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Preparation and Characterization of Mucoadhesive Loratadine Nanoliposomes for Intranasal Administration

İntranazal Uygulama için Muko yapışkanlı Loratadin Nanolipozomlarının Hazırlanması ve Karakterizasyonu

Short Title: Intranasal Mucoadhesive Loratadine Nanoliposomes
Kısa Başlık: Burun içi Muko yapışkanlı Loratadin Nanolipozomları

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ABSTRACT
Objective: The aim of this study was to formulate and evaluate the mucoadhesive intranasal liposomes of loratadine to improve drug bioavailability and efficiency. Method: Liposomes were prepared by thin-film hydration method using soybean phosphatidylecholine and cholesterol as main components. Liposomes were coated with chitosan solution in concentration of 0.05 % and 0.1% w/v and then characterized regarding particle size and polydispersity index, encapsulation efficiency, thermodynamic studies, in vitro drug release, mucoadhesivness and stability. Results: Particle size analysis showed that the vesicles were obtained in size of 193±3.3 nm, 345±4.6 and 438±7.3 nm for uncoated, 0.05% and 0.1% chitosan-coated liposomes, respectively. Size distribution was acceptable for all the formulations (PDI<0.7). Encapsulation efficiency was high enough around 80%. Coated liposomes have demonstrated slower release rate than uncoated one. Drug release kinetic model was zero order for all the formulations. Chitosan coating improved mucoadhesivness more than 3-fold compared to uncoated liposomes but there was no significant difference between 0.05% and 0.1% chitosan coating in mucin adsorption ($p > 0.05$). Stability studies focusing on particle diameter, PDI and EE% after three months storage at 4°C, indicated the coated liposomes, had no significant alternation in particles size, PDI and drug leakage. Conclusion: The results concluded that liposomes with 0.05% chitosan coating were chosen as

the optimum formulation, which can demonstrate a significant potential for overcoming the nasal drug delivery limits of short residence time, and mucociliary clearance.
Keywords: Liposomes, Loratadine, Mucoadhesive, Chitosan, Intranasal
Öz

Amaç: Bu çalışmanın amacı, ilaç biyoyararlanımını ve etkinliğini artırmak için loratadin muko yapışkan burun içi lipozomlarını formüle etmek ve değerlendirmektir. Yöntem: Lipozomlar, ana bileşenler olarak soya fasulyesi fosfatidilekolin ve kolesterol kullanılarak ince film hidrasyon yöntemiyle hazırlandı. Lipozomlar,% 0.05 ve% 0.1 w / v konsantrasyonda kitosan çözeltisi ile kaplandı ve daha sonra partikül boyutu ve polidispersite indeksi, kapsülleme etkinliği, termodinamik çalışmalar, in vitro ilaç salımı, muko yapışkanlık ve stabilite ile karakterize edildi. Bulgular: Parçacık boyutu analizi veziküllerin kaplanmamış, sırasıyla% 0.05 ve% 0.1 kitosan kaplı lipozomlar için sırasıyla 193 ± 3.3 nm, 345 ± 4.6 ve 438 ± 7.3 nm boyutlarında elde edildiğini göstermiştir. Boyut dağılımı, tüm formülasyonlar için kabul edilebilirdi (PDI <0.7). Kapsülleme verimliliği yaklaşık% 80 civarında yüksekti. Kaplanmış lipozomlar, kaplanmamış olanlardan daha yavaş salım oranı göstermiştir. İlaç salım kinetik modeli, tüm formülasyonlar için sıfır dereceydi. Kitosan kaplama, kaplanmamış lipozomlara kıyasla muko yapışkanlığı 3 kattan fazla artırdı, ancak müsin adsorpsiyonunda% 0.05 ila% 0.1 kitosan kaplama arasında anlamlı bir fark yoktu (p> 0.05). 4 ° C'de üç aylık depolamadan sonra partikül çapı, PDI ve% EE'ye odaklanan stabilite çalışmaları, kaplanmış lipozomların partikül büyüklüğü, PDI ve ilaç kaçağı açısından önemli bir değişime sahip olmadığını gösterdi. Sonuç: Sonuçlar,% 0.05 kitosan kaplamalı lipozomların, kısa kalma süresinin ve mukosiliyer klerensinin nazal ilaç verme sınırlarının üstesinden gelmek için önemli bir potansiyel gösterebilen optimum formülasyon olarak seçildiği sonucuna vardı.
Anahtar Kelimeler: Lipozomlar, Loratadin, Mukoadhesif, Kitosan, Burun İçi

INTRODUCTION

Allergic rhinitis (AR) is an inflammation in the nose with the symptoms such as sneezing, rhinorrhea, nasal congestion and nasal itching.[1, 2] Common medications for the treatment of AR are antihistamines, corticosteroids and decongestants.

Loratadine; a second generation, long lasting antihistamine, is used in allergic disorders such as rhinitis, urticarial and upper respiratory tract infections.[3] Despite fast absorption after oral administration, loratadine has poor oral bioavailability (40%) due to first-pass metabolism. On the other hand, after oral administration, loratadine can cause some systemic side effects, such as allergic reactions e.g. rash, itching, difficulty in breathing, tightness in the chest, swelling of the mouth or face and dizziness.[4] Hence, another route of administration to bypass liver metabolism as well as systemic side effects would be favorable.

Intranasal drug delivery is a convenient and interesting route with following advantages: (l) it gives an applicable area for improving systemic absorption of drugs with low solubility[5], (II) high vascularized sub-epithelial layer leads to drug rapid onset of action, (III) provides the area for bypassing first-pass metabolism, (IV) It gives higher bioavailability by lower doses of drug.[6, 7] The main drawback of the intranasal route is mucociliary clearance in this area as a defense mechanism against foreign particles. This phenomena can remove drug delivery system from nasal cavity.[8]

Among different nasal drug delivery systems, liposomes have been explored for both local and systemic purposes. Liposomes are phospholipid bilayer vehicles with the advantages such as biocompatibility, biodegradability, drug targeting and prevention of enzymatic or chemical degradation of drugs.[9, 10] Coated liposomes by mucoadhesive polymers may increase their drug residence time in nasal cavity and improve drug bioavailability.

Chitosan which is a natural cationic polymer produced by deacetylation of chitin can provide mucoadhesivness in drug delivery systems by electrostatic interaction with negative charge of mucin in the nasal cavity and hence improve liposomes' residence time that lead to the enhancement of drug bioavailability and permeation.[11]

The aim of this study was formulation of intranasal loratadine mucoadhesive liposomes to circumvent the first-pass hepatic metabolism and enhance the drug bioavailability.

MATERIALS AND METHOD

Materials

Loratadine was a gift sample from shafa® pharma Co. (Tahran, Iran). Cholesterol, chitosan, periodic acid, Schiff reagent and dialysis tubing cellulose membrane were obtained from Sigma-Aldrich (St. Louis. MO. USA). Soybean phosphatidylcholine were purchased from Lipoid GmbH (Ludwigshafen, Germany). Chloroform, methanol, acetic acid, Sodium acetate tri-hydrate, sodium monobasic and dibasic phosphate were acquired from Merck Co. (Darmstadt, Germany). All the chemicals were analytical grade.

Preparation of Liposomes

Liposomes were prepared by thin film hydration method. Briefly, soybean phosphatidylcholine and cholesterol (molar ratio 7:4) and 100 mg loratadine were dissolved in the mixture of chloroform: methanol (volume ratio 2:1). The solvent was then evaporated by rotary evaporator at 50°C until thin film formation. Film was placed in refrigerator for 24 hours to complete solvent evaporation. After 24 hours thin film was hydrated by 20 ml phosphate buffer (pH=6.5) and agitated by ultrasonic bath (ELMA, t-710 DH) for 30 minutes at 50°C. For production of chitosan-coated liposomes, chitosan solutions in concentrations of 0.1% and 0.05% w/v (in 0.1% v/v acetic acid) were added drop wise into the liposome suspension on stirrer for 1 hour. The mixtures were centrifuged at 15000 rpm, at 20°C for 45 minutes and the sediments were resuspended in phosphate buffer (pH=6.5) by vortexing at room temperature till homogeneous preparation achieved.[12]

Loratadine encapsulation efficiency

For calculation of the encapsulation efficiency, the liposome suspension was centrifuged at 15000 rpm, at 20°C for 30 minutes. The supernatant then was analyzed by UV spectroscopy at 249 nm. The encapsulation efficiency was determined by following equation:[13]

%Encapsulation= (Loratadine total amount-Loratadine amount in supernatant) ×100%/ (Loratadine total amount)

Particle Size and Polydispersity Index Analysis

The average particle size was determined by Scatterscope 1, Qudix (Seoul, South Korea). Prior to measurement, the liposome suspension was diluted using filtered deionized water (1 to 20). Each sample was read in triplicate.

Differential Scanning Calorimetry (DSC)

DSC thermogram of the lipids, chitosan and the drug, DSC were recorded on DSC 1 METTLER TOLEDO Co. Certain amount of samples were placed in aluminum pan and scanned from 20_200□C by scanning rate of 10□C min^{-1}.

In vitro Drug Release Studies

In vitro loratadine release profile was evaluated by dialysis bag diffusion technique in dissolution apparatus dt800 ERWEKA Co. (Germany). Dialysis bags (cut off 12KDa) containing formulations were placed in baskets and immersed into the flasks containing 300ml of release medium (mixture of acetate buffer (pH=5.5) and methanol (50:50v/v). The temperature and rotation speed of the baskets were set at 37°C and 100 rpm. Sampling was done after 0.5, 1, 2, 3,

4, 5, 6, and 24 hours. Volume of samples was 1 ml and was replaced with 1ml of the fresh medium. Amounts of loratadine in the samples were analyzed by UV spectroscopy methods. This test was repeated 3 times for each formulations.[14]

Mucoadhesion Test

Mucoadhesion of formulations were measured by determination of mucin (porcine stomach type II) adsorption by periodic acid/Schiff colorimetric method.[15, 16]

Standard mucin solution with following concentrations in buffer phosphate (pH=5.5) was prepared: 12.5, 6.25, 3.125, 1.625 mg/100ml. Then, 200µl of periodic acid 10% was added to 2 ml of each and the samples were incubated at 37°C for 2 hours. After that, 200 µl of Schiff reagent was added to the mixtures and UV absorbance was measured after 30 minutes.

1 ml of mucin solution was added to 1 ml of the liposome suspensions. Liposomes stirred for 1 hour at 37°C and 300 rpm. For determination of the free mucin, the samples were centrifuged for 45 minutes at 15000 rpm and 20°C. 200 µl periodic acid was added to the supernatants and incubation was done at 37°C for 2 hours. Then, 200 µl of Schiff reagent was added and after 30 minutes the absorbance was measured by spectrophotometer at 555 nm.

Stability Study

After 3 months of storage at 4°C, stability of the formulations was investigated regarding particle size, polydispersity index and encapsulation efficiency.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used for comparisons between formulations. The multiple-comparison Tukey test was used to compare the means of the different groups and $p < 0.05$ was considered as significant.

RESULTS

Characterization of the Liposomes

Results of encapsulation efficiency (EE), particle size and polydispersity index evaluations were presented in Table 1. As shown, the average size of nano-liposomes before coating were 193±3.3 nm. By addition of chitosan as the liposome's coat, this size increased to 345±4.6 nm and 438±7.3 nm (for 0.05% and 0.1% coating, respectively). So coating process caused significant increase in size of the particles ($p < 0.05$). Reasonable EE about 80% was obtained that confirmed the suitability of preparation method as well as coating process.

DSC Thermogram

The DSC thermogram of loratadine, cholesterol, chitosan, soybean phosphatidylcholine, un coated and coated liposomes with 0.05% chitosan are shown in Figure 1a,1b,1c,1d,1e,1f, respectively. The DSC curve showed an endothermic peak at 136°C for loratadine (Fig.1a), two peaks at 46.30°C and 148.56°C for cholesterol (Fig.1b), a broad endothermic peak at 54.27°C for chitosan (Fig1c) and an endothermic peak at 131°C for phosphatidylcholine (Fig.1d). The thermogram of uncoated (Fig.1 e) and coated liposomes (Fig.1 f) presented a broad endothermic peak at 80°C -100°C.

In vitro Drug Release

The in vitro drug release of the prepared liposomes is shown in Figure 2. Formulations were evaluated until 24 hours. The uncoated liposomes showed maximum drug release after 24hrs (99±0.03%). For coated liposomes, slower release rate was obtained (94±0.05% and 81±0.02% for 0.05% and 0.1% coating, respectively). Therefore, coating can lead to liposomes with more controllable drug release rate. The value of kinetics parameters and their regression are displayed in Table 2. Kinetics model was selected based on higher r^2. Analysis showed zero order was the most suitable kinetic model for all the formulation and chitosan coating does not affect kinetic model.

Stability of Formulations

The results of stability study after three months storage are shown in Table 3. Significant changes was related to uncoated liposomes. Results showed particle size increases from 193 nm to 426 nm and Polydispersity index from 0.41 to 0.65. In addition, encapsulation efficiency reduction from 83% to 49% was revealed for uncoated formulation but for coated formulations there were not any significant changes in these parameters (p >0.05).

Mucin Adsorption Study

Chitosan is a polycationic polymer that provides an electrostatic interaction with anionic groups of mucus layer such as mucin (most important component of mucus layer). Chitosan backbone flexibility make its involvement to mucus layer easier.[17] Hence, in this study mucin adsorption of nanoliposomes (uncoated and coated) was evaluated. As shown in Figure 3. Chitosan coated liposomes provide more than 3-folds higher mucin adsorption than uncoated liposomes. Although in comparison between two chitosan-coated formulations, this parameter was not significant (p >0.05).

DISCUSSION

Loratadine is a second-generation long lasting antihistamine. It is lipophilic and belongs to the class II BCS that means has low solubility and high permeability.[18] In the current study, different formulations of uncoated and coated loratadine loaded liposome were developed and evaluated for intranasal administration. Results showed thin film hydration method was a successful method for preparation of the liposomes. Submicron-sized vesicles with acceptable stability and high encapsulation efficiency were obtained for all the formulations. The Coating of liposomes by chitosan resulted in significant increase in the size by a coating layer.

The interaction between chitosan and liposomes could be due to a combination of adsorption coagulation and bridging between them. Hydrogen bonding between the chitosan and the phospholipid head groups as well as hydrophobic attraction of hydrophobic segments of chitosan and soybean phosphatidylcholine was also reported.[19, 20] Chitosan coated liposomes improved their mucoadhesivness more than 3-fold and represent a significant potential for overcoming the nasal drug delivery limits of short residence time, and mucociliary clearance. Mucoadhesion of chitosan-based delivery systems are mostly result of ionic interactions between the cationic primary amino groups of chitosan and the anionic substructures of the mucus. In addition, the hydrophobic interactions might contribute to its mucoadhesive properties.[21]

To study component interactions and thermal events, differential scanning calorimetry was performed. The DSC thermogram of loratadine showed an endothermic peak at 136°C related to melting point of the crystals. The thermogram of cholesterol first displayed a shallow endothermic peak at 46.30°C and an endothermic sharp peak at 148.56°C due to its melting point. Chitosan has broad and endothermic peak at 54.27°C, which relates to the polymer phase transient from glassy to rubbery. Phosphatidylcholine thermogram has a broad endothermic peak at 131°C that might be due to its physical change. In the thermogram of uncoated and coated liposomes only a broad endothermic peak at 80_100°C due to water evaporation is appeared.[22] Disappearance of the components peak can be related to the interaction of the liposome ingredients to form liposome's bilayer and appropriate encapsulation of loratadine inside the lipid bilayer.[23] Our findings are consistent with literature reports e.g. Alshweiat et. al. investigated the nasal delivery of loratadine in a nanosuspension form and their DSC thermograms depict a single endothermic peak at 135°C for loratadine and the formulated loratadine showed a reduced intensity and shifted peak toward a lower melting point of loratadine.[24] In another study, Singh et. al. investigated the nasal delivery of mucoadhesive in situ gel of loratadine and they interpreted

the disappearance of characteristic endothermic peak of loratadine in their formulation as inclusion of loratadine into their formulated preparation and only a broad peak for water loss was detectable for their complex.[25]

Chitosan coating of liposomes affected on the drug release rate from liposomes at different time intervals. Figure 2 clearly shows that the percentage of drug release from coated liposomes was lower in comparison with uncoated one at all examined time intervals. Chitosan coating stabilizes the liposomes membrane by adhering to the surface and making a coat layer that acts as a barrier to drug release from the surface. Data were analyzed using different fitting models of controlled release mechanisms and it was concluded that the models of controlled release mechanisms for liposomes coated with chitosan are in agreement with the release behavior of uncoated one.[20, 26]

Stability studies showed in the case of coated liposomes, little changes in the size and PDI were observed. Therefore, coating in addition to improve mucoadhesivness of the liposomes can improve their shelf life as well.

By considering the insignificant effects of high concentration of chitosan coating on the mucoadhesivness of the loratadine loaded liposomes as well as negative effects of high concentration of chitosan on particle size and PDI of the formulations, liposomes with lower coating percentages (0.05%) was selected as the optimum formulation intended to be used for the treatment of allergic rhinitis. In vivo studies would be performed in order to confirm the efficacy of selected preparation.

CONCLUSION

The Chitosan-coated liposomes, which were formulated in this study, is a beneficial delivery system for intranasal administration of loratadine with suitable release profile and improved mucoadhesiveness. Therefore, further *in vivo* studies would be done in future.

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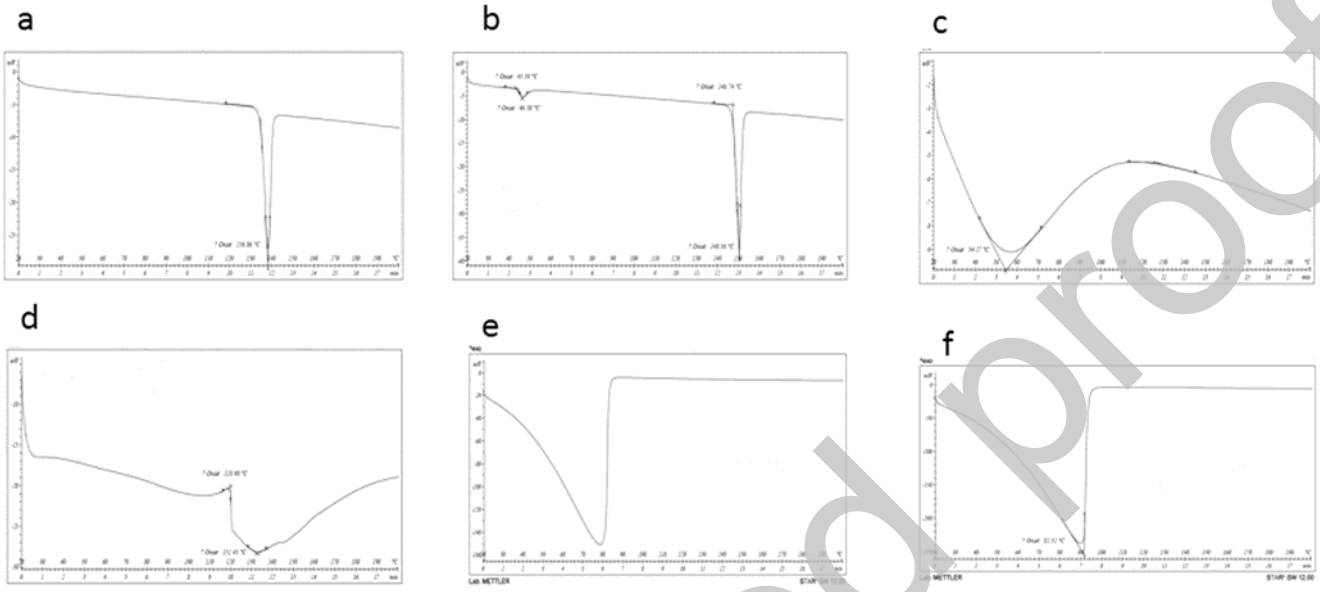

Figure 1. Differential Scanning Calorimetry of a) Loratadine, b) cholesterol, c) chitosan. d) phosphatidylcholine, e) uncoated and f) coated liposomes.

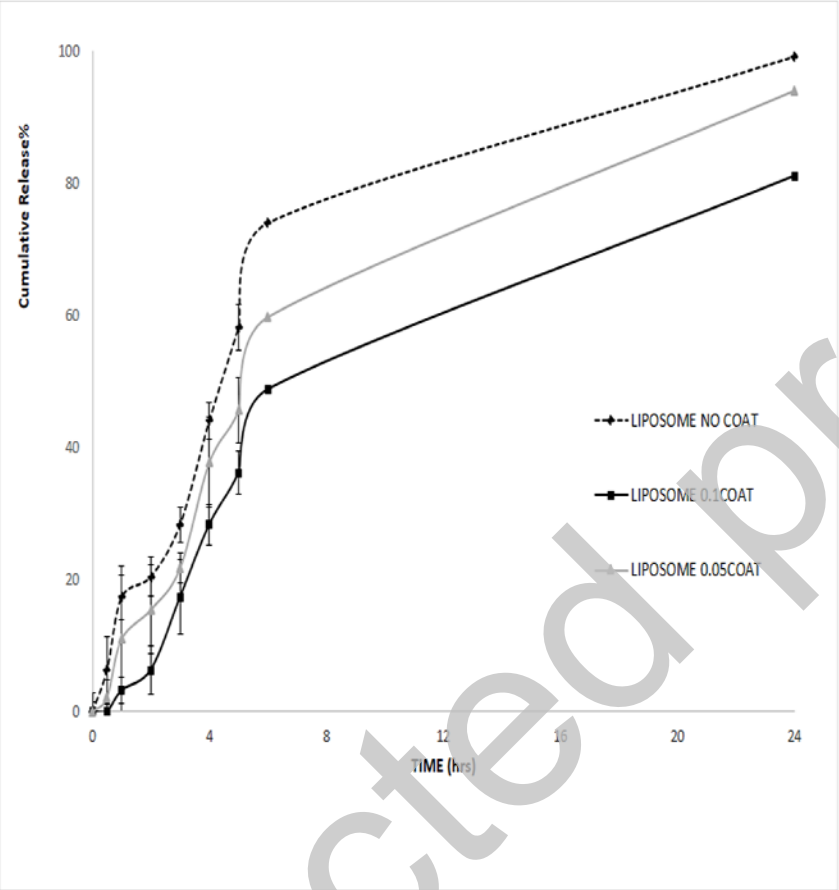

Figure 2. In vitro cumulative percent drug release vs. time (mean±SD, n=3).

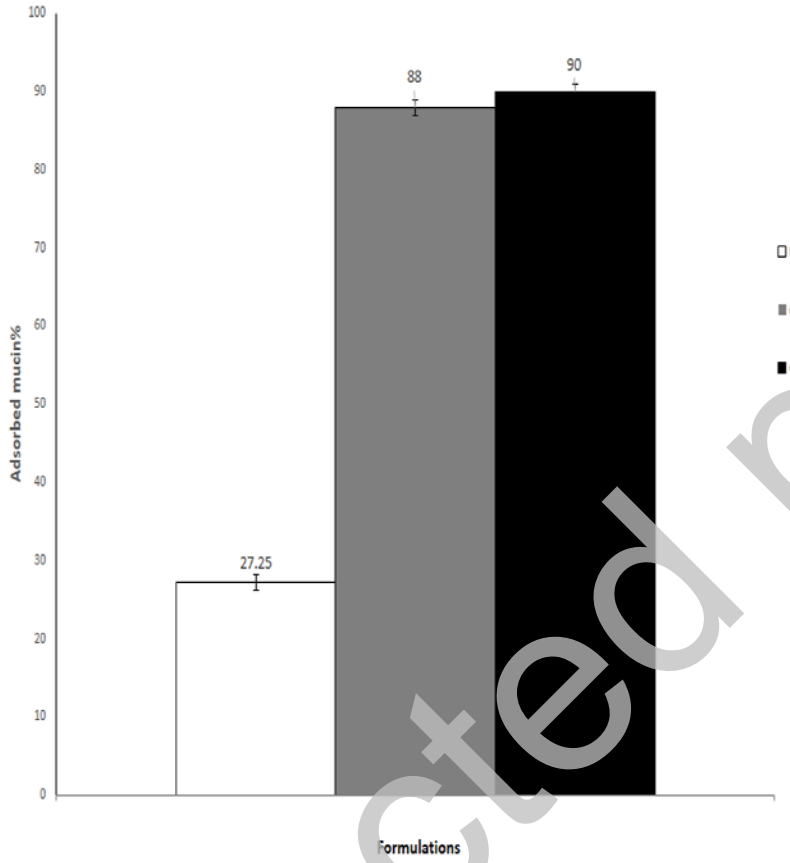

Figure 3. Mucoadhesive capacity of formulations (expressed as percentage of mucin adsorbed, mean±SD, n=3)

Table 1. Characteristics of formulations (Mean ± SD, n=3)

Formulations	Encapsulation efficiency (%)	Particle size(nm)	PDI
Un-coated liposomes	83±4.3	193±3.3	0.41±0.05
0.05% chitosan coated liposomes	78±4.6	345±4.6	0.54±0.08
0.1% chitosan coated liposomes	81±3.9	438±7.3	0.69±0.03

Table 2. Drug release Kinetics of formulations

		Liposomal Formulations		
Kinetic model	**Parameters**	**Un-coated**	**0.05%chitosan coated**	**0.1% chitosan coated**
Zero order	R^2	0.977	0.994	0.98
	K_0 (mg h^{-1})	1.3994	1.1771	1.1398
Higuchi	R^2	0.8785	0.9113	0.9212
	K_H($mgcm^2h^{-1}$)	3.4836	3.0972	3.5243
First order	R^2	0.9069	0.8326	0.9384
	K_1 (h^{-1})	-0.3905	0.4496	0.5556
Korsmeyer-Peppas	R^2	0.9566	0.976	0.9798
	K	1.546	1.0787	1.6027
	N	0.9061	1.0997	1.6027

Table 3. Characteristics of formulations after 3 months storage at 4°C (Mean ± SD, n=3)

Formulations	Encapsulation efficiency (%)	Particle size(nm)	PDI
Un-coated liposomes	49±6.6	426±6.7	0.65±0.06
0.05% chitosan coated liposomes	71±3.8	360±4.7	0.59±0.09
0.1% chitosan coated liposomes	72±8.1	450±2.7	0.73±0.07