ELSEVIER

International Journal of Pharmaceutics 108 (1994) 57–68

international journal of pharmaceutics

The design and characterization of a positively charged submicron emulsion containing a sunscreen agent

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(Received 23 November 1993; Accepted 18 January 1994)

Abstract

Stable monodispersed positively charged submicron emulsions were prepared and characterized using appropriate emulsification and homogenization processing conditions. Only the emulsions prepared with a combination of phospholipids (phosphatidylcholine (PC) and phosphatidylethanolamine (PE)), poloxamer and stearylamine, were stable enough to resist the thermic shock induced by autoclave sterilization or excessive shaking. The results suggested that a mixed interfacial film comprising the phospholipids, poloxamer and stearylamine molecules was formed at the o/w interface with an overall positive surface charge. The occurrence of the molecular interactions of stearylamine with at least the anionic phospholipidic component, PE, is revealed by the zeta potential values obtained in the experiments where the PC/PE ratio was varied. Indeed, decreasing the concentration of PE at the o/w interface led to an increase in the positive zeta potential value of the emulsions. In the presence of octyl methoxycinnamate, a sunscreen agent in the oil phase, a substantial increase in positive zeta potential was noted at 0.3% stearylamine. It appears that octyl methoxycinnamate enhanced the molecular interactions occurring between stearylamine and the phospholipidic components at the o/w interface. TEM analysis showed that most of the fractured oil droplets seemed to be surrounded by emulsifier monolayers showing the typical characteristics of an ideal submicron emulsion. Although some of the particle cores exhibited layered structures on the surfaces, no large multilamellar bilayers could be detected. Nevertheless, the formation of multilayer structures is likely to occur in the present emulsion as already reported in the investigation of negatively charged i.v. fat emulsions

Key words: Submicron emulsion; Zeta potential; Octyl methoxycinnamate

1. Introduction

Positively charged submicron emulsions were recently described as drug colloidal carriers having potential in various pharmacological applications (Elbaz et al., 1993). Davis et al. (1992) have suggested that a positively charged emulsion droplet can behave differently, when introduced into the bloodstream, than normal (negatively charged) fat emulsion droplets with respect to the uptake of plasma blood components and opsonic

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SSDI 0378-5173(94)00024-Y

factors. Positively charged emulsion droplets are expected to be sequestered by phagocytic cells from the reticuloendothelial system (RES), resulting in possible drug targeting with enhanced local drug concentration in the organs of the RES. Furthermore, it would be interesting to explore the intrinsic effect of the positively charged emulsions in accessible organs such as the skin or the cornea, which is known to carry a net negative charge at physiological eye pH (Rojanasakul and Robinson, 1989).

The main role of ultraviolet (UV) radiation in the induction of skin cancer has been confirmed by epidemiological and experimental studies (Anaise et al., 1978; Fitzpatrick and Sober, 1978). The continuous search for protective devices brought about the development of topical sunscreen preparations (TSP). Their screening effect is derived from the absorption of the biologically active wavelengths within the UV range by specific chemical ingredients comprised in these preparations (Fitzpatrick et al., 1974). When applied on the exposed skin they provide adequate protection against the immediate and late effects of solar UV radiation (Snyder and May, 1975). However, early studies (Robertson, 1968; Greiter et al., 1978) showed that the vehicle base of TSP considerably affects its efficiency and substantiality. The ideal medium in which an active ingredient is incorporated must provide not only the necessary solubility and stability, but also maintain contact between the active ingredient and the skin.

Topical administration of colloidal preparations, mainly liposomes, has received little attention until recently (Cevc and Blume, 1992). The influence of the nature of the colloidal carriers, as well as the effects of size and surface charge on drug penetration into the skin, are unknown. In particular, the type of carrier may influence drug release to a considerable extent.

The objective of the present study was to develop and to characterize an effective preparation of octyl methoxycinnamate, a well-known sunscreen agent based on a positively charged submicron emulsion, prior to its evaluation in healthy human volunteers.

2. Experimental

2.1. Materials

Octyl methoxycinnamate (Parsol MCX) was obtained from Givaudan, Geneva, Switzerland. Medium-chain triglycerides (MCT) were kindly supplied by Societe Industrielle des Oleagineux St. Laurent (Blangy, France). Phospholipids (Lipoid E-80® which comprises about 80% phosphatidylcholine (PC), 8% phosphatidylethanolamine (PE), 3.6% non-polar lipids and about 2% sphingomyelin; Lipoid E-75® which comprises 70% PC, 18% PE, 3% non-polar lipid and 2% sphingomyelin; and Lipoid E PC I® which comprises at least 98.6% PC) were purchased from Lipoid KG (Ludwigshafen, Germany). Stearylamine (SA), α-tocopherol and glycerol were purchased from Sigma (St. Louis, MO, U.S.A.). Poloxamer 188 (Pluronic F-68) was supplied by BASF, Ludwigshafen, Germany.

2.2. Methods

2.2.1. Emulsion preparation

Aqueous and oil phases were separately prepared. The aqueous phase consisted of water, glycerol, and alternatively, poloxamer; the oil phase consisted of MCT oil, the antioxidant, α-tocopherol, phospholipids, and octyl methoxycinnamate. The two phases were heated separately to 70°C. They were then combined, stirred with a magnetic stirrer and heated to 85°C. At this temperature, the coarse emulsion was further mixed using a Polytron® high-shear mixer (Kinematica Ltd, Luzerne, Switzerland) for 5 min and then rapidly cooled to below 20°C. After cooling, the emulsion was homogenized using a two-stage homogenizer (Gaulin®, APV, Hilversum, The Netherlands) for 4 min at 8000 lb/inch^2 and then cooled again. After being adjusted to a pH of 6.0 with 0.5 N HCl, the emulsion was filtered through a membrane filter (RC 55 or 60, Schuell & Schleicher-Dassel, Germany; pore size 0.45 or 1 μm, respectively) and transferred to plastic bottles packed under a nitrogen atmosphere. A typical emulsion volume ranged between 400 and 500 ml.

The influence of the concentration of the cationic lipid, stearylamine, on the overall surface charge of the emulsified droplets, while other parameters were kept constant, has previously been examined and reported (Elbaz et al., 1993).

In the present investigation, the variation of the following process parameters: poloxamer and octyl methoxycinnamate concentration, PC/PE ratio and pH on the physicochemical properties of the emulsion was examined in addition to the optimization of the manufacturing process.

2.2.2. Emulsion evaluation

2.2.2.1. Particle size evaluation. The mean droplet size and size distribution were determined by means of a photon correlation spectroscopy apparatus (Supernanosizer MD4™, Coulter Counter, 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 three size determinations were made.

2.2.2.2. Zeta potential. The zeta potential was measured with a Malvern Zetasizer™ (Malvern Instruments, Malvern, U.K.) or Delsa 440 Coultronics (Coulter, Luton, U.K.) using a phosphate buffer of pH 6.8 as diluent. It should be emphasized that two complementary methods were already used to identify and assess the positively charged nature of the actual emulsion, electrophoretic mobility, the technical details of which are described elsewhere (Benita et al., 1986) and selective adsorption of anions and cations (Elbaz et al., 1993).

2.2.2.3. Visual observation. The degree of creaming and phase separation were assessed visually at given time intervals. Any other visible changes were recorded.

2.2.2.4. Chemical emulsion evaluation. The peroxide value was determined according to UXP XXII.

The content of the sunscreen agent, octyl methoxycinnamate, was determined using UV spectroscopy. A calibration curve was constructed by dissolving various concentrations of octyl methoxycinnamate ranging from 2 to 10 μg/ml in ethanol. Various amounts (40–150 mg) of emulsions were weighed accurately and dissolved in 100 ml of ethanol. A further dilution of 1:10 in ethanol was performed. Samples of the diluted dissolved emulsions were assayed for octyl methoxycinnamate spectrophotometrically vs a corresponding blank at 310 nm, using the previous calibration curve. The other excipients of the emulsion did not interfere with the assay, since they did not absorb at 310 nm under the selected experimental conditions.

2.2.3. Stability assessment

The particle size of the emulsion was examined using short-term accelerated tests (e.g., shaking over 48 h at 100 rpm, excessive heat, sterilization by autoclave at 121°C). Furthermore, pH, particle size and peroxide value of the emulsion were assessed over long periods of storage.

2.2.4. Study of the emulsions by freeze-fracture and etching electron microscopy

The various emulsion samples prepared under different experimental conditions and process variables were subjected to freeze-fracture and etching electron microscopy according to a previously reported method (Aggerbeck and Gulik-Krzywicki, 1986). To observe a sample of emulsion through a transmission electron microscope, the following technique involving freezing, fracturing, shadowing, replica formation, cleaning and drying of the replica is needed. Finally, TEM observation is carried out on the replica. A detailed description of the technique follows.

The emulsion samples were mounted between a pair of specimen supports. Due to its good thermal conductivity, copper is used for supporting the freeze-fracture specimen. The specimen is plunged into liquefied propane (cryogen). In order to prevent ice crystal formation, freezing must be performed as quickly as possible to reach the vitreous stage. After freezing, the specimen is kept in a storage vessel containing liquid nitro-

Table 1
Description of the various emulsion formulations with their respective code names

Composition	AS.0	AS.1	AS.2	AS.3	AS.4	AS.5	AS.6	AS.7	AS.8
(1) Octyl methoxycinnemate	5	5	5	5	5	2.5	10	5	5
(2) MCT	15	15	15	15	15	17.5	10	15	15
(3) Lipoid E-80	1	1	1	1	1	1	1	–	–
(4) Stearylamine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
(5) Poloxamer 188	0	1	1.5	2	3	2	2	2	2
(6) Glycerol	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
(7) α-Tocopherol	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
(8) Lipoid E-75	–	–	–	–	–	–	–	1	–
(9) Lipoid E-PC I	–	–	–	–	–	–	–	–	1
(10) Distilled water to	100	100	100	100	100	100	100	100	100

gen. The frozen specimen sandwich is mounted onto the specimen holder while the holder is immersed in liquid nitrogen. The specimen table is then inserted, with an articulated arm, into the freeze-fracture apparatus (BAF 400, Balzers, Lichtenstein) and fixed on a precooled holder (123 K). A vacuum of 10^{-6} Torr must be reached before fracturing takes place. A precooled (123 K), single-edge scalped blade is used to realize the fracture: The knife is placed under the copper dish and this part is stripped up; shadowing and replica formation are then carried out. The

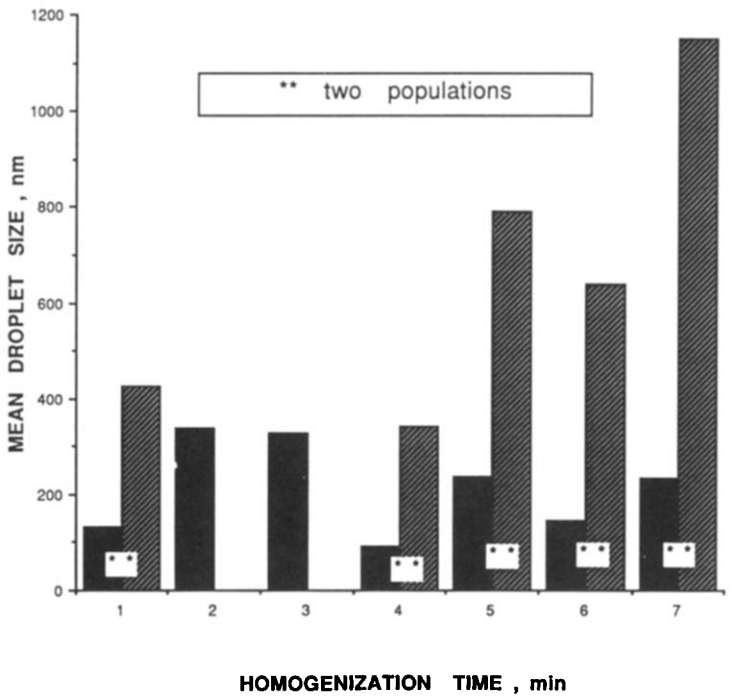

Fig. 1. Effect of the homogenization processing cycle on the mean droplet size of the standard emulsion AS.0, prepared without poloxamer and with a batch volume of 500 ml at a pressure of 8000 lb/inch^2.

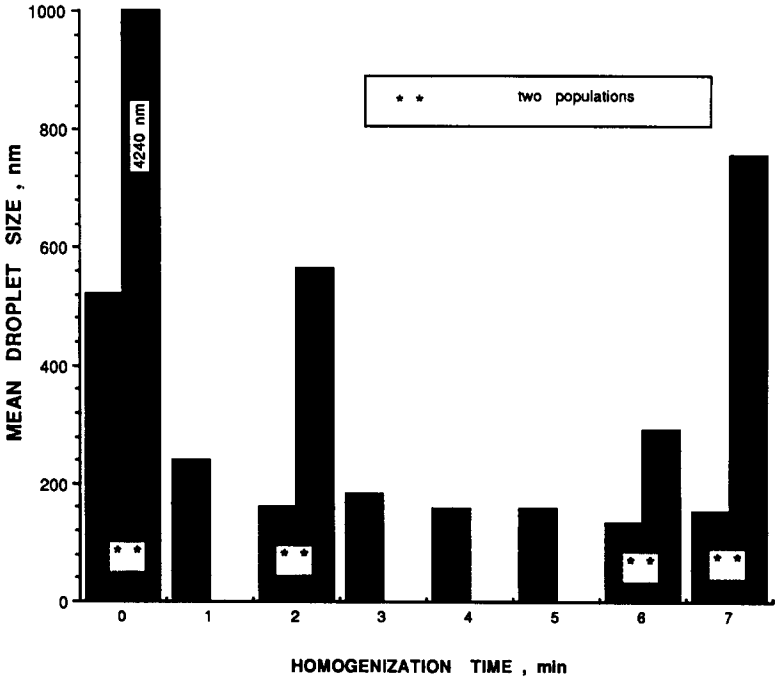

Fig. 2. Effect of the homogenization processing cycle on the mean droplet size of the standard emulsion AS.3, prepared with 2% poloxamer and with a batch volume of 500 ml at a pressure of 8000 lb/inch^2.

shadowing is made of platinum and carbon. These solids are evaporated by electron beam guns. Contrast is achieved by depositing the particles of platinum at an angle of 30°. The carbon layer is deposited vertically onto the replica. After replication of the fracture specimen, the freeze-fracture apparatus is ventilated. The specimen holder with product and replica are transferred with tweezers to a small dilution plate containing solvent. The replica is removed from the surface of the product. The replica is transferred from one cleaning solution to another. After being washed and dried on an electron microscope grid, the replica can be observed. The replica, on the spe-

Table 2
Effect of poloxamer concentration on the physico-chemical properties of the emulsion

Emulsion code name	Zeta potential (+ mV)	Initial mean droplet size (nm)	Octyl methoxycinnamate content (%, w/w)	Peroxide value	Mean droplet size after 10 months at room temperature (nm)
AS.0	46.3 ± 0.6	267 ± 45	5.47	0	277 ± 63 [b]
AS.1	35.0 ± 1.0	203 ± 90	5.35	0	216 ± 25
AS.2	43.7 ± 1.2	191 ± 47 [a]	5.24	0	195 ± 34
AS.3	45.0 ± 1.6	156 ± 39	5.31	0	185 ± 30
AS.4	45.2 ± 2.3	160 ± 61	5.32	0	178 ± 28

[a] A second population, the mean droplet size of which was 711 ± 59 nm was detected at a level of 12% from the whole population.
[b] A second population, the mean droplet size of which was above 1000 nm was detected at a level of 5% from the whole population.

Table 3
Effect of poloxamer concentration on the mean droplet size of emulsions subjected to different stress tests

Emulsion code name	Mean droplet size after 48 h shaking (nm)	Mean droplet size after autoclave sterilization (nm)	Mean droplet size after 2 weeks at 37° C (nm)
AS.0	273 ± 65	(1) 225 ± 70 (63%) (2) 527 ± 61 (37%)	phase separation
AS.1	(1) 176 ± 47 (49%) (2) 659 ± 150 (51%)	(1) 201 ± 24 (29%) (2) 812 ± 180 (71%)	245 ± 96
AS.2	(1) 133 ± 16 (27%) (2) 734 ± 88 (73%)	(1) 164 ± 43 (88%) (2) 1280 ± 300 (12%)	185 ± 59
AS.3	159 ± 37	159 ± 56	190 ± 83
AS.4	145 ± 50	(1) 137 ± 27 (95%) (2) 1470 ± 420 (5%)	148 ± 35

cial electron microscope grid, is introduced into the transmission electron microscope (TEM 100 SX, Jeol, Tokyo, Japan), and the observation can be made.

3. Results

Table 1 lists all of the formulation variations performed, with code names which facilitate identification of the parameter changed throughout the entire study. It should be emphasized that AS.0 and AS.3 represent standard formulations, without and with poloxamer, respectively.

Optimization of the homogenization process with the Gaulin® Lab 60 homogenizer was carried out in two standard emulsions designated as AS.0 and AS.3. It can be noted that a different optimization cycle pattern was obtained for the same emulsion batch volume processed (500 ml) depending on the absence or presence of poloxamer in the formulation. The most effective homogenization time range achieved without poloxamer to attain the smallest mean droplet size in the standard emulsion formulation was between 2 and 3 min (Fig. 1), while the most effective time range with poloxamer was between 3 and 5 min (Fig. 2).

The effect of poloxamer concentration on the various physicochemical properties of the emulsion stored at room temperature over 10 months is presented in Table 2. It can be noted that the variation in poloxamer concentration moderately affected the zeta potential and the content of sunscreen agent, while clearly decreasing the mean particle size and consequently increasing

Table 4
Effect of pH on zeta potential values and mean droplet size of standard emulsion prepared without poloxamer (AS.0)

Initial adjusted pH	Actual pH after 6 weeks	Zeta potential [a] (+ mV)	Mean droplet [a] size (nm)
3.09	3.31	49.0 ± 0.33	(1) 291 ± 71 (92%) (2) 1520 ± 250 (8%)
4.98	4.76	38.7 ± 2.9	(1) 212 ± 48 (70%) (2) 1860 ± 310 (30%)
6.00	5.21	46.3 ± 0.6	267 ± 45
6.98	5.60	49.2 ± 1.2	276 ± 140
8.93	6.28	50.6 ± 3.1	(1) 180 ± 32 (32%) (2) 468 ± 80 (68%)

[a] Zeta potential and mean droplet size were measured after 6 weeks storage of the emulsion.

Table 5
Effect of pH on zeta potential values and mean droplet size of standard emulsion formulation prepared with 2% poloxamer (AS.3)

Initial adjusted pH	Actual pH after 6 weeks	Zeta potential [a] (+mV)	Mean droplet size [a] (nm)
5.09	4.83	30.5 ± 2	161 ± 41
6.00	5.21	45.0 ± 1.6	156 ± 39
7.00	5.92	42.4 ± 3.3	(1) 179 ± 26 (75%)
			(2) 592 ± 100 (25%)
7.93	6.65	39.1 ± 3	(1) 144 ± 26 (70%)
			(2) 561 ± 78 (30%)
9.05	7.76	45.4 ± 1.7	(1) 145 ± 25 (40%)
			(2) 493 ± 74 (60%)

[a] Zeta potential and mean droplet size were measured after 6 weeks storage of the emulsion.

the resistance of the emulsion to various stresses as depicted in Table 3. It was interesting to note that the emulsion prepared without poloxamer revealed the presence of a second population when stored at room temperature over 10 months, indicative of instability as confirmed by the detection of phase separation when stored at 37°C over 2 weeks (Table 3). Furthermore, only the emulsion prepared with 2% poloxamer was able to maintain its physical integrity following the application of stress tests such as excessive shaking, autoclave thermic shock or storage at 37°C (Table 3).

The effect of the initial adjusted pH on the zeta potential and mean droplet size of the emulsions prepared without and with 2% poloxamer is exhibited in Tables 4 and 5, respectively. Before measuring the properties of the emulsions, it was noted that the pH decreased with time, mainly in the alkaline range. Therefore, the zeta potential and mean droplet size were measured concomitantly with the pH after 6 weeks of storage of the emulsion. No clear trend in the positive zeta potential behavior is evident with increasing pH in both emulsions (Tables 4 and 5). However, the emulsion prepared without poloxamer exhibited a homogeneous droplet population only at an initial adjusted pH of 6 and 7 (Table 4) while the same behavior was observed at an initial adjusted pH of 5 and 6 for the emulsion prepared with 2% poloxamer (Table 5).

The effect of variation in the PC/PE ratio on the physicochemical properties of the emulsions is shown in Table 6. The trend in zeta potential value is consistent with expectation, based on the concentration of anionic phospholipid components represented by PE in Table 6. An increase in PE concentration reduced the degree of positive surface charge as expected, especially in the absence of poloxamer in the emulsion. For example, emulsions prepared without poloxamer yielded the following zeta potential values (+mV): 47.5, 53 and 56 for PC/PE ratios of 8.4:1.6, 9:1 and 10:0, respectively. It appears

Table 6
Effect of variation in PC/PE ratio on the physico-chemical properties of the emulsion prepared with 2% poloxamer (AS.3)

Emulsion code name	PC/PE ratio	Zeta potential (+mV)	Initial mean droplet size (nm)	Peroxide value
AS.3	9.0:1.0	45 ± 1.6	156 ± 39	0
AS.7	8.4:1.6	45.5 ± 1.5	(1) 162 ± 29 (61%)	0
			(2) 631 ± 10 (39%)	
AS.8	10.0:0	52 ± 1	(1) 132 ± 16 (74%)	0
			(2) 390 ± 100 (26%)	

Table 7
Effect of octyl methoxycinnamate concentration on the zeta potential and mean droplet size of emulsions

Emulsion code name	Octyl methoxycinnamate content (%, w/w)	Zeta potential (+mV)	Initial mean droplet size (nm)	Peroxide value	Mean droplet size after 10 months at 25°C (nm)
AS.5	2.71	37.5 ± 1.4	198 ± 130	0	201 ± 33 [a]
AS.3	5.31	45.0 ± 1.6	156 ± 39	0	185 ± 30
AS.6	10.6	47.0 ± 2.1	166 ± 58	0	190 ± 33 [a]

[a] A second droplet population still small in size is detected as reflected by the dust value under 5 obtained during the size measurements with the nanosizer.

that the interactions occurring at the oil in water interface of the emulsified droplets are complex and require further investigation.

The effect of octyl methoxycinnamate concentration on the various physicochemical properties of the emulsion is presented in Table 7. It was observed that an increase in sunscreen agent concentration led to an increase in the zeta potential value, while no clear trend was noted with the mean droplet size as a function of the sunscreen

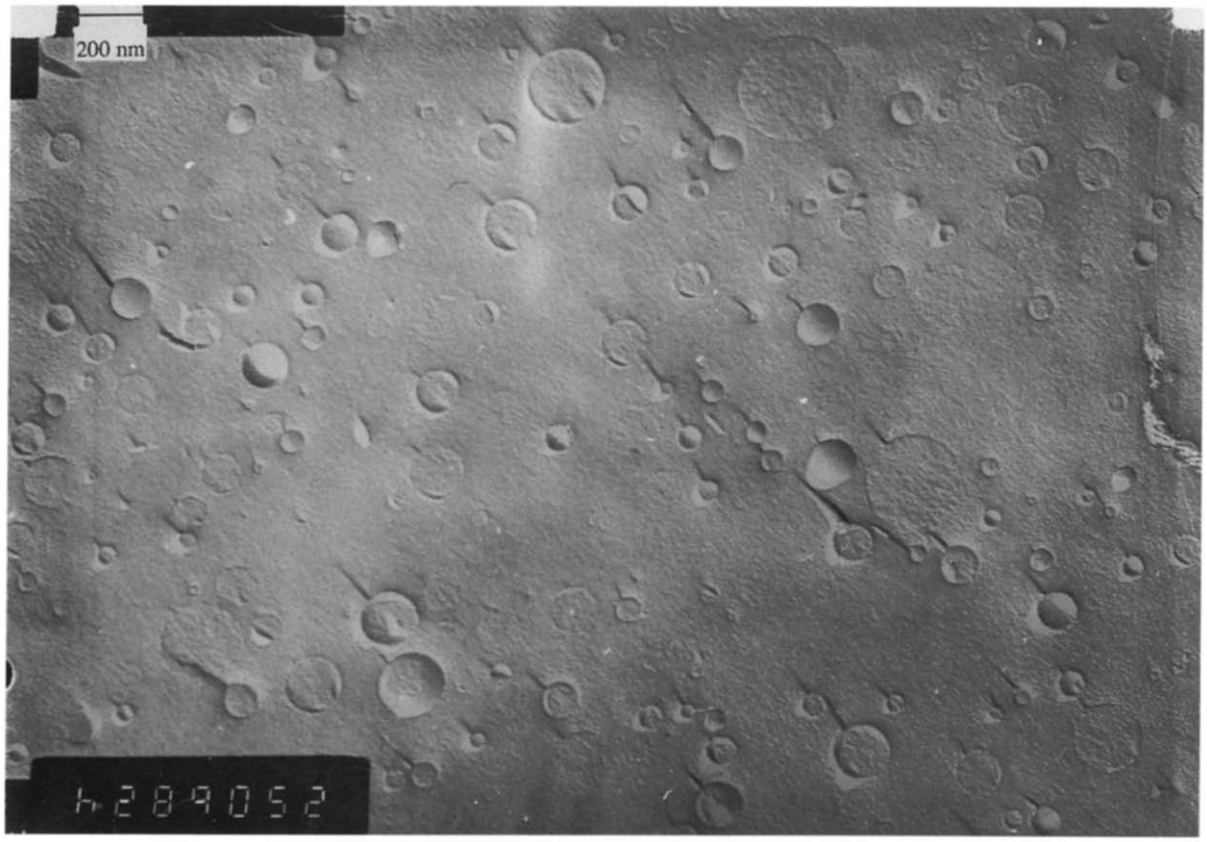

Fig. 3. Transmission electron micrograph of positively charged submicron emulsion using freeze-fracturing and etching technique. Formulation: MCT, 10; octyl methoxycinnamate, 10; phospholipids, 1.0; poloxamer 188, 2; stearylamine, 0.3; glycerol, 2.25; α-tocopherol, 0.02; and distilled water to 100.0.

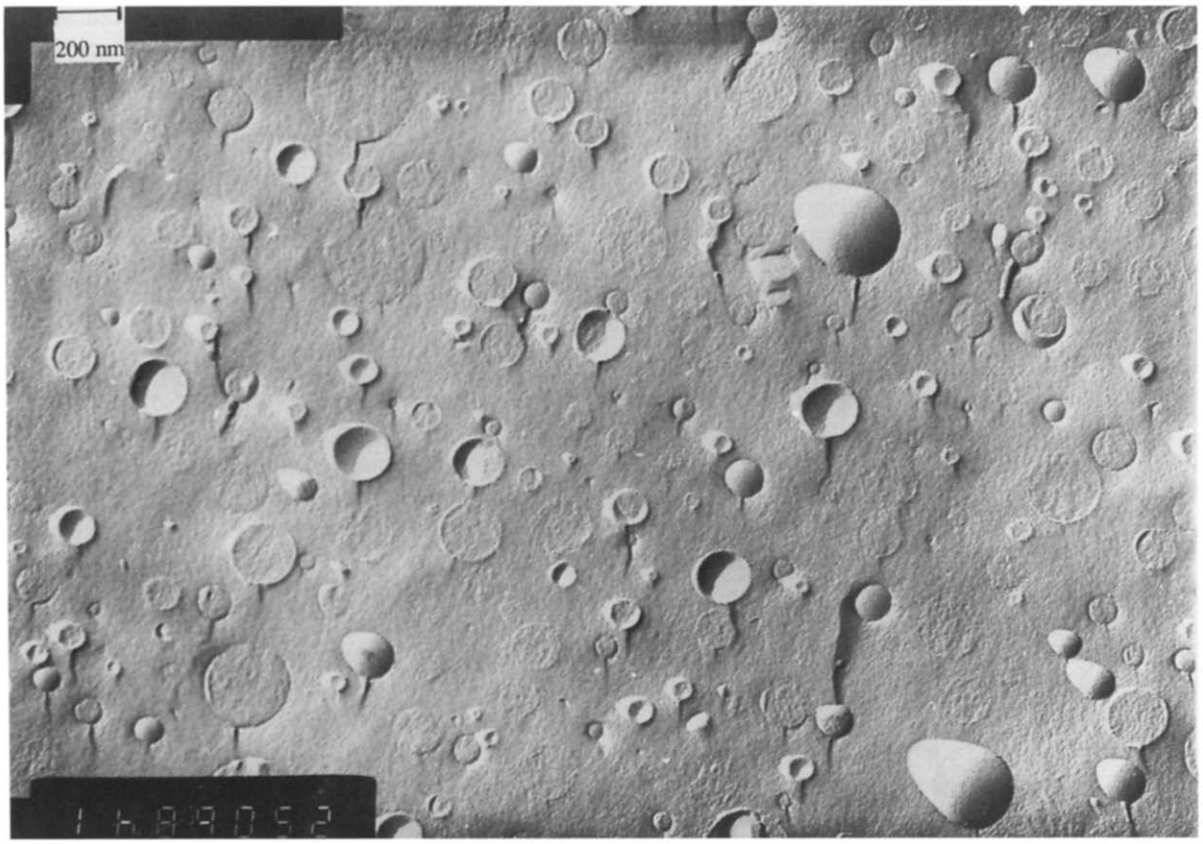

Fig. 4. Transmission electron micrograph of positively charged submicron emulsion using freeze-fracturing and etching technique. Formulation: MCT, 5; octyl methoxycinnamate, 15; phospholipids, 1.0; poloxamer 188, 2; stearylamine, 0.3; glycerol, 2.25; α-tocopherol, 0.02; and distilled water to 100.0.

agent concentration. The smallest droplet size and the most stable and resistant emulsion were obtained at a concentration of 5% sunscreen agent.

In all the tables describing the effect of the various process variables, the peroxide value was 0, indicating that no marked oxidative degradation process occurred throughout the entire series of experiments.

4. Discussion

Stable monodispersed positively charged submicron emulsions were yielded using an appropriate Gaulin® homogenization processing time. The efficiency of a dispersion process is evaluated on the basis of the ultimately measured mean droplet size achieved in the submicron emulsion droplets formed whilst maintaining the different variables constant. It should be noted that the main effect which might alter the final droplet size distribution of the emulsion during the manufacturing process is the extent of interfacial coverage of the generated droplets by the various emulsifier molecules. Partial interfacial coverage might lead to an increase in surface tension which will be compensated by an increase in droplet size. Therefore, the experimental conditions identified as yielding the smallest emulsion droplet size are

considered the optimal conditions for the manufacturing and processing of the specific emulsion formulation (Fig. 2). It was interesting to note that different homogenization processing times resulted depending on the absence or presence of poloxamer in the emulsion formulation, suggesting that some molecular interactions occurred between poloxamer and the combination of phospholipids with stearylamine at the o/w interface of the droplets (Figs. 1 and 2).

In a recent report (Elbaz et al., 1993), it was noted that while increasing stearylamine concentration did not cause a substantial change in the mean particle size, it did exert a profound effect on the zeta potential of the emulsions prepared without any drug which changed from a negative zeta potential (−14.60 mV) without stearylamine to a positive zeta potential (up to +21.8 mV) with 0.4% of the cationic lipid.

The nature of the charge was identified by comparison with Intralipid® (10% soybean oil, from Kabi-Vitrum, Stockholm, Sweden), a well-known negatively charged commercial fat emulsion which has been 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 elsewhere (Benita et al., 1986). The results showed that the present emulsion migrated in the opposite direction as compared to Intralipid® corroborating its positively charged nature. In order to further confirm the fact that the colloidal particles in the present emulsion are indeed positively charged, a study on the potential selective adsorption of two electrolytes, sodium thiocyanate and calcium chloride, as compared to Intralipid®, the negatively charged emulsion, was also carried out. The results obtained clearly confirmed the existence of a positive charge on the dispersed, very small oil droplets of the present emulsion (Elbaz et al., 1993).

Only the emulsions prepared with a combination of phospholipids, poloxamer and stearylamine at the concentrations of formulation AS.3 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 had been subjected to both accelerated tests (Table 3). 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 physico-mechanical properties of the mixed emulsifying interfacial film were strong enough to prevent any droplet coalescence upon random collision over prolonged periods of storage at different temperatures or under thermic or mechanical stresses. The probable existence of molecular interactions between phospholipids, stearylamine and poloxamer would explain the high stability of the o/w emulsion formulations prepared using the above-mentioned components. Such molecular interactions have been demonstrated between phospholipids, oleic acid and poloxamer 188 for a negatively charged submicron emulsion by carrying out monolayer studies under dynamic conditions (Levy et al., 1991). In the present investigation, the occurrence of molecular interactions of stearylamine with at least the anionic phospholipidic component, PE, is revealed by the zeta potential values obtained in the experiments where the PC/PE ratio was varied. Indeed, decreasing the concentration of PE at the o/w interface led to an increase in the positive zeta potential value of the emulsions (Table 6). This effect is more pronounced in the emulsions prepared without poloxamer (see section 3, where the zeta potential value increased from 47.5 to 56 mV, respectively), indicating that poloxamer molecules do interact with stearylamine and the phospholipidic molecules at the o/w interface. These molecular interactions appear to be complex and less quantitative than expected, especially in the presence of poloxamer molecules, since no marked difference was noted between PC/PE ratios of 9:1 and 8.4:1.6 regarding the zeta potential value as compared with a PC/PE ratio of 10:0 (Table 6). A much more quantitative behavior in increasing zeta potential value was observed with the emulsions prepared without poloxamer as probably less complex molecular interactions occurred between SA and PE.

Nevertheless, the progressive decrease in the emulsion droplet size with increasing poloxamer concentration should be attributed to the greater extent of penetration of the poloxamer molecules into the emulsifying interfacial lipid monolayer. Therefore, this pattern of a gradual decrease reflects the formation of a better close-packed mixed film of the emulsifying agents at the oil-water interface of the emulsified droplets. The interfacial film acted as a stabilizer during the earlier stage of the emulsification process by forming a high-energy barrier which caused repulsion of adjacent droplets and led to the formation of stabilized emulsified droplets of decreasing size. Varying the pH of the standard emulsions from 3 to 9 did not markedly alter the zeta potential value of the emulsions (Tables 4 and 5). This is not surprising, since the pK_a of stearylamine is 10.6 while PC, the major phospholipid component, remains zwitterionic in form and neutral, and PE remains negatively charged over the pH range tested.

In the present study, in the presence of octyl methoxycinnamate, a sunscreen agent in the oil phase, a substantial increase in positive zeta potential was noted at 0.3% stearylamine as compared to previously reported results (Elbaz et al., 1993), indicating a better coverage of stearylamine at the o/w interface under the given experimental conditions (Table 7). This is further confirmed by higher zeta potential values obtained while increasing the concentration of octyl methoxycinnamate in the oil phase. It appears that octyl methoxycinnamate enhanced the molecular interactions occurring between stearylamine and the phospholipidic components at the o/w interface.

The TEM micrographs (Fig. 3 and 4) show that the particles are randomly distributed in the fracture plane, with sizes ranging from 100 to 350 nm confirming the PCS measurements. To avoid ice crystal formation, glycerol was added to the emulsion before fast freezing was carried out in melting propane yielding clear cuts through the particles in the fracture plane. The structural information that may be obtained by TEM may be limited. However, the majority of the cores appear nearly amorphous (Fig. 3), indicating that the oil mixture of the emulsified droplet did not crystallize under the experimental conditions used. Some of the particle cores show layered structures on the surfaces. This effect being more pronounced with o/w emulsions prepared with higher octyl methoxycinnamate concentrations in the oil phase indicates probably either a different distribution pattern of the phospholipids among the various phases of the emulsion (Fig. 4), or a different crystallization pattern of the oil mixture. The formation of multilayer structures is likely to occur in the present emulsion as has previously been reported in the investigation of negatively charged i.v. fat emulsions (Groves et al., 1985; Rotenberg et al., 1991; Westesen and Wehler, 1991). Furthermore, these TEM observations made on optimal, stabilized positively charged submicron emulsions are in agreement with data reported by authors who investigated the formation of phospholipid monolayers at air/water and oil/water interfaces (Gaines, 1966; Handa et al., 1985; Handa and Nakagaki, 1992). They found that the phospholipid lowers interfacial tension and stabilizes the interface. When the monolayer was compressed to give minimum interfacial tension (maximum interfacial pressure of monolayer, e.g., maximum emulsion stability), a part of the monolayer collapsed (Gaines, 1966). The separated lipid forms bilayers in equilibrium with the monolayer (Handa et al., 1985). The coexistence of monolayer and bilayer structures thus results in maximum stabilization of the interface (Handa and Nakagaki, 1992), as can also be visualized from the present investigation. Most of the fractured oil droplets seem to be surrounded by emulsifier monolayers showing the typical characteristics of an ideal submicron emulsion as reported by others using a similar approach (Westesen and Wehler, 1992). No large multilamellar bilayers could be detected. However, as also specified by most of the investigators, the existence of small unilamellar liposomes (SUVs) cannot be ruled out by TEM studies nor can it verified because SUVs and small emulsion droplets surrounded by an emulsifying monolayer or bilayer (< 60–100 nm) could not be distinguished. It can be concluded from the present work that an optimal emulsion formulation of octyl methoxy-

cinnamate was achieved after having examined the effect of the different process variables on the physico-chemical properties of the emulsion.

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