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Research paper

In vitro adsorption of plasma proteins onto the surface (charges) modified-submicron emulsions for intravenous administration

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Abstract

Surface (charge) modified submicron emulsions (cationic and anionic) were prepared following the well established combined emulsification techniques and characterized for their droplet size distribution and surface charge. The effect of these emulsions on in vitro adsorption of plasma proteins was investigated by means of two dimensional polyacrylamide gel electrophoresis (2D PAGE). The presence of poloxamer 188 in tested emulsions effectively eliminated the adsorption of the larger proteins like immunoglobulins, fibrinogen, etc. However, depending on the type of surface charges, the smaller proteins such as apolipoproteins and albumin were almost completely adsorbed onto the submicron emulsions. Indeed, when compared to marketed lipofundin MCT 10%—and deoxycholic acid—based anionic emulsions, the adsorption of apolipoprotein, especially apoA-1, was approximately three times more on stearylamine—and oleylamine—based cationic emulsions and oleic acid-based anionic emulsions. In addition, the ratio between the apoA-1 and apoA-IV was found to be 1 for lipofundin MCT 10% whereas it was about 0.26 for deoxycholic acid-based anionic emulsion and above 5 for oleic acid-based anionic emulsions and cationic emulsions. This indicates that emulsions having similar surface/interfacial charge imparted by different anion-forming stabilizers (oleic or deoxycholic acids) exhibited markedly different protein adsorption patterns.

Keywords: Cationic; Anionic; Emulsion; In vitro; Plasma protein; Adsorption

1. Introduction

It is well known that the clearance of long-chain (LC) triglyceride emulsions such as Intralipid® closely resembles the clearance of chylomicrons [1] and is susceptible to the interaction of HDL acquiring plasma apolipoproteins [2]. Erkelens et al. [3] reported that an anionic emulsion formulation (Intralipid®) captures apolipoproteins within minutes after an infusion in man, facilitating its elimination. Once protein-free emulsions acquire apolipoproteins along with other plasma proteins like albumin, fibrinogen, etc. the opsonins and other blood proteins can promote phagocytosis by forming a 'bridge' between the emulsion particles and the phagocyte [4]. As a result, the emulsions will undergo the metabolic and transport pathways of lipoproteins and then be eliminated rapidly from the bloodstream. Studies [5,6] have shown that small changes in physical properties of fat emulsions can influence the elimination rate of these formulations from the blood. Indeed, an organ distribution study in male BALB/c mice of stearylamine-based cationic or deoxycholic acid-based anionic submicron emulsion formulations and Intralipid® was carried out [7,8]. In comparison to both of the anionic emulsions, the stearylamine-based cationic emulsion elicited a much longer retention time in the plasma, indicating clearly a long circulating half-life for cationic emulsion in the blood. To enhance the drug targeting efficacy of colloidal carriers like nanospheres and liposomes, a pegylation/cationization strategy is traditionally designed for the surface of these carriers [9]. Whereas surface pegylated colloidal carriers exhibit a prolonged plasma residence time through an

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escaping tendency from reticuloendothelial system (RES) uptake following parentral administration, surface cationized colloidal carriers can facilitate the penetration of therapeutic agents into cell surface possibly via an endocytotic mechanism. The long circulating effect of the cationic emulsion was attributed to the composition and conformation of the mixed emulsifiers film at the o/w interface, namely lipoid E80, Poloxamer 188 and stearylamine/oleylamine which created an electrostatic and steric barrier at the oil–water interface. This combined effect is well established and has been reported previously in an in vitro monolayer study [10].

To further substantiate the previously observed in vivo behaviour difference between the cationic and anionic emulsions, in vitro plasma protein adsorption patterns need to be tested onto these types of colloidal carriers to elucidate the anticipated influence of the various, mixed emulsifier combinations used while preparing these formulations.

The objective of this study was to investigate in vitro adsorption of plasma proteins onto the cationic and anionic submicron emulsions. For this purpose, the 2D PAGE technique, which was previously modified and successfully transferred to the marketed emulsion formulation [11,12], was used.

2. Materials and methods

2.1. Materials

Medium-chain triglyceride (MCT) was purchased from Societe des Oleagineux (St Blangy, France). MCT consisted of not less than 95% esterified fatty acids, comprising eight and 10 carbon atoms according to manufacturer's specifications. Lipoid E80 was purchased from Lipoid AG (Ludwigshafen, Germany). The Lipoid E80, according to manufacturer's specifications, was comprised of 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% nonpolar lipids and about 2% sphingomyelin. Polyoxyethylene–oloxypropylene emulsifier, poloxamer 188 (Pluronic F68), was furnished by BASF (Parsippany, NJ, USA). Stearylamine (SA) and deoxycholic acid were purchased from Sigma Co. (St Louis, MO, USA). Oleylamine (OA) was purchased from Fluka Chemika-BioChemika AG Industries (Buchs, Switzerland). Oleic acid was obtained from E-Merck (Darmstadt, Germany). All other ingredients were pharmaceutical grade.

2.2. Formulation development

The nonionic emulsifier (poloxamer 188) and the osmotic agent (glycerol) were dissolved in the aqueous phase and adjusted to pH 4.0. The Lipoid E80, anti-oxidant (α-tocopherol) and the cationic lipids SA or OA were dissolved in the MCT oil phase. Both phases were heated separately to 70 °C, after which the two phases were mixed and stirred with a magnetic stirrer. The resulting mixture was further heated to a temperature of 85 °C. At this temperature, the crude emulsion which was obtained, was further mixed by a high shear mixer Polytron™ (Kinematica, Luzerne, Switzerland) for 5 min and rapidly cooled to below 20 °C. After cooling, the emulsion was homogenized using a two-stage homogenizer valve assembly (Gaulin Homogenizer, APV Gaulin, Hilversum, the Netherlands) at 9000£/in.^{2} for 5 min. Due to the presence of SA or OA, the emulsion pH levels at this stage were around 9.0–9.5. Therefore, adjustment to pH 8.0 was carried out by titration with hydrochloric acid (0.1N). The emulsion was then filtered through a TE membrane filter (Schuell and Schleicher, Dassel, Germany) with a pore size of 0.45 μm. The emulsion was packed under nitrogen atmosphere in siliconized glass bottles and then sterilized by steam autoclave at 121 °C for 15 min. Again the pH was adjusted to 7.0 in order to emulate the pH change due to autoclave acidosis. The typical formulation (% w/w) consisted of MCT (8.5), Lipoid E-80 (1.2), SA or OA (0.3), α-tocopherol (0.02), poloxamer 188 (2.0), glycerol (2.25) and bi-distilled water (to 100). The anionic emulsions consisted of the same components, but instead of 0.3% SA or OA, 2.83% oleic acid or 0.5% deoxycholic acid were added. All other procedures were identical with the exception of the pH that was adjusted to 8.0 from the acidic side using sodium hydroxide (0.1N).

In total, four different formulations (two cationic and two anionic emulsions) along with a marketed emulsion lipofundin® MCT 10% (B. Braun Melsungen AG, Germany) were used to investigate and compare the protein adsorption onto emulsion droplets.

2.3. Emulsion characterization

Droplet size measurements were carried out utilizing an ALV NonInvasive Back Scattering High Performance Particle Sizer (ALV-NIBS HPPS; Langen, Germany) at 25 °C and using water (refractive index: 1.332; viscosity: 0.894543) as the solvent. A laser beam at 632 nm wavelength was used. The sensitivity range was 0.5 nm–5 μm. The lipofundin® emulsion droplet size was determined using a Zetasizer 4 in 90° angle modulus (Malvern Instruments, Malvern, UK) following appropriate dilution with double distilled water.

The zeta potential measurements were carried out using the Malvern Zetasizer 3000 (Malvern Instruments, Ltd, Malvern, UK). The samples were diluted in double distilled water and the measurements were carried out in 10 mM NaCl solution. The zeta potential of lipofundin® was measured in double distilled water with a conductivity of 50 μS/cm adjusted with NaCl solution (0.9%).

2.4. Protein adsorption experiments

Human plasma was drawn from healthy male volunteers at the DRK Blutspendedienst (Berlin, Germany) and stored

Table 1
Physical characterization of blank submicron emulsions

Emulsion types	Zeta potential[a] (mV ± SD)	Particle size[a] (nm ± SD)
Stearylamine-based	+47.8 ± 0.89	183 ± 30
Oleylamine-based	+37.2 ± 2.35	167 ± 50
Oleic acid-based	−36.1 ± 3.60	205 ± 52
Deoxycholic acid-based	−34.4 ± 0.84	192 ± 48
Lipofundin MCT 10%	−36.0 ± 0.26	250 ± 14

[a] $N = 3$.

at −30 °C. For 2D PAGE all chemicals were of analytical grade. Acrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate and piperazine diacrylamide (PDA) were purchased from Bio-Rad (Munich, Germany). All other chemicals [13,14] were supplied either from Fluka Chemie AG (Buchs, Switzerland) or Merck (Darmstadt, Germany).

Two-dimensional polyacrylamide gel electrophoresis (2D PAGE) is a powerful tool for the simultaneous detection of up to 10,000 proteins at the same time. The 2D PAGE separates the proteins according to two different parameters. In the first dimension, isoelectric focusing (IEF) is carried out for protein separation due to their isoelectric points (pI). The second dimension, a SDS-PAGE separates the proteins according to their molecular weights. This protocol enables the determination of proteins with an isoelectric point ranging from pH 4 to 10 and a molecular weight between 250 and 6 kDa. For the analysis of the in vitro plasma protein adsorption patterns on emulsion droplets with different surface charge, 2 ml of each emulsion were incubated with 6 ml citrate stabilized human plasma. Incubation of the samples took place for 5 min at a temperature of 37 °C. Emulsion droplets were separated from bulk plasma by centrifugation with 15,000*g* (Cryofuge 20-3, Hereaus, Germany). Afterwards they were washed three times with phosphate buffer pH 7.4. Protein desorption was carried out with a SDS-solution according to a previously reported method [15,16]. For the first dimension immobilized nonlinear pH gradients (IPG) ranging from 3.5 to 10 (Amershan Pharmacia, Sweden) were used. Sample entry was performed by in-sample-rehydration in a custom-made reswelling tray [14,17].

The slab gels were cast with a Model 395 gradient former and multigel casting chamber containing 9–16% acrylamide gradient and 2.6% PDA as cross-linker.

All chemicals, devices and running conditions are described elsewhere [12,15,18]. After 2D PAGE, the gels were silver stained according to Blunk [19] and scanned with an ImageScanner (Amershan Pharmacia, Sweden). Protein identification was carried out by matching the stained spots to a master map of human plasma which is accessible on the Expasy server [20]. Semi-quantitative analysis of protein spots was possible with the Melanie III software (Bio-Rad, Germany).

3. Results

Table 1 shows the mean droplet size and zeta potential values of the various emulsion formulations. Regardless of the modifications made in the compositions of the developed emulsions which render them either cationic or anionic, the mean droplet diameters of these emulsions were in the range of 167–205 nm and presented a monodispersed droplet size distribution. The data presented in Table 1 show that the zeta potential of the developed formulations was of the same order of magnitude, either positive charges for cationic emulsions or negative charges for anionic emulsions. The mean droplet size and zeta potential of Lipofundin® MCT 10% emulsion were close to the respective values of the developed anionic emulsions.

Table 2 shows the plasma protein adsorption onto the surfaces of the developed cationic and anionic emulsions as well as the marketed lipofundin MCT 10%. Fig. 1 shows the 2D PAGE gel of lipofundin MCT 10%. The proteins designated in Fig. 1 represent more than 60% of the overall protein amount detected following adsorption on the emulsion droplets. Apolipoproteins with a total amount of 45.6%, were the most dominant protein species of the detected protein pattern, i.e. apoC-II (5.6%), apoC-III

Table 2
Comparison of values (volume%) of adsorbed plasma proteins on surfaces of developed anionic and cationic emulsions and lipofundin MCT 10%

Plasma proteins	Volume percentage of detected plasma proteins after their adsorption onto				
	Anionic emulsions prepared based on		Cationic emulsions prepared based on		Lipofundin MCT 10%
	Oleic acid	Deoxycholic acid	Stearylamine	Oleylamine	
Albumin	0.0 ± 0.0	2.9 ± 0.6	2.7 ± 1.4	2.5 ± 0.5	2.0 ± 1.1
ApoA-I	25.0 ± 1.2	9.7 ± 6.2	33.2 ± 4.6	30.5 ± 2.1	9.7 ± 2.8
ApoA-II	2.5 ± 0.0	6.8 ± 0.9	6.1 ± 1.6	4.8 ± 2.7	9.2 ± 1.5
ApoA-IV	4.6 ± 4.2	36.9 ± 0.4	6.7 ± 1.8	4.5 ± 0.3	10.5 ± 3.1
ApoC-II	1.8 ± 0.3	12.0 ± 0.9	6.2 ± 1.7	4.5 ± 1.2	5.6 ± 2.1
ApoC-III	4.5 ± 0.1	14.4 ± 0.4	8.6 ± 2.7	7.9 ± 3.7	10.6 ± 3.4
ApoE	0.0 ± 0.0	1.9 ± 0.9	1.5 ± 0.9	1.2 ± 0.1	2.9 ± 0.9
ApoJ	0.0 ± 0.0	0.4 ± 0.0	0.8 ± 0.5	0.8 ± 0.3	0.5 ± 0.4
Ig-gamma-chain	2.2 ± 0.0	0.0 ± 0.0	0.9 ± 0.7	1.2 ± 0.0	8.1 ± 2.5
Ig-light-chain	6.3 ± 4.3	1.3 ± 0.0	1.5 ± 0.6	3.0 ± 0.2	0.0 ± 0.0

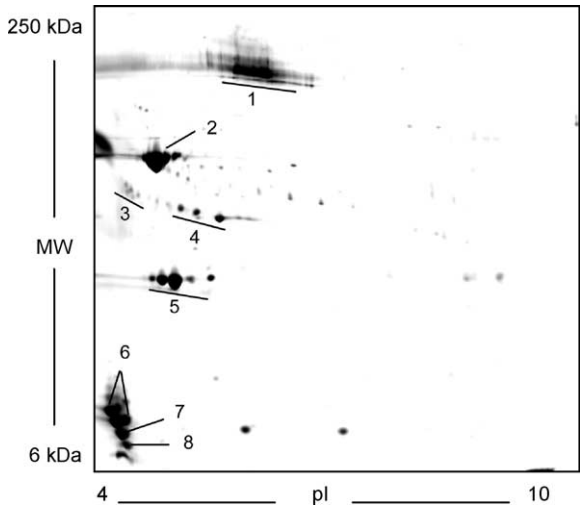

Fig. 1. 2D PAGE gel with protein adsorption pattern of lipofundin MCT 10% after incubation in human plasma. Identification: (1) albumin, (2) ApoA-IV, (3) ApoJ, (4) ApoE, (5) ApoA-I, (6) ApoC-II, (7) ApoC-III, (8) ApoA-II.

(10.6%), apoA-I (9.7%), apoA-II (9.2%) and apoA-IV (10.5%).

Fig. 2 compares the plasma protein adsorption patterns detected on the 2D PAGE gels for both the cationic and anionic emulsions. From Figs. 1 and 3 and Table 2, it can be seen that with the exception of oleic acid-based anionic emulsion formulations, all other tested emulsions showed similar protein adsorption patterns when they were qualitatively compared. For oleic acid-based anionic emulsion formulation, no albumin is adsorbed and a reduction of apoA-IV is visible, whereas light chains of Ig are clearly detectable. Fig. 3 shows the volume percentage of the major plasma proteins detected on the 2D PAGE following adsorption onto the surfaces of various emulsion formulations. These major proteins accounted for more than 60% of the total protein amount seen for all the formulations with the exception of oleic acid-based anionic emulsion formulation that had a ratio of 46.9% only. It appears as if one of the key features of deoxycholic acid-based anionic emulsion is that the major proteins as shown in Fig. 3 and Table 2 account for more than 86% of all proteins adsorbed. This is in contrast to oleic acid-based anionic emulsion with less than 47%, and all others which range between 59 and 68%. Between oleic acid—and deoxycholic acid—based anionic emulsions, a significant difference was seen among the adsorbed plasma proteins particularly in apolipoproteins and light chain Ig. The adsorbed amount of apoA-I, Ig-gamma-chain and Ig-light-chain on the oleic acid-based anionic emulsion was higher than the amount of corresponding adsorbed proteins on the deoxycholic acid-based anionic emulsion (25.0 ± 1.2 versus 9.7 ± 6.2, 2.2 ± 0.0 versus 0.0 and 6.3 ± 4.3 versus 1.3 ± 0.0,

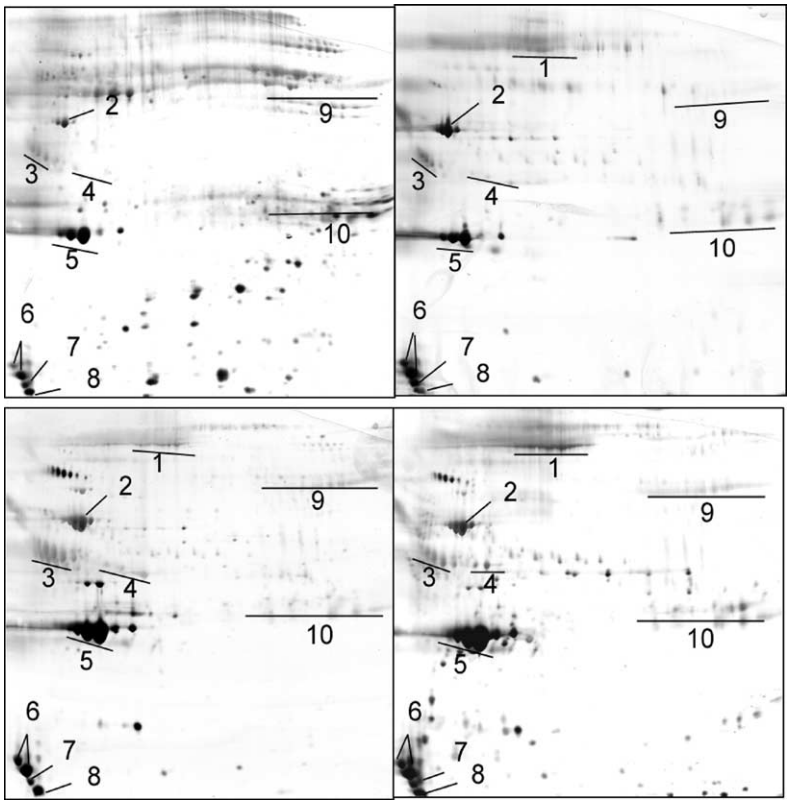

Fig. 2. Silver stained 2D PAGE gels of emulsions with different surface charges after incubation in human plasma: upper left, negative formulation I (oleic acid-based anionic emulsion); upper right, negative formulation II (deoxycholic acid-based anionic emulsion); lower left, positive formulation I (stearylamine-based cationic emulsion); lower right, positive formulation II (oleylamine-based cationic emulsion). Identification: (1) albumin, (2) ApoA-IV, (3) ApoJ, (4) ApoE, (5) ApoA-I, (6) ApoC-II, (7) ApoC-III, (8) ApoA-II, (9) IgG-gamma-chain, (10) Ig-light-chain.

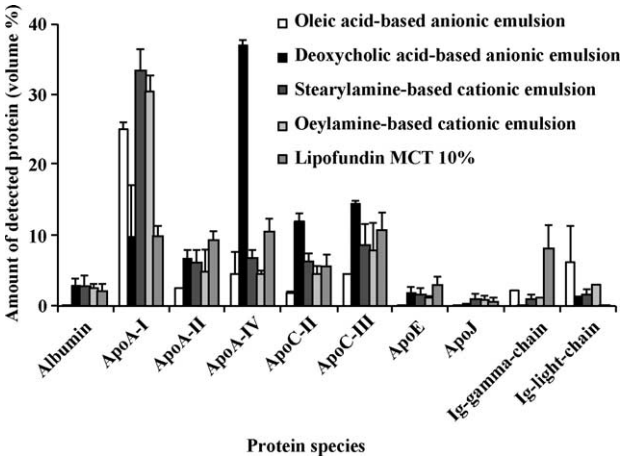

Fig. 3. Amount of major proteins on the 2D gels of plasma proteins adsorbed on emulsions with negative or positive surface charge in comparison with lipofundin MCT 10%.

respectively). However, in comparison to the oleic acid-based emulsion, higher adsorption occurred onto the deoxycholic acid-based emulsion, respectively, for the following proteins: albumin (2.9±0.6 versus 0.0), apoA-II (6.8±0.9 versus 2.5±0.0), apoA-IV (36.9±0.4 versus 4.6±4.2), apoC-II (12.0±0.9 versus 1.8±0.3) and apoC-III (14.4±0.4 versus 4.5±0.1). When compared to lipofundin MCT 10% anionic emulsion, the oleic acid-based anionic emulsion showed an increment in protein adsorption pattern only with the apoA-I (25.0±1.2 versus 9.7±2.8) and Ig-light-chain (6.3±4.3 versus 0.0). A moderate reduction in the protein adsorption pattern onto the oleic acid-based anionic emulsion as compared to the lipofundin MCT 10% anionic emulsion was, however, observed with the apoA-II (2.5±0.0 versus 9.2±1.5), apoA-IV (4.6±4.2 versus 10.5±3.1), apoC-II (1.8±0.3 versus 5.6±2.1), apoC-III (4.5±0.1 versus 10.6±3.4) and Ig-gamma-chain (2.2±0.0 versus 8.1±2.5). An increased amount in the adsorbed proteins such as apoA-IV (36.9±0.4) and apoC-II (12.0±0.9) was seen onto the deoxycholic acid-based anionic emulsion in comparison to the low amount of the respective proteins onto the Lipofundin® MCT 10% (10.5±3.1 and 5.6±2.1). Among the various emulsion formulations, the Ig-gamma-chain adsorption was very high onto the Lipofundin MCT 10% only (8.1±2.5). Both of the cationic emulsion formulations showed an almost similar protein adsorption pattern. All formulations in comparison to Lipofundin® MCT 10% showed approximately three times higher values for apoA-I, but the same order of magnitude was shown for the deoxycholic acid-based anionic emulsion formulation. On the other hand, a halving of values for apoA-IV took place for all formulations when compared to Lipofundin® with the exception of the deoxycholic acid-based anionic emulsion formulation in which three times higher values were detectable. Thus, it is remarkable that the apoA-I/apoA-IV ratio is inverted for deoxycholic acid-based anionic emulsion formulation as compared to all other formulations.

4. Discussion

It is well established that when nanoparticles and liposomes are mixed with blood, many plasma proteins, mainly the apolipoproteins, associate with the surface of these carriers. A number of factors have been reported to influence plasma protein–liposome interactions and clearance rates including surface charge, surface coatings and lipid doses [21]. It has been shown that cationic liposomes exhibit extensive interactions with plasma, resulting in immediate clot formation at charge concentrations higher than 0.5 μmol/ml [22]. The circulation time for these liposomes was in the order of minutes. These findings were further confirmed for other cationic liposome formulations showing significant serum turbidity and protein binding [23]. These results were expected since the majority of plasma proteins carry a net negative charge at physiological pH. The ability of anionic liposomes to interact with blood proteins depends on the nature of the anionic lipid, mainly the composition of the acyl chain [24]. In addition, it was found that liposomes composed of neutral saturated lipids with acyl chains lengths greater than 16 carbons bound large quantities of blood proteins and were rapidly cleared from the circulation [25]. This phenomenon was attributed to the occurrence of hydrophobic domains at the surface of the vesicles. These vesicle–blood protein interactions also depend on the lipid dose administered. Increased lipid doses result in decreased protein levels on the surface of the liposomes and longer circulation time suggesting the occurrence of a saturable protein binding mechanism [26]. Finally, the most widely used approach for enhancing the circulation time of liposomes is the inclusion of amphipathic poly(ethyleneglycols), with a typical molecular weight of 2000–5000, in the vesicle bilayers which decrease sterically the adsorption of plasma proteins onto the liposome surfaces [27,28].

To explain the potentially deviated in vivo behaviour of stearylamine-based cationic emulsion carrier that exhibited prolonged plasma residence time as compared to the deoxycholic acid-based anionic emulsion which was cleared rapidly [7,8], in vitro plasma protein adsorption experiments were carried out. In addition to the anionic emulsion prepared on deoxycholic acid basis and cationic emulsion prepared on stearylamine basis, oleic acid-based anionic emulsion, oleylamine-based cationic emulsion and Lipofundin® MCT 10%, which is close to the commercially available other anionic emulsion product Intralipid®, were also tested in the current study for comparison purpose.

Keeping the components of oil and water phases constant, the addition of primary amines like stearylamine or oleylamine and acids like oleic acid or deoxycholic acid allowed the formation of cationic and anionic emulsions, respectively, through an ionization process at the mixed o/w interfacial emulsifier film. It has been reported in a separate in vitro monolayer layer experiment that irrespective of the charges on the emulsified droplets, the interfacial steric

stabilization effect exerted by the hydrophilic polymeric moieties of poloxamer are oriented towards the external aqueous phase [10,29]. The current study shows that large proteins like gamma and light chains of Ig, fibrinogen, etc. were indeed prevented from being adsorbed on the emulsion formulations because of poloxamer hydrophilic conformation over the emulsion droplet surfaces. However, depending on the nature of electric charges of tested emulsions, smaller proteins such as various types of apoA and C, albumin, were adsorbed onto the emulsion droplet surfaces. Apolipoproteins and albumin are classified as dysopsonins which reduce the surface hydrophobicity and do not enhance particle binding or uptake by the RES. However, within the presented experimental data, the deoxycholic acid-based anionic emulsion with reported rapid clearance from plasma had by far the highest fraction of apolipoproteins ($>82\%$) adsorption patterns (Table 2). It should be emphasized that strong adsorption of apolipoproteins does not automatically result in low recognition by the RES and cannot exclude regional effects from adsorbed lipoproteins. Moreover, the rate of elimination also depends on the ratio between apoC and apoE [30]. The apolipoproteins accounted for varied between 49 and 82% of the entire protein adsorption patterns onto all of the tested formulations except for the oleic acid-based anionic emulsion. Only a value of 38.4% apos adsorption was detectable on the oleic acid-based anionic emulsion formulation.

Despite the similarities in zeta and mean particle diameter values between the developed anionic emulsions and Lipofundin® MCT 10%, the observed protein adsorption profiles on these emulsions differed markedly. Omitting the apoA-IV, apoC-II and Ig-gamma-chain adsorption volume percent, all other proteins including albumin were adsorbed in identical amounts for both deoxycholic acid-based and Lipofundin® MCT 10% anionic emulsion formulations (Table 2). Likewise, with an omission of albumin, apoA-I, apoC-II, apoC-III and both of the Ig-chains, remaining minor proteins adsorbed on the oleic acid-based anionic emulsion and both forms of cationic emulsions were in an almost equal amount (Table 2). Since, the only difference between the developed anionic emulsion formulations is in the nature of anionic lipidic acids (oleic versus deoxycholic acids), the observed variations in the adsorbed proteins could plausibly be explained as follows. The deoxycholic acid exhibits a rigid cholesterol skeleton whereas the oleic acid is linear and more labile in nature. It is possible that deoxycholic acid interferes like cholesterol with the phospholipid molecules in the mixed emulsifying interfacial layer resulting in reduced 'membrane' fluidity. In addition, deoxycholic acid could add some hydrophobic character to the mixed interfacial film accelerating the elimination process. These changes could be responsible for deviating protein adsorption patterns. The additives in the other formulations, like oleic acid, stearylamine and oleylamine are not able to vary the conditions in the same way as deoxycholic acid. Thus, the steric protective mechanism is different in the presence of deoxycholic acid as reflected by the observed protein adsorption patterns in the present study.

Based on Table 2, the observed three fold increase in the apoA-I protein adsorption on cationic emulsions in comparison to Lipofundin® MCT 10% and deoxycholic acid-based anionic emulsion formulation would be of clinical interest. In a previous report, apoA-I along with apoA-IV have been suggested to modulate the distribution of apoE between the different lipoprotein particles in the blood and thereby affect their clearance [31]. Furthermore, the attachment of apoE would greatly alter the in vivo distribution of fat emulsions since this protein is a ligand for the apoE-specific receptors on the liver parenchymal cells [32]. The higher the preferential adsorption of apoA-I onto the cationic emulsion droplets, the more intensified the displacement/redistribution of apoE would, therefore, be expected to occur on these types of cationic emulsion formulations in the blood. Indeed, the ratio of apoA-I to apoA-IV was very close to 1 for Lipofundin® MCT 10% whereas it was about 0.26 for deoxycholic acid-based anionic emulsion and above 5 for oleic acid-based anionic emulsion and both cationic emulsions. The binding of apoA-I with triolein-rich emulsion particles saturated with cholesterol was studied in vitro as a function of increasing surface concentrations of oleic acid [33]. The results indicated that oleic acid allows more apoA-I to bind with a higher affinity to cholesterol saturated emulsion particles. It has been seen that the elimination of the anionic emulsion droplets was much faster than the elimination of the cationic emulsion droplets following intravenous administration to BALB/c mice [7,8]. It can further be deduced from the data depicted in Table 2 that the nature of the cationic lipid (stearylamine versus oleylamine) has no influence since almost identical protein adsorption patterns are observed. Based on this observation, the oleylamine-based cationic emulsion should possess a similar in vivo behaviour of prolonged residence time in the blood as that of the stearylamine-based cationic emulsion following i.v. administration.

5. Conclusion

The in vitro plasma protein adsorption on cationic and anionic submicron emulsion formulations is accessible by two-dimensional electrophoresis. Fittingly, nonionic surfactant poloxamer was used in the preparation of both anionic and cationic emulsions to stabilize physically these formulations by a steric stabilization effect. In general, the addition of this steric stabilizer seemed to shield all of the emulsion droplet surfaces from the adsorption of larger proteins like immunoglobulins, fibrinogen, etc. Thus, mainly proteins with smaller molecular weight like apolipoproteins, albumin were present in the 2D PAGE pattern. Comparisons of the obtained adsorption patterns

have shown that no pronounced alterations took place between oleic acid-based anionic emulsions and cationic emulsions. However, changes in adsorbed proteins and especially the apoA-I/apoA-IV ratio can provoke a concordance for the previously observed, potentially deviant in vivo behaviour of cationic emulsion carriers that exhibited prolonged plasma clearance. On the other hand, anionic emulsion carriers (deoxycholic acid-based) which exhibited a 20 fold decrease in the apoA-I/apoA-IV ratio as compared to the cationic emulsions, were captured by the RES and eliminated rapidly following intravenous administration to BALB/c mice.

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