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Running head: *In vitro* and *ex vivo* Optimization of Lisinopril pellets

Optimization and Evaluation of Lisinopril Mucoadhesive Sustained Release Matrix Pellets: *In-Vitro and Ex-Vivo* Studies

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Abstract

Lisinopril (LIS) it is antihypertensive drug, classified as a class III drug with a high water solubility and low permeability. To overcome the low permeability, 3^2 factorial designs aimed to formulate LIS as a sustained-release (LIS-SR) matrix pellet by extrusion/spheronization. Matrix pellets were composed of wet mass containing Avicel® and polymeric matrix polymers (sodium alginate (SA) and chitosan (CS)). Evaluation the effect of two independent variables, matrix-forming units (SA and CS) on mean line torque, on pellet size, dissolution rate after 6 h, and mucoadhesion strength of the pellets were assessed using Statgraphics software. The tested formulations (F1-F9) showed that mean line torque ranged from 1.583 – 0.461 Nm, with LIS content in the LIS-SR pellets ranged from 87.9-103%, sizes varied from 1906-1404 μm and high percentages of drug released from pellets formulations (68.48 to 74.18 %), while the mean zeta potential value of mucoadhesive range from -17.5 to -22.9 mV.

The selection of optimized formula must have the following desirability: maximum peak torque, maximum pellets' particle size, and minimum % LIS release after 6hr. LIS optimized sustained release pellet formula composed of 2,159 % SA and 0.357 % CS was chosen as optimized formula. It's showed a 1.055 Nm mean line torque was responsible for the increased pellet size to 1830.8 μm with decreased release rate 56.2 % after 6 hr, and -20.33 mV average mucin zeta potential.

Ex-vivo mucoadhesion studies revealed that that the optimize formula, exhibited excellent mucoadhesive properties, after 1 h, about 73% of the pellets were still attached to the mucus membrane. Additionally, *ex-vivo* permeation determination of LIS from the optimized LIS-SR formulation was found to be significantly higher (1.7-folds) as compared to free LIS.

In conclusion: LIS-SR matrix pellets, prepared with an extrusion/spheronization have desirable excellent characteristics *in-vitro and ex-vivo* sustained-release pellet formulation of LIS-SR was able to sustain the release of LIS for up to 8 h.

Keywords:

Lisinopril; Oral delivery; pellets; extrusion/spheronization; *in vitro* and *ex vivo* characterization

1. Introduction

Two general categories can be used to classify oral sustained release dose forms: single (Tablets or capsules),and multiparticulate (beads, granular and pellets) dosage forms (Sivalingan, GNK et al. 2020). Administration of oral dose forms of LIS may have a limited bioavailability of around 25%, although it is highly water soluble, but has low permeability, characterized by a high inter-subject variability (6–60%) and slow absorption (Fernandez-Carballido, Barcia et al. 2014; Palepu and Therapeutics 2019). Different formulation approaches such as formulation of a tablets (Ijaz, Qureshi et al. 2015; Ahmad, Jamshaid et al. 2020; Tandon, Jangra et al. 2021), microspheres (Sudha 2012; Shelake, Mhetre et al. 2018), and nanoparticles (Varshosaz and Soheili 2008; Fernandez-Carballido, Barcia et al.

2014) have been established to maintain the LIS released orally. Unfortunately, there is no study on the use of the traditional palletization technique for the development of LIS that has been published in the literature to produce a mucoadhesive sustained release LIS matrix pellets as oral delivery system.

Designing sustained release dosage formulations primarily aims to change the normal behavior of drug molecule in a physiological environment and maintain drug plasma concentrations over extended periods of time, which reduces dosage intervals and side effects that are connected to the dose (Nasiri, Yousuf et al. 2016; Garg and Mishra 2021). Coating and matrix technologies are the two methods most frequently used when developing sustained release dosage forms. Sustained release matrix systems are typically simpler to produce (Vergote, Vervaet et al. 2001). The drug is contained within a carrier substance (polymers, sodium alginate (SA), and chitosan (CS)) in these matrix systems. The matrix's physicochemical composition impacts the drug's underlying release mechanisms, release rates, and resulting release patterns (Gandhi, Kaul et al. 1999; Siepmann, Muschert et al. 2006). To promote sustained release, two different types of matrix systems—hydrophilic and hydrophobic—are frequently utilized (Tapia, Buckton et al. 1993).

Pellets can be produced by blending fine powders with a binder solution. Regarding therapeutic and formulation benefits, the multi-particulate structure of pellets provide various advantages over single-unit dose forms. To produce sustained release matrix pellets, a variety of methods have been used, including spay drying, spay congealing, fluidized bed technology, rotary spheronization, rotary shaker pelletization, layer building method and extrusion/spheronization techniques (Ibrahim and Alanazi 2012). The most well-known technique for developing spherical pellets is extrusion and spheronization, which is a simple, quick, and best procedure that can be easily scaled up.

The focus in matrix pellets for sustained drug delivery strategies has been increasing exponentially. This is because of its efficient dispersion in the gastric mucosa; they increase drug absorption, decrease potential side effects, reduce peak plasma fluctuations, and achieve this without lowering drug bioavailability. Matrix pellets are being produced using extrusion and spheronization as such technique.

Indeed, several research articles investigated the formulation of LIS as mucoadhesive tablets to improve its gastric residence time, and hence enhance the drug intestinal permeation, which would result in improving its oral bioavailability (Abdelbary 2003). Other articles investigated formulation of Lisinopril as mucoadhesive microspheres (Radhika 2011; Singh 2013). It is worth mentioning that the previous studies did not discuss the formulation of LIS-SR matrix pellets containing mucoadhesive agents incorporated in enteric coated capsules using statistical design.

In the present study, LIS-SR matrix pellets containing mucoadhesive agents (sodium alginate and chitosan) will be formulated based on statistical design to optimize the pellet formulations. In addition, the pellet formulation was protected from gastric release by incorporation inside enteric coated capsules to force the drug release in intestinal medium only. Development and evaluation of oral LIS-SR mucoadhesive pellets were the goals of this investigation. The pellets were manufactured based on using a 3^2 factorial design, and utilizing variable factors, including sodium alginate (X1) and chitosan as binder solution (X2) concentrations. The manufactured pellets were optimized, and evaluated for their mean torque line in Nm (Y1), average particle size (Y2), *in vitro* drug release after 6 hours (Y3), and *in-vitro* drug mucoadhesion (Y4). The optimized pellet formula was loaded

in enteric-coated hard capsules to study the *in-vitro* drug release in the intestinal medium. Additionally, the *ex-vivo* mucoadhesion and permeation studies were done also.

2. Materials and methods

2.1. Materials

LIS was obtained from Aljazeera Pharmaceutical Industry (Riyadh, Saudi Arabia). Sodium alginate (SA) was obtained from Fluka Chemie (Buchs, Switzerland). Chitosan (CS) low molecular weight Poly (D-glucosamine) deacetylated chitin was obtained from Sigma Aldrich (St. Louis, MO, USA). Microcrystalline cellulose (MCC; Avicel® PH101) was purchased from Serva Feinbiochemica (Heidelberg, Germany). Mucin of bovine submaxillary glands was obtained from Sigma Aldrich (St. Louis, MO, USA). Hydroxypropyl methylcellulose (HPMC) enteric-coated capsules were purchased from DR T&T health (Corby, UK). The rest of the supplies and solvents were of the reagent or analytical grade, didn't need to be further purified, and were utilized exactly as they were delivered.

2.2. Preparation of Lisinopril sustained release mucoadhesive matrix pellets

Pellets of the LIS-SR mucoadhesive matrix were produced utilizing the extrusion/spheronization procedure. Two factors three levels (3^2) full factorial design was utilized in this study, independent variables; SA (X1) and CS solution (X2), were examined for their effects on the properties of LIS-SR mucoadhesive matrix pellets. Statistical analysis was conducted using the Statgraphics software (version 17.2.02.; Statgraphics Centurion). Table 1, showed the production of pellets containing SA in three different concentrations (0.5, 1.75, and 3%) in addition to different CS solutions concentrations (0.2, 0.6, and 1%), which dissolved in 1% acetic acid. Avicel® PH 101, was added as a pellet excipient and 5% w/w of the drug (LIS). The highest torque value recorded by the mixed torque rheometer served as the basis for choosing the binder ratio necessary for wet massing. In accordance with the binder ratio estimated from mixer torque rheometry (MTR) studies, the powder combination was moistened with the binder solution. The resulting wet mass was extruded through a 2.0 mm-pored screen at a speed of 90 rpm. The generated extrudates were then spheronized using a spheronizer that had a rotating plate with a regular cross-hatch shape. at a speed of 700 rpm, for 10 min. Pellets were then dried on a tray in a hot oven at 50–60 °C for 4.0 h (Abou Obaid, Al-Jenoobi et al. 2020).

Table 1. Variables in three level full factorial design and composition of LIS matrix pellet formulations.

2.3. Mixer torque rheometry (MTR) for wet mass characterization:

As a pre-formulation step, MTR was used to measure the rheological properties of wet masses before extrusion/spheronization procedures. The MTR used in this investigation is made up of a stainless steel bowl with a 135 mL capacity and two mixing blades with rotating speeds ranging from 20 to 150 rpm. A sample of 15–30 g of dry powder material is sufficient to cover the mixer blades, depending on the bulk density. With the aid of a torque arm connected from the mixer's main body to a calibrated load transducer, the torque was directly measured on the mixer bowl. The mixer is set to run at 50 rpm. On a personal computer, the data acquisition system and software package provided by the equipment vendor were used to complete the data acquisition and processing.15 g of solid pellet excipients (LIS, SA, and Avicel; Table 1) were combined in the turbula mixer for 5 minutes before being added to the MTR bowl. Throughout the course of seven wet massing periods, five milliliters of binder solution were applied in various amounts, and vortexed for 1.0 min., data logging (collecting) period made up each wet massing interval. Throughout the wet massing procedure, the mean line torque, Nm, a measure of wet mass consistency, was taken (Ibrahim, Zayed et al. 2019; Ibrahim and Alshora 2021).

2.4. HPLC analysis

Chromatographic separation was achieved by modified method of Dawud et al., 2019 (Dawud and Shakya 2019), using a suitable validated reversed phase-HPLC method. It was carried out using a Waters ™ high performance liquid chromatography system (HPLC) with a variable UV absorbance detector and an autosampler. Optimum separation of LIS was achieved by utilizing a Hypersil-Gold C18 column (150, 4.6 mm, 5 µm particle size, Thermo Fisher Scientific, USA), Using an optimized mobile phase made up of water and methanol (7:3) at 25 °C, the pH was adjusted to 3 ± 0.02 using orthophosphoric acid. The solvent system flow rate was 1.2 mL/min, the injection volume was 20 µl and the analysis was carried out at λ max of 205 nm. Drug content, *in-vitro* drug release, and *ex-vivo* permeability characteristics were assessed using this technique.

2.5. Drug content

The drug content in the produced pellets (F1-F9) as well as the optimized pellet formula was assessed in triplicates by utilizing high performance liquid chromatography. A specific weight (25 mg) of the pellets formula was grinded, and suspended in 50 ml solution of methanol-phosphate buffer mixture (pH 7.4) in a ratio 1:4. After being sonicated for 20 minutes, the dispersion was filtered using a cellulose nitrate filter with a 0.22 m pore size (Germany). The LIS content of the filtrate was then determined by HPLC at 205 nm.

2.6. Measurement of pellet size

The size of manufactured pellets was determined using a micrometer (Mittotuyo Micrometer, NSK Co., Japan) and computed as the average value of 10 pellets. As well as pellets size was confirmed from the SEM studies.

2.7. *In-vitro* drug release studies

Dissolution test, equipment-1(Erweka DT 700, Heusenstamm, Germany) was used to accomplish the *in-vitro* dissolving of LIS-SR from the pellet formulations in accordance with the USP dissolution basket technique. A calculated amount of the pellets equivalent to 20 mg LIS (400 mg formulation pellets) was placed into the basket immersed in 500 mL of phosphate buffer (pH 7.4). The dissolving media was rotated at a speed of 50 rpm while maintaining a temperature of 37± 0.5 °C. Five mL samples were taken out at intervals of 0.25, 0.5, 1, 2, 3, 4 and 6 hours, and then filtered through a Bulk Acrodisc® 25 mm syringe filter (with a 0.22 m Supor® Membrane, Germany).). The drug concentration will be determined using the previously stated validated HPLC method following the appropriate dilution with mobile phase. To keep the volume consistent, the removed samples were replaced with the same volume of fresh dissolving media. The amount of LIS dissolved from each formulation was calculated by use the average of three measurements.

2.8. *In-vitro* mucoadhesion

The *in-vitro* mucoadhesion potential of the pellets was determined by their mucin binding efficiency. Average of the mucin zeta potential of the LIS-SR matrix pellets was evaluated using Malvern Zetasizer (Nano ZS90, Malvern instruments). Bovine mucin powder was suspended in a phosphate buffer (pH 7.4) in a concentration of 1% w/v. Fifty mg of pellets was added to 3.0 ml of the mucin suspension and mixed by vortexing for 1 min at room temperature. The zeta potential of the mucin suspension containing the formulation was measured after incubation for 2 h using laser light diffraction and compared to zeta potential of pure mucin suspension. The samples were placed in polystyrene cuvette at 25°C after dilution (1:100) with deionized water and the readings were measured at fixed angle (Piao, Lee et al. 2009; Cheng, Cui et al. 2021). Every measurement was made three times.

2.9. Optimization and characterization of the optimized pellet formula

The software statistical program was used to find the optimal LIS-SR matrix pellet formula based on the following criteria: maximum peak torque, maximum pellet particle size, minimum percentage of LIS release after 6 hours, and maximum mucoadhesion.

2.9.1. Scanning electron microscopy (SEM)

Using scanning electron microscopy (SEM), the morphological properties of LIS-SR matrix pellets were studied. A gold sputter module in a high vacuum evaporator was used with samples were sputter-coated with a thin gold palladium layer while being exposed to an argon environment. After that, photomicrographs were taken.

2.9.2. Differential scanning calorimetry (DSC)

DSC analysis was used to determine the thermal characteristics of LIS, SA, CS, and the chosen optimal formulation. The 3 mg dried powder samples will be precisely weighed and put into conventional aluminum pans before being hermetically sealed. As a guide, an empty pan will be utilized. Samples will be heated from 30 to 300^{o} C at a rate of 10 °C/min while being surrounded by a stream of nitrogen gas.

2.9.3. *In-vitro* release of optimized LIS pellet formula filled in enteric coated capsule

In-vitro release study for LIS-SR from enteric-coated capsules containing the optimized mucoadhesive LIS-SR matrix pellet formula was conducted. Enteric coated capsules were filled with a determined quantity of LIS-SR matrix pellets equal to 20 mg LIS (400 mg pellets), which were then put into a basket that was submerged in 750 mL of 0.1N HCl (pH 1.2) for two hours. After that, 250 mL of 0.2 M trisodium phosphate was added to the released medium to bring the pH level to (pH 6.8). With a rotational speed of 50 rpm and a further 6 hours of testing, the release medium temperature was maintained at 37.0± 0.5 °C. A five mL samples were taken out at intervals of 0.25, 0.5, 1, 2, 3, 4, and 8 hours, and then filtered through a Bulk Acrodisc® 25 mm syringe filter (with a 0.2 m Supor® Membrane, Germany). The drug concentration will be determined using the developed HPLC method following the appropriate dilution with mobile phase. To keep the dissolution medium's volume constant, the removed samples were substituted with an equal volume of fresh dissolution media. The amount of medication dissolved from each formulation was calculated as the mean of three measurements. Additionally, Kinetic modeling of the *in vitro* release of LIS from LIS-SR mucoadhesive matrix pellets will be carried out based on the main kinetic models (the zero-order model, the first-order model, the Higuchi diffusion model).

2.10. *Ex-vivo* studies

2.10.1. Animals

The study employed healthy male New Zealand white rabbits weighing 1.8–2 kg. Water and a regular laboratory meal were given to the animals, who were kept at a temperature of 25 ± 2 °C and a relative humidity of 55 ± 2%. In King Saud University's college of pharmacy, experiments were conducted using the center's laboratory animals (Riyadh, Saudi Arabia). The study's protocol was authorized for use with animals by the King Saud University Ethics Committee (IRB approval number SE-19-153).).

2.10.2. *Ex-vivo* mucoadhesion

The experiment was performed utilizing a modified wash off test method (Martins, de Oliveira et al. 2017) where the *ex-vivo* evaluation of the mucoadhesive properties of the optimized LIS pellets was performed and compared with the nonpareil sugar seeds. The freshly excised pieces of intestinal mucosa of male New Zealand rabbits (1.8-2 kg) (5 ×1.5 cm) was washed with Krebs solution as a physiological saline and opened longitudinally, attached onto basket of dissolution apparatus type I using a fine cotton thread, in a way that the inner mucus layer of tissue faced the outside (Alhowyan, Altamimi et al. 2019). Briefly, known quantities of the optimized LIS-SR pellet formula, (10 pellets) was put in contact onto each wetted specimen of intestinal tissue (Figure 1), and pressed gently to attach to the mucosal surface. The mucoadhesiveness of the pellets measured by connecting the prepared slide with the gut to the USP dissolution apparatus I (Erweka DT 700, Heusenstamm, Germany). The pellets were forced to wash off under the reciprocating motion of dissolution apparatus with 50 rpm in 500 mL of Krebs buffer solution (pH 6.8).,At regular intervals (2, 5, 15, 30, 45, 60, 75, and 120 min) and the quantity of pellets remained adherent to the tissue was quantified. As previously noted, a control experiment was conducted with nonpareil sugar seed. The number of pellets left in each time interval was used to calculate the percentage of mucoadhesion using the formula in the following equation. (Equation 1):

$$\%\ of\ pellets\ remaining = \frac{remaining\ number\ of\ pellets}{initial\ number\ of\ pellets\ added} * 100 \qquad \text{(Equation 1)}$$

Figure 1. *In-vitro* intestine wash off test, (A) for nonpareil sugar seed, (B) for LIS-matrix pellets; attached to the mucosal intestinal wall.

2.10.3. *Ex-vivo* permeation study

Apparent *ex-vivo* intestinal permeability of LIS powder and optimized LIS-SR matrix pellets was determined using modified gut sac method (Alhowyan, Altamimi et al. 2019). The freshly excised pieces of rabbit intestinal mucosa (7 cm) were washed with Krebs solution as a physiological saline. Each segment was opened by gently pushing a glass rod through the intestine and tied from one side, Figure 2. The intestinal sac was then filled with 2 mL of Krebs solution containing 5 mg of untreated LIS, or 100 mg of the optimized LIS-SR pellet formula (equivalent to 5 mg drug). Thereafter, the other end of

intestinal sac was gently closed with a fine cotton thread, as shown in Figure 2. Each sac was immersed in 10 mL Krebs solution at 37 ± 0.5 °C.

At specified intervals (0.5, 1, 2, 3, 4, 6, 8 and 24 h), one mL sample was removed from the acceptor media and replaced with a fresh Krebs solution. After dilution with methanol at a ratio of 1:1 as previously stated, the concentration of LIS at various time points was measured by HPLC analysis.

The apparent permeability was then determined using the following equation. (P_{app}):

$$P_{app} = \frac{dQ/dt}{A * C_o} \qquad \text{(Equation. 2)}$$

Where: (dQ/dt) is the slope of the change in concentration with the change in time at steady state, A is the area of the tissue and C_0 is the initial concentration.

Figure 2. Intestinal segment before permeation study, (A) and after filling with the tested formulation (B).

3. Results

3.1. HPLC assay for Lisinopril:

The average retention times for LIS peak was 2.3 ± 0.2 min with no interfering with the mobile phase peak as shown in Figure 3, This is an indication the specificity of the HPLC assay method. It should be mentioned that there were no interfering peaks from the pellets ingredients during i*n-vitro* studies co-eluted with LIS peaks which further confirming the specificity of the method. The regression equation was found to be Y = (35305) x + (45280) with correlation coefficient (r^2) of 0.9974, for the peak area ratios of LIS, versus different concentrations (5 to 30 ng/ml) using mobile phase consisting of methanol: water (3:7, pH 3 ± 0.1) at λ_{max} 205 nm.

Table 2, described the precisions either within-run or between-run were done six times a day, or in sex consecutive days, the values were less than 11.4 and 12.3% respectively. While the accuracy was calculated as more than 94.3%, the recovery was demonstrated as 87.3% as in Table 2. These calculations were an indication for validation and sensitivity of the developed LC MS/MS methods for determination of LIS.

Figure 3. HPLC chromatograms of LIS in HCl (chromatogram A), in phosphate buffer (chromatogram B), Krebs solution (chromatogram C) and water: methanol in ratio (7:3).

Table 2. Estimated recoveries, accuracies and precisions for determination of lisinopril at different concentrations (n = 6)

3.2. Drug content

LIS content in the pellets ranged from 87.9-103% of the theoretical claim (Table 3), indicating consistence drug distribution during formulations.

Table 3 Properties of extruded/spheronized LIS mucoadhesive SR matrix pellets. Properties of extruded/spheronized LIS mucoadhesive SR matrix pellets

3.3. Effect of independent parameters on LIS mucoadhesive SR matrix pellets attributes

3.3.1. Effect on pellet wet mass

It is evident from Table 3, Figure 4, that the pellet formula F2, which contains the highest levels of both SA (3%) and CS (1%), recorded the highest value of pellet wet mass mean line torque (1.584 Nm), followed by F4, which contains the highest levels of SA (3%) and medium level of CS (0.6%), in which a mean line torque of 1.524 Nm was observed. When mixed with Avicel®, pellet formula F6 comprised the lowest concentrations of both SA (0.5%) and 0.2% CS solution, and the lowest value of wet mass mean line torque (0.461 Nm) was measured. These findings are consistent with data gathered by a number of researchers (Alshora, Ibrahim et al. 2020), who discovered that increasing the polymer

concentration increased the mean line torque of wet mass when producing pharmaceuticals as sustained-release matrix pellet formulations. The effects of the two independent parameters (SA and CS solution concentration) on the mean line torque of pellet wet mass were investigated, and the data were summarized in a Pareto chart (Figure 5A). The peak torque of the pellets' wet masses is significantly agonistically influenced by SA concentration (p-value = 0.0457). Moreover, the CS binder solution, quadratic effect, quadratic effects of both independent parameters exhibited agonistic, but insignificant effect on pellet wet mass peak torque as the p-value were recorded as 0.6563, 0.628 and 0.949 and 0.653, respectively.

By graphically representing maxima and minima, a response surface plot enables visual observation of the relevance of the regression equation. Figure 6A shows the 3D response surface plot showing the impact of independent parameters on the pellet wet mass peak torque.

Figure 4. Mean line torque of wet masses for tested Lisinopril SR pellets formulations.

Figure 5. Standardized Pareto chart estimating the effect of independent formulation factors on (A); mean line torque value of pellet wet masses, (B); pellet sizes and (C); % Lisinopril release from SR pellets 6h after and (D); mucoadhesion studies.

Figure 6. Response surface plot estimating the effect of SA (X1) and CS solution (X2) on (A); the mean line torque value of pellet wet masses (Y1), (B); pellet sizes and (C); % Lisinopril release from SR pellets 6h after and (D); mucoadhesion studies.

3.3.2. Effect on pellets' sizes (Y2)

The influence of the two independent variables concentrations of SA and CS binder solution on the pellets' particle size (Y_2; μm) are display in Pareto chart in Figure 5B, both SA(X1) and CS (X2) have an agonist effect on pellet size, however the effect of SA (p = 0.0594) is higher than the effect of CS binder solution (p = 0.3115) but their effect is minimal or in insignificant. Meanwhile, a significant antagonistic interactive effect (X1X2) (p = -0.0200) on the pellets' particle size. There was also insignificant antagonistic effect of SA (X1X1) and CS binder solution (X2X2) quadratic effect (p = -0.4821) and (p = -0.7062) on the pellets' particle size (Y_2), respectively. The 3D response surface plot (Figure 6B) is graphically showed that by increasing the concentration of both SA and CS solution, an increase in the pellet sizes was observed.

The mean pellets size range from 1442.20 ± 138 to 1906.80± 194 µm, Table 2. The small SD value indicates a narrow particle size distribution (Ibrahim and Alanazi 2012). For pellets with low concentration of CS binder solution (0.2%), increasing the SA concentration from 0.5% to 1.75% to 3% resulted in pronounced increase in the mean pellets size from 1442.20 to 1655.40 and to 1906.80, for pellet formulations F6, F7 and F8, respectively.

3.3.3. Effect on the *in vitro* LIS release of (Y3)

Table 3, shows the impact of SA (X1) and CS binder solution (X2) on the LIS-SR release after 6 h in% (Y3) of the produced LIS SR pellets. The standardized ANOVA Pareto chart (Figure 5C) demonstrated that the CS binder solution had a discernible, though negligible, antagonistic, influence on the rate of LIS release ($p = 0.5407$). At 6 hours, the interaction impact (X1X2) showed an agonistic influence on the percentage of LIS release, but it was also negligible ($p=0.5252$). While the agonist effects of the quadratic effects (X1X1) and (X2X2) are negligible ($p = 0.9953$ and 0.8680, respectively). The effect of SA and CS binder solution on the percentage of LIS released after 6 hours is also graphically depicted in Figure 6C, and it can be seen that increasing the concentrations of both SA and CS binder solution marginally increased the percentage of LIS released after 6 hours.

The release of untreated drug powder was very fast in which 100% of untreated LIS was released in about 30 min, due to its higher water solubility, 97 mg/ml (DeMarco and Brooks 1992). In contrast, incorporation of LIS in SR pellet formulas resulted in a pronounced retardation of drug release in which only less than 75% % of the loaded LIS was released after 6 h, Table 3. The highest release rate (74.18 % after 6 h) was documented in F3, which was consisted of 0.5% SA and 0.6% CS, while, the slowest release rate (61.89 % after 6 h) was recorded in F9, which was composed of 1.75% SA and 0.6% CS, as shown in Table 3.

Kinetic modeling the *in vitro* release of LIS from SR mucoadhesive matrix pellets

The highest correlation coefficient was used to calculate the release kinetics. The findings demonstrated that the Higuchi model was followed in the release of LIS from the LIS-SR pellet, with the highest correlation coefficient values when compared to both zero and first order kinetics. The computed n values for all matrix pellet formulations were found to be less than 0.45 in Table 4, which further supports the anomalous or non-Fickian drug release from the produced mucoadhesive matrix pellets. Several researchers showed that non-Fickian or irregular diffusion happens when the rates of liquid diffusion and polymer relaxation (erosion) are equivalent (Sinclair and Peppas 1984; Ritger and Peppas 1987; Peppas and Brannon-Peppas 1994).

Table 4. Kinetic analysis of LIS release from various mucoadhesive LIS-SR matrix pellets.

3.3.4. Effect on *in-vitro* pellets' mucoadhesion (Y4):

The *in-vitro* mucoadhesion of different LIS-SR pellet preparations was determined by the interaction with mucin solution and measuring its zeta potential to determine their mucoadhesion strength (Dodero, Alberti et al. 2021; Niculescu and Grumezescu 2022).

ANOVA analysis represented by standardized Pareto chart (Figure 6D), illustrate there was a significant agonistic effect interactive effect (X1X2) between SA and CS solution on the pellets' mucoadhesive properties (p =0.0262). Additionally, the quadratic effect (X2X2) of CS binder solution exhibited an agonistic effect on pellets' mucoadhesive properties, but the difference (p = 0.2700) was negligible. However, there were minor antagonistic quadratic effect of SA (X1X1), and SA (X1) and CS (X2) was as p = -0.4041, -0.7317 and 0.9834, respectively. 3D response surface plot (Figure 6D) indicates no significant influence of both SA and CS solution individually.

The mean zeta potential value of mucin suspension after contacting pellet formulations ranged from -17.5 to -22.9 mV (Table 2), while the measured zeta potential of control mucin suspension was -14.8 ± 0.87 mV. For pellets prepared with high concentration of SA (3%), increasing the CS binder solution concentration from 0.2% to 0.6% to 1% generated a significant decline in the negativity of zeta potential from -22.9 to -19.9 to -18.1, as the case of F8, F4 and F3, respectively, indicating lowering mucoadhesion power.

3.4. Optimization of mucoadhesive SR pellet formulation

The selection of optimized pellet formulation was based on the following desirable criteria (Table 4): maximum mean line torque, maximum particle size, minimum% drug release, and maximum mucoadhesive properties (maximum ZP of mucin solution when stirred with pellets). These parameters together should be synchronized so as to produce matrix pellets of controlled drug release and high mucoadhesion properties. The statistical program used several response optimizations to estimate and advise the composition of the optimal pellet formula based on these parameters and the prior data gathered from the created formulations. The optimized formula was composed of 2.159% SA (X1) and 0.378% CS (X2). The estimated and real values of peak torque, binder ratio, pellets' particle size, and percentage LIS release after six hours of the optimized LIS-SR pellets were shown in Table 5 to be in close agreement with each other. A mean line torque value of 1.055 Nm at a binder ratio of 1.33 mL/g was displayed by the optimized pellet formulation at this optimal level, which was close to the projected value (1.045 Nm) as shown in Figure 7. Moreover, a particle size of 1830.8 205 m as opposed to 1751 m was noted for the optimized formula (predicted). After 6 hours, the medication was released 56.2 5.6% less from the improved pellet formula than was anticipated (67.45%). Moreover, the observed ZP of mucin solution after mixing with optimized pellet formula was -20.33± 1.07mV, which was close to the predicted value of the response (-19.381 mV)).The drug content for the optimized formulation was as 98.47± 0.04 % (n=3).

Table 5. Peak torque, binder ratio, pellet size, and percentage of LIS release after 8 hours for the optimized LIS-SR matrix pellets: predicted and observed values

Figure 7. The composition suggested by the statistical software of the optimize formulation of the LIS-SR matrix pellets.

3.5. Characterization of the optimized mucoadhesive SR matrix pellet formula

3.5.1. *In vitro* release of LIS from enteric coated capsule containing the optimized SR mucoadhesive pellet formulation

A specific weight of the optimized SR mucoadhesive pellet formulation equivalent to 20 mg LIS was filled into enteric coated HPMC hard gelatin capsules to study the drug release in both acidic and alkaline pH media. Figure 8 shows the in vitro release profile of LIS from the optimized pellet formula over two pH values relevant to GIT conditions. It was observed that the drug exhibited a slow release rate from pellet formula at the acidic pH value, in which only 6.07± 1.29% of the drug was released within the first hour, and almost 11.8± 2.95% after two hours. This is due to the slow dissolution of the enteric-coated HPMC capsules containing the pellet formulation that could withstand intact in the acidic condition. Thereafter, the drug exhibited faster but controlled release profile upon changing the medium pH to 7.4 pH due to the dissolution of the enteric coat carrying the optimized pellet formula. The drug showed a release rate of 23.64± 1.37% 15 min after pH change. In addition, the drug exhibited controlled release manner after changing pH during the remainder 6 h, in which 71.56± 0.16% of the loaded LIS was released after 6 h in the intestinal pH medium (7.4). In addition to processing the pellet formula to have good mucoadhesive properties, the controlled release profile of LIS from the optimized pellet formula in the alkaline (simulated intestinal) pH value is taken into consideration as a base for improving drug contact with intestinal wall and enhancing its permeation. In addition to processing the pellet formula to have good mucoadhesive properties, the controlled release profile of LIS from the optimized pellet formula in the alkaline (simulated intestinal) pH value is taken into consideration as a base for improving drug contact with intestinal wall and enhancing its permeation.

Figure 8. Release profile of LIS from enteric-coated capsule containing the optimized pellet formula (F10) in 0.1 N HCL for 2 hours then in (pH 6.8) for 8 h.

3.5.2. Physicochemical characterization of the optimized LIS SR matrix pellet formula:

Pellets' shape and morphology (SEM)

The surface morphological analysis of the optimized LIS-SR matrix pellets is demonstrated in Figure 9. The manufactured pellets were spherical with average size around 1800 μm. In addition, the pellets' surfaces were wrinkled and rough.

Figure 9. Scanning electron micrographs of optimized LIS-SR matrix pellet formulation (A) and scanning electron micrographs of its surface (B).

Differential scanning calorimetry (DSC):

The DSC graph for the LIS showed in Figure 10, which has two endothermic peaks at 70.8-118.4 and 180.4 ^{0}C. The first two peaks do not show up after cooling to room temperature; this is proof that the water molecules have been lost. According to the plot's scale, the crystal's total energy absorption during the first and second transitions was H = -256 J/g, and the transition temperatures were 70.8–118.4 C and 180.4 C, respectively. The total energy absorption during the third transition was H = -95.63 J/g and the transition temperature was 180.4 ^{0}C, which corresponds to the melting point of the drug. It's also important to point out that the process of losing water molecules is irreversible, meaning that even though the highest temperature achieved is lower than 171 0 C, the dehydrated crystal cannot return to the hydrated form through cooling since there isn't any water remaining. (Hinojosa-Torres, Aceves-Hernandez et al. 2008).The DSC thermographs of pure LIS, SA, CS, Avicel®, optimized pellet formula and the corresponding physical mixture, which are identical with the reported data (Jagdale, Suryawanshi et al. 2014). These findings indicate that the melting point of the medication in its pure form is 180.4 °C and that of the drug in its pellet formulation is rather close to that temperature.

Figure 10. DSC curve of untreated LIS, SA, CS, Avicel® and optimized LIS-SR matrix pellet formulation.

3.5.3. *Ex- vivo* mucoadhesion

The number of pellets remained attached to the rabbits' intestinal mucosa was used as an indication for the mucoadhesion properties of the optimized LIS-SR matrix mucoadhesive pellet formulation. The percentage of pellets adhering to the mucosa as a function of time is displayed in bar chart in Figure 11. The optimized formula pellets showed an enhanced mucoadhesive properties were 100, 90, 86 and 73% of the particles remained attached to the mucosal membrane after 15, 30, 45, and 60 min, respectively. It can be seen that the optimized pellets exhibited excellent mucoadhesive properties. After 1 h, about 73% of the pellets were still attached to the mucus membrane. In contrast, the nonpareil sugar seeds exhibited a weak mucoadhesion to the mucosa and for a short period, only 5% of which remained attached to the mucosal surface for not more than 5 min.

Figure 11. The *ex-vivo* mucoadhesion of the optimized LIS-SR matrix pellet formula in compared with sugar seed particles represented by the time for pellets remaining attached to the intestinal mucosa (n=6).

3.5.4. *Ex-vivo* permeation

Ex-vivo permeability study for LIS from the optimized LIS-SR matrix pellet formula as compared to free powder LIS by using the everted sac technique is shown in Table 6 and Figures 12. Permeation of LIS from the studied pellets formula was observed at each time point and the P_{app} for the LIS-matrix pellets was found to be 3.5×10^{-3} cm·min^{-1}, which was significantly higher, approximately 1.7-folds as compared to free LIS (2×10^{-3} cm·min^{-1}), Table 5. Furthermore, around 50% of LIS permeated across the intestinal membrane after 24 h, which was higher as compared to free LIS, which was only 38.4% at the same time point. The obtained data indicate higher apparent permeability (P_{app}) of LIS from its-loaded mucoadhesive SR matrix pellet optimized formula in comparison to the untreated drug.

Table 6. *Ex-vivo* apparent permeation parameters of LIS from optimized LIS-SR mucoadhesive matrix pellet formula compared to untreated drug (n=6).

Figure 12. Permeation profiles of LIS from the optimized LIS-SR matrix pellet formula compared to untreated drug (n=6).

Discussions

The extent of the pellet powder mass wetting and spreading, as well as the substrate-granulating liquid interacting with the binder solution, can be quantified mathematically using the mean line torque peak values. In this study increasing concentration of SA and CS, led to increase the peak torque significantly. This was attributed to the increment in the network of liquid channel in the pendular and funicular phases, which thus increased the cohesiveness of powder mass and the mean torque line. A progressively increasing network of liquid bridges characterizes the pendular and funicular states. Both of these stages will increase the cohesiveness of the powder mass and therefore an increased torque on the mixer (Chatlapalli and Rohera 2002). Moreover, Ibrahim and Mahrous (Ibrahim and Mahrous 2019) clarified that according to the MTR profiles of carbopol-avicel PH101 and HPMC LV-Avicel PH101 systems, wet mass peak torques can be increased by maximizing the ratio of both polymers in the pellet mass.

On the other hand, the increase in pellet sizes by increasing SA concentrations might be due to increasing wet mass torque with increasing the level of SA that caused an increase in polymer viscosity. The increase in binder ratio from 1 mL/g in case of F6 to 1.333 mL/g in case of F7 resulted in a pronounced increase in peak torque from (0.461 Nm) to (1.286 Nm) and consequently in the particle size. This is consistent with the information from Ibrahim et al., who discovered that for all formulations, increasing torque values resulted in increased water content and larger particle size. (Ibrahim, Hassan et al. 2016). In case of 0.6% and 1% CS binder solution, increasing the SA concentration led to an increase in the

size of the pellets, but not to the same extent in case of low concentration of CS solution (0.2%). This might be due to the hydrophobic nature of CS.

In general, inclusion of the hydrophilic polymer SA and binding the pellets wet masses by CS solution resulted in retarding drug release. The might be due to the cross-linking interaction between SA and CS, which resulted in increasing wet mass torques and, in turn, increased pellet size, which slowed the drug release rate. In addition, the increased wet masses' torque due the interaction between SA and Cs (especially at higher SA levels) produced compact pellet structures that slowed the drug release rate. Between 68.48 and 74.18% of the drug was released from the pellets formula. As the formulation that almost gave a slow release than other were also distinguished as has a high peak torque value and larger pellet size. Vice versa, formula distinguished as having a smaller particle size it also has a smaller peak torque and a faster %release. Loading drugs into SA and CS matrices has been reported to enhance the drug encapsulation efficiency, increase drug stability, gives sustained release, reduce the burst release of the incorporated therapeutic agents, prolonged contact time in the gastrointestinal tract and increase the bioavailability of the loaded drugs (Arora and Budhiraja 2012).

Several factors could contribute in controlling drug release rate from sustained matrix pellets, including pellets' composition, nature of pellet forming polymers as well as the binder type and concentrations. These factors affect peak torque of wet mass before extrusion/spheronization procedures as well as pellet size, which, in turn, impact drug release rate. Alshora et al. (2020) showed that the release profile od flurbiprofen from SR matrix pellets was dependent on pellet composition, which impacted pellet wet mass mean line torque and pellets' sizes. Moreover, Ibrahim et al. (2019) explained that the weight ratios of hydrophilic and hydrophobic excipients in the pellet composition modified the drug release profile from these pellet formulations.

Moreover, in case of zeta potential, the increase in negativity by increasing CS binder solution might be due to increasing the cross-linking interaction between CS and SA, which could reduce the available carboxyl groups in SA for ionization in alkaline media. Furthermore, the highest zeta potential value (-22.1 mV) was obtained in case of pellet formula F3, which composed of lowest SA level (0.5%) and highest CS solution level (0.6%). This pellet formula showed lowest peak torque value (0.557 Nm), smallest size (1610 μm), Table 1. These data indicated the effects of wet mass peak torque on pellet size, which in turn affect their interaction with mucin. Several investigators showed that CS and SA polymer have been proposed to mediate mucoadhesive characteristics interactions with mucin. The interaction of CS with mucin in the presence of various additives showed that hydrophobic and hydrogen bonding forces are present in addition to electrostatic interactions when CS and mucin are combined in an aqueous environment (Menchicchi, Fuenzalida et al. 2014; Haugstad, Håti et al. 2015).

As the crosslinking occurred between SA and CS binder solution caused an increase in the wet mass peak torque, which caused in difficulty in extrusion procedure, resulting in pellets with roughness in their surfaces. Also, surface roughness could be caused by partly collapsing the polymeric gel network during drying. Similar descriptions of the microstructure utilizing various drugs loaded with SA-CS examined by SEM have been reported by other authors (Souza, Caldas et al. 2014; Gatiganti, Srimathkandala et al. 2016; Kulig, Zimoch-Korzycka et al. 2016; Gomathi, Sudha et al. 2017; Moganti and Shivakumar 2017; Nalini, Basha et al. 2019).

The results obtained by DSC indicate no interaction between the drug and the pellets excipients as supported by both FTIR and XRPD studies (Jagdale, Suryawanshi et al. 2014). The results of DSC studies for LIS, optimized pellet formula, as well as the corresponding physical mixture showed that no probability of the interaction between the drug and pellet excipients indicating the compatibility between LIS and the pellet excipients (Guthmann, Lipp et al. 2007).

Ex-vivo mucoadhesive results can be attributed to the nature of the polymers used in the pellet formula; CS and SA, which is, according to literature, strongly associated with improved mucoadhesion (Bernkop-Schnürch, Schwarz et al. 1999). In addition, many studies showed that viscosity is directly proportional to the mucoadhesion properties of different preparations (Vijayabhaskar, Venkateswarlu et al. 2016). When compared to non-mucoadhesive beads, the pellets made of Avicel® and the mucoadhesive polymers SA and CS had improved mucoadhesive qualities that were seen in the in vitro wash-off experiment (i.e. sugar seed). *Ex-vivo* wash-off test results revealed that the pellets had good mucoadhesive properties, which may boost the drug's bioavailability by lengthening their residence time and bringing their absorptive membranes closer together. This will allow for extended drug release of LIS in the gut, which serves as the location for the medication's absorption window. (Prajapati, Tripathi et al. 2008). The presence of hydroxyl groups in both of the hydrophilic mucoadhesive polymers (SC and SA) included in the polymeric matrix, enhanced the capability to form hydrogen bonds with the mucous membranes, leading to the mucoadhesion of the pellets to the mucosal wall. Wetting, diffusion, and fracture theories, as well as the Van der Waals and hydrogen bond theories of electrostatic interaction, also were included in the interpretation of mucoadhesive efficacy of these polymers (Mortazavi and Smart 1995). Additionally, the hydrophilic polymers such as SA and HPMC-K4M also have the ability to form noncovalent bonds such as Van der Waals forces or ionic interactions, resulting in mucoadhesion as reported by Morgan et al. (Moganti, Shivakumar et al. 2021). These results have proven that the optimized mucoadhesive SR LIS pellet formula with its mucoadhesive properties are suitable for formulating LIS sustained release drug delivery system for enhancing intestinal contact time, and in turn, playing a crucial rule in enhancing drug permeability.

LIS's permeability was increased when it was included into mucoadhesive matrix pellets. This may be explained by the pellets' capacity to stick to mucus and deliver the medication directly onto the cell membrane surface, that’s enhancement of the permeation. In addition, CS polymer can act as potent permeation enhancer (PE) in gastro intestinal tract for the mucous layer (Kotze, Luessen et al. 1999). The key events controlling oral drug absorption are the dissolution/solubility of the drug in the GIT environment, in addition to its permeability through the GIT membrane (Dahan, Miller et al. 2009). Transcellular diffusion of substances from the luminal to serosal side has to partition the drug from the aqueous luminal area to the nonpolar lipid bilayers of the cell membrane. (Balimane, Chong et al. 2000).

Conclusion

Three factorial two independent levels design was used to optimize formulation of LIS from LIS-SR mucoadhesive matrix pellets; containing the mucoadhesive polymers (SA and CS). By adjusting the concentrations of both SA and CS solution, which affected pellet wet mass consistency, pellet size, *in-vitro* mucoadhesion efficacy, and the *in-vitro* release, the

optimized LIS-SR pellets was formulated as combination of both SA and CS 2,159 and 0.357 % respectively. The optimized formula exhibit a 1.055 Nm mean line torque was led 1830.8 μm pellet particle size, had decreased release rate 56.2 % after 8 hr and -20.33 mV as average mucin zeta potential. The *Ex-vivo* mucoadhesion studies revealed after 1 h, about 73% of the pellets were still attached to the mucus membrane. Moreover, the *ex-vivo* permeation was found to be significantly higher (1.7-folds) as compared to free LIS. In conclusion: LIS-SR matrix pellets, prepared with an extrusion/spheronization have desirable excellent characteristics *in-vitro and ex-vivo* sustained-release pellet formulation of LIS-SR was able to sustain the release of LIS for up to 8 h.

Conflict of interest
There is no conflict of interest to be disclosed by the authors other than what has been recognized above.

Availability of information and resources
Upon request, all information and materials are available.

Conflicts of interest
The authors state that there are no interests at odds with one another.

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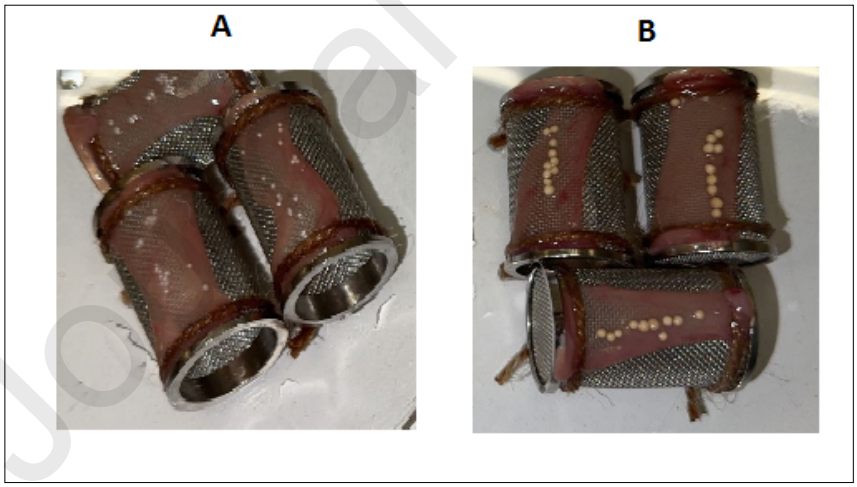

Figure 1. *In vitro* intestine wash off test, (A) for nonpareil sugar seed, (B) for LIS-matrix pellets; attached to the mucosal intestinal wall.

Figure 2. Intestinal segment before permeation study, (A) and after filling with the tested formulation (B).

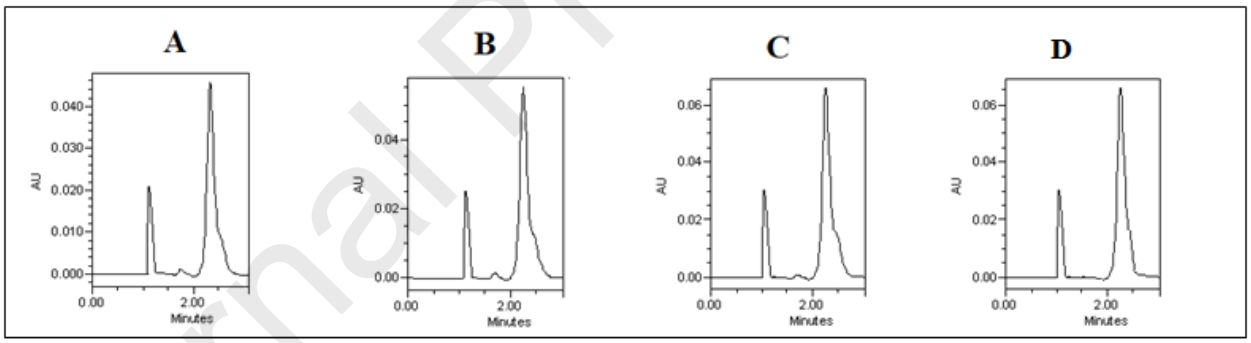

Figure 3. HPLC chromatograms of LIS in HCl (chromatogram A), in phosphate buffer (chromatogram B), in Krebs solution (chromatogram C) and in water:methanol in ratio (7:3).

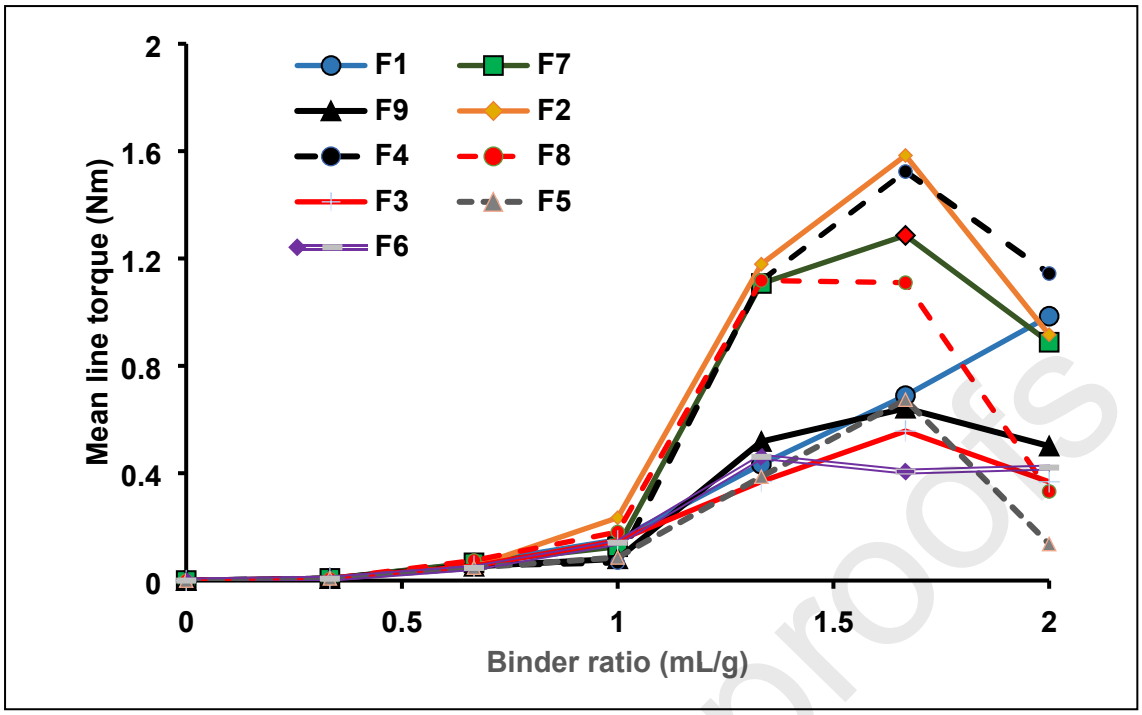

Figure 4. Mean line torque of wet masses for tested Lisinopril SR pellets formulations.

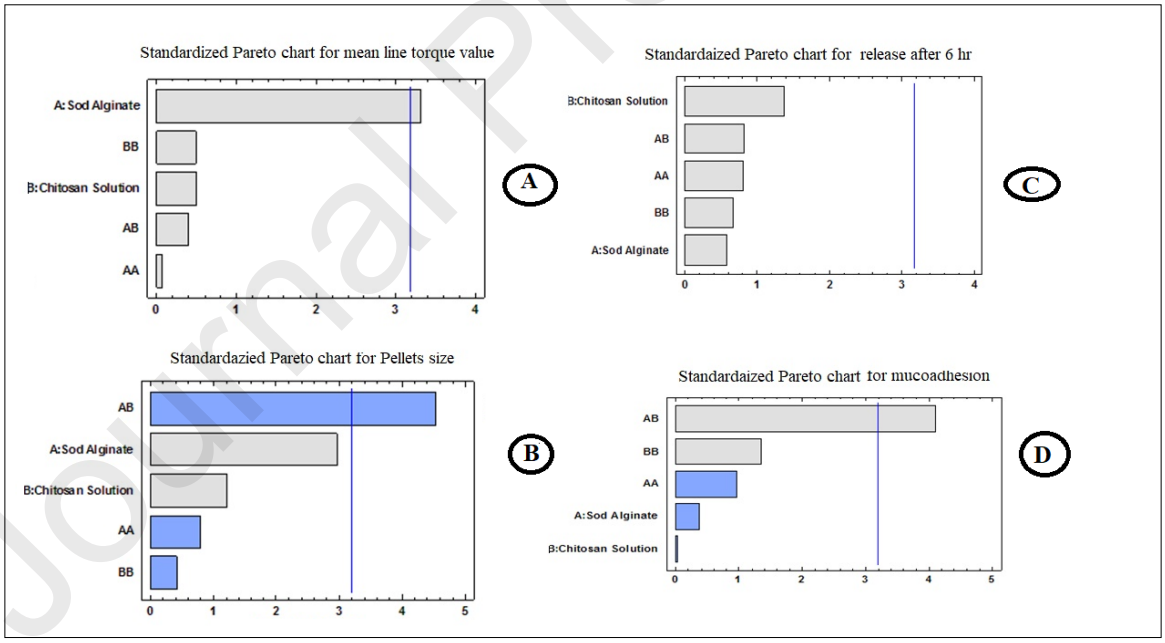

Figure 5. Standardized Pareto chart estimating the effect of independent formulation factors on (A); mean line torque value of pellet wet masses, (B); pellet sizes and (C); % Lisinopril release from SR pellets 6h after and (D); mucoadhesion studies.

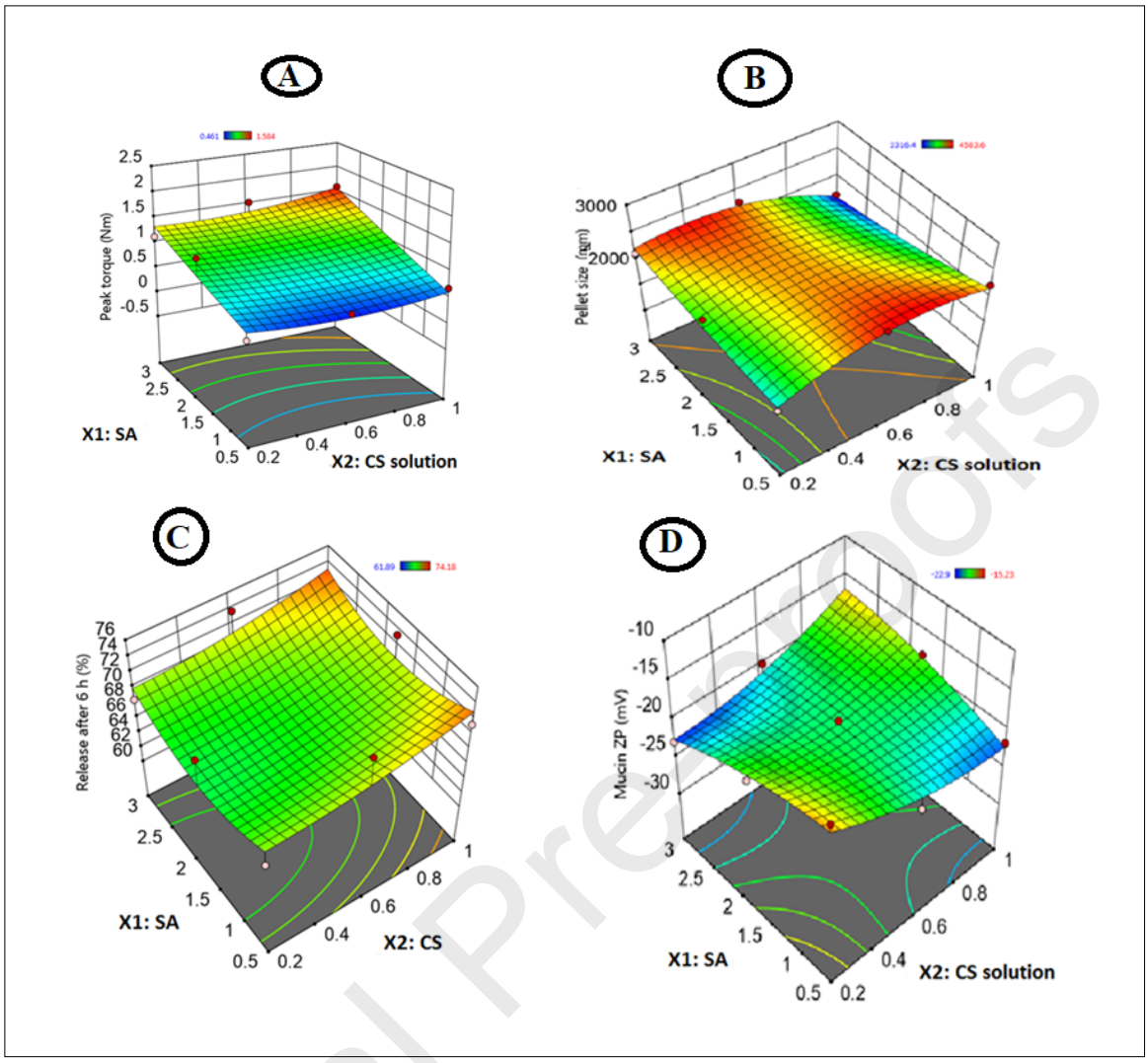

Figure 6. Response surface plot estimating the effect of SA (X1) and CS solution (X2) on (A); the mean line torque value of pellet wet masses (Y1), (B); pellet sizes and (C); % Lisinopril release from SR pellets 6h after and (D); mucoadhesion studies.

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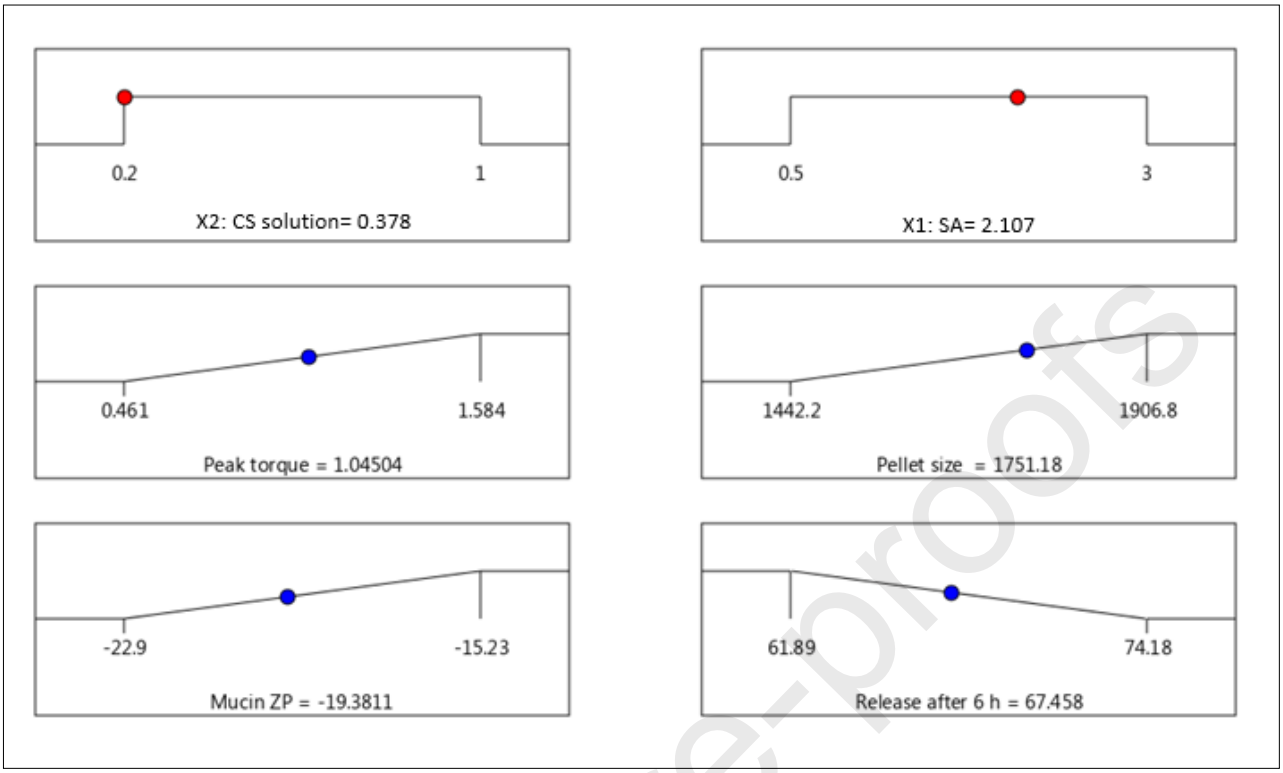

Figure 7. The composition suggested by the statistical software of the optimize formulation of the LIS-SR matrix pellets.

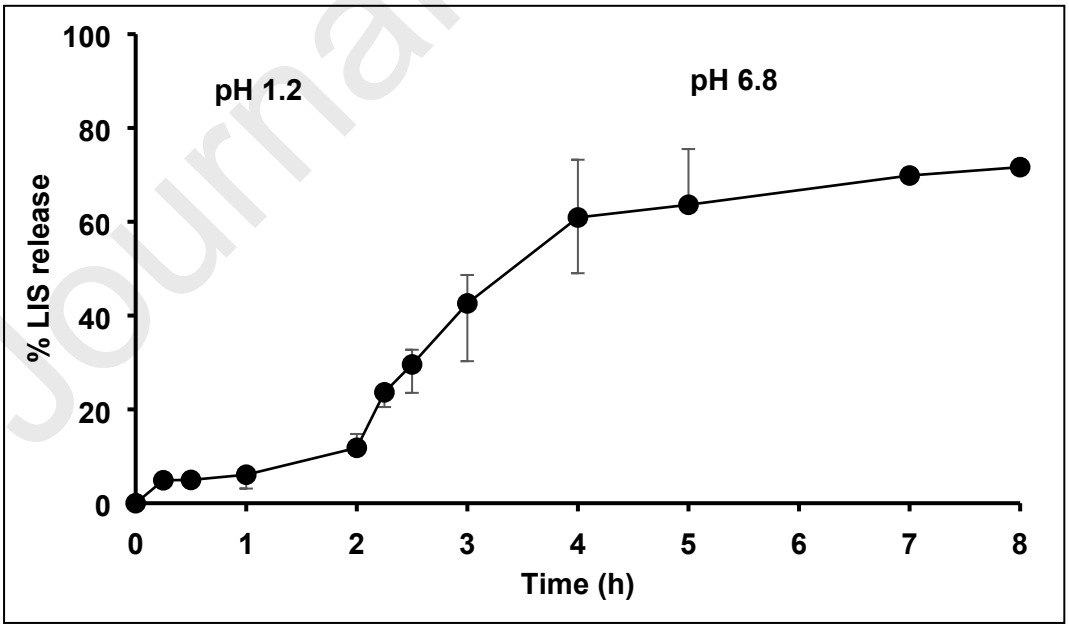

Figure 8. Release profile of LIS from enteric-coated capsule containing the optimized pellet formula (F10) in 0.1 N HCL for 2 hours then in (pH 6.8) for 8 h.

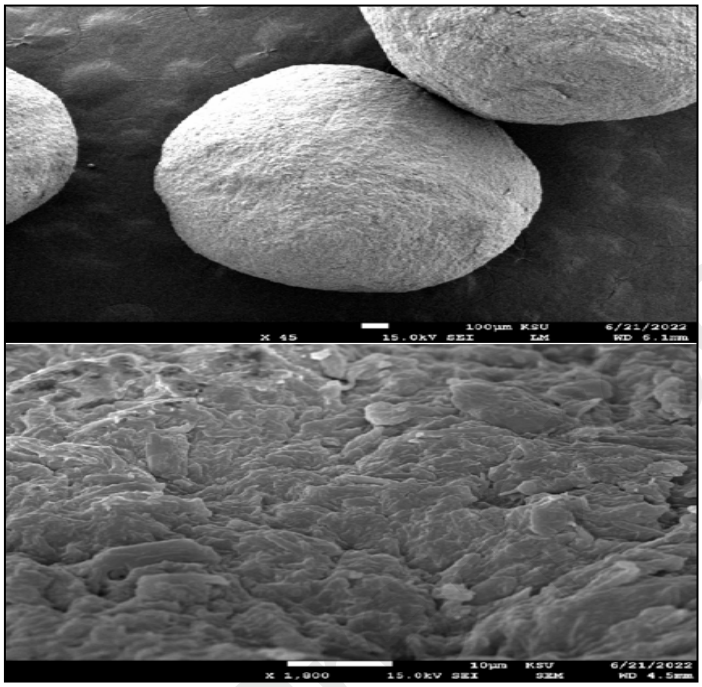

Figure 9. Scanning electron micrographs of optimized LIS-SR matrix pellet formulation (A) and scanning electron micrographs of its surface (B).

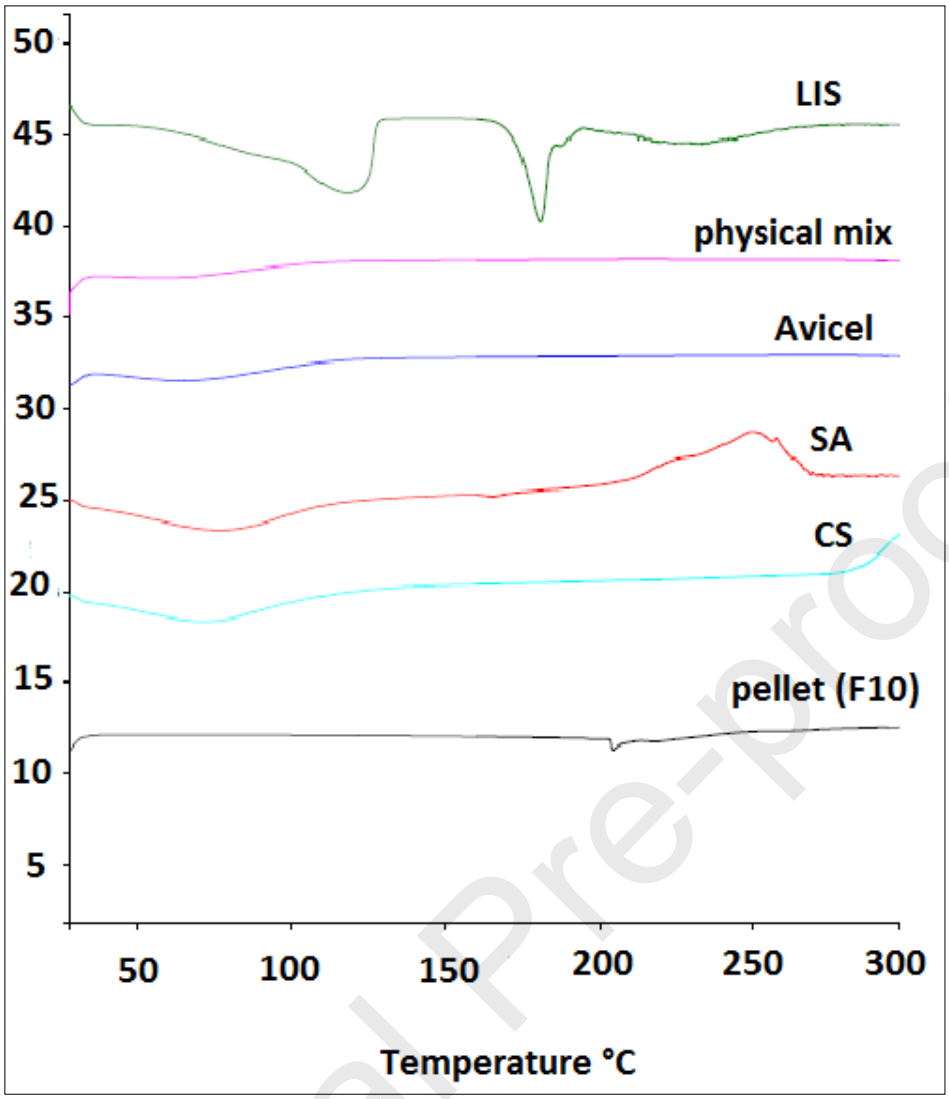

Figure 10. DSC curve of untreated LIS, SA, CS, Avicel® and optimized LIS-SR matrix pellet formulation.

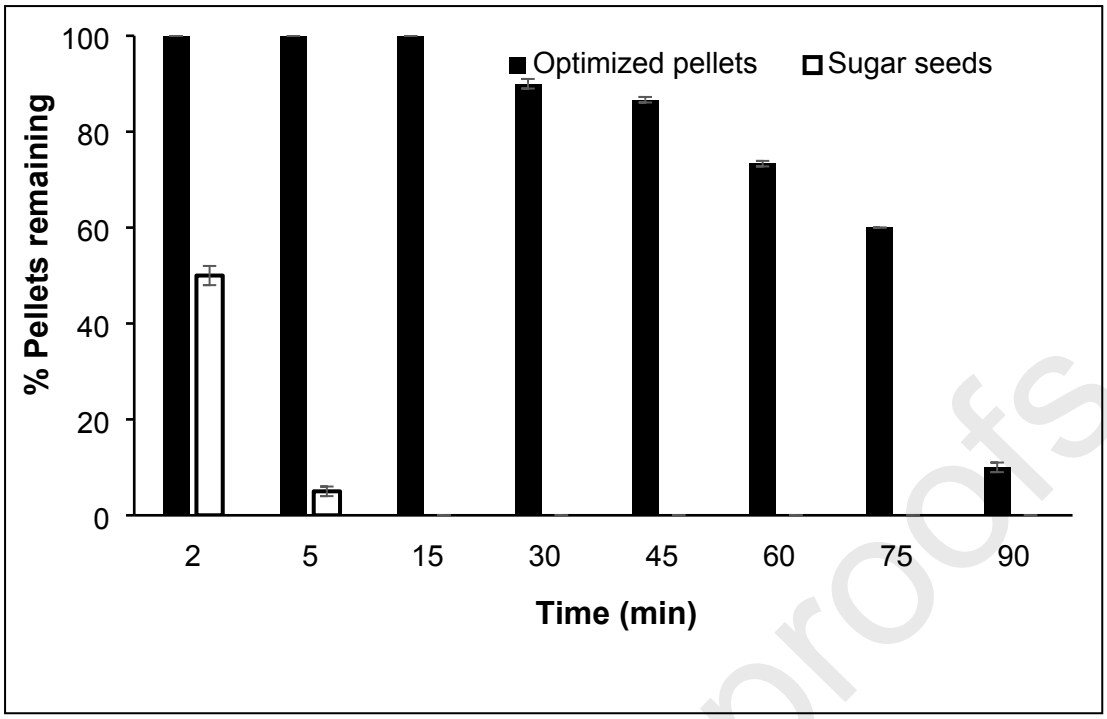

Figure 11. The *ex-vivo* mucoadhesion of the optimized LIS-SR matrix pellet formula in compared with sugar seed particles represented by the time for pellets remaining attached to the intestinal mucosa (n=6).

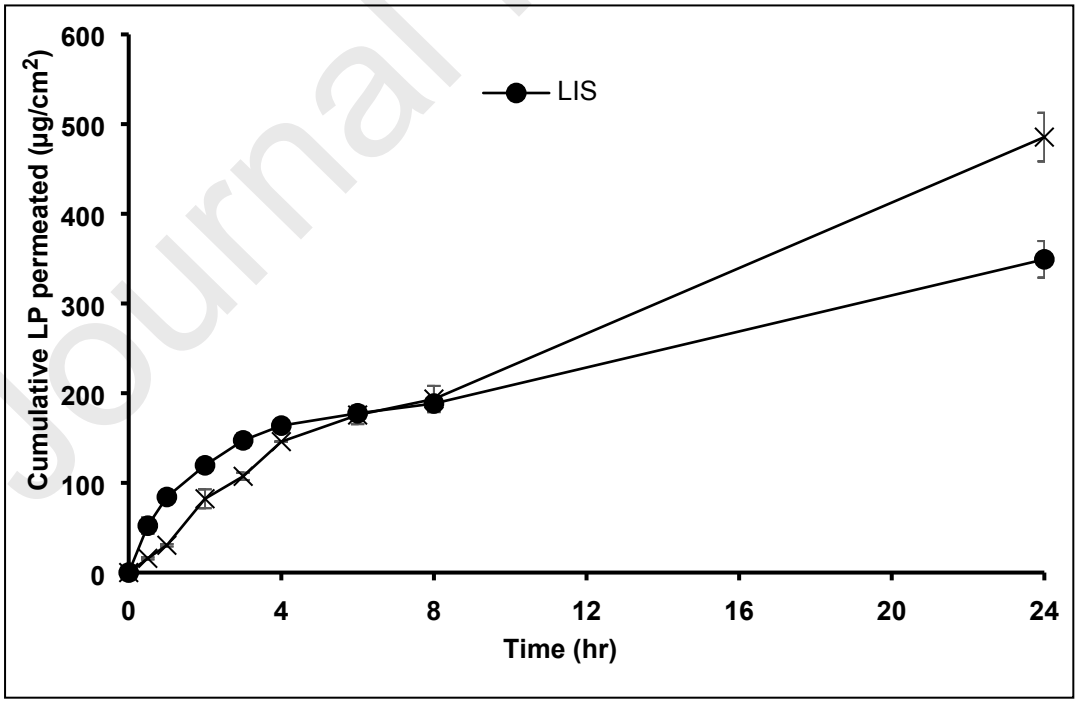

Figure 12. Permeation profiles of LIS from the optimized LIS-SR matrix pellet formula compared to untreated drug (n=6).

Table 1. Variables in three level full factorial design and composition of LIS matrix pellet formulations.

Independent variables (factors)			
	Low (-1)	Medium (0)	High (+1)
X1: SA (%)	0.5	1.75	3
X2: CS solution (%)	0.2	0.6	1
Dependent factors (responses)			
Y1: Peak torque (Nm)			
Y2: Pellets' particle size (μm)			
Y3: LIS percentage release after 6 h (%)			
Y4: Mucoadhesive (zeta potential, mV)			

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Avecil PH 101 (%)	93.25	92	94.5	92	94.5	94.5	93.25	92	93.2
X1: SA (%)	1.75	3	0.5	3	0.5	0.5	1.75	3	1.75
X2: CS solution (% w/v)	1.0	1.0	0.6	0.6	1.0	0.2	0.2	0.2	0.6
LIS (%)	**5**								

Table 2. Estimated recoveries, accuracies and precisions for determination of lisinopril at different concentrations (n = 6)

sample	Nominal conc.(ng/ml)	Within run			
		Calculated Conc.(ng/ml)	Precision (CV %)	Accuracy (%)	Estimated recoveries (%)
Q1	125	120 ± 4.3	11.4	95.4	87.3
Q2	500	485 ± 7.3	8.3	97.2	90.3
Q3	1000	983 ± 7.1	4.7	98.1	94.5
		Between run			
Q1	125	118 ± 3.6	12.3	94.3	88.7
Q2	500	509 ± 4.5	9.5	96.2	92.3
Q3	1000	1023 ± 3.2	5.6	98.7	94.7

Table 3. Properties of extruded/spheronized LIS mucoadhesive SR matrix pellets.

Formula	Mean line torque (Nm)	Pellet size (µm)	LIS content (%)	*In vitro* release after 6 h (%)	*in vitro* drug mucoadhesion (ZP of mucin suspension; mV)
F1	0.985	1721.00 ± 133	87.9±1.09	73.26 ± 4.01	-18.1 ± 0.92*
F2	1.584	1677.40 ± 121	91.5±0.25	71.95 ± 1.61	-17.5 ± 1.06
F3	0.557	1610.40 ± 150	87.4±0.54	74.18 ± 3.49	-22.1 ± 2.02
F4	1.524	1731.00 ± 278	90.2±0.68	73.58 ± 4.35	-19.9 ± 1.68
F5	0.674	1796.60 ± 125	89.9±0.95	71.28 ± 0.65	-21.6 ± 1.33
F6	0.461	1442.20 ± 138	93.7±1.72	67.88 ± 0.15	-15.3 ± 0.45
F7	1.286	1655.40 ± 153	95.8±0.73	70.61 ± 2.07	-19.1 ± 1.94
F8	1.118	1906.80 ± 194	98.7±0.89	68.48 ± 2.17	-22.9 ± 1.51
F9	0.642	1814.20 ± 212	103±1.73	61.89 ± 1.67	-19.4 ± 0.95

* Zeta potential of control mucin suspension is -14.8 ± 0.87 mV.

Table 4. Kinetic analysis of LIS release from various mucoadhesive SR release matrix pellets.

Formula	Zero order model		First order Model		Higuchi diffusion model		Peppas model	
	r^2	Slope	r^2	Slope	r^2	Slope	r^2	n*
F1	0.822	4.84	-0.867	-0.054	0.909	15.80	0.972	0.135
F2	0.828	4.04	-0.866	-0.046	0.915	13.17	0.952	0.123
F3	0.782	4.84	-0.828	-0.057	0.879	16.04	0.934	0.13
F4	0.737	4.05	-0.779	-0.047	0.844	13.67	0.909	0.101
F5	0.752	4.36	-0.783	-0.048	0.859	14.69	0.906	0.126
F6	0.660	2.60	-0.687	-0.027	0.767	8.91	0.829	0.053
F7	0.667	3.11	-0.698	-0.035	0.781	10.76	0.873	0.065
F8	0.709	3.89	-0.760	-0.039	0.811	13.14	0.939	0.086
F9	0.725	3.79	-0.752	-0.033	0.833	12.84	0.881	0.11

*n: the release exponent was calculated from Korsmeyer-Peppas equation.

Table 5. Peak torque, binder ratio, pellet size, and percentage of LIS release after 8 hours for the optimized LIS SR matrix pellets: predicted and observed values

Optimized Independent factors	**Dependent Variable**			
	Responses	**Desirability**	**Predicted**	**Observed**
SA (X1): 2.107 %	**Peak torque (Nm)**	Maximum	1.045	1.055
	Pellet size (µm)	Maximum	1751.18	1830.8 ±205
CS solution (X2): 0.378 %	***In vitro* Release after 6 h (%)**	Minimum	67.458	56.2±5.6
	Mucin ZP (mV)	Maximum	-19.381	-20.33± 1.07

Table 6. *Ex vivo* apparent permeation parameters of LIS from its-loaded optimized mucoadhesive SR matrix pellet formula (F10) compared to untreated drug (n=6).

Sample	**Amount permeated/cm² ± SD (µg/cm²) after 24 h**	**P_{app} ± SD (cm/min)**	**Permeation enhancer ratio**
Free LIS	349 ± 20	2×10^{-3} ± 0.6	
LIS-pellets	486 ± 27	3.5×10^{-3} ± 0.5	1.72