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Rapid Communication

Positively charged submicron emulsions – a new type of colloidal drug carrier

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Summary

A positively charged submicron emulsion was prepared and stabilized by forming a mixed film comprising phospholipids, poloxamer and stearylamine at the o/w interface of the oil droplets. This was confirmed concomitantly by the selective adsorption of the thiocyanate anion and the lack of adsorption of Ca^{2+} onto the emulsified oil droplets of the actual emulsions. The incorporation of various drugs in the new type of submicron emulsions altered neither the nature of the surface charge nor the mean droplet size. These results clearly demonstrated that the positively charged submicron emulsion can be used as a new type of colloidal drug carrier.

Lipid submicron emulsions are receiving more attention as colloidal drug carriers for various potential therapeutic application. They are well accepted as i.v. delivery systems for either lipophilic or hydrophobic drugs such as cytotoxic drugs (Prankerd et al., 1988; Stella et al., 1988) or amphotericin B (Davis et al., 1987; Kirsh et al., 1988; Levy et al., 1993). The last authors showed that, irrespective of the formulation used, the incorporation of amphotericin B in the emulsion extended the survival time of mice infected with *Candida albicans* when compared to Fungizone®, the marketed corresponding product. Other authors (Rubinstein et al., 1991; Meyers and Stella, 1992) have used the submicron emulsion delivery system approach to prolong the pharmacological effect of drugs with short biological half-lives or poor bioavailability following oral administration. Finally, Muchtar and colleagues (1992) have demonstrated that submicron emulsions can also be used as an ocular delivery system for a lipophilic antiglaucoma drug, tetrahydrocannabinol (THC). They showed that the emulsion was able to elicit a long-lasting antidepressant effect on the intraocular pressure of rabbits following a single instillation. These overall results underline the promising properties of emulsion drug carriers as therapeutic delivery systems for a variety of drugs.

Until now, all submicron emulsion formulations reported in the literature were based on lecithins which are a mixture of phospholipids of

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varied composition combined with nonionic or anionic emulsifiers. The lecithins may comprise phosphatidylcholine as the major component, zwitterionic in form and neutral over a wide pH range, together with negatively charged phospholipids such as phosphatidylethanolamine, phosphatidylserine and phosphatidic acid which confer the negative charge to the emulsified oil droplets. The other surfactants in the emulsions are non-ionic or also negatively charged, leading to submicron emulsions with high negative zeta potential value, which effectively prevents coalescence of the droplets upon random collision.

We have designed emulsion formulations bearing a positive charge instead of a negative charge. To the best of our knowledge, there is no report in the literature of submicron emulsions prepared de novo bearing a positive charge.

The subject of this communication is the presentation of the novel submicron emulsion formulation which is prepared according to the following procedure:

Aqueous and oily solutions were separately prepared. A typical aqueous solution consisted of distilled water (up to 100 g), poloxamer 188 (2.0 g, Pluronic F-68, BASF, Ludwigshafen, Germany), and glycerin (2.25 g). A typical oil solution consisted of MCT (Mid Chain Triglyceride, 8.0 g Societe des oleagineux, St. Blangy, France), stearylamine (0.2 g, Sigma, MO, U.S.A.), the antioxidant α-tocopherol (0.1 g), and the drug at the appropriate concentration (the following drugs were already incorporated in the emulsion: miconazole, physostigmine, pilocarpine, diazepam, HU-211, a non-psychotropic cannabinoid derivative supplied by Professor R. Meshoulam, Dept of Natural Products, School of Pharmacy, Hebrew University of Jerusalem). The pH of the aqueous solution was adjusted to 6.8 and each of the two solutions was filtered (TE and BA filter types, Schull & Schleicher, Dassel, Germany), and heated separately to 70°C. Prior to the heating process, the phospholipids (1.0 g, Lipoid E-80, Lipoid AG, Ludwigshafen, Germany which comprise about 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% non-polar lipids and about 2% sphingomyelin) were first dissolved in a few millilitres of absolute alcohol and dispersed in the aqueous phase following removal of the alcohol by heat evaporation. The two phases were then mixed and stirred with a magnetic stirrer and the resulting mixture was further heated to a temperature of 85°C. At this temperature, the coarse emulsion which was obtained was further mixed by a high shear mixer polytron™ (Kinematic, Luzern, Switzerland) for 3 min, and then rapidly cooled to below 20°C. After cooling, the emulsion was homogenized by a one-stage homogenizer (Rannie, Albertsland, Denmark) for 5 min at 10000 lb/inch^2 and then cooled again. After adjusting the pH to 6.8–7.0 where the drug was HU-211, miconazole and diazepam and to 5.0–5.50 where the drug was physostigmine or pilocarpine, the emulsion was filtered through a membrane filter (TE, Schull & Schleicher), having a pore size of 0.45 μm and transferred to plastic bottles that were sealed under a nitrogen atmosphere.

The emulsions which did not contain any drug and those containing HU-211, diazepam or miconazole, were sterilized by a steam autoclave at 121°C for 15 min.

The emulsions containing pilocarpine and physostigmine (which are heat sensitive) were sterilized by a double-stage membrane filtration, first filtration through a 0.45 μm filter, followed by filtration through a 0.22 μm (both filters were TE, Schull & Schleicher).

The influence of the cationic lipid, stearylamine, concentration on the overall surface charge of the emulsified droplets was examined while other parameters were kept controlled.

The physico-chemical characterization of the emulsion was carried out by the determination of mean particle size and the zeta potential for each emulsion as follows:

Particle size evaluation: The mean droplet size and size distribution were determined by means of a computerized laser light scattering apparatus (Coulter Counter Supernanosizer MD4™, Luton, U.K.). Each emulsion sample was diluted to the appropriate concentration with a filtered isotonic solution (2.5% w/v glycerol in water). The measurement was carried out at 25°C. Each emulsion system was analyzed twice, and for each diluted sample ten size determinations were made.

Zeta potential: The zeta potential was measured with a Malvern Zetasizer™ (Malvern, U.K.). It should be emphasized that the Malvern Zetasizer is unable to identify the nature of the surface charge of the droplets. Therefore, two complementary methods were used to assess the positive charge of the actual emulsion:

Electrophoretic mobility: The nature of the charge was identified by comparison with Intralipid® (10% soybean oil), a well known negatively charged commercial fat emulsion widely investigated, using the moving boundary electrophoresis technique (Shaw, 1969). Appropriate experimental conditions able to yield accurate electrophoretic mobility data have already been established and described elswhere (Benita et al., 1986).

Selective adsorption of anions and cations: In order to confirm the fact that the colloid particles in the present emulsion are indeed positively charged, the potential selective adsorption of two electrolytes, sodium thiocyanate and calcium chloride, as compared to Intralipid®, the negatively charge emulsion, was examined. Solutions of thiocyanate and of calcium chloride at concentrations of 2 and 1 mM, respectively, were prepared. 15 ml of these solutions were mixed with 15 ml of either the emulsion, or Intralipid®, resulting in final thiocyanate and calcium chloride concentrations of 1 and 0.5 mM, respectively. The thiocyanate diluted emulsions were allowed to stand for 1 h at room temperature and then were filtered through an Amicon stirred filtration cell. The calcium chloride diluted emulsions were immediately immersed in the filtration cell and samples were ultrafiltered at given time intervals for 10 min over 1 h.

The ultrafiltration procedure was carried out as follows: YM-10, 62 mm Amicon ultrafiltration membranes were soaked in deionized water, with several changes of water, for at least 1 h to remove water-soluble contaminants. The membranes were placed into a stirred filtration cell (Model 8200, Amicon, Danvers, MA, U.S.A.) operated at room temperature. 30 ml of the thiocyanate solution mixed with the emulsion were placed into the stirred vessel. Samples of approx. 1 ml of the clear filtrate were collected at given time intervals until 15–20% of the liquid was ultrafiltered. This was done by applying low pressure (20–40 lb/inch^2) using nitrogen gas. Each sample was then assayed for thiocyanate using a colorimetric method which will be described below, and for calcium chloride by atomic absorption technique.

Prior to the use of the ultrafiltration technique to determine the selective potential adsorption of thiocyanate and calcium, the technique required validation as already performed by others (Teagarden et al., 1988). Membrane adsorption and rejection have to be accounted for in order to accurately measure aqueous concentrations of thiocyanate or calcium. The Ultrafiltration membranes were specifically selected for their low non-specific binding. The effects of membrane binding and rejection of thiocyanate and calcium were studied by ultrafiltering an aqueous solution of sodium thiocyanate and calcium chloride at concentrations of 1.26 and 0.5 mM, respectively. The recovery curve of thiocyanate from the aqueous solution is shown in Fig. 1. The membrane appears to be nearly saturated after approx. 5–7% of the total volume has been filtered, as is evident in the leveling off of the curve. The percentage recovery was 96% of theoretical, indicating that rejection was negligible. Based on these rejection data, ultrafiltration data for thiocyanate solution and emulsion formulations required only a slight correction provided that at least 7% of the total volume was filtered to saturate the membrane. The recovery results for calcium showed that no calcium at all was adsorbed by the membrane.

The method used for the thiocyanate assay was a modification of a well-established colorimetric reaction technique used for ferric chloride determination (Kolthoff, 1969). 5 ml of ferric nitrate solution at a concentration of 0.01 M were added to 1 ml of unknown thiocyanate samples. Volume was adjusted to 10 ml with 1% nitric acid solution and the intensity of the orange color formed was immediately monitored at 480 nm and calculated against a calibration curve. A calibration curve was constructed using known concentrations of sodium thiocyanate ranging from 0.01 to 1 mM. A linear relationship ($r^2 = 1$) was obtained. The calcium concentration in the filtrates was mea-

sured using atomic absorption against a calibration curve constructed using standard solutions of $Ca(NO_3)_2$. A linear relationship ($r^2 = 1$) was observed over the range of Ca^{2+} concentrations from 0 to 6 ppm, achieved using 1% lanthanium oxide solution for appropriate dilution. Filtrate samples were diluted (1:5) with 1% lanthanium oxide solution prior to assay.

Stable monodispersed submicron emulsions were yielded using the manufacturing process described above. It was noted from the results presented in Table 1 that while increasing stearylamine concentration did not cause a substantial change in the mean particle size, it had a profound effect on the zeta potential of the emulsions prepared without any drug which changed from a negative zeta potential (−14.60 mV) with no stearylamine, to a positive zeta potential (up to +21.8 mV) with 0.4% of the cationic lipid. The positive surface charge of the emulsions was confirmed first using the electrophoretic mobility technique which showed that both Intralipid® and the emulsion prepared without stearylamine

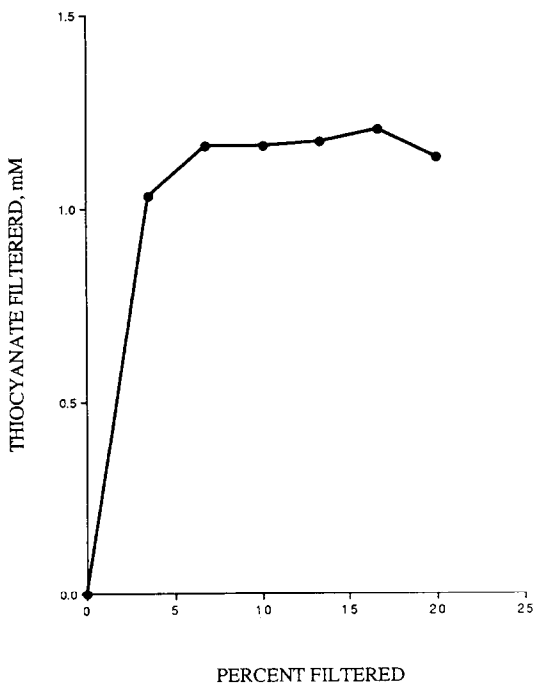

Fig. 1. Recovery curve for thiocyanate anions from aqueous solutions using the ultrafiltration technique at low pressure.

TABLE 1

Influence of stearylamine concentration on the mean droplet size and zeta potential of the emulsion

Stearylamine concentration (% w/w)	Mean particle size (nm)	Zeta potential (mV)
0.0	136 ± 51	−14.60
0.1	151 ± 43	+8.51
0.2	139 ± 39	+14.91
0.3	144 ± 46	+20.91
0.4	143 ± 37	+21.80

moved in the opposite direction than the emulsion containing stearylamine, irrespective of its concentration (Table 1). Furthermore, no substantial increase in positive zeta potential is noted above 0.3% stearylamine, indicating the probable occurrence of a saturation coverage process of stearylamine at the o/w interface under the given experimental conditions. These emulsions were stable enough to resist the thermic shock induced by autoclave sterilization or excessive shaking at 100 rpm over 48 h as indicated by the lack of difference in the droplet size distribution after the emulsions were subjected to both accelerated tests. These results clearly suggested that a mixed interfacial film comprising the phospholipids, poloxamer and stearylamine molecules was formed at the o/w interface with an overall resulting positive surface charge. The physicomechanical properties of the mixed emulgator interfacial film were strong enough to prevent any droplet coalescence upon random collisions or under thermic or mechanical stresses. To confirm the existence of a positive surface charge, the emulsions prepared with increasing stearylamine concentration were diluted with two distinct electrolyte solutions, the ions of which are known to adsorb selectively. It was already reported in the literature that thiocyanate ions (SCN^-) are adsorbed selectively on positively charged microcapsules (Sawaya et al., 1987) while divalent cations such as Ca^{2+} are known to be adsorbed on negatively charged emulsified droplets reducing the negative zeta potential rapidly, resulting in phase separation (Burham et al., 1983; Muchtar et al., 1991).

The results of these experiments revealed that the negatively charged emulsion (0% stearylamine) did not adsorb thiocyanate whereas an increase in adsorption was noted with increasing stearylamine concentration (Fig. 2), suggesting that stearylamine conferred a positive charge to the emulsified droplets which interacted with the negative charge of thiocyanate. No thiocyanate was adsorbed by Intralipid®, corroborating the negative charge of this commercial emulsion. These results were clearly confirmed by the adsorption studies of $CaCl_2$ which showed that the positively charged emulsion containing stearylamine did not adsorb any Ca^{2+} while the negatively charged emulsion (0% stearylamine) adsorbed 18% of the initial Ca^{2+} concentration and Intralipid® adsorbed 30% of initial Ca^{2+} concentration. Both negatively charged emulsions were shown to be unstable in the presence of Ca^{2+}. These results emphasize the great advantage of the positively charged emulsions which are not sensitive to the presence of cationic electrolytes

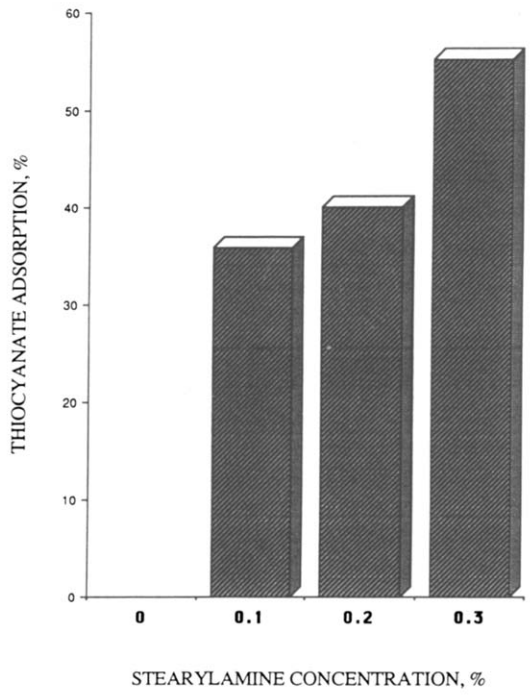

Fig. 2. The extent of thiocyanate anion adsorption as a function of stearylamine concentration in the emulsions.

TABLE 2

Physicochemical properties of emulsions containing various drugs

	Drug concentration (% w/w)	Mean droplet size (nm)	Zeta potential (mv)
Miconazole	1.0	164 ± 43	+10.1
Pilocarpine	1.0	103 ± 27	+8.63
Diazepam	0.5	151 ± 65	+8.9
Physostigmine	0.1	131 ± 29	+5.7
HU-211	0.1	131 ± 87	+5.45

generally encountered in the physiological environment.

The incorporation of various drugs in the new type of submicron emulsion altered neither the nature of the surface charge nor the mean droplet size, as shown in Table 2. Physostigmine and HU-211 emulsions exhibited lower positive zeta potential values than other medicated emulsions (Table 2).

This should be attributed to the presence of oleic acid which was added to the oil phase of these emulsions in the ratio of 1:4 to increase the drug oil solubility. Oleic acid is known to localize at the o/w interface and probably interacted with other emulsifying agents resulting in a decrease of the positive surface charge of the oil droplets. All the medicated emulsions were both chemically and physically stable over 3 months at different storage temperatures of 4, 20 and 37°C.

It should be pointed out that the absolute zeta potential value of the various emulsions was relatively low, ranging from +8 to +20 mV, quite small compared to the absolute value of the negatively charged submicron emulsions which ranged from −35 to −60 mV. The small zeta potential value of the positively charged emulsion can be attributed either to the method of phospholipid incorporation in the aqueous phase or to the molecular ratio between the various emulsifiers at the o/w interface. Inter- and intra-formulation modifications are currently being investigated to increase the positive zeta potential value of the present emulsions. Nevertheless, these results clearly demonstrated that the positively charged

submicron emulsion can be used as a new type of colloidal drug carrier.

Furthermore, it is well known from liposomal studies that the surface charge and the size of the colloidal carrier may affect the biofate of the drug in the various organs of the body following i.v. administration (Gregoriadis and Neerunjum, 1974; Juliano and Stamp, 1975). Positively charged submicron emulsions containing appropriate drug might bind to negatively charged sites on the skin or cornea resulting in prolonged residence time thereby enhancing lipophilic drug biodisposition and influencing drug release to a considerable extent. Therefore, the development of positively charged emulsions opens pioneering work in the search for new submicron emulsion formulations for various medical applications.

Our group is currently undertaking such investigations which will be reported in the near future.

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