Acta Pharm. Sci. Vol 63:(3), 2025
Original article
DOI: 10.23893/1307-2080.APS6333

A novel multi-layer mucoadhesive buccal film containing liposomal Sumatriptan: Development and *in vitro* drug release kinetics evaluation

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ABSTRACT

This study introduces a new three-layered buccal film for the controlled drug delivery of Sumatriptan. Sumatriptan was loaded in cationic liposomes and embedded in a hyaluronic acid film. This film was sandwiched between a mucoadhesive layer of carbopol® 934P/HPMC K4M and an ethylcellulose backing layer. The systems' characteristics were evaluated, including thickness, weight uniformity, swellability, mucoadhesive strength, etc. Also, the in vitro release kinetics of Sumatriptan were assessed using DDSolver software. The nanoliposomes showed a spherical shape with average size, zeta potential, and entrapment efficiency of 138.3 ± 3.99 nm, 17.3 ± 2.7 mv and 75% ± 4.16, respectively. The final system exhibited a suitable mucoadhesive strength (1225 Pascal) by altering the swelling and disintegration of layers, the backing layer facilitated the unilateral drug release toward the mucus, resulting in prolonged drug release. About 90% of Sumatriptan was released within 24 h through predominant diffusion and polymer relaxation mechanisms.

Keywords: buccal drug delivery, drug release, liposomal Sumatriptan, mucoadhesive film

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(Received 8 Apr 2024, Accepted 24 Sept 2024)

INTRODUCTION

Oral drug delivery is one of the most preferred medicine administration routes; however, it must be formulated in a way to overcome absorption restrictions. There are advantageous strategies that assist with this. Among them are oral mucoadhesive films, which enhance the chance of drug absorption through adhesion to the oral cavity mucosa and increase the drug residence time in the absorption site[1]. They enable extended or rapid drug release for local or systemic activities[2-4]. These dosage forms can also be single- or multi-layered to fulfill diverse medicinal purposes. Besides, they help the drugs to bypass the hepatic first-pass effect or prevent them from degradation by enzymatic activity and severe pH changes in the gastrointestinal tract, which leads to increased bioavailability of susceptible medications[5,6]. The ease of drug administration and improved patient compliance are their other benefits[7,8]. The limited surface area in the mouth, the continuous washing by saliva, and the low penetration of hydrophilic molecules into biological membranes are among the main challenges with oral mucoadhesive systems. So, increasing the loading capacity of the drug into the dosage form, providing enough adhesion strength, and exploiting penetration-enhancing strategies must be considered.

The restricted surface area in the mouth, the continuous washing by saliva, and the low penetration of hydrophilic molecules into biological membranes are among the main challenges with oral mucoadhesive systems. So, increasing the loading capacity of the drug into the dosage form, providing enough adhesion strength, and exploiting penetration-enhancing strategies must be considered.

Generally, mucoadhesive polymers have a hydrophile nature with multiple polar functional groups that interact with mucus components through physical entanglements and/or secondary chemical bonds, creating weak mutual networks. These interactions maintain long-term contact between the formulation and the oral mucosa. For example, the carboxyl and sulfate functional groups have shown high mucoadhesive performance because they can make hydrogen bonds with mucin oligosaccharide chains[9,10].

Polyacrylic acid (PAA) is a well-known mucoadhesive polymer containing carboxylic groups. Polycarbophil and carbopol are PAA derivatives used as mucoadhesive platforms for drug delivery. They also have advantages in sustained-release drug delivery systems due to their ability to form a good gel[9-11].

Hydroxypropyl methylcellulose (HPMC) is a polysaccharide polymer. The high presence of –OR groups in HPMC plays a significant role in mucoadhesive

strength through hydrogen bond interactions. Many studies have demonstrated that HPMC-containing films had appropriate mucoadhesive and adhesion times on oral membranes while also displaying the desired release of drugs at the appropriate time[10,12,13].

Hyaluronic acid (HA) is a natural anionic polymer made of repeating units of glucuronic acid and N-acetylglucosamine. Due to its high biocompatibility and low immunogenicity, it has gained significant attention in the pharmaceutical industry, particularly in film systems, over the last few years. HA is an excellent choice for oral drug administration because of its significant adhesive properties, which enable the loaded drug to be delivered in a continuous pattern[14-16].

When systemic drug absorption is required guiding the drug molecules toward the mucosa will increase the therapeutic yield[17,18]. For example, by placing a backing layer, the bilateral medication release can be limited to unilaterally toward the mucosal membrane[19]. Ethylcellulose (EC) is a water-insoluble derivative of cellulose; having hydrophobicity, moderate flexibility, and drug impermeability, EC can be employed as a polymer for constructing the backing layer in bioadhesive formulations to ensure unidirectional drug release[20,21].

Nanoparticulate vehicles are shown to assist drug penetration through mucous membranes. They can be tailor-made to offer benefits such as excellent cellular crossing, deep tissue penetration, and sustained drug release[4,22,23]. These colloidal systems, such as micelles, liposomes, nanoemulsions, and polymeric nanoparticles, can alter the drugs' distribution in the body, increasing their effectiveness and decreasing their toxicity[24].

Among nanoparticles, liposomes (LPs) have received significant attention for their ability to carry both lipophilic and hydrophilic drugs[25,26] and their easy crossing through the cell membranes due to their similarity to biological membranes[27]. By incorporating liposomes in mucoadhesive films, the advantages of both can be taken; i.e., mucoadhesive buccal films extend retention time and modify the drug release profile, and liposomes enclose the drug and improve their release and permeability[3,4].

Different mechanisms and rates are involved in drug release which can affect the absorbed amounts of drug per time unit and duration of therapeutic effect. The release assessment is among the main tests that must be done for a newly designed product to ensure its quality. Generally, a constant and extended release is desired, while varied release rates make systemic concentration predictions difficult.

In this study, a three-layer mucoadhesive system was designed, synthesized, and characterized. It includes the carbopol® 934P-HPMC mucoadhesive layer, the hyaluronic acid middle layer containing Sumatriptan nanoliposomes, and the ethylcellulose impermeable backing layer. The three layers were separately synthesized and characterized in appearance, morphology, thickness, surface pH, mucoadhesion, folding endurance, swellability, film disintegration time, and content uniformity. Finally, the *in vitro* release mechanism of the Sumatriptan from the final three-layer formulation was evaluated by fitting the data to different kinetics models using DDSolver software.

METHODOLOGY

Material

Carbopol® 934P and ethylcellulose (EC) were gained from Sigma, Germany. Hydroxypropyl methylcellulose (HPMC) K4M was acquired from Alfa Aesar, United Kingdom. Hyaluronic acid (HA), octadecyl amine (stearyl amine), propylene glycol, glycerol, acid citric, chloroform, acetone, methanol, and agar were purchased from Merck, Germany. Tehran Chemie Pharmaceutical Co., Tehran, Iran, provided Sumatriptan. Other reagents and chemicals were of the analytical grade.

Preparation of nanoliposomes

For preparing liposomes, the thin film hydration method was used. Briefly, lipophilic compounds, namely phosphatidylcholine (17 or 25 mg), stearyl amine (2 or 7 mg), and cholesterol (3 or 13 mg), were dissolved in a 4 mL solvent mixture of 3:1 chloroform: methanol and delivered to a round bottom flask. To form a thin lipid film on the flask wall, the organic solvent was evaporated in a rotary evaporator (IKA® RV 10 basic/digital) under the condition of 58°C, 100 rpm, and slow declining pressure from 470 to 50 mbar to completely remove all the solvent[28,29]. The dried lipid layer was then hydrated by a 4 mL aqueous phase (phosphate-buffered saline, PBS, pH=7.4) containing Sumatriptan at different concentrations of 0 (blank), 0.1, 0.55, 1, or 1.25% w/v for one hour under slowly rotating in the rotary evaporator (temperature 58°C; 50 rpm; with opened vacuum screw to reach the ambient pressure). Subsequently, this dispersion was sonicated in an ultrasonic bath for 10 minutes to become homogeneous. It was then kept at room temperature for about one hour to allow intermolecular forces to form and strengthen the liposome membrane. For further size reduction, the liposomal solution was sonicated for a 10-second cycle by an ultrasonic probe (120 W) and freeze-thawed for at least ten cycles. Afterward, the liposome dispersion was centrifuged (15,000 rpm for 1.5 hours

at four °C); the supernatant was used in the next steps to determine the encapsulation efficiency and drug loading, and the precipitate (nanoliposomes) was washed three times by distilled water and collected freshly for formulation preparation.

Based on the liposomes evaluation, the formulation consisting of 17 mg phosphatidylcholine, 7 mg stearyl amine, 13 mg cholesterol, and 0.55% w/v Sumatriptan was chosen.

Characterization of nanoliposomes

Liopsome size, PDI, and zeta potential studies

The surface charge, hydrodynamic diameter, and polydispersity index (PDI) of drug-loaded nanoliposomes were assessed upon investigation with a zeta sizer (DLS; HORIBA Scientific SZ-100, CA, USA).

Evaluation of the liposome morphology

Nanoliposome morphology and size were evaluated using scanning electron microscopy (FE-SEM, TESCAN MIRA3, and the Czech Republic).

Entrapment efficiency (EE%) and drug loading (DL%) measurement

The supernatant obtained from nanoliposome centrifugation was subjected to the determination of the unloaded drug. The concentration of Sumatriptan was measured using spectrophotometry at a wavelength of 227 nm. The calibration curve for Sumatriptan was acquired by plotting the absorbance against the different drug concentrations in artificial saliva (pH=6.8).

Entrapment efficiency (EE%) and drug loading (DL%) were calculated using equations 1 and 2, respectively:

$$EE\,(\%) = \frac{W_t - W_f}{W_t} \times 100 \quad \text{(Equation 1)}$$

$$DL\,(\%) = \frac{W_t - W_f}{W_s} \times 100 \quad \text{(Equation 2)}$$

Where W_t, W_f, and W_s are the initial amount of drug, the amount of unloaded drug measured in the supernatant, and the total amount of the nanoliposomal system, respectively.

Stability evaluation of liposomes

The stability of prepared liposomes was examined at three temperatures of -20, 4, and 25 for three weeks. Briefly, liposomes, with or without (blank) drug, were kept in a freezer (-20), refrigerator (4), and room temperature (25) for a defined duration, namely three weeks, and the amounts of Sumatriptan that were released in this period measured. Then the percentage of the drug remaining in liposomes was statistically compared between these three groups to find out the effect of storage temperature on premature drug release.

Preparation of multi-layer mucoadhesive film

The designed final dosage form consisted of three layers: a mucoadhesive layer, a layer containing drug-loaded nanoliposomes, and an impermeable backing layer. Each layer was prepared using the solvent casting and then attached to construct the final system.

The mucoadhesive layer was prepared according to the following steps. First, carbopol® 934P (0.5, 1.5, or 3% w/v) and HPMC K4M (0.5, 1, or 1.5% w/v) polymers were dispersed in 7 mL distilled water. The mixture was stirred until it became a perfectly homogeneous solution. Propylene glycol (300 µL) and glycerol (200 µL) with a ratio of 3:2 v/v were then added to the solution as plasticizers. The resulting solution was held stationary until all of its bubbles had disappeared, then it was poured into a glass Petri dish with a diameter of 7 cm and dried for 24 hours in a 40°C oven[30]. Based on the produced mucoadhesive film's physical appearance, elasticity, and homogeneity, the optimal concentrations of 1.5% w/v and 0.5% w/v were ultimately chosen for carbopol® 934P and HPMC, respectively.

The middle layer was constructed by homogeneously dispersing various concentrations of HA polymer (1.5 or 2% w/v) in 7 mL of distilled water, followed by adding Sumatriptan-containing nanoliposomes (containing an equivalent of 16.5 mg of Sumatriptan) and 300 µL propylene glycol and 200 µL glycerol. The bubble-free solution was then poured on top of the formed mucoadhesive layer and dried entirely for 24 hours in an oven set at 40°C.

HA in a 1.5% w/v concentration was chosen based on superior physical characteristics, flexibility, and consistency.

In order to create the EC impenetrable layer, different amounts of the polymer (1.5, 3, or 5% w/v) were dissolved in 7 mL of acetone, and the polymer solution was supplemented with 300 µL castor oil and 100 µL propylene glycol, as plasticizers; it was then poured onto the previously prepared two-layer and then

dried in the oven for 24 hours. The EC concentration greatly influences the creation of a uniform impermeable barrier and the homogeneity of the final formulation. It was determined that the 3% w/v EC was the best concentration based on its physical characteristics, flexibility, and uniformity compared to other concentrations.

Characterization of mucoadhesive system

The physical appearance of the film's layers

The appearance of films was evaluated visually for having a smooth surface and flexibility and being free of bubbles and wrinkles.

Weight and thickness uniformity

As layers must be uniform throughout, they undergo weight and thickness uniformity. A digital scale (Mettler Toledo, ME303, Switzerland) was used to weigh at least three different pieces of each film with an area of one cm^2; the mean weight SD was then calculated.

A digital micrometer was used to measure the thickness of three separated pieces, one in the center and two in the corners, of a film with a one cm^2 surface area.

Folding endurance

The film's folding strength was measured by manually folding a one cm^2 piece several times and counting the number of folds until cracking happened.

Surface pH

An agar plate (1% w/v of agar in artificial saliva, pH=6.8, as solvent) was used to measure the film's surface pH.

The artificial saliva consisted of 1.2 g of potassium chloride, 0.85 g of sodium chloride, 0.05 g of magnesium chloride, 0.13 g of calcium chloride, and 0.13 g of di-potassium hydrogen orthophosphate in one liter of distilled water; the final pH was set to 6.8[31].

Agar powder was dissolved in the artificial saliva at 100°C; it was then put onto a petri dish and allowed to cool down and gelled. Then, one cm^2 piece of the film was placed on the agar gel's surface, and after 10 minutes, the pH was determined by placing the pH indicator paper on the swollen film's surface.

Disintegration time

The films were placed in a beaker containing 10 mL of artificial saliva (pH=6.8) and shaken at a rate of 400 rpm at 37°C. The disintegration time was verified visually when the film started to fragment.

Swellability study

The degree of swelling of the films was also determined in the agar plate (1% w/v). The initial weight (W_1) of one cm² piece of film was first measured. The agar plate's surface was moistened with artificial saliva, and the samples were then placed on it. The excess water was then removed from the surface of the films with filter paper after ten minutes, and the weight of the swollen films was measured (W_2). The swelling percentage of the films was calculated using Equation 3:

$$\text{Swelling}(\%) = \frac{W_2 - W_1}{W_1} \times 100 \qquad \text{(Equation 3)}$$

In vitro mucoadhesive strength study

The mucoadhesion strength was tested for the final three-layered film and the mucoadhesive layer alone. The examination was handled using a self-built instrument (Figure 1)[32]. Sodium alginate (10% w/v) gel was employed as a mucosal model. As illustrated in Figure 1, a piece of thread with appropriate length was affixed to the surface of carbopol® 934P -HPMC film (or, in the case of three layers, to the backing layer), and the other end was attached to a light plastic cup (container). This connection was at a perpendicular angle using a glass roller connector. The film's surface was moistened with artificial saliva, gently adhered to the gel, and then allowed to set for two minutes. After releasing the container from the lab jack stage, water was slowly added at a constant rate until the film separated from the sodium alginate gel's surface. The weight of the water-containing container was determined, and the film's mucoadhesive strength was calculated using Equation 4:

$$\text{Mucoadhesive strength}\left(N/cm^2\right) = \frac{W\,(Kg) \times g\left(m/s^2\right)}{A\left(cm^2\right)} \qquad \text{(Equation 4)}$$

Where the W is the weight of the container plus the weight of water, g is the acceleration of gravity (9.8 m/s²) and A is the surface area of the films.

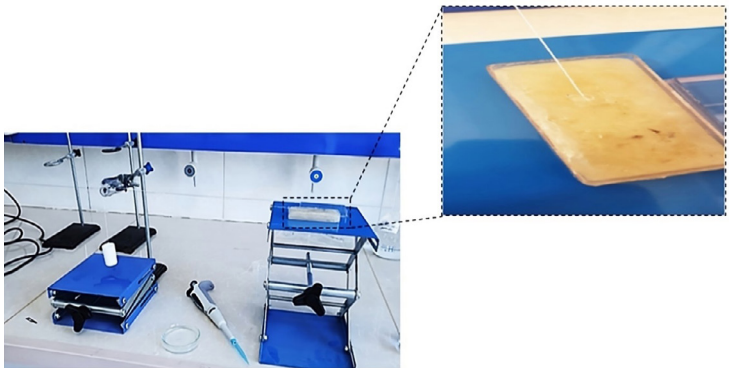

Figure 1. The apparatus designated for measuring mucoadhesive strength. Sodium alginate gel (10% W/V) was molded into a rectangular plate and moistened with artificial saliva. The film was then adhered to sodium alginate and connected to a thread. A lightweight container is fastened to the thread at a perpendicular angle. The container is then gradually filled with water until such a point that the film becomes detached. Ultimately, the weight of the container and water is measured to determine mucoadhesive strength.

In vitro drug release evaluation

The drug release test was studied using a two-sided modified Franz cell and dialysis bag (cut off=12 kDa). The apparatus consisted of two identical chambers between which the holding area is located (Figure 2). A piece of film with an area equal to the cross-sectional area of the sample hold area (4.5 cm^2 containing an equivalent of 1.9 mg of Sumatriptan) was sandwiched between two dialysis bags with the same dimensions. Each chamber was fully filled with 10 mL of artificial saliva. The device was placed in a shaker incubator and shaken at 100 rpm at 37°C. During 48 h in defined intervals, 500 µL of the release medium was withdrawn from both chambers and replaced with the same amount of artificial saliva. The released Sumatriptan amounts at each time point were plotted graphically to find the release behavior of the drug.

The final formulation without the drug was used as a blank sample to measure Sumatriptan concentration more accurately.

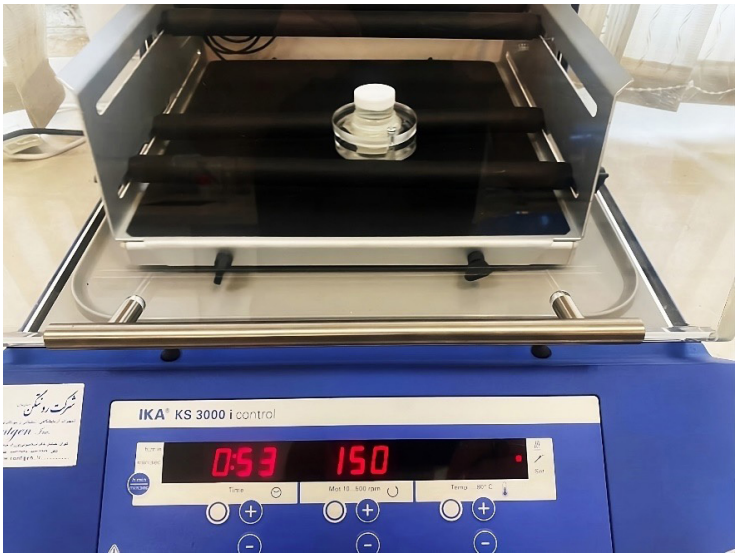

Figure 2. The modified Franz cell instrument for drug-release test

The mechanism of drug release

Sumatriptan release kinetics was analyzed by fitting the release data into different kinetic models: zero-order, first-order, and Higuchi. Other models, namely Makoid-Banakar, Korsmeyer-Peppas, Weibull, and Peppas-Sahlin, were also employed to find the involved mechanisms in Sumatriptan release. The untransformed release results were analyzed using DDSolver (v 1.0; an Excel add-in software). The best model was selected based on the highest adjusted and model selection criterion (MSC) and the lowest Akaike information criterion (AIC).

Statistical analysis

All experiments were repeated thrice, and the findings were presented as mean ± SD. The ANOVA was used to compare groups, with p-values≤0.05 indicating statistically significant differences. SPSS software (SPSS 16.0 Version) was utilized for Statistical analysis.

RESULTS and DISCUSSION

Characterization of Sumatriptan-loaded nanoliposomes

Sumatriptan-loaded nanoliposomes were characterized based on their morphology (Figure 3), size, polydispersity index (PDI), zeta potential, drug loading (DL%), and entrapment efficiency (EE%) (Table 1).

Table 1. Characteristics of the optimized Sumatriptan-loaded nanoliposomes

Characteristic	Hydrodynamic size ± SD	PDI ± SD	Zeta potential	DL* (%) ± SD	EE* (%) ± SD
Value	138.3 ± 3.99	0.34 ± 0.01	17.3 ± 2.7	33% ± 1.83	75% ± 4.16

*DL and EE stand for drug loading and entrapment efficiency, respectively.

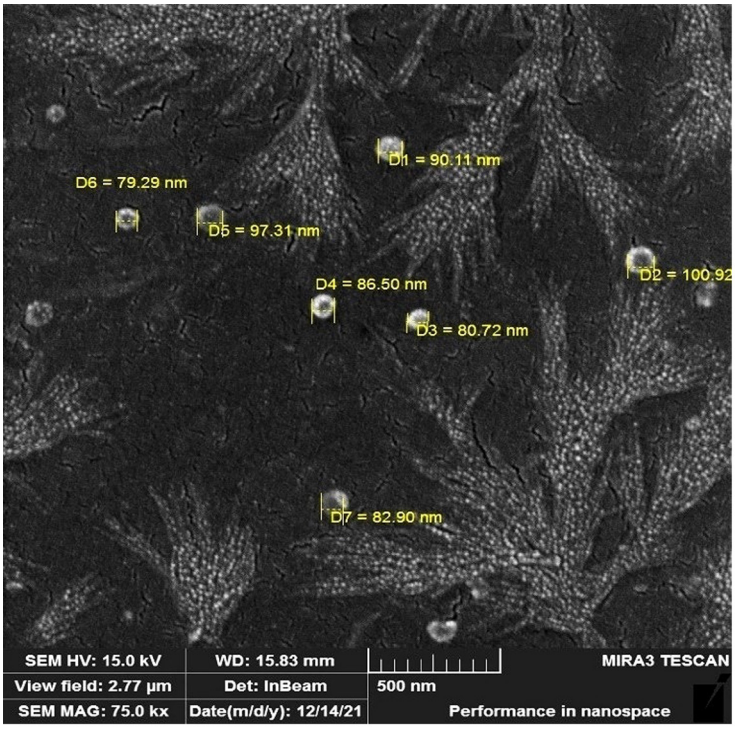

Figure 3. SEM image of Sumatriptan-loaded nanoliposomes

The Sumatriptan calibration curve (Figure 4) was used to measure its concentration.

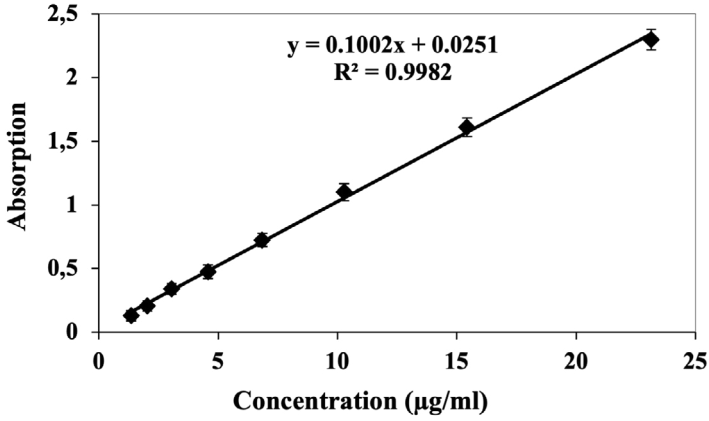

Figure 4. Calibration curve of Sumatriptan in artificial saliva (pH=6.8)

Data from the dynamic light scattering (DLS) method showed that the liposomal Sumatriptan had an average particle size (z-average) ± SD of 138.3 ± 3.99. The nanoliposome's observed size and morphology under the scanning electron microscope (SEM) photograph (Figure 3) displayed the particle's average size was 88.25 nm with spherical shapes. The difference between the DLS and SEM results is due to the presence of the double layer of charge on the surface of nanoliposomes formed in the aqueous medium, i.e., the hydrodynamic diameter is determined through DLS, whereas the projected area diameter can be seen in SEM image[1].

The value of PDI is an index for determining nanoliposome size uniformity. This parameter is important because, in nanometre size, the behavior of particles profoundly depends on their size and effective surface; in this sense, a wide PDI reduces the control over the drug release rate and penetration[19]. The PDI of 0.34 ± 0.01 is acceptable for our Sumatriptan-loaded nanoliposome. This size uniformity can also be seen in SEM images. Therefore, the release of Sumatriptan from all particles is expected to be uniform.

The vesicle charge is an important parameter in drug permeability. Generally, positively charged compounds interact more with the biosurfaces compared to neutral or negatively charged ones. For instance, it was shown that coating phosphatidylcholine and cholesterol-based liposomes with chitosan can enhance Sumatriptan nasal absorption[33,34].

The optimum entrapment efficiency (EE%) and drug loading (DL%) were 75% and 33%, respectively. The key parameters in nanoliposome synthesis are the water-to-oil phase ratio, temperature, drug concentration, the ratio of the different types of lipids, and other manufacturing process conditions[29]. In the

case of water-soluble drugs, high EE% depends on the ability of liposomes to entrap them during the vesicle formation. Actually, the hydrophilic drugs can enter the interior compartment of the liposome along with the aqueous phase, so this compartment's volume plays an important role in imported medicine. For example, single-wall liposomes have more water core volume than multilamellar vesicles of the same size. It was shown that lamellarity can also affect Sumatriptan EE% and unilamellar liposome was favored[29,35]. The freeze-thawing can reduce the number of walls and lead to an increased internal volume.

For increasing the EE%, the temperature comes to the transition temperature of amphiphiles during liposome manufacturing. This elevated temperature gives energy to lipids and disrupts their interactions, so lets more drugs enter. However, the temperature must be decreased again then.

On the other hand, the liposome must be able to confine and keep the entrapped drug. The drug's log p, the interactions between liposome membrane components (lipid constituents), and the production temperature are of important parameters for drug retention inside the vesicle[36]. Drugs with affinity to both lipid and water can easily escape the liposome, while drugs with little partitioning to the lipid membrane can retain more. The lamellae packing depends on the interaction of its components. Based on the therapeutic goal, the type and ratio of the constituents must be chosen. For instance, cholesterol can increase the lipoid molecules' interactions and rigidify the membrane, reducing the bilayer permeability and hence drug scaping. However flexible vesicles are favorable when it comes to penetrating the biological membrane. So, the cholesterol content must be optimized.

The interaction of the enclosed drug with liposome constituents is also important. By changing the membrane components, the affinity and distribution of the drug would also be changed. For example, it was shown that depending on the liposome charge, Sumatriptan EE% varies. Compared to neutral or negatively charged liposomes, the positively charged liposome containing stearylamine (SA) had the highest EE%. Albeit the charge density is important, by increasing the SA concentration, the repulsive forces can reduce the membrane packing and reduce the EE%. Sumatriptan is mainly protonated in neutral pH and below, which can electrostatically interact with anionic compounds like the phosphate group in phosphatidylcholine or diacetyl phosphate (DCP). It can also form hydrophobic bonds with the tails of phospholipids. The interactions can cause the drug to be both in the core and bilayer membrane; the presence of the drug in the membrane can perturb the bilayer and lower the EE%. Their results also confirmed that positively charged liposomes had smaller sizes, narrower size distribution, and more stability[29,35].

Stability of liposomes at different temperatures

The percentage of drug remaining in liposomes after three weeks of storage in -20, 4, and 25 is presented in Figure 5. As is evident in Figure 5 in three groups more than 90% of the drug remained in liposomes.

Statistical evaluation (using GraphPad Prism® 9 software) revealed that there are no significant differences between these three groups; in other words, liposomes can be stably stored in these three temperatures.

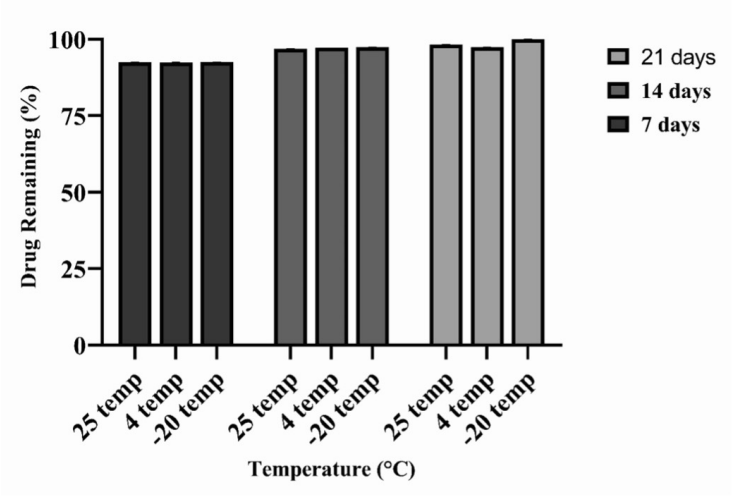

Figure 5. The percentage of Sumatriptan remaining in liposomes after three storage at -20, 4 and 25

Characterization of the film layers

The weight, thickness, folding endurance, surface pH, swelling, and disintegration time were evaluated for the mucoadhesive system; Table 2 shows the physical characteristics of its three layers.

Table 2. Physical characteristics of the three layers and final formulation

Layer	Weight (mg)	Thickness (μm)	Folding endurance	Surface pH	Swellability (%)	Disintegration time (min)	Mucoadhesion strength (N/cm^2)
Mucoadhesive	18.42 ± 2.07	23.09 ± 1.47	No cracks seen	5	192.42 ± 4.80	38.25 ± 2.2	1225 ± 40
Nanoliposome-loaded hyaluronic acid	14.33 ± 0.82	60.21 ± 2.5	No cracks seen	6	203.05 ± 31.2	60 ± 1.46	-
Impermeable	15.26 ± 0.57	19.66 ± 0.47	No cracks seen	7	0	Not disintegrated	
Final formulation	45.37 ± 0.58	99.67 ± 4.73	No cracks seen	7	-	-	1274 ± 80.01

According to the results, all three layers of the optimized formulation have desirable physical features. The required dose should be placed in the intended dimensions; to reduce the sensation of foreign bodies on the oral mucosa and to enhance patient compliance, oral mucoadhesive films should be flexible and have appropriate thickness. Therefore, it is necessary to determine the thickness of the mucoadhesive film. The final thickness of the synthesized mucoadhesive film was 99.67 ± 4.73 μm. The mucoadhesive film prepared in our study has less than 100 μm thickness which is in the range of patient acceptance[5].

The oral mucoadhesive film must have strong strength and endurance against the mechanical stresses in the mouth. The folding endurance test in this investigation demonstrated the appropriate films' flexibility.

Having a similar pH to the oral environment is another characteristic of an ideal mucoadhesive film[7,8,37]. The surface pHs of the films were between 5 and 7, which is within the range of saliva's pH (5.6 to 7.4) and is compatible with the buccal mucosa with no irritation or mucosal damage.

The swellability of the layers also has a favorable value. As EC is an insoluble polymer, it cannot absorb water to its chains and swell; it cannot be disintegrated in an aqueous medium. Our data is consistent with this; no signs of degradation were seen, even after three days of testing, nor did this layer swell. These features of EC help this layer prevent the release of the drug in the oral environment, and the whole drug diffusion is toward the mucosa[2]. The swelling of the mucoadhesive layer shows the tendency of polymers to absorb water; this is required because by drawing water to their strands, polymers can extend in the aqueous medium and expose their functional groups for binding with the mucin. In addition, the water can easily diffuse into hydrophilic polymers, dissolve the drug, and facilitate its release out of the system. Although exces-

sive water absorption might cause the loosening of the film attachment and wash it away with water, so highly hydrophilic polymers are not ideal for being mucoadhesive[3,4]. The strength of the mucoadhesion has a significant effect on the retention of the system on the mucus. The mucoadhesive strength should be sufficient without damaging the mucosal membrane. Using a modified balancing method, the mucoadhesive strength of the three-layered mucoadhesive film and the carbopol® 934P -HPMC layer were studied separately and compared. Based on the findings, the mucoadhesion was strong enough. Besides, no statistically significant difference was seen between the single-layer carbopol® 934P-HPMC and the final three-layer system mucoadhesive strength. Therefore, adding HA and EC layers to the carbopol® 934P -HPMC mucoadhesive layer did not affect its mucoadhesive strength (Table 1).

Sumatriptan release from the three-layered mucoadhesive film

The release of Sumatriptan from the final multi-layer system in artificial saliva (pH=6.8) was followed up for 48 hours. Both the film matrix and the liposome features could affect the release. As a carrier, the liposome can aid the bioabsorption of the drug molecules. The drug-containing liposomes can interact with the mucus and resist washing by saliva which can enhance the chance of the drug, alone or encapsulated in the liposome, to cross biological membranes[36]. So, the drug should remain inside the vesicle when it reaches the mucosa.

Generally, extended drug release is preferred by patients because the frequency of dose uptake is reduced. However, for this issue, zero-order release with a constant rate is preferential. The Sumatriptan release data demonstrate a biphasic behavior in which 67% of the drug was released during the first four hours, followed by a sustained release phase. As shown in Figure 6, approximately 90% of the drug is released from the system within 24 h and reaches the maximum (100%) after 48 h. It means the drug release rate is fast at first and reduced gradually. The fast release of surface-bound drugs and those in the bilayer membrane can be the reasons for the biphasic nature of the release[38]. The rate constant (k) determines the maximum cumulative drug release time. It is governed by the drug permeability across the liposome, which itself relies upon the liposome constituents and drug physicochemical features.

In this study, the drug did not release from the EC layer side, which indicates the optimal performance of this layer as an impermeable back layer of the film.

Based on the disintegration results, the carbopol® 934P -HPMC and HA layers degradation are almost fast, however, the backing layer can change the disin-

tegration behavior of the final film and pronounce the roll of film matrices in drug release as well.

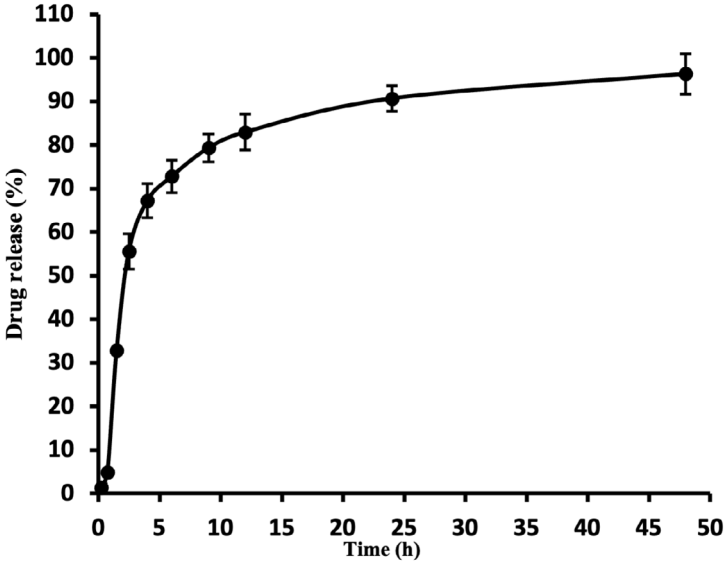

Figure 6. *In vitro* release profile of Sumatriptan from the three-layered mucoadhesive film

Data was further fitted in various kinetics models to better understand the release mechanism.

Drug release kinetics

As drugs must reach the desired place in an appropriate and predictable amount, realizing the drug release mechanism and rate for each newly designed drug delivery system is necessary. A well-defined behavior of drug release kinetics is necessary for drug dosing. Drug release depends on the system's characteristics and environmental conditions, like temperature, pH, contents, etc. So, for the in vitro study of drug release, the experiment conditions must be strictly selected in a way to be similar to the real situation in the body.

System parameters, like the dosage form geometry, the accessible surface for drug release, uniformity of the drug distribution in the entire system, interactions between ingredients, and the way the dosage form is introduced to the body, all contribute to drug release behaviour[39]. In polymeric matrices, polymer microstructure, and crystallinity, swelling potency, polymer chain relaxation, polymer chain disentanglement (especially in non-cross-linked polymers), and polymer dissolution can control drug release.

Generally, diffusion, erosion, and swelling, alone or in combination, are the main drug release mechanisms. For example, in hydrophilic matrices, swelling is supposed to play a role in drug transport, and the water content of the system can affect drug diffusivity. Still, other factors must also be considered. For instance, the presence of a backing layer in mucoadhesive films can prevent water diffusion into the system, hamper the swelling of the matrix, and cause a unidirectional release of the article through a diffusion mechanism. In this case, the swelling and polymer dissolution contribution may be less substantial.

In our study, Sumatriptan is loaded in the liposome, and this nanoparticle is embedded in a hydrophilic matrix, i.e., HA film. Therefore, the drug must pass through the liposome bilayer and polymer gel to be released. A backing layer was also applied to make the molecules' movements unilateral toward the mucous.

To find the kinetics of drug release, data were graphically fitted in zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, Figure 7. The best kinetic model was determined based on the coefficient of determination (R^2).

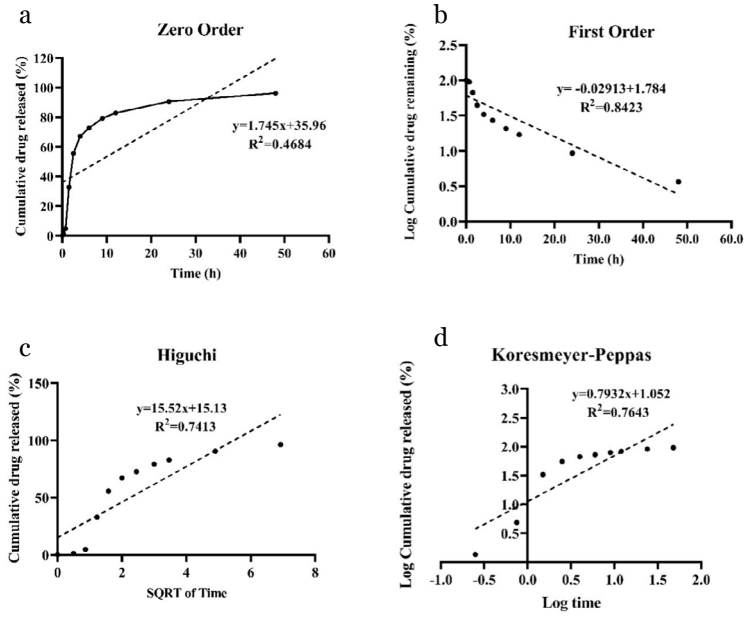

Figure 7. The Sumatriptan release data fitted in (a) Zero-order, (b) Higuchi, (c) First-order, and (d) Korsmeyer-Peppas kinetic models; GraphPad Prism is used for data graphing.

Zero-order, first-order, and Higuchi are related to diffusion and Fickian release. If other mechanisms are involved, other models, like Korsmeyer-Peppas and Weibull, can reveal them. Generally, Higuchi governs the molecule release from polymeric insoluble matrices where the dimensions of the polymer remain constant, for example, in non-eroding polymeric films. The penetrated medium into the matrix can dissolve the embedded drug hence Fickian drug release happens.

As mentioned, polymer chain glassy/rubbery transitions, relaxation, disentanglement, polymer dissolution, and surface erosion can also happen.

When a membrane (like a liposome bilayer) is in the way of molecule movement, the zero- or first-order is possible, depending on the molecule concentration. Zero-order rate is commonly seen in depot systems, where the amount is beyond release ability and the concentration gradient remains constant. In the first-order phenomenon, the rate depends on the concentration gradient; so the rate would be reduced as the drug is released over time.

In our designed system, all of these phenomena are likely to occur, which must be evaluated.

The best kinetic model was chosen based on the highest R^2 values, determined by the regression. According to Figure 7, R^2 had the highest value in the first-order model in which the rate is concentration gradient-dependent.

If the polymeric film does not limit the drug release and the release of Sumatriptan from the nanoliposome is the rate-limiting step, then it can be concluded that the drug concentration decreases inside the nanocarrier with time; consequently, the rate of drug release from the system decreases. Indeed, the R^2 is not satisfactorily large, i.e., less than 0.9. It can be concluded that the simple Fickian diffusion does not lonely govern the drug release, and probably the HA matrix also collaborates in drug release.

DDSolver (v 1.0; an Excel add-in software) software was used to analyze the untransformed data. Zero-order, first-order, Higuchi, Makoid-Banakar, Korsmeyer-Peppas, Weibull, and Peppas-Sahlin models were considered. The best model was selected based on the highest adjusted R^2 and model selection criterion (MSC), and lowest Akaike information criterion (AIC). As shown in Table 3, among the diffusion-based models, drug release behavior is based on the first-order mechanism (adjusted R^2=0.95, AIC=72.69, and MSC=2.76), which is in accordance with the graphical result.

Table 3. Sumatriptan release evaluation by fitting release data to mathematical kinetics models; the model parameters are calculated using DDSolver to find out the goodness of fit.

Model	Equation*	Rate constant	Model parameters
Zero Order	$F = k_0 \times t$ $D_t = D_0 + k_0 t$	$k_0 = 2.972$	R^2_adj = -0.22 AIC = 108.98 MSC = -0.61
First-Order	$F = 100\ (1-e^{-k\_1})$ $\log C = \log C_0 - k_1\ t/2.303$	$k_1 = 0.234$	R^2_adj = 0.95 AIC = 72.69 MSC = 2.68
Higuchi	$F = k\_{H} \times t^{\wedge 1/2}$	$k_H = 19.287$	R^2_adj = 0.66 AIC = 94.58 MSC = 0.69

*k_o, k_1 and k_H are zero-order, first-order, and Higuchi rate constants, respectively; t is the time; F is the fraction of the drug that is released at the time t; D_t is the amount of the drug that is released at the time t; D_o is the initial amount of the drug; C is the drug concentration at the time t and C_o is the initial concentration of the drug in the medium.

Among the models for finding the underlying release mechanism, Table 4, the Weibull model showed the best data fitting (R^2_adj=0.95, AIC=73.69, and MSC=2.59). Since the shape parameter (β) in the Weibull model is 0.864, which is between 0.75 and one, it indicates the release rate is reduced over time, and a combined mechanism, swelling along with diffusion, plays a role in drug release[40,41]. The presence of the backing layer and the mucoadhesive layer can delay the fairly fast disintegration time of the HA film and reinforce its effect on drug release. This finding was also confirmed by n=0.56 in the Makoid-Banakar model (R^2_adj=0.91). The n=0.563 (between 0.5 and one) implies the anomalous mechanism.

Table 4. The used mechanism models for Sumatriptan release evaluation; DDSolver calculated the model parameters to find out the goodness of fit.

Model	Equation*	Rate constant	Other parameters	Model parameters
Makoid-Banakar	$F = k_{MB}\, t^n e^{-kt}$	$k_{MB} = 27.719$	n= 0.563 k= 0.021	R^2_adj = 0.91 AIC = 81.06 MSC = 1.92
Korsmeyer-Peppas	$F = k_{KP}\, t^n$	$k_{KP} = 34.787$	n= 0.303	R^2_adj = 0.76 AIC = 80.84 MSC = 1.18
Weibull	$F = 100\ (1-e^{-\frac{t^\beta}{\alpha}})$		α=3.655 β=0.864	R^2_adj = 0.95 AIC = 73.69 MSC = 2.59
Peppas-Sahlin	$F = k_1\, t^m + k_2 t^{2m}$	$k_1 = 30.741$ $k_2 = -2.313$	m= 0.558	R^2_adj = 0.91 AIC = 81.5 MSC = 1.88

*k_{MB}, k_{KP}, are Makoid-Banakar, and Korsmeyer-Peppas rate constants, respectively; k_1 and k_2 are Peppas-Sahlin constants; t is the time; F is the fraction of the drug that is released at the time t; n is exponent power; m is Fickian diffusional coefficient; α is the scale factor, and β is the shape factor.

The data did not satisfactorily fit in the Korsmeyer-Peppas (R^2_adj=0.76).

Peppas-Sahlin (R^2_adj=0.91) approximates the contribution of diffusion and polymer relaxation in an anomalous drug release. The model equation consists of two terms: $F = k_1t^m + k_2t^m$; the m exponent in this equation is the Fickian diffusion exponent. The contribution of diffusion (F) and polymer relaxation (R) can be calculated using the model equation or $\frac{R}{F} = \frac{k_2 t^m}{k_1}$. For our system, the k_1 and k_2 coefficients were 30.741 and -2.313, respectively, and m was equal to 0.558[42]. Figure 8 shows the contribution of each mechanism over time; the diffusion is prominent in the early hours and reduced to 60% at the end of drug release. As approximately 80% of the drug is released during nine hours, the involvement of diffusion at this time is still about 80%.

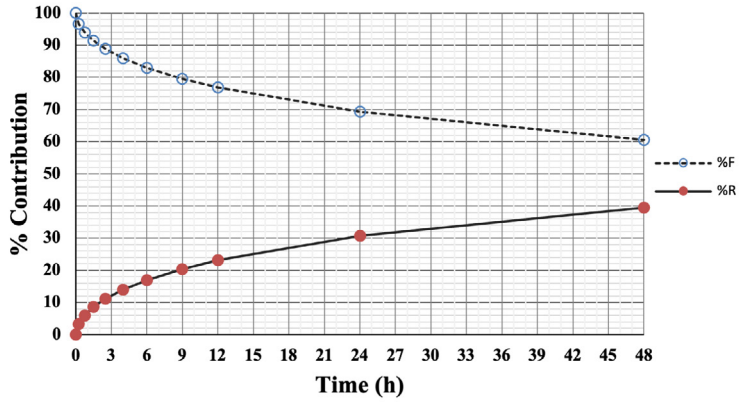

Figure 8. The % contribution of diffusion and polymer relaxation in drug release, based on the Peppas-Sahlin model.

The correlation between the observed amount of Sumatriptan released and the anticipated amounts by different kinetics models are shown in Figure 9 which is in line with mathematical analysis data.

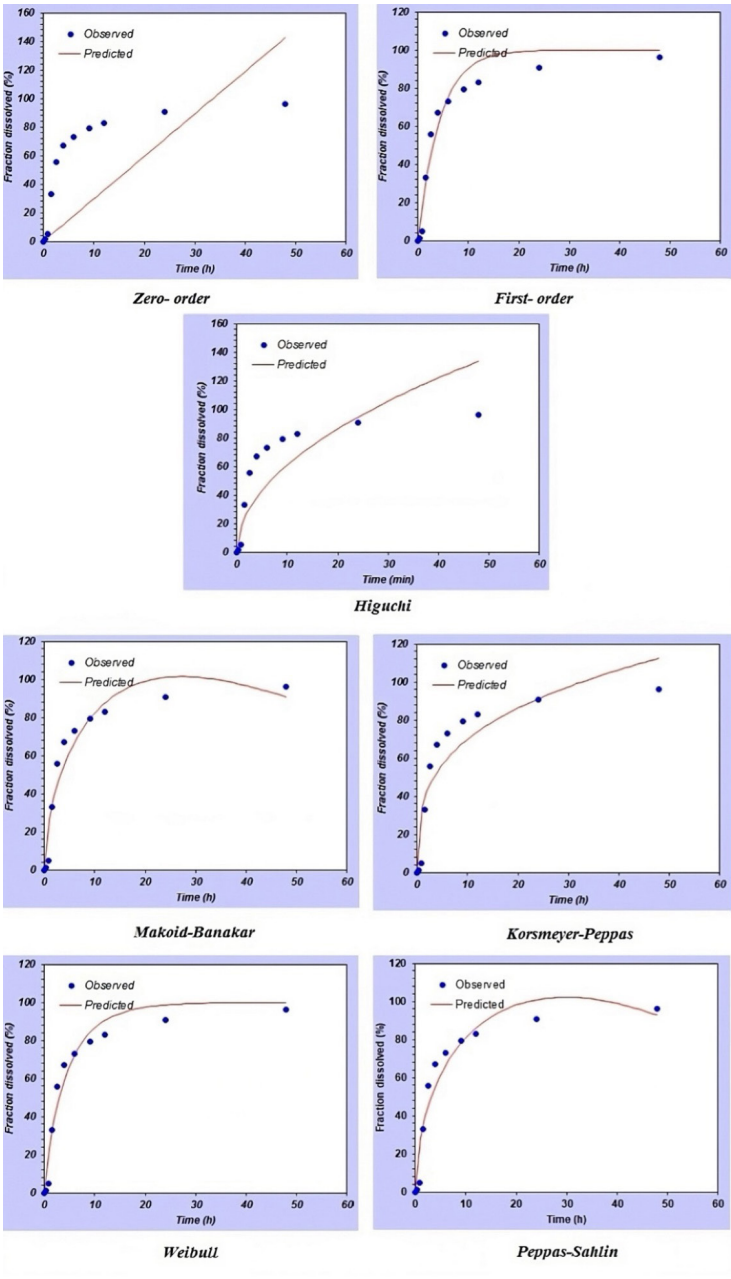

Figure 9. The correlation between the observed amount of Sumatriptan released, and the anticipated amount is based on different kinetics models.

From all these observations, it can be deduced that both the film and liposome can control the drug transport; probably drug was released from the liposome by diffusion (possibly by first-order mechanism), and film swelling at the next step let the drug leach out. As the role of diffusion is more predominant, exiting from liposome is the rate-limiting step. It is preferred the drug does not leave the liposome readily; this is confirmed by extended drug release, which is more dependent on the drug leaving the liposome.

It must be noticed that the liposomes with unreleased drugs can also penetrate biomembranes, while in our release test, the dialysis film was in the way of liposomes, and only the released drugs were evaluated.

In this study, a three-layered mucoadhesive buccal film for the delivery of Sumatriptan was designed and characterized. A positively charged liposome was used as the drug carrier as it can interact with mucus and enhance drug permeation. The system showed a good mucoadhesive feature which is helpful to keep the drug in contact with the mucus and give it enough time to be absorbed. The backing layer made the drug transport unilateral and also affected drug release behavior. The results showed that a predominant diffusion and polymer relaxation mechanisms are involved in drug release which is mainly dependent on the drug diffusion through the liposome bilayer (by first-order mechanism) rather than being controlled by the polymeric matrices. In other words, the system can help keep the drug sufficiently in contact with the mucosa, whereas liposomes can control the drug release and aid its penetration. As the zero-order mechanism has the advantage of releasing drugs at a constant rate, changing the first-order to zero-order can be achieved by increasing the drug loading in liposomes and turning them into drug depots.

As our system showed desirable features, it can also be used for other hydrophilic drugs. Although ex vivo and in vivo studies, like cytotoxicity, mucoadhesion using animal models, and drug penetration through epithelial cells, need to be performed to empower the data, which are now underway in our laboratory.

STATEMENT OF ETHICS

This study has received the Ethics Code of IR.LUMS.REC.1400.147 from the Ethics Committee of Lorestan University of Medical Sciences on September 9, 2021.

CONFLICT OF INTEREST STATEMENT

The authors confirm that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: NT; Methodology: MHA; Formal analysis and investigation: MHA, NA; Writing - original draft preparation: ZA; Writing - review and editing: NT; Supervision: NT.

FUNDING SOURCES

No funding was received for this study.

ACKNOWLEDGMENTS

The authors declare that no acknowledgments are applicable for this work.

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