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RESEARCH PAPER

Chitosan coating of anionic liposomes containing sumatriptan succinate: A candidate for nasal administration

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ABSTRACT

Objective(s): Sumatriptan is a routine medication in the treatment of migraine and cluster headache that is generally given by oral or parental routes. However, a substantial proportion of patients suffer severe side effects. Nasal administration is significantly effective in case of oral administration of drug gives an undesirable side effect. So, the purpose of the present study was to develop intranasal delivery systems of Sumatriptan succinate using nanoliposomes as container of a water-soluble drug and chitosan as a mucoadhesive polymer.

Materials and Methods: Liposomal formulations containing Sumatriptan as well as chitosan-coated liposomal formulations with different phospholipids and different concentrations were prepared. The formulations were evaluated for their physicochemical properties, stability and Cytotoxicity on BEAS-2B cells.

Results: The prepared liposomal formulations coated with chitosan containing Sumatriptan had a size range of 165±9.4to 258±6.4 nm, and the surface charge of the obtained formulations was measured between 32±6 and 40±5 mV. Also, the encapsulation efficiency of the formulations was also observed between 14.2±2.7% and 19±3.4%. Based on the obtained results of physicochemical studies, liposomes F2 was also tested for stability and toxicity and showed that the F2 liposomes retained its physicochemical properties for up to 3 months. Finally, the toxicity test of the mentioned formulation showed relatively low toxicity on BEAS-2B cells.

Conclusion: In the presents study, stable liposomal formulations coated with chitosan containing Sumatriptan were prepared and studied. Based on the obtained, these formulations can be used in preclinical and animal studies for the nasal administration of Sumatriptan.

Keywords: *Chitosan, Intranasal Administration, Nanotechnology, Liposomes, Sumatriptan*

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INTRODUCTION

Nowadays, nasal drug delivery is a challenging subjects for pharmaceutical scientists. Nasal administration of drugs and other therapeutic compounds is significantly effective whereas oral administration gives an unwanted side effect [1,2]. The bioavailability and efficacy in nasal administration have been improved compared to the oral route, because of avoiding first-pass metabolism and incomplete absorption of drugs in the gastrointestinal tract [3,4].

Sumatriptan (ST), an agonist of 5-HT1D (5-hydroxy tryptamine 1D)-receptor from triptans family, is usually given in the therapy of acute migraine and cluster headache. Generally, ST is used by parental or oral routes. Nevertheless, it seems that nausea or vomiting during the migraine attacks in a substantial proportion of cases may negatively affect the outcomes

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of oral administration. Furthermore, a lower bioavailability was observed in human volunteers after oral administration of ST (15%). While the subcutaneous route of ST is an alternative approach, this method cannot be acceptable treatment for those who dislike injections or are unable to perform self-administration. Therefore, the nasal administration of drugs is more popular alternative in the case of self-administration due to lack of such limitations [5].

Although the nasal delivery systems are going to attract further attention, some problems are associated with this route of ST solution, including a decrease in retention time (15 minutes) in the nasal cavity, leading to a reduced bioavailability and lower brain absorption of ST via olfactory pathways [6].

More recently, novel drug delivery systems with mucoadhesive properties have exerted more desirable consequences, as they can raise the residence time of medications at the site of action/absorption. Thus, these systems prepare sustained delivery of the drug and diminish the drug degradation in different sites of individual body [7]. Among different cationic polysaccharide, chitosan exerts mucoadhesive features that has been broadly utilized in the formulation of mucoadhesive dosage forms [8,9].

So, we decided to prepare a sustained formulation of chitosan-coated mucoadhesive liposomes to increase the retention time, absorption and bioavailability of ST for future studies in animals. So, the aim of this study was to establish intranasal delivery systems of ST using nanoliposomes as container of a water-soluble drug and chitosan as a mucoadhesive polymer.

MATERIALS AND METHODS

Materials

ST as succinate salt was supplied from Natco Fine Pharmacy Pvt Ltd ,Hydrogenated soya phosphatidylcholine (HSPC) and Cholesterol were purchased from Lipoid (Ludwigshafen, Germany). Dipalmitoylphosphatidylcholine (DPPC), 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG) and 1,2-Distearoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DSPG) were obtained from Avanti Polar Lipids (Alabaster, AL). Trypan blue, and chloroform were purchased from Merck. 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) was supplied from Sigma-Aldrich. All other reagents and chemicals utilized in the present research were of analytical grade.

Preparation of nanoliposomes

In order to prepare of Sumatriptan for liposomes synthesis, Sumatriptan succinate was solubilized in dextrose 5 %. Liposome formulations in the present study were provide by thin film hydration and extrusion method [10, 11]. In summary, proper amounts of phospholipids and cholesterol were added as chloroform stock solutions in a round bottom balloons and subjected to solvent evaporation under vacuum in a rotary evaporator followed by overnight freeze-drying. The resulting film was hydrated with an appropriate volume of ST solution (250 mM) to achieve final phospholipid concentration of 11 mM and then, sonicated for further 10 min under argon atmosphere at 60 °C. Subsequently, the newly formed liposomal preparation was extruded through polycarbonate nanopore membranes of 200, 100 and 80 nm pore size (Avestin, Canada) [12]. To purification of the prepared liposomal formulations from the unencapsulated ST, the liposomes were then dialyzed three times against dextrose 5 % in dialysis cassettes (Pierce, Rockford, Illinois) with molecular weight cut off of 12-14 kDa. The lipid composition and nomenclature of the liposomes are shown in Table 1.

Table 1. Composition and molar ratio of liposomal formulations containing ST

Liposomal formulations	Lipid composition	Molar ratio
F1	DPPC/ Cholesterol /DPPG	70/15/15
F2	DPPC/ Cholesterol /DPPG	70/18/12
F3	DPPC/ Cholesterol /DPPG	70/21/9
F4	DPPC/ Cholesterol /DSPG	70/15/15
F5	DPPC/ Cholesterol /DSPG	70/18/12

Coating of nanoliposomes with chitosan

To provide the chitosan-coated liposomes (CCLs), suspension of liposome in dextrose 5 % was added dropwise into the chitosan solution (0.001% [w/v] in acetic acid 0.1 M with adjusted pH of 4.5 via dilute NaOH). These steps of CCLs preparation was performed under stirring (250 rpm) in a volume ratio of 1:4 at room temperature. Then, the suspension was kept overnight at room temperature. Finally, CCLs were extracted from the mixture via centrifugation (15,000 ×g for 30

min) at 4 ◦C and resuspended in dextrose saline (5 %). This washing process was done twice [13]. CCLs were transferred to an Amicon-Ultra centrifuge filter device (Millipore Billerica, MA) with MWCO of 12-14 kD and centrifuged for 30 min at 4000 × g and after depletion of acetic acid, CCLs resuspend in dextrose 5 % [14].

Characterization of nanoliposomes and CCLs

Size and zeta potentials of nanoparticles

Measurements of particle size and zeta potentials as well as polydispersity index (PDI) of nanoliposomes containing ST and CCLs were done via a Dynamic Light Scattering Instrument (Nano-ZS; Malvern, UK) and Transmission electron microscopy [4].

Liposomal formulation's shape and formation were also evaluated by TEM. Briefly, a carbon-coated copper grid was used to fix the samples via applying a drop of the mixture on their surfaces. After 2 min of allowing the attachment of nanoparticles onto the carbon substrate, the extra dispersion was cleaned via a piece of filter paper and in the next step, a drop of phosphotungstic acid solution (1%) was used for 1 min and allowed the surface to air dry [15]. The images were prepared by a LEO 912AB Omeg TEM (Zeiss, Jena, Germany) at a voltage of 120 kV.

Determination of encapsulation efficiency

Stock solution and standards

In order to encapsulation efficiency determination, stock solutions of ST was prepared by mixing of ST with methanol to a final level of 1.0 mg/ml [16]. The final calculation of ST levels was expressed on the base form amount. The stock solutions were stored in stable condition for at least 1 month at -20±5°C.

A seven point non-zero calibration standard (with a range of 1 to 100 ng/ml) was provided via drug free spiked EDTA-containing human plasma with a chosen ST amount. Six different lots human plasma samples (blank) was tested before spiking to confirm that no endogenous interference was observed at ST retention times.

Chromatography and quantitation

The chromatographic system consisted of an Exformma HPLC system with UV detector (Ex1600, china). The separation was achieved by using a 250 mm ×4.6 mm, Eclipse XDB C18 column (Agilent Technologies, Palo Alto, CA, USA) with the mobile phase consisting of water, acetonitrile, formic acid (85:15:0.2v/v/v). Mobile phase was delivered at a flow rate of 1.0 ml/min. The UV detector wavelength was set at 282 nm. A peak area was used for quantitation [17]. ST was quantitated via a peak height ratio method. Encapsulation Efficiency (EE %) was then estimated by determination of the ST concentration before removal extra ST and after that by dialysis cassette according to the following equation:

EE%= (Initial ST concentration/removal of excess ST Initial concentration) ×100

Stability of liposomes and CCLs

The liposome stability during the storage period was assessed for three months. On this aim, nitrogen gas was used to fill the vials containing samples. Then these samples were stored at 4 ◦C for three months and collected at day 0, 1, 3, 7, and 15, as well as month 1 and 3. Finally, the characterization studies including encapsulation efficiency and particle size of each sample were done as described above [18].

Cytotoxicity assay of liposomes and CCLs

Cell line and culture

To evaluate the cytotoxicity of CCLs, BEAS-2B cells were cultured in a standard condition (at 37 ◦C, 5% CO2, and 95% air humidified atmosphere). The cultured medium solution of DMEM (Dulbecco's Modified Eagle Medium) composed of 2 mM l-glutamine supplemented with heat-inactivated fetal bovine serum (FBS, 10%), penicillin (100 IU/mL) and streptomycin (100 mg/mL, Gibco). The BEAS-2B cells (~1 × 104 cells) were seeded in a 96- well plate, each well containing 200 µl of the prepared culture medium and incubated at 37 ◦C. After 24 hours of adhesion, a serial of doubling dilution of the ST, uncoated liposomal containing ST (SL), CCL and empty liposomes was added to four replicate wells and incubated for 24 and 48 hours.

The cell viability was then assessed through MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.

After 24 and 48hr of incubation times, 10 µl of stock solution MTT was added to each well. Then, the plates were incubated at temperature of 37 ◦C for more than 4 hours. Then, the medium was removed and the formazan blue, which formed in the cells, was dissolved via 200 µl dimethyl sulfoxide (DMSO).

Table 2. Characteristics and encapsulation efficiencies of Chitosan coated liposomes and uncoated liposomes containing ST

	Z-Average (nm) Mean±SD	Zeta potential (mV) Mean±SD	Polydispersity index (PDI) Mean±SD	Encapsulation efficiency (%) Mean±SD
Uncoated liposome (F2)	135±5.5	-35±3	0.2±.018	22.3±.2.7
F1	210±7.1	38±3	0.34±0.017	14.2±2.7
F2	165±9.4	32±6	0.47±.025	21.5±2.1
F3	205±6.7	37±1	0.37±0.1	19±1.4
F4	258±6.4	38±5	0.23±0.008	17±2.1
F5	197±4.3	40±3	0.39±0.04	16.3±0.2

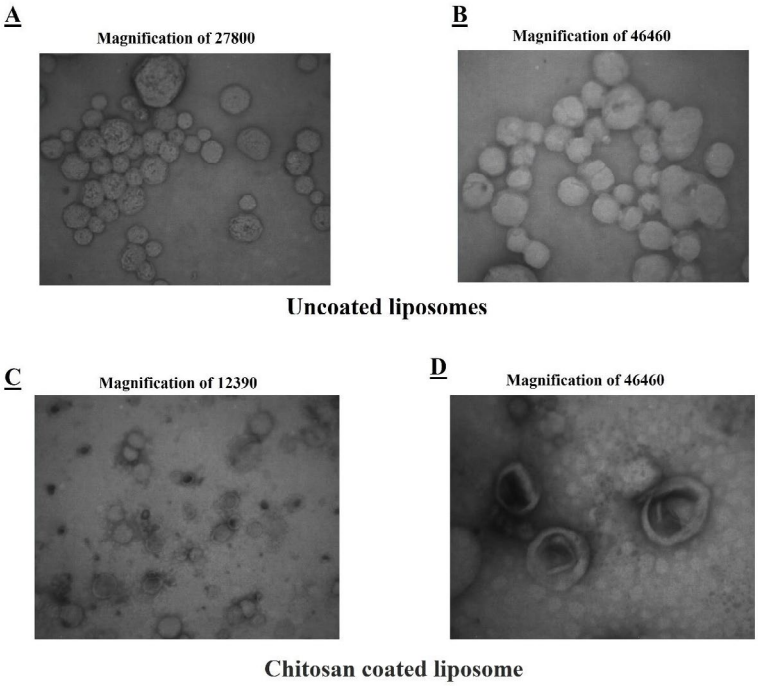

Fig 1. TEM images of liposomes containing ST. A and B: Uncoated liposomes at magnifications of ×27800 and ×46460, respectively. C and D: Chitosan coated liposomes at magnifications ×12390 and ×46460, respectively. Both formulations have a spherical and smooth shape. Polymer adsorption increases the size of liposomes(C, D). In addition, chitosan-coated liposomes are spaced apart, which may be due to repulsion from chitosan charge and spatial barrier

Finally, the absorbance measurements of wells was performed at 550 nm with a microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT). The mean absorbance values obtained from negative and positive control wells as the lower and upper range of the well absorbance were used to estimate the half maximal inhibitory concentrations (IC50) by GraphPad Prism software ver. 6 (GraphPad Software, San Diego, CA) [19].

RESULTS AND DISCUSSION

Preparation and characterization of liposomes

The physicochemical properties especially solubility, permeability and partition coefficient, are important determinants for the extent of its liposomal incorporation [20]. Considering the high solubility of ST in aqueous solution, anionic liposomes were prepared by dissolving ST in dextrose 5 % with DPPC, DPPG, DSPG and cholesterol. Afterwards, the obtained anionic liposome was coated with chitosan to produce mucoadhesive liposome [13]. The characterization results including particle size and PDI, zeta potential and encapsulation efficiency of the obtained liposomes were summarized in Table 2.

As seen in Table 2, before the procedure of ST

coating via chitosan, the liposomes with negative charges were obtained as relatively small size vesicles with distribution of narrow size, while the mean size of liposomes after coating with chitosan were about 165±9.4 to 258±6.4 nm with the PDI ranged between 0.23±0.008 to 0.47±.025. The chitosan binding to the surface of the liposomes increased the mean particle size and PDI. Moreover, addition of chitosan to the surface of liposomal nanoparticles caused the net surface charge of the liposomes to shift from negative to positive, which is a confirmation for the chitosan coating on the surface of the liposomes [21]. The zeta potential proposed that the ionization of the phosphate groups of DPPG and DPPC phospholipids formed liposomes with negative charges. These characteristics made the liposomes to be able to have electrostatic interaction with positively charged chitosan, and then, the liposomal surface were coated by this mucoadhesive layer [13]. Also based on the table 2, the encapsulation efficiency of uncoated liposomes was 22.3% ±.2.7, which was seen between 14.2%±2.7 and 21.5%±4.1 in F1 to F5 chitosan coated formulations. The highest encapsulation rate was also observed in F2 formulation (Table 2).

Transmission electron microscopy

The spherical form of the prepared liposomes was characterized using TEM, which also confirmed its narrow size distribution (Fig.2). TEM results showed that chitosan-coated liposomes and uncoated liposomes have a spherical shape and are consistent with the results in Table 2. There was also no significant difference between the shape of uncoated and chitosan-coated liposomes. This result can be due to the strong adsorption of the polymer by the liposome, which leads to its smooth shape and thus makes it difficult to observe on the liposome [22]. In addition, according to the results of Table 2 and TEM images, polymer adsorption increases the size of liposomes. In addition, as seen in Fig 1, chitosan-coated liposomes are spaced apart, which may be due to repulsion from chitosan charge and spatial barrier. These free polymers can also be effective in stabilizing liposomes [23]. In general, according to the results, liposomes containing 12% DPPG phospholipid coated with chitosan at a concentration of 0.001% w/v and in a volume ratio of lipid to chitosan 1: 4, were selected as the optimal formulation, because they have the optimal size, zeta potential and stability.

The effect of lipid concentration

The effect of lipid concentration and liposome size in this study showed that increasing the lipid concentration of liposomes increased the percentage of encapsulation in them. Internal volume of liposomes is directly related to lipid concentration and liposome size. With increasing lipid concentration as a result of increasing the size of liposomes, the internal volume of liposomes increases and as a result the percentage of encapsulation should increase [24].

The effect of lipid composition on the properties of liposomes

The presence of various additives in the lipid membrane significantly affects the thickness, fluidity and polarity, which in turn can affect the percentage of entrapment of hydrophilic liposomes [25].

Cholesterol, as one of the main components of biological membranes, has an important role in the organization, function and dynamics of membranes. Cholesterol affects the vesicle diameter and thus the percentage of drug enclosure. Studies have also shown that adding cholesterol to liposomes reduces the volume of the aqueous phase within liposomes and thus reduces the percentage of encapsulation [26]. Finally, the length of the hydrophobic carbon chain of phospholipids is an important factor influencing drug loading in liposomal formulations, and studies have shown that when using phospholipids such as 16-carbon DPPC compared to 18-carbon DSPG, the liposome membrane thickness decreases. As a result, the rate of confinement increases [27].

The effect of Zeta potential charge

In general, molecular interfacing of drug with the lipid bilayer membrane exerts an essential role in the formulation of liposomal formulation and the encapsulation of drugs. Negatively or posititvely charged lipids are used to induce electrostatic interaction with the drug with the opposite charge and increase the percentage of confinement. For small drugs such as ganciclovir, there is a clear relationship between liposome load and percentage of encapsulation that has been reported in the past [28].

In our present study, anionic formulations were used to encapsulation of ST.

Table3. Stability profile of Chitosan coated liposomes and uncoated liposomes

		Z-Average (nm) Mean±SD	Zeta potential (mV) Mean±SD	Polydispersity index (PDI) Mean±SD	EE(%)
Uncoated liposomes	day 0	142.33±8.38	36.25±4.58	0.21±0.03	22.1±1.6
	day 1	143.00±5.19	35.55±5.21	0.21±0.03	22.3±0.9
	day 3	141.33±5.03	37.66±2.51	0.23±0.04	22.1±1.1
	day 7	144.31±6.50	35.37±4.28	0.23±0.3	21.7±0.3
	day 15	142.33±6.11	33.12±2.64	0.21±0.04	20.8±0.8
	1 month	145.33±7.76	34.66±4.04	0.20±0.02	20.5±0.4
	3 months	148.33±9.07	34.76±3.22	0.23±0.03	19.8±.1.0
F2 chitosan coated liposomes	day 0	167.33±3.39	-35.25±0.02	0.20±0.06	21.5±0.2
	day 1	170.33±1.52	-36.12±7.93	0.15±0.11	21.4±0.6
	day 3	168.66±2.08	-34.18±4.35	0.12±0.10	21.6±0.8
	day 7	170.66±3.51	-31.66±1.52	0.21±0.02	20.9±1.2
	day 15	168.00±3.00	-34.66±2.88	0.16±0.12	20.7±0.7
	1 month	171.00±3.00	-37.66±7.50	0.14±0.10	20.2±0.1
	3 months	169.66±3.51	-41.66±1.52	0.18±0.03	19.9±0.5

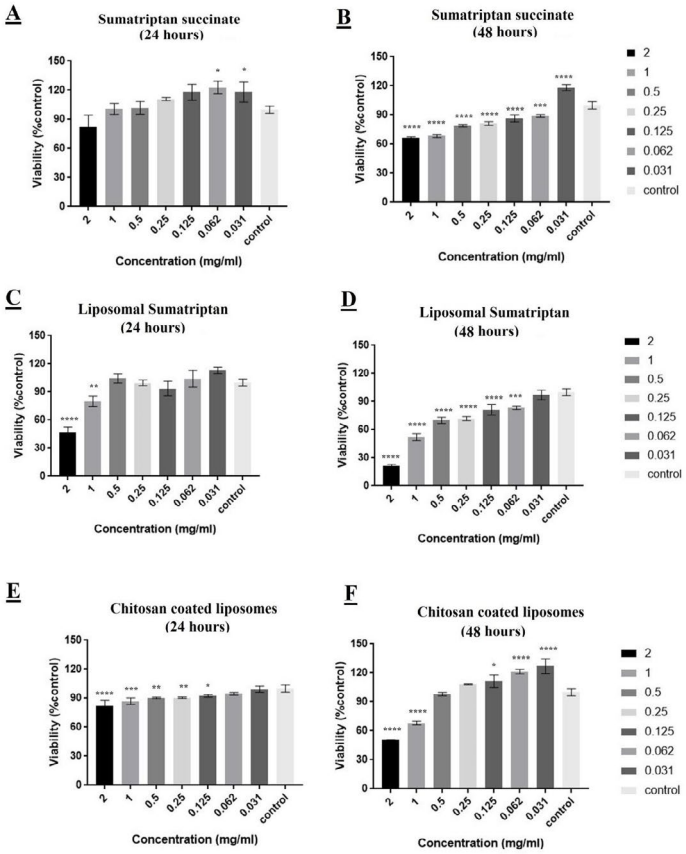

Fig 2. Cytotoxicity study of different concentrations of ST, liposomal formulations containing ST, and chitosan-coated liposomes containing ST at 24 and 48 h on BEAS-2B cells

As seen in results, liposomes without chitosan coating had a negative zeta potential charge which could encapsulate the ST. Also, after coating with chitosan, the liposomes charge were changed to positive, which had relatively no effect on the encapsulation efficiency of the ST.

Stability of liposomes and Chitosan coated liposomes

As can be seen in the table 3, the stability of the uncoated formulation and the chitosan-coated F2 formulation maintained their stability in terms of particle size and surface charge for 3 months at 4 ° C.

Cytotoxicity assay of liposomes and CCLs

As seen in the Fig 2, different concentrations of ST, liposomal formulations containing ST, and chitosan-coated liposomes containing drug were examined for toxicity at 24 and 48 h on BEAS-2B cells. According to the results, in 24 hours, the highest cytotoxicity was observed in the liposomal ST group with a concentration of 2 mg/ml. In comparison between the ST liposomal formulation and the chitosan-coated liposomal formulation containing ST, the toxicity of chitosan coated liposomes was lower than that of the liposomal formulation in the first 24 hours. Also, in most cases, the increase in toxicity was dose-dependent, which increased with increasing the dose of ST. Finally, in all doses, the lowest cytotoxicity was observed in the ST group at 24 hours and the highest in the ST liposomal formulation group at 48 hours. (Fig 2).

As has shown in the in Fig 2, in both 24 h and 48 h, the concentration of 2 mg/ml of ST has a relative toxicity and in the other concentrations has seen lower inhibitory growth. The results showed that adding chitosan on the surface of nanoliposome reduced the cell toxicity. Overall we concluded that the two upper concentrations of drug (2& 1 mg/ml) wasn't suitable for BEAS-2B cells and can damage to the cells. But the reducing effect of chitosan on liposomes after coating of nanoparticles has shown in these concentrations too.

CONCLUSION

In this study, we designed chitosan-coated liposome formulations and examined their physicochemical properties, stability and cytotoxicity. According to the results of this study, stable liposomal formulations coated with chitosan containing ST were prepared and studied. These formulations can be used in preclinical and animal studies for the nasal administration of ST. Among polymers, chitosan is a natural cationic polymer that has good biocompatibility and adhesive mucosa. Chitosan is able to load hydrophilic and hydrophobic drugs in drug delivery systems. Its super-sticky mucus makes it suitable for use in vaccines as well as for overcoming tight intercellular connections. One way to increase the adhesive mucosa of drugs is to coat the nanoparticles with adhesive mucosal polymers such as chitosan. Moreover, due to the presence of the ciliary system in the nasal mucosa, which causes the rapid elimination of drugs, the bioavailability of drugs decreases after intranasal administration, although the presence of a liposomal system can increase the rate of drug entry into the systemic circulation. Further studies should be done in order to investigate and synthesis of the appropriate drug delivery system for similar drugs to ST to obtain the optimum efficacy of the drugs.

The aim of the present study was to create a stable nanoliposomal formulation of ST with negative charge and good loading efficiency. In the following the stable nanoparticles has been coated with chitosan polymer. Eventually among different prepared formulations the proper ratio of DPPC, Cholesterol and DPPG have selected. This formula has the highest loading and stability. The result of cytotoxicity study has indicated of low toxicity of ST and nanoliposomes.

In conclusion concerning good encapsulation efficiency, proper positive charge and suitable stability of formulation along with mucoadhesivity of chitosan, this product can be used for nasal administraion in preclinical and clinical studies.

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