Drug Development and Industrial Pharmacy, 2010; 36(6): 666–675

informa
healthcare

ORIGINAL ARTICLE

Positively and negatively charged liposomes as carriers for transdermal delivery of sumatriptan: in vitro characterization

Sheila Villasmil-Sánchez[1,2], Wijdane Drhimeur[1], Sirce Cecilia Salas Ospino[1], Antonio M. Rabasco Alvarez[1] and M.L. González-Rodríguez[1]

[1]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Seville, Seville, Spain and*
[2]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Jos Andes, Mérida, Venezuela*

Abstract

Background: The influence of liposome composition, lamellarity, preparation method, and charge on the encapsulation efficiency, size, polydispersity, and surface charge of sumatriptan liposomes was studied. For this purpose, we studied multilamellar, unilamellar, and frozen and thawed liposomes. *Method:* Positively or negatively charged liposomes were obtained using both phosphatidylcholine and cholesterol, in combination with stearylamine or dicetylphosphate. Liposomal formulations were characterized by confocal laser scanning microscopy and optical microscopy for vesicle formation, morphology, and lamellarity, by dynamic laser light scattering for size distribution and polydispersity, and electrophoretic mobility for zeta potential determination. To obtain more information about the sumatriptan encapsulation, dynamic dialysis technique was employed. The sumatriptan amount was quantified by high-performance liquid chromatography. *Results:* Overall obtained results showed that liposomes may be interesting carriers for sumatriptan succinate. Statistical analysis evidenced that the preparation method does not affect the evaluated characterization parameters. However, the presence of charge inducer agents modified these characteristics. Highest loading efficiency of sumatriptan was exhibited for positively charged liposomes containing 6.58:10.34:3.73 mmolar ratio for phosphatidylcholine : cholesterol : stearylamine. The mean size was affected by the charge inducer, being smaller in positively charged liposomes. Logically, surface charge of liposomes varied as a function of the employed charged agent. Also, interesting results were obtained when vesicles were loaded with sumatriptan, showing a statistical significance between all pairs, comparing the formulations with and without drug. *Conclusion:* Results obtained revealed that the presence of sumatriptan in the vesicles has a different behavior in negative and positively charged liposomes.

Key words: *Dicetylphosphate; liposome; migraine; stearylamine; sumatriptan*

Introduction

Sumatriptan is a selective serotonin 5-HT agonist at the 5-HT_{1B} and 5-HT_{1D} receptors used in the treatment of acute migraine episodes[1,2]. It was the first of the so-called triptan drugs that had a significant impact on the treatment of acute attacks[3], and it is available in several dosage forms including products for oral, nasal, and rectal delivery[4–6]. However, some clinical studies underscore the drug limited efficacy, slow onset, and incomplete prevention of recurrence as major shortcomings of current therapies[7].

Subcutaneous injection of sumatriptan yields the most rapid response at the 30-minute time point and complete relief at the 2-hour time point. In addition to the reluctance for self-injection, there have also been reports of skin site reactions in more than 50% of patients[8].

Transdermal delivery offers a convenient alternative particularly in the case where nausea prevents administration of an oral dosage form[9]. In addition, sumatriptan

Address for correspondence: Dr. M.L. González-Rodríguez, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Seville, C/ Prof. García González, 2, 41012 Seville, Spain. Tel: +34 95 4556397, Fax: +34 95 4556085. E-mail: malugoro@us.es

(Received 24 Mar 2009; accepted 13 Oct 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040903419640

has a relatively poor oral bioavailability (14% only) and a relatively short half-life ($t_{1/2} \sim 2$ hours). This route avoids the hepatic first-pass metabolism and permits to achieve constant plasma levels over an extended period of time[10–13]. Liposomal systems can enhance skin permeation of drugs[14,15]. In this way, sumatriptan liposomes could be an interesting strategy to improve the drug bioavailability, and considering that the sumatriptan succinate permeates through the skin[16–18], it would be interesting to apply them by this route.

Moreover, these vesicles as drug delivery systems have the potential to provide controlled release of the administered drug and to increase the stability of the labile drugs such as sumatriptan[19]. Encapsulating a sufficient amount of the therapeutic agent is one of the desirable properties for their use[20]. Factors affecting the encapsulation efficiency of the drug in liposomes are numerous and they come from the properties of both liposomes and encapsulated drugs. Concerning the encapsulated drug, the entrapment efficiency is affected by hydrophilic or lipophilic properties and its tendency to interact with the lamella[21,22]. As for the liposome properties, aqueous volume, membrane rigidity, surface area, and preparation methods are reported to have an influence on the encapsulation efficiency[23–25].

The lipid lamellae of the *stratum corneum* of the skin contain a high ratio of negatively charged lipids, which are expected to interact with cationic liposomes. Transfer of some of the bilayer components of the liposomes to the skin is then induced. Several studies have demonstrated that positively charged liposomes have a remarkable effect in enhancing the penetration of drugs across the skin as the latter is negatively charged and favors the electrostatic attraction for positively charged liposomes[26]. For this reason, stearylamine (SA), a positively charged lipid, has been included in the formulation of the different liposomes in this work.

On the other hand, several formulations have been developed by using dicetylphosphate (DCP) as an anionic agent that favors the ionic interaction with the drug, which is partially positively charged in the formulations.

The aim of this work was to develop a liposomal formulation to encapsulate sumatriptan succinate for topical administration. This article covers the analysis of different production techniques on several characterization parameters such as encapsulation efficiency, vesicle size, polydispersity index (PI), zeta potential, and lamellarity. The influence of SA and DCP on the above parameters has also been studied. With regards to the evaluated parameters, the best formulation was selected for further release and permeation studies.

Materials and methods

Materials

Sumatriptan succinate was received as a gift sample from GlaxoSmithKline (Brentford, UK). Egg yolk phosphatidylcholine (PC) (60%, w/w, purity), SA, and DCP were purchased from Fluka (Steinheim, Germany). Cholesterol (CH) was obtained from Sigma-Aldrich (Barcelona, Spain). Chloroform, diethyl ether, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were received from Panreac Chemistry (Barcelona, Spain). The buffer solution used for the preparation of liposomes was HEPES 10 mM to pH 5.5 that corresponds to skin pH value. All other reagents used in this study were of analytical grade.

Preparation of sumatriptan formulations

Liposomes were prepared using a slightly modified protocol according to our previously published method[24,27]. Liposomes were prepared using three different methods: thin-layer evaporation (TLE), reverse phase evaporation (REV), and freezing and thawing multilamellar vesicles (FATMLV).

- *Thin-Layer Evaporation (TLE)*. Thin lipid film-hydrated vesicles were prepared according to the method described previously[21,23]. Briefly, different ratios of PC, CH, and the charged substance (SA or DCP) were dissolved in 10 mL of chloroform in a round-bottom flask. The organic solvent was then removed under reduced pressure in a rotary evaporator (Büchi, R-200) at 58°C (temperature above the critical transition temperature of lipids, T_c), thus obtaining a thin film of dry lipid on the flask wall. The film was then hydrated by adding 10 mL of buffer HEPES (10 mM, pH 5.5) with and without 0.67% (w/v) (16.11 mM) sumatriptan succinate. Multilamellar liposomes were formed by periodic vortexing cycles consisting of 2 and 6 minutes in a thermostatized bath at 58°C for five times[15,28–30].
- *Freezing–Thawing (FAT)*. A thin lipid film was prepared from the lipid solution in chloroform, as described above. Ten milliliters of 16.11 mM sumatriptan in HEPES buffer solution (pH 5.5) was then added to the lipid film in the round bottom. The lipid film was gradually hydrated by gently shaking the flask at 58°C for 30 minutes. The liposome dispersion was then subjected to nine FAT cycles, by freezing the sample in liquid nitrogen and then thawing the suspension in a thermostatized bath at 58°C[31].
- *Reverse phase evaporation (REV)*. REV liposomes have been prepared according to the method of Skoza and Papahadjopoulos[32], with some modifications.

Lipids were dissolved in 10 mL chloroform–ether mixture (8:4) into a 100-mL round-bottom flask. The buffer HEPES with and without the drug was added at this point. The resulting two-phase system was sonicated (*Branson 3510*) by 10 minutes until obtaining a water/oil emulsion (homogeneous opalescent dispersion). The organic solvent was then removed in a rotary evaporator at 58°C. During this process, the material first forms a viscous gel, subsequently the gel collapses, and becomes an aqueous suspension[33]. At this moment, the dispersion was introduced to a vortexing cycle for 5 minutes to obtain the liposomes[32].

Liposomal formulations containing different amounts of SA and DCP have been obtained. For SA formulations, 10 (3.71 mM), 20 (7.42 mM), and 30 mg (11.13 mM) and for DCP, 10 (1.83 mM), 12.5 (2.29 mM), 15 (2.74 mM), 20 (3.66 mM), and 30 mg (5.49 mM) were used to analyze the effect of lipid charge on sumatriptan encapsulation efficiency, polydispersion index, vesicle size, and zeta potential of liposomes. In Table 1, the different prepared formulations are described.

Table 1. Composition of the different liposome formulations.

Batch	Method	Inducer charge agent	Amount of inducer charge agent (mg)
1	TLE	SA	10
2	TLE	SA	20
3	TLE	SA	30
4	TLE	DCP	10
5	TLE	DCP	12.5
6	TLE	DCP	15
7	TLE	DCP	20
8	TLE	DCP	30
9	REV	SA	10
10	REV	SA	20
11	REV	SA	30
12	REV	DCP	10
13	REV	DCP	12.5
14	REV	DCP	15
15	REV	DCP	20
16	REV	DCP	30
17	FAT	SA	10
18	FAT	SA	20
19	FAT	SA	30
20	FAT	DCP	10
21	FAT	DCP	12.5
22	FAT	DCP	15
23	FAT	DCP	20
24	FAT	DCP	30

Each batch was prepared with a ratio in milligrams PC : CH (50:40), as lipid vesicles formed, and sumatriptan succinate 66.67 mg. Batches without sumatriptan of each formulation were prepared (placebo). DCP, dicetylphosphate; SA, stearylamine.

Measurement of liposome size and surface charge

The average diameter of the vesicles and their zeta potential were determined using a Zetamaster apparatus (Malvern Instruments, Malvern, UK) at a temperature of 25°C. To measure the size of vesicles, a 100 μL volume of liposome dispersions was suitably diluted with 20 mL of purified water to avoid multiscattering phenomena. The sample was sized immediately by photon correlation spectroscopy and the intensity of the laser light scattered by the samples was detected at an angle of 90° with a photomultiplier. For each batch of liposomal suspension, three independent samples were taken, each of which was measured at least twice and up to five times. The data accumulation was done in a series of subruns, 10 subruns by each run. Finally five runs were analyzed using a Contin method, which is a lower resolution algorithm[34] (Malvern Instruments manual[35]). Based on this analysis, the *z*-average value was obtained enabling the approximation of the hydrodynamic diameter (nm) of liposomes. The PI allows us to determine the level of homogeneity between different sizes of particles. A small value of PI (<0.2) indicates a homogeneous vesicle population, whereas a larger PI (>0.3) indicates heterogeneity.

To measure zeta potential, liposome dispersions (3 mL) were diluted with purified water and then injected into a photoelectric cell. The liposome movement within the electric field permitted to determine their electric charge. This determination was applied upon three samples for each formulation.

Chromatographic system

The chromatographic apparatus consisted of a Hitachi system manager D-7000, equipped with a quaternary pump L-7100, a diode array detector L-7455, an automatic injector L-7200, and an interface D-7000. For data collection and calculation, HSM System Manager Software was used. The chromatographic conditions were optimized using a column C18 (LiChrospher 100 RP-18; 125 × 4 mm, 5 μm; Merck, Barcelona) and following a modified methodology previously described by other authors[36]. The mobile phase consisted of phosphate buffer (0.05 M) acetonitrile (80:20, v/v) adjusted to pH 6 with sodium hydroxide (0.05 M). The mobile phase was filtered through a 0.22-μm nitrocellulose-membrane filter (Millipore, Barcelona, Spain) and degassed under vacuum prior to use. Absorbance was measured at 227 nm and the flow rate was 1 mL/min. The injection volume was 20 μL. Peak areas were measured and high-performance liquid chromatography analysis was conducted at room temperature.

Determination of drug entrapment efficiency in liposomes

Liposome encapsulation efficiency was performed by a dynamic dialysis technique, monitoring the drug concentration in the receiver solution, corresponding to the nonentrapped drug from liposomes[15,23,37]. In this method, 3 mL of drug-loaded liposomal dispersion was dropped into a cellulose acetate dialysis bag (Spectra/Por®, MW cutoff 12.000; Spectrum, Canada) immersed in 150 mL of cold buffer, HEPES 10 mM (pH 5.5), and magnetically stirred. Samples, taken at time intervals from the receiver solution, were replaced with equal volumes of fresh solvent. Sumatriptan succinate was assayed by high-performance liquid chromatography at 227 nm[36]. The experiment was stopped when constant drug concentration values were obtained in subsequent withdrawals from the receiver phase. The percent of encapsulation efficiency (EE%) was then calculated according to the following equation:

$$\%EE = \frac{\text{total drug-diffused drug}}{\text{total drug}} \times 100.$$

Each result was the mean of at least three separate experiments of each sample.

Lamellarity

This parameter was evaluated by confocal laser scanning microscopy (CLSM). The equipment used was a Leica TCS SP II confocal unit (Leica, Heidelberg, Germany) equipped with a kryton–argon–helium/neon laser and mounted on a Leica DM IRE 2 inverted microscope (Leica) using HC PL Fluotar Leica lens with magnifications of 10, 20 × (dry) and HCX PLAN APO Leica lens 40 × (0.85 multi immersion objective). To observe the liposomes, we added a fluorescent marker, Rhodamine 6G, in the aqueous phase (buffer HEPES) to each liposomal dispersion and the images were obtained by fluorescence emission. For the probe excitation, 488 nm laser line was used and the fluorescence emission was detected above 520 nm.

Statistical analysis

Mean differences in preparation method and SA or DCP concentrations were compared using one-way analysis of variance (ANOVA). Post hoc Scheffé´s *F*-test was used to obtain levels of statistical significance while adjusting for multiple comparisons. The nonparametric Mann–Whitney *U*-test was used to study the influence of sumatriptan on the formulations. Statistical significance was established at $P < 0.05$. All experiments were repeated at least three times and results are expressed as mean ± SD.

Results and discussion

Entrapment efficiency

By inspection of Table 2 and Figure 1, it is obvious that sumatriptan encapsulation efficiency would vary with the preparation method and the type and concentration of charge inducer used in the prepared liposomes.

The highest encapsulation efficiency (60.10%, w/v) was observed with the positively charged unilamellar liposomes (REV) composed of PC:CH:SA (6.6:10.3:3.71) mmolar ratio (i.e., the positive liposomes with the lowest SA content), while negatively charged liposomes with the lowest DCP content (6.6:10.3:1.83) molar ratio exhibited the lowest encapsulation efficiency (12.83%, w/v).

Concerning the effect of type of charge inducer on the encapsulation efficiency of sumatriptan, results evidenced that the percentage of drug encapsulated increased in SA formulations. The one-way ANOVA

Table 2. Characterization parameters of sumatriptan succinate formulations: liposome size, polydispersity index (PI), zeta potential, and drug incorporation efficiency (EE%).

Batches	Size (nm)	PI	Zeta	EE (%)
1	408.6 ± 279.1	0.56 ± 0.01	35.40 ± 1.23	53.68 ± 9.09
2	311.4 ± 154.6	0.25 ± 0.09	26.26 ± 1.11	43.05 ± 1.19
3	348.7 ± 100.3	0.28 ± 0.24	37.90 ± 3.67	39.16 ± 3.53
4	812.4 ± 115.4	1.00 ± 0.00	−72.0 ± 0.9	12.83 ± 1.26
5	731.9 ± 15.3	1.00 ± 0.00	−61.3 ± 0.7	29.79 ± 7.25
6	635.9 ± 13.6	0.817 ± 0.103	−59.8 ± 0.9	41.87 ± 2.76
7	617.8 ± 29.9	0.634 ± 0.23	−68.4 ± 1.3	27.90 ± 0.62
8	549.2 ± 10.3	0.37 ± 0.09	−68.1 ± 0.4	32.54 ± 0.66
9	313.5 ± 97.8	0.30 ± 0.15	24.42 ± 2.33	60.10 ± 5.55
10	376.5 ± 160.7	0.18 ± 0.17	31.27 ± 0.35	56.67 ± 0.48
11	603.7 ± 180.5	0.34 ± 0.31	33.24 ± 0.70	54.27 ± 10.33
12	671.5 ± 3.20	0.802 ± 0.04	−70.1 ± 0.9	29.11 ± 3.14
13	613.9 ± 19.7	0.701 ± 0.16	−67.3 ± 0.4	35.25 ± 2.36
14	649.8 ± 6.18	0.95 ± 0.04	−73.0 ± 0.7	34.52 ± 2.50
15	672.5 ± 13.1	1.00 ± 0,00	−77.6 ± 0.6	49.81 ± 1.34
16	860.6 ± 54.7	1.00 ± 0.00	−78.5 ± 0.8	54.56 ± 1.88
17	360.0 ± 220.3	0.48 ± 0.12	46.30 ± 1.26	19.37 ± 5.64
18	351.1 ± 121.8	0.31 ± 0.15	40.70 ± 0.56	52.78 ± 4.31
19	317.1 ± 232.8	0.26 ± 0.14	56.70 ± 15.44	50.0 ± 3.53
20	531.8 ± 41.7	0.375 ± 0.13	−63.7 ± 0.7	35.29 ± 11.08
21	521.5 ± 19.7	0.37 ± 0.11	−63.0 ± 0.6	28.07 ± 10.69
22	599.0 ± 7.6	0.67 ± 0.09	−71.5 ± 0.8	37.57 ± 8.29
23	445.1 ± 23.0	0.186 ± 0.05	−68.7 ± 0.8	29.14 ± 2.39
24	505.1 ± 17.6	0.256 ± 0.05	−67.8 ± 0.7	29.66 ± 0.69

Each value represents the mean ± SD, $n = 3$. The composition of each batch is given in Table 1.

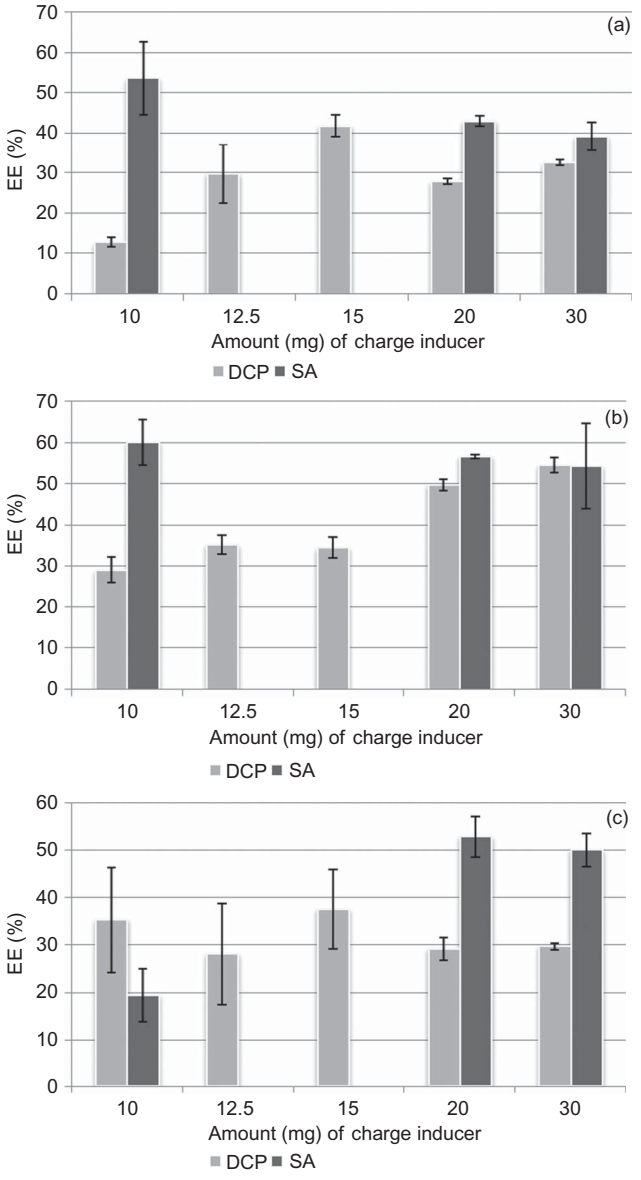

Figure 1. Encapsulation efficiency (%) of sumatriptan as a function of the charge inducer amount (mg) added into the formulations. (a) TLE method, (b) REV method, (c) FAT method. DCP, dicetylphosphate; SA, stearylamine. Each data represents the mean ± SD for $n = 3$.

showed a significant difference between all pairs at $P < 0.05$. This increase in the entrapment efficiency occurs because SA may affect specific characteristics such as the membrane permeability or the electric charge density[38]. Also, some authors have demonstrated that the presence of the positive charge in the lipid bilayer produces a change in the lateral packing of the liposome bilayer causing a decrease in the entrapment efficiency[39]. The hydrophobic part of the sumatriptan molecules may interact with the hydrophobic tails of the phospholipid bilayers of the liposomes[40,41], which may be involved in the binding of sumatriptan into liposomes, in fact higher concentrations of SA produce a lower encapsulation efficiency.

On the other hand, the decrease in encapsulation efficiency was more evident for liposomes presenting negatively charged substances in their composition. In fact, at pH 5.5, sumatriptan is partially protonated. In this way, in negative liposomes, the sumatriptan entrapment occurs basically by electrostatic attraction between the ionized sumatriptan and DCP. By this reason, DCP charge does not generate important repulsive interactions in the lipid bilayer. Indeed, with higher concentrations of DCP, the entrapment efficacy was increased. The drug encapsulation in negative liposomes has been lower than positive liposomes, probably because in negative liposomes the entrapment is directly related to the ionization of the sumatriptan succinate. Sumatriptan contains more than one functional group that may become charged by protonation or deprotonation at pH 5.5. The presence of these groups greatly affects both the rates of diffusion and the final equilibrium distribution of this compound across the membrane bilayer. Therefore, there is certain affinity of drug for the phospholipid bilayer, which contributes to the drug encapsulation.

In addition, despite results, and using the ANOVA analysis, there was no significant difference in the percentage of sumatriptan using different charge inducer concentrations ($F = 0.76$, $P = 0.563$) and changing the preparation method of liposomes ($F = 2.56$, $P = 0.101$).

Vesicle size measurement

Vesicle sizes were measured for freshly prepared positively charged and negatively charged TLE, REV, and FAT liposomal dispersions with lipid components in the molar ratio PC:CH:SA or DCP 6.6:10.3:X, where X corresponds to SA or DCP different ratios used to prepare the formulations previously described. CONTIN method analysis confirmed the results obtained previously[24], that is, that vesicle size is dependent on preparation method and bilayer composition.

The particle size was estimated to be between 311 and 408 nm for positively charged liposomes. Negatively charged liposomes showed a mean diameter between 505 and 860 nm (Table 2). These results can be attributed to the inclusion of a charge inducer in liposomes, which modified the spacing between the adjacent bilayers. The negatively charged lipid electrostatically attracts the drug cation, which may be expected to push phospholipids' head groups apart, hence increasing the particle diameter[42]. However, the positively charged liposomes tend to stabilize the colloidal dispersion, giving rise to vesicles with smaller size and a narrow polydispersion index. The one-way ANOVA showed a significant difference between all pairs ($F = 24.102$; $P < 0.05$).

Concerning the influence of the preparation method on vesicle size, globally the comparison between the mean vesicle diameter of REVs and multilamellar vesicles (MLVs), liposomal formulations evidenced that the multilamellar liposomes are larger in size than REV liposomes prepared with the same lipid molar ratio[43].

We used the confocal microscopy to demonstrate the lamellar multiplicity. CLSM experiments were carried out by using liposomes labeled with rhodamine, which present a high affinity to the lipid bilayer. The photomicrograph of sumatriptan REV liposomes showed in Figure 2a reveals the presence of homogeneous population of unilamellar vesicles with one phospholipid bilayer and oligolamellar vesicles consisting of a few concentric bilayers. The presence of unilamellar vesicles implies a higher aqueous compartment to encapsulate the hydrophilic drug; the prepared liposomes are well-identified spheres that have a large internal aqueous space relative to the sphere diameter. Figure 2b shows how the rhodamine interacted with the lipid bilayers and was distributed in the whole structure of MLVs, in accordance with several authors[30,44]. The photomicrograph of sumatriptan multilamellar liposomes reveals the presence of well-defined multilamellar vesicles that consist of many concentric phospholipid bilayers. It is to be noted that MLV liposomes are larger in size than REV liposomes.

As a conclusion, the size and number of lamellae of the vesicles are important for the encapsulation of drugs in liposomes. Because MLVs contain large lipid surface and numerous lamellae, they are more suitable than the other types of liposomes for the encapsulation of hydrophobic drugs via interacting with the lipid layers. However, water-soluble drugs, such as sumatriptan succinate, are better

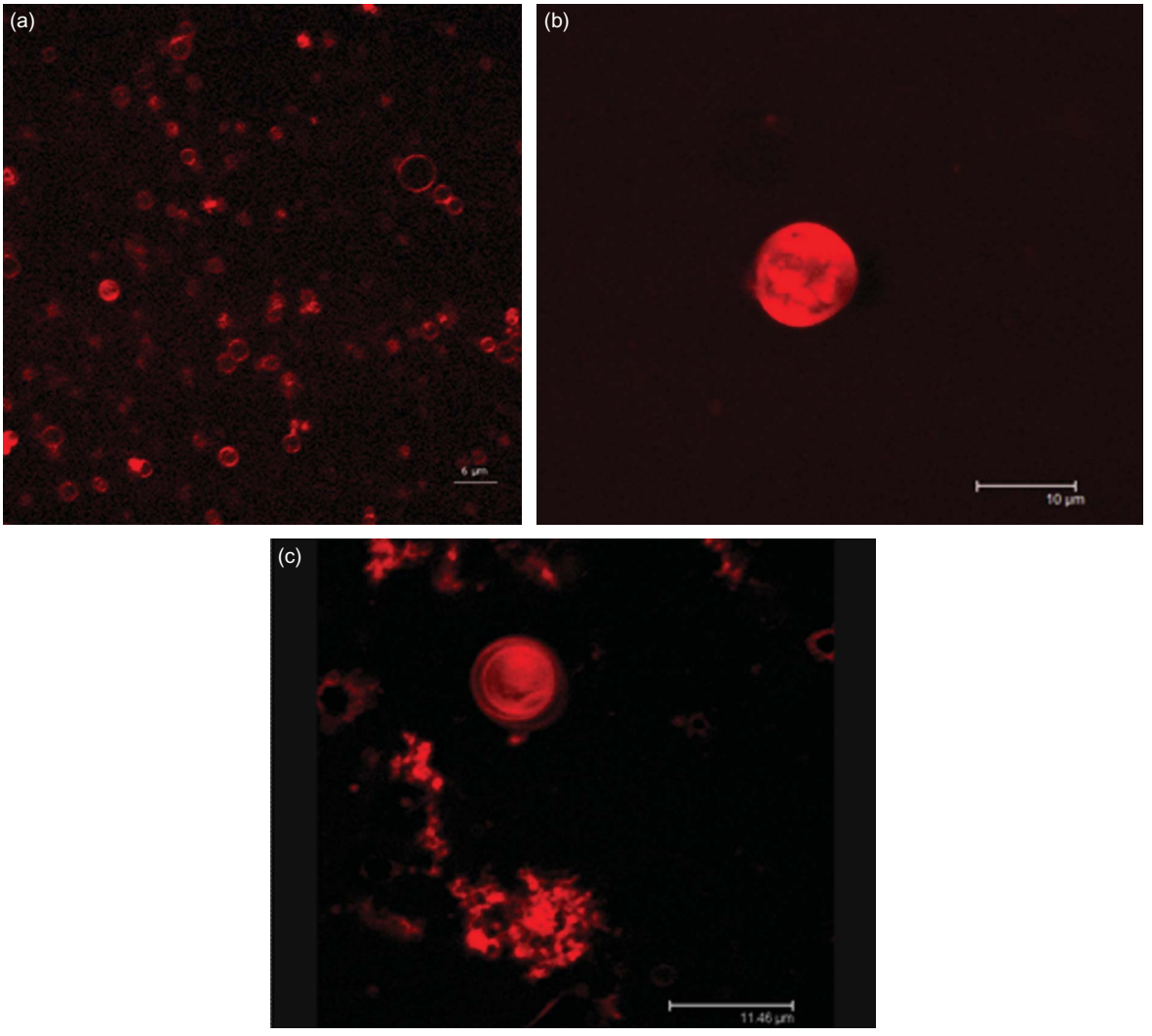

Figure 2. Confocal microscopy images of liposomes. (a) REV method, (b) TLE method, (c) FAT method. The composition of all formulations was 6.6:10.3 PC:CH molar ratio, with 10 mg of SA. Each data represents the mean ± SD for $n = 3$.

encapsulated with REV method, because the large unilamellar vesicles (LUVs) obtained lead to more aqueous internal compartment.

In the case of FATMLV, the confocal images demonstrate that the vesicles were not completely formed. In Figure 2c, we can observe some vesicles and remaining lipids, because the freezing–heating treatment destroys part of the sample.

Zeta potential measurement

Zeta potential was measured by electrophoretic light scattering at room temperature. Liposome dispersions were diluted with HEPES buffer before the measurement to adjust the intensity.

The independent analysis of positively and negatively charged liposomes showed a significant difference in the zeta potential of the different positively charged colloidal systems as a function of the preparation method (ANOVA, $F = 6.756$, $P = 0.029$). Despite their highest vesicle size, TLE liposomes have similar positively charged surface than REV liposomes (using the same SA concentration), as we can see in Figure 3. Because three levels of this variable have been considered, the Scheffé test can be used for making pairwise comparisons. To do this, we calculate the F ratio for the difference between the means of two preparation techniques, and then we test the significance of this F value. The summary of Scheffé test results is shown in Table 3.

Based upon the obtained results, the existence of statistical difference between groups has been demonstrated. The comparison between REV and FAT techniques showed a mean difference of 18.25 ($P = 0.037$), indicating that the average value of zeta potential in REV formulations is less positive than in FAT samples. This conclusion could be relevant and should be taken into account for further studies, because this difference of the zeta potential values might affect the vesicle stability and the skin permeation behavior.

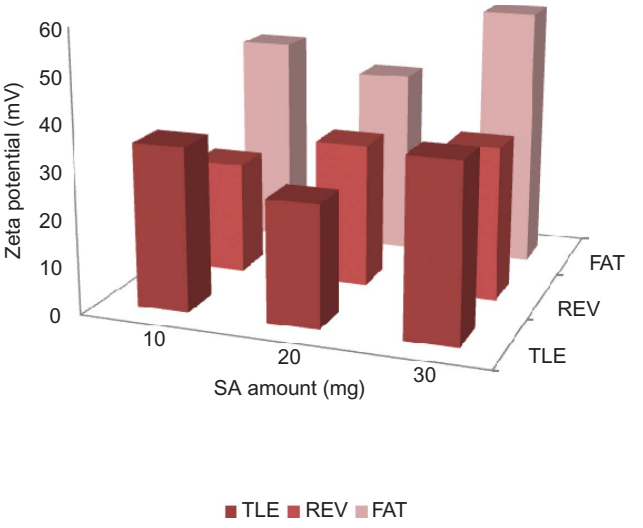

Figure 3. Zeta potential (mV) values as a function of the SA amount in the formulations for the three preparation techniques used. Each data represents the mean ± SD for $n = 3$.

Concerning the effect of the charge inducer agent on the zeta potential values between negatively and positively charged liposomes, statistical significance has been obtained when ANOVA test was applied ($F = 2035.61$, $P < 0.05$). On the basis of zeta potential measurements, several authors have concluded that DCP incorporated in liquid-crystalline PC bilayers is randomly distributed on the plane of the bilayer. Furthermore, the distribution of this negatively charged phospholipid between the two halves of the bilayer is uniform. However, this pattern was not reproduced with liposomes containing SA.

At pH 5.5, the protonation of SA would be theoretically complete. Then, as the SA increased in the formulations, the zeta potential should also be increased. However, results indicated a possible asymmetrical distribution of SA in the bilayer because a nonlinear relationship between the SA content and the zeta potential values was obtained (Figure 4a–c). In addition, several authors have reported the capacity of SA to escape easily from the lipid

Table 3. Comparison of zeta potential values of the different preparations of sumatriptan liposomes between the three preparation methods, comparing them by pairs.

						Confidence level at 95 %	
Variable	(I) Method	(J) Method	Mean difference (I – J)	Standard error	P	Lower limit	Higher limit
ZETA	TLE	FAT	−14.71333	5.26599	0.082	−31.6027	2.1761
		REV	3.53667	5.26599	0.805	−13.3527	20.4261
ZETA	FAT	TLE	14.71333	5.26599	0.082	−2.1761	31.6027
		REV	18.25000(*)	5.26599	0.037*	1.3606	35.1394
ZETA	REV	TLE	−3.53667	5.26599	0.805	−20.4261	13.3527
		FAT	−18.25000(*)	5.26599	0.037*	−35.1394	−1.3606

$P \leq 0.05$ significantly different as evaluated by Scheffé's F-test.

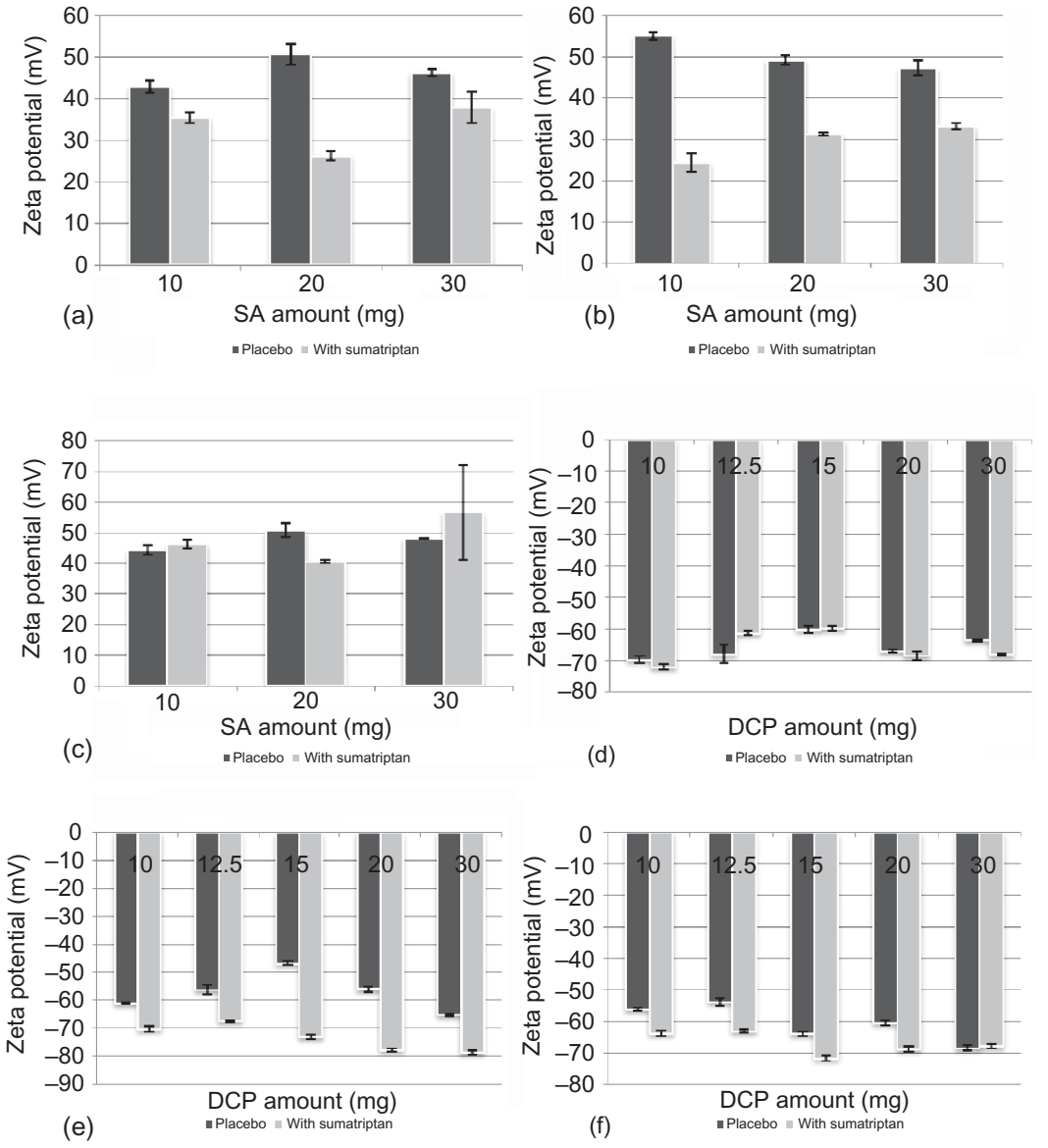

Figure 4. Comparison between the zeta potential (mV) values obtained in formulations with and without sumatriptan. (a–c) Formulations containing SA as charge inducer agent (a, TLE method; b, REV method; c, FAT method). (d–f) Formulations containing DCP as charge inducer agent (d, TLE method; e, REV method; f, FAT method). Each data represents the mean ± SD for $n = 3$.

bilayer and to protect its hydrocarbon chain from the hydrophilic environment: SA is organized in micelles, undergoing a rapid segregation into the medium, which change the surface charge density[45]. Probably in REV, micelles formation is higher than TLE, because zeta potential value is lower in these preparations.

On the other hand, the effect of sumatriptan on the zeta potential has been confirmed by applying Mann–Whitney *U*-test for independent samples ($P = 0.036$). Sumatriptan succinate is a weak salt of succinic acid (pK_a: 4.2). When this substance was dissolved in aqueous solution (pH 5.5), it ionized in equilibrium:

$$C_{14}H_{21}N_3O_2S\cdot C_4H_6O_4 + H_2O \leftrightarrow C_{14}H_{21}N_3O_2S^+ + C_4H_6O_4^-.$$

SA is an amphiphilic compound, which may ionize when it will be incorporated into the liposomes and aqueous environments, as shown in the following equation[46]:

$$C_{18}H_{39}N + H_2O \leftrightarrow C_{18}H_{40}N^+ + OH^-.$$

With the obtained results in the above studies, we can assume a displacement of SA from the bilayer because of a stronger interaction between sumatriptan and the lipid heads. Therefore, an increase in the encapsulation efficiency of sumatriptan gives rise to lower zeta potential, as we can see in Figure 4a–c.

Several authors have revealed that, in slightly acidic solutions, a decrease of negative charge occurs (DCP without sumatriptan, see Figure 4d–f). When the drug was incorporated, succinate stayed ionized, increasing the negative charge of vesicles[38]. However, in the case of SA, which was ionized at pH 5.5, an increase in positive charge occurs in the absence of sumatriptan, compared with the surface charge in drug formulations. At pH 5.5, succinate can react with SA, giving rise to a decrease in the zeta potential.

Conclusion

In this work, we have shown that sumatriptan succinate can be encapsulated in liposomes. Both preparation method and charged inducer have no statistical influence on the studied parameters. Only sumatriptan succinate has been a significative influence above zeta potential. It was found that positively charged REV liposomes, prepared with PC:CH:SA (6.6:10.3:3.71) mmolar ratio, showed vesicles with smaller size, unilamellar morphology, and high drug encapsulation. Therefore, we have chosen this formulation to realize future studies with the aim to apply them by transdermal route.

Acknowledgments

We are grateful to the Electron Microscopy Service from University of Seville for providing the CLSM. We also thank Mrs. Asunción Fernández and Mr. J. Luis Ribas for their valuable contribution to this work.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Peroutka S. (1993). 5 – hydroxytriptamine receptor subtypes and the pharmacology of migraine. Neurology, 43:34–8.
2. Silberstein S, Hargreaves R. (2000). The history and pharmacology of ergotamine and dihydroergotamine. In: Fisher M, Christopher H, eds. Clinical neuroscience, vol. 17. Switzerland: Karger, 52–65.
3. Arulmozhi D, Veeranjaneyulu A, Bodhankar S. (2005). Migraine: Current concepts and emerging therapies. Vascul Pharmacol, 43:176–87.
4. Tepper SJ, Rapoport A. (1999). The triptans: A summary. CNS Drugs, 12:403–17.
5. Callenbach MCP, Pels LPM, Mulder PGH, Linssen HJP, Gooskens HJM, van der Zwan JL, et al. (2007). Sumatriptan nasal spray in the acute treatment of migraine in adolescents and children. Eur J Paediatr Neurol, 11:325–30.
6. Weir RAP, Oldroyd KG, Walters MR. (2007). Myocardial infarction after oral sumatriptan use in a woman with normal coronary arteries. Am J Med, 120:7–8.
7. Weidmann E, Unger J, Blair S, Friesen C, Hart C, Cady R. (2003). An open – label study to assess changes in efficacy and satisfaction with migraine care when patients have access to multiple sumatriptan succinate formulations. Clin Ther, 25:235–46.
8. Krämer HH, Lundblad L, Birklein F, Linde M, Karlsson T, Elam M, et al. (2007). Activation of the cortical pain network by soft tactile stimulation after injection of sumatriptan. Pain, 133:72–8.
9. Patel SR, Zhong H, Sharma A, Kalia YN. (2007). In vitro and in vivo evaluation of the transdermal iontophoretic delivery of sumatriptan succinate. Eur J Pharm Biopharm, 66:296–301.
10. Biruss B, Valenta C. (2006). Skin permeation of different steroid hormones from polymeric coated liposomal formulations. Eur J Pharm Biopharm, 62:210–9.
11. El-Maghhraby G. (2008). Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: Effects of cosurfactants. Int J Pharm, 355:285–92.
12. El-Laithy H. (2009). Novel transdermal delivery of timolol maleato using sugar esters: Preclinical and clinical studies. Eur J Pharm Biopharm, 72:239–45.
13. Cassano R, Trombino S, Muzzalupo R, Tavano L, Picci N. (2009). A novel dextran hydrogel linking trans-ferulic acid for the stabilization and transdermal delivery of vitamin E. Eur J Pharm Biopharm, 72:232–8.
14. Fang J, Hong C, Chia W, Wang Y. (2001). Effect of liposomes and niosomes on skin permeation of enoxacin. Int J Pharm, 219:61–72.
15. Mura P, Maestrelli F, González-Rodríguez ML, Michelacci I, Ghelardini C, Rabasco AM. (2007). Development, characterization and in vivo evaluation of benzocaine-loaded liposomes. Eur J Pharm Biopharm, 67:86–95.
16. Femenía-Font A, Balaguer-Fernández C, Merino V, Rodilla V, López-Castellano A. (2005). Effect of chemical enhancers on the in vitro percutaneous absorption of sumatriptan succinate. Eur J Pharm Biopharm, 61:50–5.
17. Femenía-Font A, Balaguer-Fernández C, Merino V, Rodilla V, López-Castellano A. (2006). Combination strategies for enhancing transdermal absorption of sumatriptan through skin. Int J Pharm, 323:125–30.
18. Balaguer-Fernández C, Femenía-Font A, Del Rio-Sancho S, Merino V, López-Castellano A. (2008). Sumatriptan succinate transdermal delivery systems for the treatment of migraine. J Pharm Sci, 97(6):2102–9.
19. Arora R, Jain CP. (2007). Advances in niosome as a drug carrier: A review. Asian J Pharm, 1:29–39.
20. Mainardes RM, Silva LP. (2004). Drug delivery systems: Past, present and future. Curr Drug Targets, 5:449–55.
21. Maestrelli F, González-Rodríguez ML, Rabasco AM, Mura P. (2005). Preparation and characteristion of liposomes encapsulating ketoprofen-cyclodextrin complexes for transdermal drug delivery. Int J Pharm, 298:55–67.
22. Zhang JA, Anyarambhatla G, Ma L, Ugwu S, Xuan T, Sardone T, et al. (2005). Development and characterization of a novel Cremophor® EL free liposome-based paclitaxel (LEP-ETU) formulation. Eur J Pharm Biopharm, 59:177–87.
23. López Pinto JM, González-Rodríguez ML, Rabasco AM. (2005). Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. Int J Pharm, 298:1–12.
24. Maestrelli F, González-Rodríguez ML, Rabasco AM, Mura P. (2006). Effect of preparation technique on the properties of

liposomes encapsulating ketoprofen–cyclodextrin complexes aimed for transdermal delivery. Int J Pharm, 312:53–60.
25. Song YK, Kim CK. (2006). Topical delivery of low-molecular-weight heparin with surface-charged flexible liposomes. Biomaterials, 27:271–80.
26. Kitagawa S, Kasamaki M. (2006). Enhanced delivery of retinoic acid to skin by cationic liposomes. Chem Pharm Bull, 54(2):242–4.
27. González-Rodríguez ML, Barros LB, Palma J, González-Rodríguez PL, Rabasco AM. (2007). Application of statistical design to study the formulation variables influencing the coating process of lidocaine liposomes. Int J Pharm, 337:336–45.
28. Bangham A, Standish M, Watkins J. (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol, 13:238–52.
29. Vyas S, Goswami S, Singh R. (1995). Liposomes based nasal delivery system of nifedipine: Development and characterization. Int J Pharm, 118:23–30.
30. Lasic D, Weiner N, Riaz M, Martin F. (1998). Liposomes. In: Lieberman A, Rieger M, Banker G, eds. Pharmaceutical dosage forms: Disperse systems, vol. 3. New York: Marcel Dekker, 43–86.
31. Abraham S, Waterhouse D, Mayer L, Cullis P, Madden T, Bally M. (2005). The liposomal formulation of doxorubicin. Methods Enzymol, 391:71–96.
32. Skoza F, Papahadjopoulos D. (1978). Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse – phase evaporation. Proc Natl Acad Sci USA, 75:4194–8.
33. Vyas T, Shahiwala A, Marathe S, Misra A. (2005). Intranasal drug delivery for brain targeting. Current Drug Deliv, 2:1–11.
34. Puziy A, Matynia T, Gawdzik B, Poddubnaya O. (1999). Use of CONTIN for calculation of adsorption energy distribution. Langmuir, 15:6016–25.
35. Sreeram K, Nidhin M, Nair B. (2009). Synthesis of aligned hematite nanoparticles on chitosan-alginate films. Colloids Surf B Biointerfaces, 71:260–7.
36. Shirsat V, Gabhe S, Deshpande S. (1997). High performance liquid chromatographic determination of sumatriptán succinato from pharmaceutical preparation. Indian Drugs, 35:404–7.
37. Trotta M, Peira E, Debernardi F, Gallarate M. (2002). Elastic liposomes for skin delivery of dipotassium glycyrrhizinate. Int J Pharm, 241:319–27.
38. Kotynska J, Figaszewski ZA. (2007). Adsorption equilibria at interface separating electrolyte solution and phosphatidylcholine-stearylamine liposome membrane. Biophys Chem, 127:84–90.
39. Pavelic Z, Skalko-Basnet N, Filipovic-Grcic J, Martinac A, Jalsenjak I. (2005). Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir. J Control Release, 106:34–43.
40. Sezer AD, Akbuga J, Bas AL. (2007). In vitro evaluation of enrofloxacin-loaded MLV liposomes. Drug Deliv, 14:47–53.
41. Mohammed A, Weston N, Coombes A, Fitzgerald M, Perrie Y. (2004). Liposome formulation of poorly water soluble drugs: Optimisation of drug loading and ESEM analysis of stability. Int J Pharm, 285:23–34.
42. Hathout RM, Mansour S, Mortada ND, Guinedi AS. (2007). Liposomes as an ocular delivery system for acetazolamide: In vitro and in vivo studies. AAPS PharmSciTech, 8(1):E1–2.
43. Puglisi G, Fresta M, Mazzone G, Purneri PM, Tempera G. (1995). Formulation parameters of fluoroquinolone-loaded liposomes and in vitro antimicrobial activity. Int J Pharm, 118:65–76.
44. Koynova R, Tarahovsky Y, Wang L, McDonald R. (2007). Lipoplex formulation of superior efficacy exhibits high surface activity and fusogenicity, and readily releases DNA. Biomembranes, 1768:375–86.
45. Casals E, Soler M, Gallardo M, Estelrich J. (1998). Electrophoretic behaviour of stearylamine containing liposomes. Langmuir, 14(26):7522–6.
46. Manosroi A, Podjanasoonthon K, Manosroi J. (2002). Development of novel topic tranexamic acid liposome formulations. Int J Pharm, 235:61–70.