples were analyzed by 2-D-PAGE analysis as described in the literature [9]. The protein spots were detected by densitometric measurements after silver staining.

2.7. Experimental design

A central composite design and a 3^2-design were used for conducting the experiments. The exact conditions (coded and real values) are listed in Tables 1 and 2.

All experiments were conducted as randomized blocks. Each experiment was performed twice except for the central point which was conducted four times to obtain an estimate of the experimental error. The following equation was used to calculate the surface plots:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2$$

where y is the response parameter of interest; x_1 the component 1; x_2 the component 2; and β_i the fit parameters resulting from multiple linear regression.

Table 1
Coded and real values of the central composite design

	Coded values		Real values	
	Component 1	Component 2	Phospholipon 80® in (μmol/ml)	Solutol HS 15® in (μmol/ml)
1	1	0	20	10.6
2	0	1	15	21.2
3	− 1	0	10	10.6
4	0	− 1	15	0
5	√0.5	√0.5	18.5	18.1
6	√0.5	√0.5	11.5	18.1
7	√0.5	√0.5	11.5	3.1
8	√0.5	√0.5	18.5	3.1
9	0	0	15	10.6
10	1	1	20	21.2
11	− 1	1	10	21.2
12	− 1	− 1	10	0
13	1	− 1	20	0

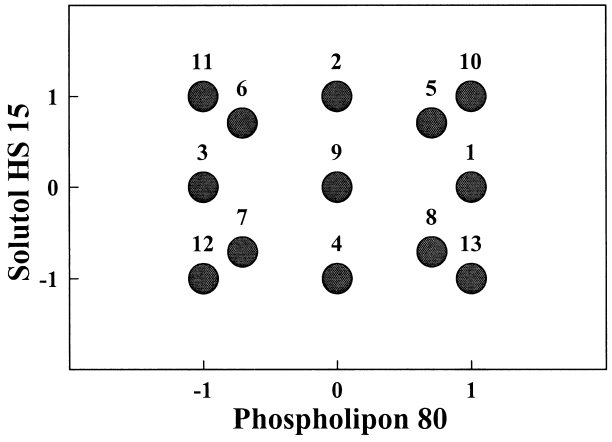
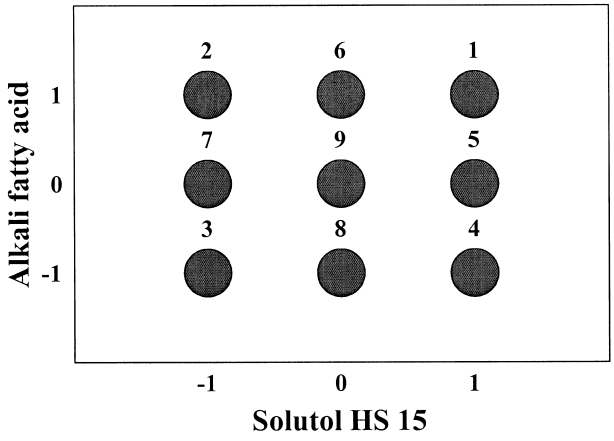


Table 2
Coded and real values of the 3^2-design

	Coded values		Real values	
	Component 1	Component 2	Solutol HS 15® (μmol/ml)	Alkali fatty acid (μmol/ml)
1	1	1	21.2	10
2	− 1	1	0	10
3	− 1	− 1	0	2
4	1	− 1	21.2	2
5	1	0	21.2	6
6	0	1	10.6	10
7	− 1	0	0	6
8	0	− 1	10.6	2
9	0	0	10.6	6



The model chosen takes into account linear and quadratic dependencies as well as interactions of the components. Lack of fit was not significant at $\alpha = 5\%$. The r^2 ranged between 0.93 and 0.99.

3. Results and discussion

3.1. Incorporation of Solutol HS 15®

The addition of polyethylene glycol-660-12-hydroxy stearate (Solutol HS 15®) led to a significant decrease of mean particle size (D 50%-value) reaching a minimum at an incorporation efficiency of 15 μmol/ml. By further increasing the amount of Solutol HS 15® the droplet sizes increased .again (Fig. 1). This might be due to the spacious hydrophilic parts of the co-emulsifier [10]. The fatty acid chain is bound up into the interface whereas the hydrophilic polymer remains at the surface of the oil droplet.

After autoclaving it was observed that the emulsion formulations containing 40–50 μmol/ml of the non-ionic co-emulsifier exhibited an irreversible phase separation whereas emulsions containing 30 μmol/ml Solutol HS 15® and less maintained their particle size distribution pattern. This might be correlated to the exceeding of the clouding point of aqueous solutions of Solutol HS 15® which was measured to be 75–80°C. At higher temperatures Solutol HS 15® becomes more and more dehydrated and tends to leave the interfacial layer and to form micelles. So with regard to an increasing share of Solutol HS 15® of the mixed emulsifier interface, the leakage of Solutol HS 15® from the interfacial layer results in a lower packing density of the remaining emulsifier molecules which are not sufficient to stabilize the whole interface [10].

With increased amounts incorporated a reduced stability not only in autoclaving, but also in long-term stability resulted. After 6 months storage the D50%-value of emulsion preparations containing 20–30 μmol/ml Solutol HS 15® shifted to 5% higher values. So for the physical stability the amount added was determined to be the most important

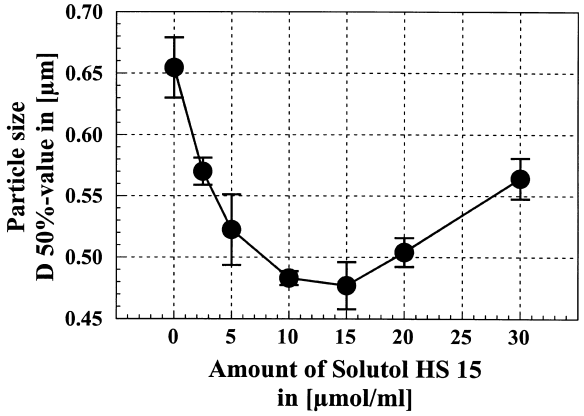


Fig. 1. Influence of increasing amounts of Solutol HS 15® on the mean particle size (D50%-value) of O/W emulsions ($n = 4$, confidence intervals, $\alpha = 5\%$).

factor. The optimum co-emulsifier load was detected to be 15 µmol/ml.

When increasing the incorporated amount of the nonionic Solutol HS 15® the negative zeta potential decreased as shown in Fig. 2. The zeta potential is determined by the presence of the negatively charged SPC. As described above, the incorporation of a defined amount of Solutol HS 15® lead to a proportional displacement of SPC in the interfacial layer. This results in a shielding of the negative surface charge provided by SPC and in a less dense interfacial film due the spacious co-emulsifier share. Altogether, this leads to a decrease in surface potential [11] and therefore in zeta potential as well. Taking the critical micelle concentration (CMC) of Solutol HS 15® into account which was determined to be 0.021% (w/v) by surface tension measurements using the Wilhelmy plate method, it might be also likely that the amount of SPC available is reduced when SPC is solubilized in Solutol HS 15® micelles [12,13]. Regarding the long-term stability the negative zeta potential of a stable O/W emulsion should not fall below −30 mV. Even though all measurements were well above

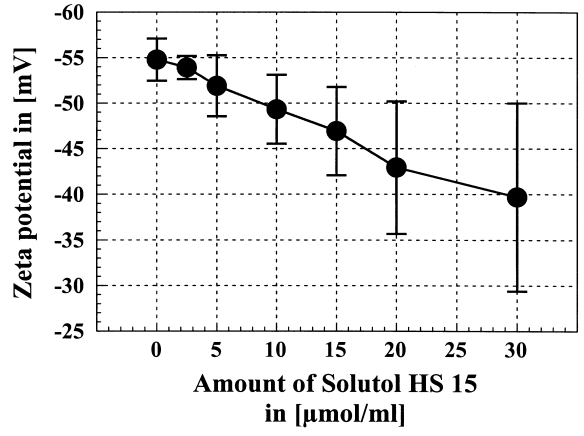


Fig. 2. Influence of increasing amounts of Solutol HS 15® on the zeta potential of O/W emulsions ($n = 4$, confidence intervals, $\alpha = 5\%$).

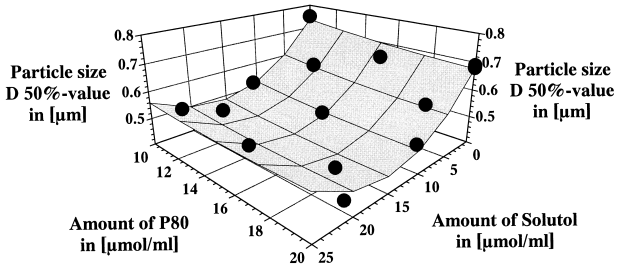


Fig. 3. Influence of Phospholipon 80® and Solutol HS 15® on the mean particle size (D 50%-values) of O/W emulsions ($n = 2$).

this critical value, the results showed growing standard deviations when greater amounts of Solutol HS 15® were incorporated. This indicates a rising instability as well.

For further optimizing the formulation the amounts of SPC and Solutol HS 15® were varied according to a central composite design. It was intended to study whether the observed effect was due to the total amount of the emulsifier/co-emulsifier mixture added or due to a certain ratio of both components involved in the stabilization of the interfacial layer.

The experimental design is shown in Table 1. As can be seen in Fig. 3 the surface plot of the mean particle size (D 50%-value) of the emulsion droplets showed a minimum for emulsion formulations which were formulated with 10–15 µmol/ml Solutol HS 15® regardless of the amount of SPC added. Accordingly the statistical analysis revealed a statistically significant effect of the amount of Solutol HS 15® added (x_2 and x_2^2). However, when the D 99%-values (data not shown) were more closely examined, it was observed that the interaction term had a significant influence as well. For further evaluation of the optimum area of small particle sizes which can remain stable over a long period of time the PCS data were analyzed (Fig. 4). The results showed again the optimum load of Solutol HS 15® being 15 µmol/ml regardless of the amount of SPC added.

3.2. Addition of alkali fatty acids to O/W emulsions modified with Solutol HS 15®

The addition of alkali fatty acids led to a mixed emulsifier

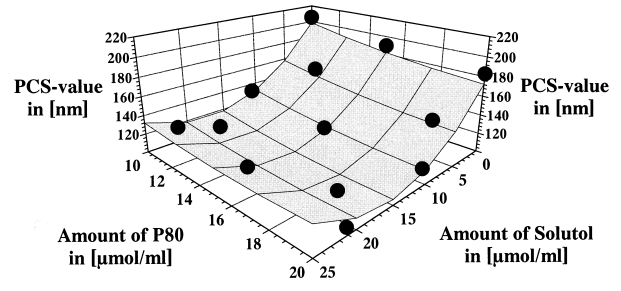


Fig. 4. Influence of Phospholipon 80® and Solutol HS 15® on the PCS data of O/W emulsions.

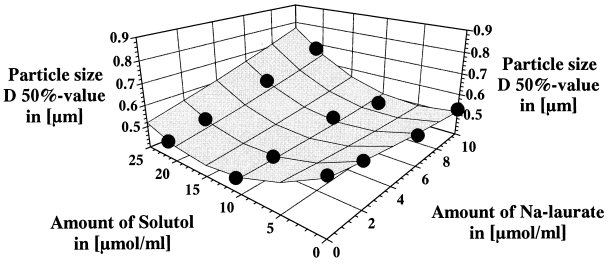


Fig. 5. Influence of Solutol HS 15® and sodium stearate on the mean particle size (D 50%-value) of O/W emulsions ($n = 3$).

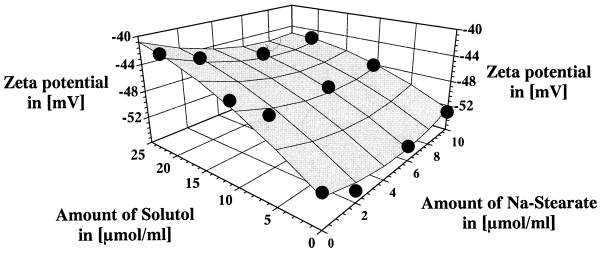


Fig. 7. Influence of alkali fatty acids on the zeta potential of Solutol HS 15®-modified O/W emulsions ($n = 2$).

layer with increasing packing density [7,14]. The statistic analysis of the D 50%-values of sodium laurate-modified emulsion systems revealed the significant impact of the co-emulsifiers themselves and their interaction on the droplet size. As Fig. 5 shows, the mean particle sizes of emulsions, which were only stabilized with one of the components are inverse to those which were stabilized with a mixture of both co-emulsifiers. Thus the D 50%-values of emulsions prepared with increasing amounts of sodium laurate decreased while those of emulsion formulations co-stabilized with sodium laurate in the presence of Solutol HS 15® increased. An analogous behaviour was found for Solutol HS 15®-modified preparations by addition of sodium laurate. When the total amount of the mixture of both co-emulsifiers was more closely examined, minimum D 50%-values were measured for emulsion systems which were stabilized with 20 µmol/ml of an equimolar mixture of both components (Solutol HS 15®:sodium laurate = 10 µmol/ml:10 µmol/ml). By incorporating a long chain fatty acid derivative, sodium stearate, instead of sodium laurate the deviations in mean particle sizes were detected to be negligible. After autoclaving the impact of the chain length of the fatty acid derivative turned out to be more pronounced (Fig. 6). The results showed sodium stearate to be most favourable.

Improved stability properties conferred on emulsions by alkali fatty acids could be attributed to the negative zeta potential increase even in the presence of Solutol HS 15® (Fig. 7). Adding up to 10 µmol/ml sodium stearate to an O/W emulsion modified with 21.2 µmol/ml Solutol HS 15® increased the surface charge of the droplets by about 5 mV.

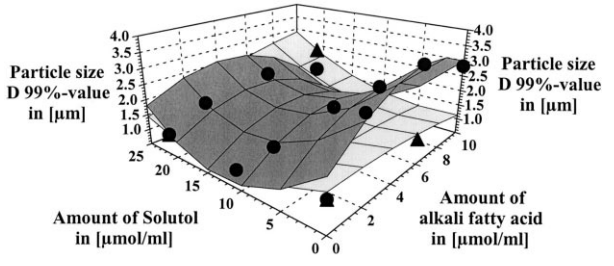


Fig. 6. Influence of Solutol HS 15® and alkali fatty acids on the particle size (D99%-values) of O/W emulsions after autoclaving. Key: ●, sodium laurate; ▲, sodium stearate.

Consequently the ionized fatty acids determined the interface charge.

Alkali fatty acids are commonly added to emulsions for total parenteral nutrition to adjust the pH of the formulations to physiological values. As Fig. 8 shows, the resulting pH of emulsions modified with Solutol HS 15® and alkali fatty acids was strongly dependent on the ratio of co-emulsifiers used. Again, equimolar co-emulsifier mixtures could be shown to result in pH-values very well within an acceptable physiological range. Furthermore it could be noticed that a slightly alkali pH had a positive influence on the chemical stability of emulsion formulations.

3.3. Plasma protein adsorption onto emulsion droplets

The incorporation of a non-ionic co-emulsifier was intended to result in a hydrophilization of the particle surface. Thus it could be expected that the amount of adsorbed plasma proteins onto the emulsion droplets would be diminished. However, when incorporating Solutol HS 15® the total amount of adsorbed plasma proteins was tremendously increased. The increase was less pronounced in the presence of alkali fatty acids (Fig. 9).

For a more detailed analysis of the influence of the co-emulsifiers on the plasma protein adsorption pattern the amount of apolipoproteins and of immunoglobulins were evaluated separately. By incorporating alkali fatty acids into emulsion formulations the total apolipoprotein adsorption was decreased by about 10% in comparison to the reference emulsion. An analogous effect was observed in the presence of the non-ionic co-emulsifier Solutol HS 15®. However, when comparing the adsorbed amount of

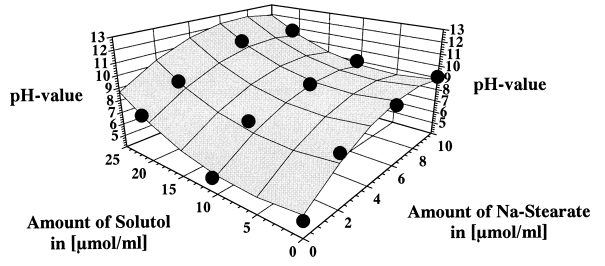


Fig. 8. Influence of Solutol HS 15® and sodium stearate on the pH of O/W emulsions.

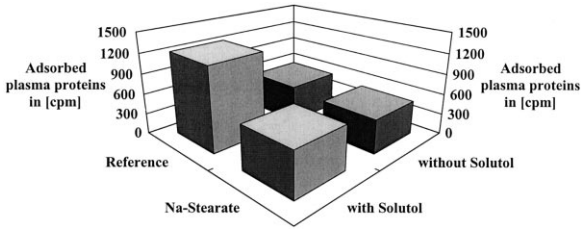


Fig. 9. Influence of Solutol HS 15® and sodium stearate on the adsorbed amount of plasma proteins onto O/W emulsion droplets.

apolipoproteins onto alkali fatty acid modified droplet surface the presence of Solutol HS 15® led to an additional decrease by about 20%. The data are shown in Fig. 10.

Concerning the immunoglobuline adsorption it was noticed that the presence of Solutol HS 15® led to a significant increase in IgD and IgG γ adsorption. On the other hand, the incorporation of alkali fatty acids had a profound effect on the amount of adsorbed IgD as well (Fig. 11). It has been described in the literature that the prononced adsorption of IgD onto emulsion droplet surfaces is a common feature of these colloidal systems [15]. The reasons for it as well as the implications for the biodistribution of emulsion carriers are still under investigation.

4. Conclusions

The mean particle size of O/W emulsions can markedly be decreased by addition of the non-ionic co-emulsifier Solutol HS 15®. However, the resulting lower packing density of the mixed interfacial layer had a negative contribution to the long-term stability of those emulsion preparations. The optimum load was found to be 15 μmol/ml Solutol HS 15®.

With regard to enhancing the mechanical strength of the interface the addition of alkali fatty acids turned out to be suitable. The incorporation of alkali fatty acids had a positive influence on the surface charge of Solutol HS 15®-modified emulsion droplets. The zeta potential increased by about 5–10 mV leading to an improved electrostatic stabilization of the colloidal systems. When comparing different chain length the long chain fatty acid derivative, sodium stearate, seemed to be superior in strengthening the interfacial layer. The pH of the alkali fatty acid modified

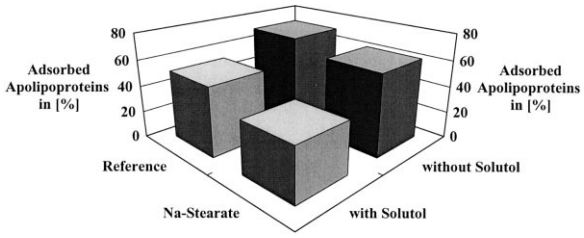


Fig. 10. Influence of Solutol HS 15® and sodium stearate on the adsorbed amount of apolipoproteins onto O/W emulsion droplets.

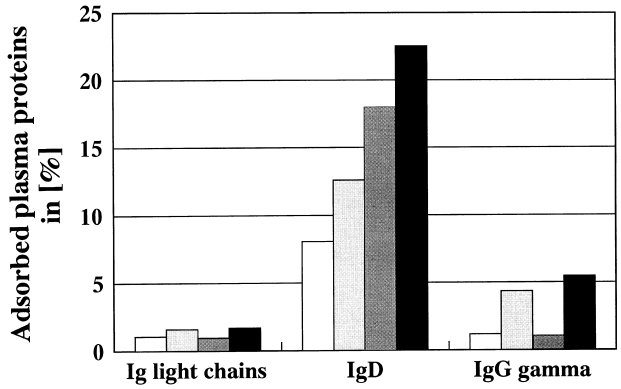


Fig. 11. Influence of Solutol HS 15® on the adsorbed amount of immunoglobulins onto sodium stearate-modified O/W emulsion particles ($n = 2$). Key: first column, reference; second column, 10.6 μmol Solutol HS 15®; third column, 4 μmol sodium stearate; fourth column, 4 μmol sodium stearate, 10.6 μmol Solutol HS 15®.

emulsion formulations was measured to be in the range between 8 and 9 leading to an acceptable biocompatibility.

For the evaluation of the in vivo behaviour of different emulsion formulations the plasma protein adsorption has been taken as an indicator. It was observed that the presence of Solutol HS 15® led to a tremendous increase in total plasma protein adsorption. This effect was less pronounced in the presence of alkali fatty acids. The plasma protein adsorption pattern showed a reduce in apolipoprotein adsorption by 20% in the presence of Solutol HS 15®. However, the adsorbed amount of immunoglobulins, especially of IgD and of IgG γ, was increased. It can be concluded that the incorporation of the non-ionic co-emulsifier showed an influence on the pattern of the plasma protein adsorption. However, the implications for an in-vivo biodistribution behaviour are still unknown.

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