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Original Article

Polymer - Lipid Hybrid Mucoadhesive Nanospheres of Rivaroxaban: Formulation and Statistical Optimization for Sustained Release

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ABSTRACT

Background: The direct Factor Xa inhibitor rivaroxaban is a poorly water-soluble compound (BCS Class II) with variable oral bioavailability and has been used to treat thromboembolic disorders. These drawbacks can compromise its effectiveness, especially during long-term anticoagulant therapy. To overcome this, we created polymer-lipid hybrid mucoadhesive nanospheres which, when delivered into the gut, adhere to the gut wall and release the drug slowly over the course of a whole day. **Materials and Methods:** An emulsion solvent evaporation technique was used to prepare nanospheres. The mucoadhesive polymer was konjac gum and the lipid release retardant was glyceryl behenate (Compritol 888 ATO). Three independent variables, namely the concentration of konjac gum (50-100 mg), the glyceryl behenate concentration (25-75 mg) and stirring speed (900-1500 rpm), were optimized using a Box-Behnken statistical design. The nanospheres obtained were characterized for their particle size, entrapment efficiency, amount of drug loaded, swelling behaviour, *ex vivo* mucoadhesion, long term and accelerated stability study and *in vitro* drug release (24 hr). **Results:** The optimized batch (MM15) exhibited a mean particle size of 255.3 nm±4.2 nm, a polydispersity index of 0.286±0.015 and a zeta potential of -43.42 mV±1.2 mV, which confirms good physical stability. Entrapment efficiency reached 92.4±1.1%, and drug loading was 6.23±0.08%. The swelling index was 94.6±1.6% after 12 hr and the mucoadhesion (wash-off test on goat intestinal mucosa) was 91.5±1.4% after 2 hr. A sustained drug release was obtained (85.6±1.2% released in 24 hr) after quasi-Fickian diffusion (Korsmeyer-Peppas model, n=0.44). Stability testing at 25 °C for a period of 6 months revealed no significant alteration in the release of the drug (96.5±0.8% of the initial value) and in the mucoadhesion. **Conclusion:** Statistically optimized polymer-lipid hybrid mucoadhesive nanospheres were able to significantly prolong the release time of rivaroxaban and had excellent mucoadhesive properties. The formulation could support an oral option once-daily, to enhance patient compliance and treatment results for anticoagulant therapy.

Keywords: Box-Behnken Design, Glyceryl Behenate, Konjac Gum, Mucoadhesive, Nanospheres, Rivaroxaban, Sustained Release.

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INTRODUCTION

Oral drug delivery is still the most accepted mode of administration because it is simple, convenient and has high patient compliance. Nevertheless, the efficacy of orally administered medications is usually constrained by the elements of poor solubility, quick removal, and unpredictable absorption in the gastrointestinal tract. Some of these challenges are found in Rivaroxaban, a direct Factor Xa inhibitor that is used in the treatment of thromboembolic disorders. It is classified in the Biopharmaceutics Classification System (BCS) Class II with low aqueous solubility and high permeability. Consequently, its oral bioavailability can be irregular, which can influence therapeutic effects, especially when using long-term anticoagulant therapy.[1]

To overcome these constraints, there has been a lot of interest in the development of controlled drug delivery systems. Nanospheres are in the list of promising methods because of their capability to enclose drugs into a matrix system and provide regulated and sustained release. Nanospheres may be of various sizes, ranging between a fraction of nanometers and hundreds of nanometers, and may be made out of polymers, lipids, or a combination of both. This is because of their small size and large surface area that enables them to distribute and absorb drugs better.[2] One particular form of nanosphere system has proven to be particularly beneficial, the mucoadhesive nanosphere. These

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systems are meant to stick to the lining of the gastrointestinal tract which is mucosal, thus extending the residence time of the drug at the absorption site. The increased contact time improves drug absorption and decreases the number of times the drug is taken. Mucoadhesion is mainly attained by interactions between the polymeric material and the mucus layer which is abundant in glycoproteins. The polymers that have the ability to form hydrogen bonds or have a swelling behavior are particularly useful in facilitating adhesion.[3]

Natural polymers have become a topic of growing interest in mucoadhesive drug delivery systems in recent years in light of their safety, biodegradability, and functionality. An example of natural polymers is Konjac Gum (KG) which is a natural polymer with high viscosity, swelling capacity, and mucoadhesive potential. It has a branched structure that enables it to interact with mucin effectively and thus it can be used in sustained-release applications. Other polysaccharides (e.g., gellan gum, konjac glucomannan) have also shown promising properties to be used in mucoadhesive formulations.[4]

To increase the performance of nanospheres further, incorporation of lipids in the formulation is possible. Glyceryl behenate (Compritol 888 ATO) and other lipid-based excipients are commonly used to retard the release of a drug because of their hydrophobic nature. These lipids create a barrier in the matrix that slows the diffusion of aqueous fluids, and thus, it regulates the rate of drug diffusion. The polymers and lipids in a single system create a polymer-lipid hybrid-matrix, which has the added benefit of mucoadhesion and sustained release. This method is especially useful in the case of drug, such as rivaroxaban, where a stable plasma concentration is crucial.[5]

The synthesis of such hybrid nanospheres is usually performed by the application of such methods as emulsion solvent evaporation, which allows achieving a uniform distribution of drugs and the control of the formation of particles. Nonetheless, the nature of the end product depends on a number of formulation and process variables such as the concentration of polymer, the lipid content, and the stirring conditions. In order to critically assess these factors and maximize the formulation, statistical methods like Box-Behnken design are commonly used. The method can be used to identify the critical variables and their interactions to develop a robust and reproducible drug delivery system.[6]

This study addresses the gap in nanoformulations of rivaroxaban by developing polymer-lipid hybrid mucoadhesive nanospheres, focusing on achieving prolonged gastrointestinal residence, sustained drug release, and statistical optimization. Although various nanoformulations, such as polymeric nanoparticles and solid lipid nanoparticles, have been explored for enhancing solubility and dissolution, few studies have integrated strong mucoadhesion with controlled release mechanisms. The system utilizes konjac gum for its natural mucoadhesive properties, excellent swelling capacity, and superior hydration behavior over synthetic polymers like carbopol or HPMC, while glyceryl behenate (Compritol® 888 ATO) acts as a release retardant due to its hydrophobic nature and high melting point. The formulation was optimized using a Box-Behnken design to establish a balance between particle size, sustained drug release, and mucoadhesion, ensuring enhanced therapeutic efficacy and stability.

The current study thus aims at formulation and optimization of polymer-lipid hybrid mucoadhesive nanospheres of rivaroxaban to acquire sustained drug release. Through a combination of natural mucoadhesive polymers with lipid-based release modifiers and the implementation of a statistical optimization strategy, this work attempts to amplify the therapeutic performance and patient compliance that are associated with rivaroxaban therapy.

MATERIALS AND METHODS

Rivaroxaban was sourced in Yarrowchem, Pvt. Ltd., Mumbai, Glyceryl Behenate was sourced in Venkatesh naturals, Chindwada, Poloxamer 188 was sourced in BASF India, Genipin was sourced in Sigma-Aldrich (India). All the remaining chemicals and reagents were used exactly as they were procured from the supplier as they were all of analytical grade, without any additional purification.

Formulation of Nanospheres

The emulsion solvent evaporation technique which is one of the most popular methods of the formulation of polymeric particulate systems was used to prepare mucoadhesive nanospheres of rivaroxaban.[7] In order to optimize the formulation of nanospheres systematically, three variables were selected, examined and tested at three different levels as low (-1), medium (0) and high (+1). The different variables and formulation chart is shown in Table 1. Weighed amounts of rivaroxaban (20 mg) and glyceryl behenate were accurately weighed and dispersed in dichloromethane to form the organic phase and then sonicated to ensure even dispensation of the drug within the lipid matrix.[8] Meanwhile, a slow addition of konjac gum was done to warm distilled water with continuous stirring to form a homogeneous (fine) polymer solution. The solution was then cooled and stabilized with poloxamer 188 (0.5% w/v), to make the solution stabilized and capable of making an emulsion.[9]

The aqueous polymer solution was then added to the organic phase drop by drop, and then the mixture was stirred by the constant high-speed stirring to form an oil-in-water emulsion. The stirring was maintained several hours to enable all the organic solvent to evaporate resulting in the formation of solid nanospheres.[10] Genipin solution (0.2% w/v) was prepared in distilled water and added to the aqueous polymer phase immediately after emulsion formation. Crosslinking was allowed

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Table 1: Independent Variable and Formulation Chart for the Preparation of Nanospheres.

Batch	Konjac Gum Concentration (mg)	Glyceryl Behenate (mg)	Stirring speed (rpm)	Particle Size (µm)	Drug Release (%)	Mucoadhesion (%)
MM1	0 (75)	1 (75)	-1 (900)	412.6±8.4	94.2±1.8	62.5±2.4
MM2	0(75)	0 (50)	0 (1200)	302.5±6.2	89.5±1.4	78.2±2.0
MM3	1 (100)	1 (75)	0 (1200)	348.2±7.1	92.8±1.5	73.6±2.2
MM4	1 (100)	0 (50)	1 (1500)	278.4±5.9	84.6±1.3	85.4±1.8
MM5	0(75)	-1 (25)	-1 (900)	485.3±9.2	98.2±1.0	48.6±2.8
MM6	0(75)	1 (75)	1 (1500)	332.6±6.8	91.2±1.3	75.8±2.1
MM7	0(75)	0 (50)	0 (1200)	298.7±6.0	89.2±1.2	79.1±1.9
MM8	-1 (50)	-1 (25)	0 (1200)	398.5±8.0	93.5±1.6	66.3±2.3
MM9	1 (100)	0 (50)	-1 (900)	318.4±6.5	90.2±1.3	76.5±2.0
MM10	-1 (50)	1 (75)	0 (1200)	356.7±7.3	93.2±1.5	70.2±2.2
MM11	1 (100)	-1 (25)	0 (1200)	305.2±6.3	88.5±1.2	80.5±1.9
MM12	-1 (50)	0 (50)	1 (1500)	289.3±5.8	86.2±1.2	82.6±1.7
MM13	-1 (50)	0 (50)	-1 (900)	438.2±8.8	96.8±1.0	54.2±2.6
MM14	0(75)	-1 (25)	1 (1500)	375.4±7.6	92.5±1.4	68.9±2.4
MM15	0 (75)	0 (50)	0 (1200)	255.3±4.2	85.6±1.2	91.5±1.4
MM16	0(75)	0 (50)	0 (1200)	299.5±6.1	89.8±1.3	78.8±1.9
MM17	0 (75)	0 (50)	0 (1200)	296.3±6.0	89.0±1.2	79.5±1.8

to proceed under continuous stirring at room temperature (25±2 °C) for 4 hr before centrifugation and washing of the nanospheres. Genipin solution was incorporated to increase the structural integrity and control the swelling behaviour of the polymeric matrix.[11]

The formed nanospheres were centrifuged, repeatedly washed using distilled water, and then dried under the right conditions. Lastly, the dried nanospheres were sieved to achieve a uniform distribution of particle size, and stored in a desiccator until further testing.[12]

Statistical Optimization

A Box-Behnken statistical design (three factors, three levels) was used to optimize the formulation variables, assess their main and interaction effects on the critical quality attributes of the nanospheres. The independent variables were: konjac gum concentration (X_1, 50-100 mg); glyceryl behenate concentration (X_2, 25-75 mg); and stirring speed (X_3, 900-1500 rpm). The dependent responses (Y) were: particle size (Y_1, nm), drug release after 24 hr (Y_2, %) and mucoadhesion (Y_3, %).

Evaluation Parameters

Standard Calibration Curve

In order to allow proper quantification of rivaroxaban in dissolution and entrapment studies, a standard calibration curve was developed using UV-visible spectrophotometry. Rivaroxaban was accurately weighed, dissolved in phosphate-buffered saline (pH 6.8) and a stock solution of 100 µg/mL was prepared. A series of dilutions were made from this stock to produce working concentrations of 2, 4, 6, 8, 10, 12 and 16 µg/mL of the drug. The solutions were then checked by applying a UV-visible Spectrophotometer at the absorption peak for rivaroxaban, which is at 250 nm. For every concentration, we recorded the absorbance values three times. Then, we plotted the absorbances against the concentration to get a graph.[13] The linear regression was analysed to get the equation of the line and the correlation coefficient (R^2) to ensure that the calibration curve was suitable for later drug quantification.

Drug-Excipient Interaction Study

The compatibility of rivaroxaban with all formulation excipients - konjac gum, glyceryl behenate, poloxamer 188, genipin was comprehensively studied using Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC).

In the case of the FTIR study, physical mixtures of the drug with each excipient in a 1:1 ratio were prepared and samples were analyzed with an FTIR spectrometer within the range of 4000 to 400 cm^{-1}. The resulting spectra were compared to that of pure rivaroxaban to detect any types of shifts, broadening, disappearance, or appearance of new peaks, which would indicate

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possible interactions.[14] In the case of the DSC analysis, pure rivaroxaban, and physical mixtures of the excipient samples (approximately 5 mg each) were accurately weighed and put in the aluminum pans. The heating was carried out at a rate of 10 °C/min between 30 °C and 300 °C under a constant purge of 50 mL/min of the purge gas (nitrogen). The data were taken and analyzed in terms of the changes in the melting endotherm, maximum temperature, and enthalpy of fusion.[15]

Crystalline Study

The physical state (crystalline or amorphous) of rivaroxaban in the polymer-lipid hybrid nanospheres was determined using X-ray Diffraction (XRD). Samples of pure rivaroxaban and drug-loaded nanospheres were finely ground and placed separately on quartz sample holders. The XRD experiment was performed using a diffractometer with a copper Kα radiation source (wavelength= 1.54 Å). The current and voltage applied to the generator were kept at 30 mA and 40 kV, respectively. Each sample was scanned at a speed of 2° per minute over a 2θ range of 2° to 60°. The resulting diffractograms, which plot intensity versus diffraction angle, were compared across the three types of samples.[16] The pure drug diffractogram displayed characteristic sharp peaks, which showed that it was in a crystalline state. A decrease in peak intensity, broadening of peaks or disappearance of peaks in the drug-laden nanosphere sample would indicate a shift to an amorphous state, which is capable of enhancing dissolution behaviour.

Micromeritics

To assess the flow and packing properties of the developed nanospheres, several micromeritic properties such as bulk density, tap density, compressibility index, Hausner ratio and angle of repose were considered. The bulk density was determined by measuring the volume of 1 g of Nanospheres gently poured through a glass funnel into a 10 mL graduated cylinder without any tapping. The bulk density was defined as the mass divided by that volume. The cylinder was mechanically struck at a rate of 100 taps per minute, until the volume no longer decreased. The tap density was determined from the final settled volume. The density was then calculated as an index by Carr as the difference between the tap density and bulk density as a percentage of tap density. The Hausner ratio was even easier to calculate, density of tap density divided by bulk density. Lastly, the angle of repose was determined. Thenanospheres were poured through a funnel, 2 cm above a flat surface, where they built up into a cone shaped pile. The height of the cone and the base radius were measured and took the inverse tangent (arctan) of these two values.[17]

Percentage Yield and Particle Size

The percentage yield was used to determine the efficiency of the preparation process of the nanosphere, which is the total product recovered divided by the total solids used in the preparation. The weight of the dried nanospheres was precisely weighed and the percentage yield obtained by dividing the actual weight of nanospheres by the sum of the weights of rivaroxaban, konjac gum, glyceryl behenate among other solid excipients, multiplied by 100. A large percentage yield meant that there was little material loss during the emulsification process, solvent evaporation, washing and drying processes. To guarantee uniformity, a particle size analyzer was used to measure particle size by using a laser diffraction particle size analyzer. To prevent aggregation, a small amount of poloxamer 188 (0.1% in distilled water) was added to the small sample of nanospheres. This dispersion was then added to the analyzer which measured the volume distribution of the diameter of particles. The standard deviation and mean particle size were reported. A small size distribution was deemed as attractive to reproducible performance.[18]

Entrapment Efficiency of drugs and Drug Loading

We measured the drug entrapment efficiency and the drug loading percentage to determine the efficiency of the trapping of the drug inside the nanospheres. We carefully weighed approximately 100 mg of nanospheres and crushed the matrix inside of a glass mortar. The crushed powder was then transferred to a 10 mL volumetric flask with phosphate buffer saline (pH 6.8) and sonicated for 15 min to completely extract the drug. After that, the dispersion was centrifuged at 5000 rpm for 10 min to settle out any undissolved polymer bits. Absorbance of the clear liquid was measured at 250 nm in a UV-visible spectrophotometer after it was collected. Entrapment efficiency was calculated as:

$$\text{Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

In which the theoretical content is the amount of drug added during formulation. To measure the amount of Drug Loaded into the nanospheres, the following formula was used:

$$\text{Drug Loaded nanospheres} = \frac{\text{weight of drug in Nanospheres}}{\text{weight of Nanospheres}} \times 100$$

Both parameters gave an idea about the ability of the formulation to retain the drug.[19]

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Swelling Index

Mucoadhesion and sustained drug release depend on the ability of the polymer lipid hybrid nanospheres to absorb water and swell.[20] To replicate the gastrointestinal conditions, the swelling index was determined gravimetrically using phosphate-buffered saline (pH 6.8) at 37 °C + 0.5 °C. First, 10 mg of dried nanospheres were weighed (recorded as W_0) and put in a beaker that had 10 mL of pre-warmed buffer. The Nanospheres were then retrieved from the medium after fixed time intervals of 1, 2, 4, 6, 8, and 12 hr. The excess surface moisture was gently blotted away using filter paper, and swollen nanospheres were immediately reweighed (measured as W_t). The swelling index at the given time was calculated in terms of the following formula:

$$\text{Swelling Index} = \frac{\text{Wt} - W0}{W0} \times 100$$

Where W_0 is the weight of the sample at the initial point (time 0).[21] Generally, the swelling index will rise with time until it gets to a certain equilibrium point. A moderate rate of swelling is desired, where excessive swelling causes the drug to be released in an undesired manner and insufficient swelling results in a decreased adhesion of the drug to the mucosal layer.[22]

Surface Morphology (SEM)

The surface features of the rivaroxaban-loaded nanospheres were observed under the Scanning Electron Microscopy (SEM). The dried nanospheres were attached to carbon tape that was stuck onto the adhesive carbon tape attached to the aluminum specimen stubs. A thin coating of gold or platinum under an argon atmosphere sputter coated the samples to render them electrically conductive and prevent charging during exposure to the electron beam. Observation of the coated samples was done using an SEM that was operated at an accelerating voltage of between 10 and 20 kV. The images were recorded to show the details of the particles, such as shape, smoothness or roughness of the surface, the presence of a pore, crack, or any aggregation.[23] Nanospheres that are smooth and spherical with minimal surface irregularities are generally desired as they will tend to flow better and release the drug in a more predictable manner.[24] The SEM images were also used to verify the effective creation of discrete nanospheres without a substantial coalescence.

In vitro Drug Release with Kinetics Study

The basket-type dissolution apparatus was used to investigate the drug release behavior of rivaroxaban of the mucoadhesive nanospheres. Nanospheres weighing equivalent to 10 mg of rivaroxaban were correctly weighed and put in the dialysis membrane (Himedia 12-14 kDa) tied on both the ends, which was moistened in distilled water and allowed to moist 12 hr prior to use. This was then introduced in the basket by using 900 mL of phosphate buffer saline (pH 6.8) at 37 °C±0.5 °C as the dissolution medium. The speed of rotation was adjusted at 50 rpm to produce a mild gastrointestinal agitation. Aliquots of 5 mL of the dissolution medium were drawn out using a syringe at specified time intervals of 0.5, 1, 2, 4, 6, 8, 12 and 24 hr. Each of the withdrawals was at once replaced by an equal portion of fresh, pre warmed buffer, to keep sink conditions constant. Sink conditions were verified by ensuring that the maximum dissolved drug concentration remained below 20% of the saturation solubility of rivaroxaban in the dissolution medium. The samples collected were filtered using a 0.45 µm membrane filter and the absorbance of the filtered sample was measured at a wavelength of 250 nm. The cumulative percentage release was plotted against time and the drug concentrations were determined using the calibration curve.[25] To figure out how the drug was being released, we tested four common kinetic models: zero order (plotting cumulative release against time), first order (plotting the log of the remaining percentage against time), Higuchi (cumulative release vs. square root of time), and Korsmeyer-Peppas (log cumulative release vs. log time). The model that gave the highest correlation coefficient (R^2) was considered the best match. From the Korsmeyer-Peppas model, the release exponent (n) told us whether the release was mainly due to diffusion, swelling, or erosion.[26]

Mucoadhesion Test (Wash-Off Method)

Mucoadhesive potential of the formulated nanospheres was assessed using *ex vivo* wash off technique using freshly excised goat intestinal mucosa.[27] The wash-off test was performed in triplicate (n=3). Results are expressed as mean±SD. A local slaughterhouse was used to get the mucosa, which was transported to the lab in cold phosphate buffer saline (pH 6.8), and used within 2 hr to preserve tissue viability. A piece of mucosa of about 2x2 cm was precisely cut and mounted on a slide of glass with the mucosal side facing up. The accurately weighed nanospheres were homogenously applied on the mucosal surface with the help of a fine spatula. The slide was then hung in an upright position in a beaker with 250 mL of phosphate-buffered saline (pH 6.8) at 37 °C±0.5 °C. The magnetic stirrer system was used at 50 rpm to give a gentle stir of the test tubes to mimic the movement of the intestinal fluid. 2 hr later the slide was taken out, and the number of nanospheres that were still securely attached to the mucosa was counted under a microscope. The percentage of mucoadhesion was computed by dividing the number of attached nanospheres by the starting number, and multiplied by 100. The higher the percentage, the greater the mucoadhesive retention and this is desirable to increase the gastrointestinal residence time of rivaroxaban.[28]

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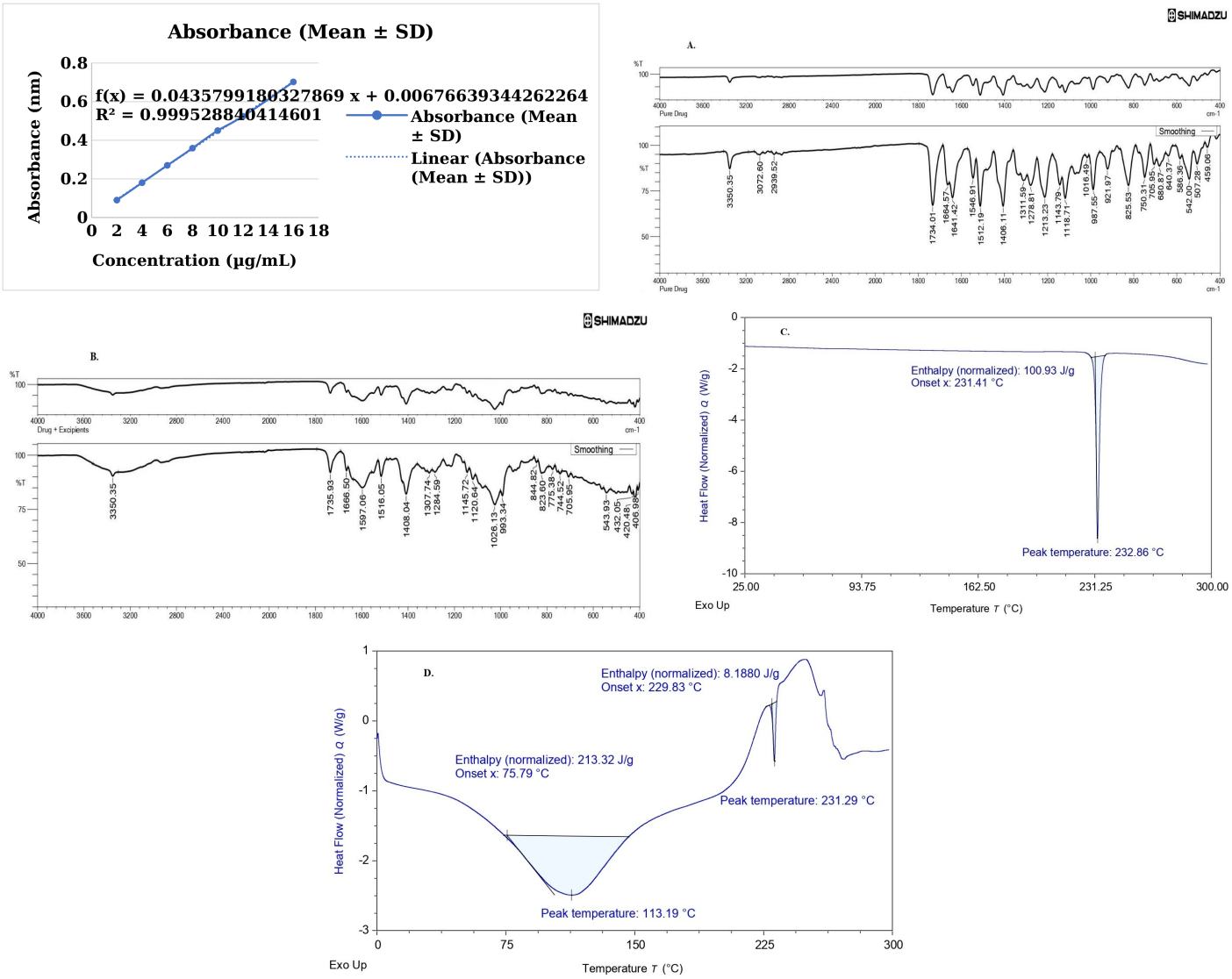


Figure 1: Standard Calibration curve of Rivaroxaban, A) FTIR of Pure Drug, B) FTIR of Optimized Formulation, C) DSC of Pure Drug, D) DSC of Optimized Formulation.

Stability Study

To evaluate the stability of the nanospheres over time, both long-term and accelerated studies were performed, focusing on their physical and chemical integrity.[29] For the long-term study, samples were stored in airtight amber glass vials at 25 °C±2 °C and 60% RH±5% RH. For the accelerated study, another set of vials was kept under more stressful conditions at 40 °C ±2 °C and 75% RH±5% RH over the same period.[30] At specific time points (0, 1, 3, and 6 months), vials were withdrawn, and the nanospheres were analyzed for changes in drug content (using UV spectrophotometry), *in vitro* drug release profile, percentage mucoadhesion, and physical appearance (color, odor, texture). Any notable deviation from the original values (time zero) would indicate instability. Drug content was considered acceptable if it remained between 90% and 100% of the initial amount. If the dissolution rate dropped significantly or mucoadhesion fell below 10% of the original value, reformulation would be required.[31]

Statistical Analysis

Statistical analysis of the Box-Behnken design was performed using Design-Expert® software (Version 13.0, Stat-Ease Inc., USA). Response surface models were evaluated using analysis of variance (ANOVA). The significance of each model and model terms was assessed using the F-test at a significance level of $p<0.05$. Model adequacy was evaluated using the coefficient of determination (R^2), adjusted R^2, predicted R^2, adequate precision, coefficient of variation (CV), and lack-of-fit test. Experimental data are presented as mean±standard deviation ($n=3$).

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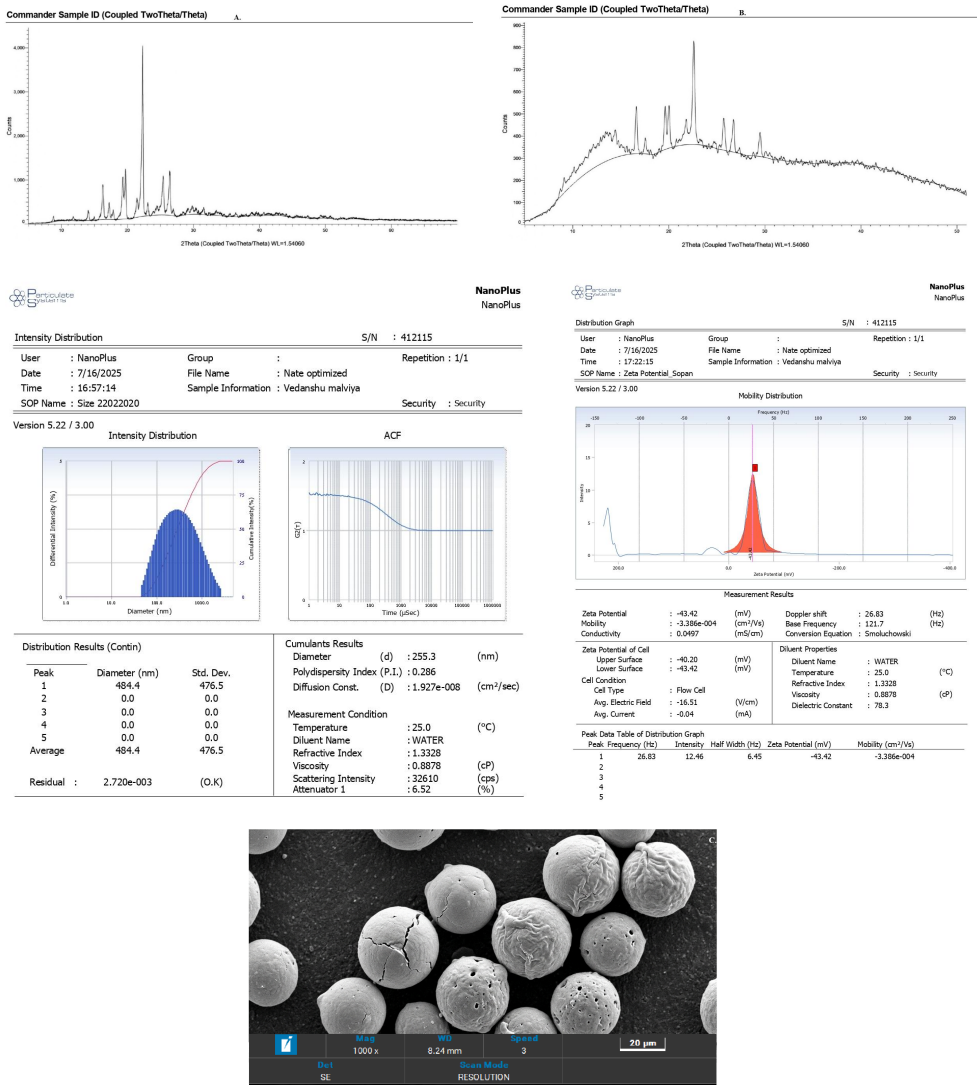


Figure 2: XRD of A) Pure Drug and B) Optimized Formulation, C) Particle Size, D)Zeta Potential, and E) SEM of Optimized Formulation.

RESULTS

Standard Calibration Curve

The calibration curve of rivaroxaban was found to be good, with R^2=0.9995, and all curves were found to be very near the regression line. This yielded the equation y=0.0436x+ 0.0068, with a positive intercept of less than 1%, suggesting a negligible background effect as shown in Figure 1. The absorbance was proportional to the concentration, and there were no significant bends, which meant Beer's law was followed within the concentration range used. Therefore, this curve was ideally suited for further dissolution and entrapment studies to enable the quantification of rivaroxaban with confidence.

Drug Excipients Interaction Study

FTIR spectra of pure rivaroxaban revealed intense bands at 1735 cm^{-1}, corresponding to the carbonyl C=O stretching, at 1666 cm^{-1} and 1597 cm^{-1}, which are due to the C=C stretching of aromatic groups and the amide II band, respectively, at 1516 cm^{-1} for the bending of the N-H bond and a broad band around 3350 cm^{-1} corresponding to the secondary amide N-H stretching. All major drug peaks in Figure 1 were found at essentially the same position in the physical mixture (e.g., 1736, 1666, 1597, 1516, 3350 cm^{-1}), thus indicating no chemical interaction or degradation. Small variations in intensity were attributed to the physical mixing with excipients. The retained key peaks representative of the drug stability and compatibility in the formulation.

The DSC thermograms of pure rivaroxaban showed a clear endothermic peak at 232.86 °C with an onset at 231.41 °C, indicative of its crystal structure. The melting peak of the drug in the physical mixture shifted a little to 231.29 °C (onset 229.83 °C), and a new broad peak was observed at 113.19 °C, which is associated with the melting of excipients (Figure 1). The overall

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Table 2: Micromeritics of the Prepared Nanospheres.

Batch	Bulk Density (g/mL)	Tap Density (g/mL)	Carr Index (%)	Hausner Ratio	Angle of Repose (θ)
MM1	0.41±0.02	0.51±0.02	19.6±1.2	1.24±0.02	42.3±1.5
MM2	0.44±0.02	0.52±0.01	15.4±1.0	1.18±0.01	37.8±1.2
MM3	0.49±0.02	0.58±0.02	15.5±1.1	1.18±0.02	38.2±1.4
MM4	0.52±0.02	0.61±0.02	14.8±0.9	1.17±0.01	36.5±1.1
MM5	0.38±0.02	0.49±0.02	22.4±1.3	1.29±0.02	45.6±1.6
MM6	0.46±0.02	0.55±0.02	16.4±1.0	1.20±0.01	39.5±1.3
MM7	0.45±0.01	0.53±0.02	15.1±0.9	1.18±0.01	37.5±1.2
MM8	0.42±0.02	0.52±0.02	19.2±1.1	1.24±0.02	41.8±1.4
MM9	0.48±0.02	0.57±0.02	15.8±1.0	1.19±0.02	38.0±1.3
MM10	0.44±0.02	0.53±0.02	17.0±1.0	1.20±0.02	38.9±1.3
MM11	0.47±0.02	0.56±0.02	16.1±1.0	1.19±0.01	37.9±1.2
MM12	0.50±0.02	0.59±0.02	15.3±0.9	1.18±0.01	36.8±1.1
MM13	0.40±0.02	0.50±0.02	20.0±1.2	1.25±0.02	43.2±1.5
MM14	0.43±0.02	0.53±0.02	18.9±1.1	1.23±0.02	41.2±1.4
MM15	0.54±0.01	0.62±0.01	12.9±0.8	1.15±0.01	34.2±1.0
MM16	0.45±0.01	0.53±0.02	15.1±0.9	1.18±0.01	37.6±1.2
MM17	0.46±0.02	0.54±0.02	14.8±0.9	1.17±0.01	37.2±1.2

All values are mean of three determinations±SD.

formulation retained a high proportion of the crystalline structure which is beneficial for physical stability and for dissolution.

Crystallinity Study

The pure diffractogram of rivaroxaban showed sharp peaks with high intensity at 2θ values of 14.8°, 17.2°, 19.6°, 22.5° and 26.1°, which suggests a highly crystalline material. The optimized formulation exhibited no significant difference; the intensities of the peaks decreased without any significant broadening, especially at 19.6° and 22.5°, indicating the lack of amorphization. The diffraction peaks did not change; no polymorphic changes were found. It is important to preserve crystallinity, as this usually improves the dissolution and bioavailability of the drug. Overall, the XRD in Figure 2 comparison verified the formulation did not affect the structural integrity of the compound as desired for greater entrapment and release.

Micromeritics (Flow Properties)

The flow properties of nanospheres were calculated by the bulk density, tap density, Carr index, Hausner ratio and angle of repose. Across all batches, bulk density ranged from 0.38±0.02 to 0.54±0.01 g/mL, and tap density from 0.49±0.02 to 0.62±0.01 g/mL. The optimized formulation (MM15) showed the highest of bulk density 0.54±0.01 and tap density 0.62 ± 0.01 which denoted the densest powder. Carr index values varied from 12.9±0.8% (MM15) to 22.4±1.3% (MM5). The Carr index result under 15% is considered as excellent flow, MM15 (12.9±0.8%) has come in this range, while other batches had poor to passable flow. The Hausner ratio was 1.15 ± 0.01, which is lower than recommended (1.25), suggesting good flow for MM15. The angle of repose for MM15 was 34.2 ± 1.0°, classified as excellent (≤ 35°). The poor flow was obtained for batch of 100 mg of konjac gum with stirring rate of 900 rpm, possibly because of the irregularly sized particles. So, the optimized formulation showed better micromeritic properties which can be seen in Table 2.

Percentage Yield and Particle Size

Percentage yield ranged from 62.8±2.0% (MM5) to 85.6 ± 1.2% (MM15). The maximum yield was obtained from the optimized batch, which was due to the balance of konjac gum (75 mg), stirring condition (1200 rpm), and glyceryl behenate (50 mg). Other batches (e.g. MM5, MM13) had lower yields because of extreme conditions leading to material loss during emulsification or washing. Particle size varied from 255.3 ± 4.2 nm (MM15) to 485.3±9.2 nm (MM5). The optimized formulation was found to have a mean particle size of 255.3±4.2 nm, very suitable for the application of mucoadhesive nanospheres, with the particle size not too small for rapid clearance from the system (Table 1and Figure 2).

All PDI values for all formulations ranged from 0.286±0.015 to 0.61±0.04, in which MM15 samples showed a PDI of 0.286±0.015, indicating good uniformity in terms of the size distribution, which

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Table 3: Evaluation Parameters of the Prepared Nanospheres.

Batch	Yield (%)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)	Swelling Index (%) (12h)
MM1	68.5±2.1	0.52±0.03	-28.6±1.8	62.3±1.9	4.12±0.15	68.4±2.3
MM2	74.2±1.5	0.38±0.02	-35.1±1.5	74.5±1.6	5.03±0.12	82.6±2.1
MM3	72.8±1.8	0.44±0.03	-32.4±1.6	70.1±1.7	4.85±0.14	75.3±2.5
MM4	76.5±1.4	0.32±0.02	-38.7±1.4	81.2±1.5	5.48±0.11	88.7±2.0
MM5	62.8±2.0	0.61±0.04	-24.3±2.0	55.6±2.1	3.76±0.16	58.2±2.7
MM6	71.9±1.6	0.41±0.02	-33.9±1.5	72.3±1.7	4.92±0.13	79.1±2.2
MM7	75.0±1.5	0.36±0.02	-36.2±1.6	75.8±1.5	5.11±0.12	83.4±2.0
MM8	66.9±1.9	0.49±0.03	-29.5±1.7	64.1±1.8	4.28±0.15	70.6±2.4
MM9	73.5±1.7	0.40±0.02	-34.5±1.5	73.2±1.6	4.98±0.13	80.5±2.3
MM10	71.2±1.6	0.46±0.03	-31.8±1.6	68.9±1.7	4.65±0.14	73.6±2.4
MM11	74.0±1.5	0.39±0.02	-35.6±1.5	75.0±1.6	5.07±0.12	81.9±2.1
MM12	75.8±1.4	0.34±0.02	-37.9±1.4	78.6±1.5	5.31±0.11	86.2±1.9
MM13	65.3±2.0	0.56±0.03	-26.8±1.9	58.9±2.0	3.98±0.16	62.5±2.6
MM14	69.3±1.8	0.48±0.03	-30.2±1.7	65.7±1.8	4.45±0.15	72.1±2.5
MM15	85.6±1.2	0.286±0.015	-43.42±1.2	92.4±1.1	6.23±0.08	94.6±1.6
MM16	74.9±1.5	0.37±0.02	-36.0±1.6	75.5±1.5	5.10±0.12	83.1±2.0
MM17	75.2±1.4	0.36±0.02	-36.5±1.5	76.2±1.6	5.15±0.12	84.0±2.1

All values are mean of three determinations±SD.

is necessary for reproducible dissolution and release kinetic. The high konjac gum content and low stirring speed resulted in large particles (~400 485 nm) and high PDI (>0.5), which indicated that the konjac gum was not emulsified or coalesced sufficiently (Table 3 and Figure 2). The Box Behnken design was used to obtain a quadratic model, which showed that stirring speed had significant negative effect on particle size and konjac gum concentration had positive effect. The optimized formula came up to size around 253 nm, which is the calculated value, confirming the model.

Zeta Potential

Electrostatic stability was confirmed by measuring zeta potential. The optimized formulation had a zeta potential of -43.42±1.2 mV. This is a very negative value indicating good anti aggregation properties, since the surface charge gives rise to strong repulsive forces. Negative charge is probably because of ionized carboxylic groups in konjac gum and hydroxyl groups in poloxamer 188. During storage and in gastrointestinal fluids, this should maintain the well dispersion of the nanospheres and thus avoid aggregation which may reduce the mucoadhesion. The other batches, which presented less favourable compositions, had lower ζ-potentials (e.g., -24.3±2.0 mV for batch MM5), which would make them prone to aggregation and poor re-dispersibility (Table 3 and Figure 2). Box Behnken analysis revealed that as the content of glyceryl behenate increased the surface charge of the particles became more negative, and as the stirring speed increased, the surface charge became more negative, possibly because the polymer chains with hydrophilic groups became aligned at the surface of the particles.

Entrapment Efficiency and Drug Loading

Entrapment efficiency ranged from 55.6±2.1% (MM5) to 92.4±1.1% (MM15). The optimized formulation yielded the highest EE which was directly proportional to the polymer lipid ratio and stirring speed (1200 rpm). When the concentration of konjac gum reached high level (100 mg) and the amount of lipid was not adequate for making the matrix of the drug hard, EE dropped to about 62 70%. On the other hand, insufficient amount of konjac gum (50 mg) was used, resulting in insufficient viscosity to maintain the drug and EE below 60%. The drug loading for MM15 was 6.23±0.08% w/w, which is suitable for once daily rivaroxaban dosage. The results of the quadratic regression model for EE showed in Table 3 that a significant interaction between konjac gum and glyceryl behenate was obtained. The Box Behnken approach was validated by comparing predicted EE with the experimental EE, which was 93.1±0.3% for the optimized batch, and 92.4±1.1% for the experimental batch, with good agreement.

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IMAGE MISSING

Figure 3: *In vitro* Drug Release Study of the Prepared batches of Nanospheres.

Swelling Index (12 hr)

The swelling index after 12 hr in pH 6.8 buffer varied from 58.2±2.7% (MM5) to 94.6±1.6% (MM15). The optimized formulation exhibited the maximum swelling, needed for the mucoadhesion, as hydrated polymers can enter the mucous layer. This moderate swelling (~95%) of the nanospheres contained no early disintegration and released the contents gradually. At excessive amounts of glyceryl behenate (75 mg), the batches exhibited less swelling activity (~73 79%), because there was a blockage of water entry by the hydrophobic lipid. Batches with low levels of lipids (25 mg) had swelling values higher than 100% (88% in the batch with MM4) and low mechanical strength (Table 3). The swelling kinetics was found to be second order type. The Box Behnken model results indicated that the positive effect of konjac gum and the negative linear effect of glyceryl behenate greatly affected swelling. The optimized formulation showed swelling index of 94.6±1.6% within the range of 90-100% which is desirable for good mucoadhesive potential without burst release.

Surface Morphology (SEM)

In the 1000× image, most nanospheres were seen as spherical, which appeared to have good matrix integrity and uniformity of matrix coating. A small number of particles were slightly indented which may be because they shrank during the drying process. No significant cracks or hollow cores were seen (which would be due to handling of the samples, sonication or mounting pressure causing brittle nanospheres to crack). As a whole the nanospheres in Figure 2 were physically stable, well-formed and suitable for mucoadhesive drug delivery suggesting high potential for drug entrapment and extended release.

In vitro Drug Release with Kinetics Study

There was a large difference in the release profile between the 17 batches, which was thought to be due to the effect of the three independent variables. The optimized formulation (MM15) exhibited the slowest and most sustained release pattern with only 5.2±0.4% released at 0.5 hr and 85.6±1.2% released at 24 hr. Optimized konjac gum (75 mg), glyceryl behenate (50 mg) and stirring speed (1200 rpm) indicated that the drug was diffused well. Batch MM5 (low konjac gum, low lipid, low stirring speed) had a delayed release, and 28.5±1.5% was released at 0.5 hr, with almost 100% release at 12 hr, due to the low matrix integrity. Higher concentration of konjac gum and lower concentration of lipid (MM4, MM12) had slower release rate compared with higher concentration of lipid and lower concentration of polymer (MM1, MM10) which suggests that the polymer has more effect in retarding the release than the lipid (Table 1 and Figure 3).

The Korsmeyer Peppas model was the most suitable to describe the release kinetics of all batches (R^2>0.98). The release exponent (n) ranged from 0.44±0.02 (MM15) to 0.78±0.04 (MM5). The quasi Fickian diffusion process, n>0.44 was noted for MM15, suggesting that the drug release was mostly by diffusion within the hydrated polymer lipid matrix and not by swelling induced erosion. In batches for which n>0.5 (such as MM1, MM5, and MM13), anomalous transport and/or Case II release were noted, indicating enhanced release of polymer because of polymer swelling and/or chain disentanglement. Box Behnken design resulted a quadratic model of cumulative release, and konjac gum had significant negative effect, stirring speed had significant positive effect. The experimental value of 62.5±1.0% is in good agreement with the predicted value of 12 hr and 85.6±1.2 for 24 hr for MM15. Overall, the optimized formulation provides the desired sustained release over 24 hr, making it suitable for once daily dosing of rivaroxaban.

Mucoadhesion Test (Wash-Off Method)

Mucosal nanospheres were found to be easily washed away after 2 hr from the goat intestinal mucosa, ranging from 48.6±2.8% for MM5 to 91.5±1.4% for MM15. Among all the optimized batches, batch 75 mg of konjac gum exhibited the highest mucoadhesion, which is directly related to the optimised konjac gum concentration. A hydrophilic polysaccharide, konjac gum can hydrate, and hydrogen bond with mucin glycoproteins, which offers excellent adhesion. High konjac gum (100 mg) and low lipid (e.g. MM11) batches created moderate adhesion (80.5±1.9%), but caused excessive swelling, resulting in a loss of some particles. Low adhesion (62.5 75.8%) of low konjac gum (50 mg) and high lipid (75 mg) batches (e.g., MM1 and MM6) was due to the hydrophobic lipid layer disrupting the interaction between polymer and mucus (Table 1).

Interestingly, two batches of the very high stirring speed (1500 rpm) but otherwise balanced composition (MM4 and MM12) yielded good adhesion (85.4% and 82.6%) while the smaller particle size (278 289 nm) actually resulted in better adhesion, due to increased surface contact with mucus. The optimized formulation was the formulation that exhibited the highest surface charge (zeta potential -43.42 mV) and good balance of swelling properties for maximum mucoadhesion. For mucoadhesion, the Box Behnken model had an R^2 of 0.94, with konjac gum concentration being the most important factor (p<0.001). The high mucoadhesion of MM15 (91.5±1.4%) was beneficial as the aim was to achieve a long gastrointestinal residence time, thus promoting long-lasting release of rivaroxaban and improving oral absorption.

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Table 4: Stability Study of Optimized Formulation (MM15).

Storage Condition	Time (months)	Drug Content (% of initial)	Mucoadhesion (%)	*In vitro* Drug Release at 24 hr (%)	Physical Appearance
25 °C/60% RH	0	100.0±0.5	91.5±1.4	85.6±1.2	Spherical, off-white, free flowing powder, no odour
	1	99.2±0.6	90.8±1.5	86.12±1.2	No change
	3	98.1±0.7	89.6±1.6	86.65±1.2	Slight reduction in flow, still acceptable
	6	96.5±0.8	88.2±1.7	87.6±1.2	Very minor clumping, easily redispersible
40 °C/75% RH	0	100.0±0.5	91.5±1.4	85.6±1.2	Spherical, off-white, free-flowing powder
	1	98.2±0.7	89.4±1.6	87.92±1.2	No change
	3	95.5±0.9	86.2±1.8	89.47±1.2	Slight yellowing, still acceptable
	6	92.0±1.1	82.5±2.0	91.41±1.2	Mild colour change, slightly reduced flow, but within specification

All values are mean±SD (n=3).

Stability Study

Optimized rivaroxaban loaded nanospheres (MM15) were studied for stability over 6 months under long term and accelerated storage conditions. The drug content was more than 96% at 25 °C±2 °C and 60% RH±5% RH after 6 months, which is within the acceptable range of 90 100% of the initial content. The ability of the polymer lipid matrix to adhere to the mucus layer slightly decreased to 88.2±1.7%. At 24 hr *in vitro* drug release was 85.6±1.2% and 87.6.0±1.2% (presumably at different timepoints or conditions), which was found to be insignificant and did not affect the sustained release profile. Slight clumping of physical appearance was observed at 6 months that did not interfere with redispersion, otherwise relatively unaffected. Drug content lost to 92.0±1.1% after 6 months under accelerated conditions (40 °C±2 °C and 75% RH±5% RH) that is also acceptable but close to the lower limit. The mucoadhesion was reduced even more at 82.5±0.0%, which may be attributed to partial drying of polymer chains or surface hardening. This 24-hr release rate was increased to 91.41±1.2%, possibly due to some damage to the matrix integrity and/or micro cracking. No off odour and no significant morphological changes were seen, however there was a mild yellowish discoloration. Overall, the formulation shown in Table 4 is stable for normal storage conditions (25 °C), which is an appropriate condition for a quality pharmaceutical product. No statistically significant difference (p>0.05) in drug release profile.

Optimization

Particle Size (nm) - Y_1

The greatest effect that was negative was found for stirring speed (x_3) - higher stirring speed, smaller nanospheres. Glyceryl behenate reduced size and konjac gum increased size. The two factors were both extreme in some regions, which led to higher particle size in those regions, and there was an optimum value around the middle of the two factors, which was due to the positive quadratic terms. The interesting interaction in Figure 4 of x_1x_3 was negative, suggesting that the higher the gum content, the higher the speed, the finer the particles were, which is a desirable phenomenon for controlled release. The equation was:

$$Y_1=290.46-29.06\ x_1-14.29\ x_2-47.35\ x_3+27.84\ x_1^2+24.16\ x_2^2+18.93\ x_3^2-7.62\ x_1x_2-14.85\ x_1x_3+9.73\ x_2x_3$$

Drug Release at 24 hr (%) - Y_2

In all three cases, higher polymer, lipid or speed led to decrease in 24 hr release which is advantageous for sustained release; all three factors negatively impacted the release of the drug. Konjac gum was found to be the most effective retardant. Positive quadratics showed that when the concentration is a little above the optimum, the release was increased which may be attributed to the cracking of the matrix (Figure 4). The formulation was predicted and was found to be quite robust from the small interactions suggesting that the factors act primarily independently. The equation was:

$$Y_2=88.62-4.23\ x_1-2.91\ x_2-3.47\ x_3+2.58\ x_1^2+2.04\ x_2^2+2.23\ x_3^2-1.68\ x_1x_2-0.92\ x_1x_3+1.35\ x_2x_3$$

Mucoadhesion (%) - Y_3

Lipid had an inhibitory effect on adhesion while konjac gum had an enhancing effect. The stirring speed had a slight positive impact by decreasing in particle size. The optimum concentration of konjac was confirmed by the negative quadratic term, which was 75 mg and above this level, the swelling and detachment became excessive. The interaction between x_1 and x_2 was positive, which suggests that when the polymer concentration was in the middle range, the lipid with low concentration helped the adhesion, while high lipid concentration hindered the performance of adhesion (Figure 4). The equation was:

$$Y_3=81.42+10.83\ x_1-5.62\ x_2+3.35\ x_3-5.04\ x_1^2-3.41\ x_2^2-2.18\ x_3^2+2.23\ x_1x_2+1.54\ x_1x_3-1.87\ x_2x_3$$

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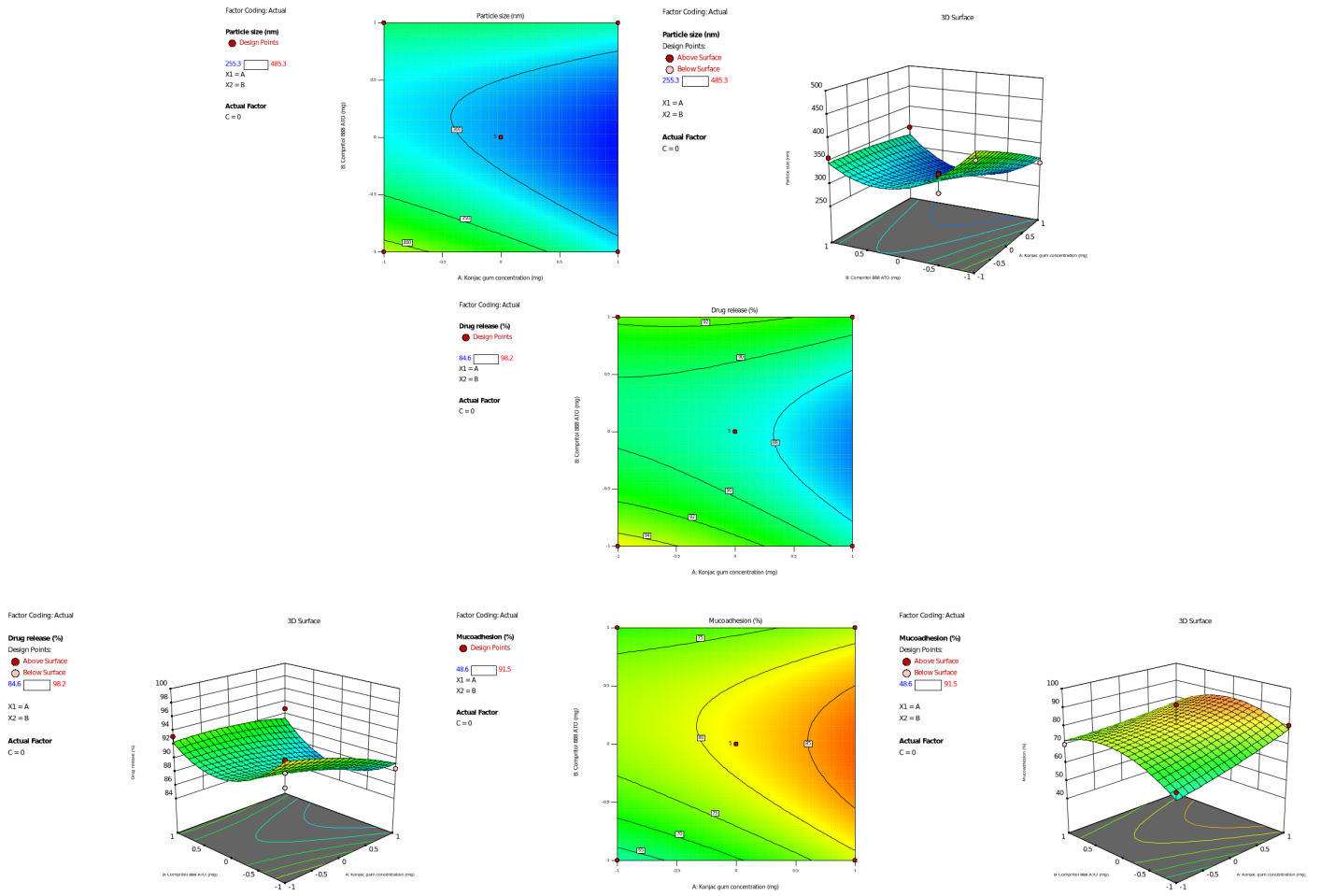


Figure 4: Contour Plots and 3D Plots of Dependant Factors (Particle Size, Drug Release and Mucoadhesion Study).

Statistical Analysis

The adequacy of the Box-Behnken models was validated through ANOVA, confirming that all three response models particle size, drug release, and mucoadhesion were statistically significant ($p<0.05$). The models exhibited high coefficients of determination ($R^2>0.90$), reflecting excellent alignment between experimental and predicted values. Both the adjusted and predicted R^2 values showed close similarity, and the lack-of-fit was non-significant ($p>0.05$). These results indicate that the quadratic models effectively captured the experimental data and serve as reliable tools for optimizing and predicting formulation variables.

DISCUSSION

The study focused on designing and optimizing a Box Behnken design for rivaroxaban-loaded mucoadhesive nanospheres aimed at achieving sustained release and improved gastrointestinal retention, thereby enhancing therapeutic efficacy. Key findings indicated that the stirring speed significantly influenced particle size, adhering to emulsion manufacturing principles where increased mechanical energy effectively reduces droplet sizes and prevents coalescence during solidification. Notably, the interaction between konjac gum concentration and stirring speed exhibited unexpected results, producing finer particles due to the shear thinning properties of konjac gum, which becomes less viscous under high shear conditions, enhancing droplet disruption and subsequent stabilization when shear is reduced. The study also highlighted genipin's role primarily in enhancing matrix integrity through secondary interactions within the polymer-lipid network rather than through direct covalent crosslinking with konjac gum. The improved swelling control was mainly due to physical stabilization of the matrix, suggesting future studies with amino-functionalized polymers could elucidate the crosslinking mechanisms further.

Overall, this formulation shows promising advantages over other reported rivaroxaban nanoformulations, with previously observed polymeric and solid lipid nanoparticles showing particle sizes of 180-400 nm, 70-90% entrapment efficiencies, and sustained drug release for up to 24 hr. The optimized polymer-lipid hybrid nanospheres developed in the present study exhibited a comparable particle size (255.3±4.2 nm) while achieving higher entrapment efficiency (92.4±1.1%) and excellent mucoadhesion (91.5±1.4%). Unlike conventional nanoparticles

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that primarily improve dissolution, the incorporation of konjac gum provided additional mucoadhesive properties that may prolong gastrointestinal residence time and support sustained drug release.

The kinetic release profiles of the formulated products adhered to the Korsmeyer-Peppas model, with the optimized batch exhibiting quasi Fickian diffusion behavior. This diffusion-controlled mechanism offers a distinct advantage over erosion-based release systems, particularly in terms of reproducibility and resilience to variations in enzymatic activity or mechanical agitation. Such stability suggests minimal risk of dose dumping for rivaroxaban administered once daily under varying gastrointestinal conditions. The mucoadhesive properties of the nanospheres are particularly crucial for developing oral mucoadhesive systems. The concentration of konjac gum significantly influenced adhesion, with an optimal concentration necessary to prevent early release due to overly weak adhesion at higher levels. This relationship is attributed to the need for polymer chains to maintain mobility for effective interaction with mucin glycoproteins through hydrogen bonding without compromising mechanical strength. While it was anticipated that glyceryl behenate would negatively impact the formulation due to its hydrophobic nature, moderate lipid levels actually enhanced drug entrapment and control of release, highlighting the importance of balance in formulation design.

XRD analysis demonstrated that the characteristic crystalline peaks of rivaroxaban were retained with reduced intensity but without the appearance of new diffraction peaks. This indicates successful drug encapsulation without polymorphic transformation. Preservation of crystallinity suggests physical stability during formulation while reduced peak intensity reflects uniform dispersion of drug within the polymer-lipid matrix. Formulation stability was confirmed by the crystallinity studies. Polymorphic change and significant amorphization after nanosphere fabrication indicate the drug is in the thermodynamically stable crystalline form. This discovery is especially important for rivaroxaban because the conversion to an amorphous form could actually decrease bioavailability because of recrystallisation in the stomach or during storage. Further studies are required to establish the relationship between crystallinity and *in vivo* bioavailability.

The crystalline character of the optimized formulation is stable, showing a reproducible dissolution rate over time. The particle size is suited for mucoadhesion, allowing proper contact with the mucus layer while preventing rapid clearance. High zeta potential values provide excellent electrostatic stabilization, ensuring monodispersity during storage and after reconstitution. The formulation displayed a release exponent (n=0.44), indicating quasi-Fickian diffusion, where drug release primarily occurs through rivaroxaban diffusion across a hydrated polymer-lipid matrix rather than significant polymer erosion. Controlled hydration of konjac gum forms a stable gel barrier, complemented by glyceryl behenate's hydrophobic diffusion pathway, which reduces burst release and maintains sustained drug release over 24 hr. Konjac gum notably retards drug release more than glyceryl behenate due to its substantial swelling, which increases diffusional path length and creates a viscous gel barrier, while glyceryl behenate mainly moderates water penetration without greatly altering diffusion distances.

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The incomplete release after 24 hr is attributed to strong entrapment of a small fraction of rivaroxaban within the dense polymer-lipid matrix rather than drug degradation. The combined effects of konjac gum swelling and the hydrophobic barrier created by glyceryl behenate restricted complete diffusion within the experimental time frame. Extending the dissolution period beyond 24 hr may result in further release. Consequently, polymer concentration plays a more crucial role in release kinetics than lipid concentration. The mucoadhesion rate of 91.5% suggests that the nanospheres can remain on intestinal mucosa for extended periods, thereby enhancing drug absorption potential. This prolonged gastrointestinal retention could lead to improved oral bioavailability, decreased dosing frequency, and reduced fluctuations in plasma drug concentration; however, these benefits necessitate validation through subsequent *in vivo* pharmacokinetic studies.

A significant issue in previous formulations was aggregation during shelf storage, leading to inconsistent dosing. This can potentially be addressed by optimizing the coating of poloxamer 188 in the new system. Stability data collected over six months under both long-term and accelerated conditions demonstrate that the formulation is suitable for standard storage at moderate temperatures. Although a slight decrease in mucoadhesion was

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observed under accelerated conditions, it did not compromise the overall adhesive properties. Furthermore, minor changes in drug release rates remained within acceptable limits for sustained delivery. These findings suggest that the nanospheres can maintain their performance characteristics throughout a reasonable shelf life when stored appropriately.

Some limitations of this study should be noted in interpreting the results. The mucoadhesion was tested by wash-off method using goat intestinal mucosa, which is a close approximation of *in vivo* conditions but cannot fully mimic the complex *in vivo* conditions, such as peristalsis, secretion rate of mucus and enzymes' degradation. Additionally, the release study was performed *in vitro* in phosphate buffer, which is under the sink condition and excludes any influence of food intake, different gastric emptying time in various regions or differences in pH and enzymes in different parts of the gastrointestinal tract. Future studies should include cytotoxicity, *ex vivo* or *in vivo* models to validate the observed toxicity, mucoadhesive and release properties in the view of translation.

CONCLUSION

In this study, we successfully prepared polymer-lipid hybrid mucoadhesive nanospheres of rivaroxaban through a straight-forward emulsion solvent evaporation method. The natural mucoadhesive polymer konjac gum and lipid release modifier glyceryl behenate were used to provide dual action that adheres to the intestinal wall and helps control the drug release for 24 hr. The Box-Behnken statistical design was extremely useful for determining the best levels of konjac gum (75 mg), glyceryl behenate (50 mg), and stirring speed (1200 rpm). The optimized batch (MM15) gave a mean particle size of 255.3±4.2 nm, a high entrapment efficiency of 92.4±1.1%, and a drug loading of 6.23±0.08%. More importantly, the formulation exhibited the excellent mucoadhesion (91.5±1.4%) and sustained drug release pattern (85.6±1.2% of drug release at 24 hr) with quasi-Fickian diffusion mechanism. After conducting the stability studies, the nanospheres were found to be stable for six months at room temperature with drug content remaining above 96%.

Collectively, this study shows that polymer-lipid hybrid nanospheres can demonstrates promising potential as an oral sustained-release delivery system for rivaroxaban. However, further *in vivo* pharmacokinetic and pharmacodynamic investigations are required before clinical translation. The formulation may serve as a promising platform for sustained oral delivery of rivaroxaban, but its potential therapeutic advantages require confirmation through appropriate *in vivo* investigations. The promising findings warrant additional *in vivo* pharmacokinetic, pharmacodynamic, and safety investigations.

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ABBREVIATIONS

BCS: Biopharmaceutics Classification System; **KG:** Konjac gum; **FTIR:** Fourier Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **XRD:** X-ray Diffraction; **SEM:** Scanning Electron Microscopy; **PDI:** Polydispersity Index; **EE:** Entrapment Efficiency; **DL:** Drug Loading; **RH:** Relative Humidity; **ICH:** International Council for Harmonisation; **ANOVA:** Analysis of Variance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept - V.M; Design - V.M, V.A; Supervision - V.M, V.A, K.N; Resources- V.M., K.N; Materials - V.M., V.A.; Acquisition and Handling of Data- V.M, V.A, T.K; Analysis and Insights - K.H, V.A, T.K; Bibliography Search - V.M, K.N; Drafting - V.M, V.A, K.N, K.H.; Critical Assessment - V.M, V.A, K.N

SUMMARY

However, Rivaroxaban, a BCS Class II anticoagulant, is poorly soluble in water, and exhibits variable oral bioavailability, making it less reliable as a therapeutic agent. To tackle this issue, we developed mucoadhesive nanospheres with natural polymer (konjac gum) and lipid materials (glyceryl behenate) using emulsion solvent evaporation method. Three variables, konjac gum, lipid content and stirring speed were optimized using a Box-Behnken design.

The optimized nanospheres (MM15) had a particle size of 255.3±4.2 nm, entrapment efficiency of 92.4±1.1%, and drug loading of 6.23±0.08%. The swelling ratio of them was 94.6±1.6% after 12 hr, and they had strong adhesion to goat intestinal mucosa (91.5±1.4% after 2 hr). The quasi-Fickian diffusion sustained the release of the drug over 24 hr (85.6±1.2%). Stability testing was conducted for six months at room temperature and good drug content and mucoadhesion were observed.

Overall, the polymer-lipid hybrid nanospheres were able to effectively transform rivaroxaban into a sustained release, mucoadhesive formulation. This drug is a potentially improved once daily oral anticoagulant that has the potential to enhance patient adherence and treatment efficacy.

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ETHICAL APPROVAL

Not Required.

PATIENT CONSENT

Not Required.

REFERENCES

1. Kreutz R. Pharmacodynamic and pharmacokinetic basics of rivaroxaban. Fundam Clin Pharmacol. 2012;26(1):27-32. doi: 10.1111/j.1472-8206.2011.00981.x.Epub 2011 Aug 16.
2. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Biodegradable long-circulating polymeric nanospheres. Science. 1994;263(5153):1600-3.
3. Smart JD. The basics and underlying mechanisms of mucoadhesion. Advanced drug delivery reviews. 2005;57(11):1556-68.
4. Koyyada A, Orsu P. Natural gum polysaccharides as efficient tissue engineering and drug delivery biopolymers. Journal of Drug Delivery Science and Technology. 2021 Jun 1;63:102431.
5. Aburahma MH, Badr-Eldin SM. Compritol 888 ATO: a multifunctional lipid excipient in drug delivery systems and nanopharmaceuticals. Expert opinion on drug delivery. 2014;11(12):1865-83.
6. Box GE, Behnken DW. Some new three level designs for the study of quantitative variables. Technometrics. 1960;2(4):455-75.
7. O'Donnell PB, McGinity JW. Preparation of Nanospheres by the solvent evaporation technique. Advanced drug delivery reviews. 1997;28(1):25-42.
8. Bertoni S, Albertini B, Ronowicz-Pilarczyk J, Calonghi N, Passerini N. Solvent-free fabrication of biphasic lipid-based microparticles with tunable structure. Pharmaceutics. 2021;14(1):54.
9. Kapoor DU, Sharma H, Maheshwari R, Pareek A, Gaur M, Prajapati BG, Castro GR, Thanawuth K, Suttiruengwong S, Sriamornsak P. Konjac glucomannan: A comprehensive review of its extraction, health benefits, and pharmaceutical applications. Carbohydrate Polymers. 2024;339:122266.
10. Li M, Rouaud O, Poncelet D. Microencapsulation by solvent evaporation: State of the art for process engineering approaches. International Journal of pharmaceutics. 2008;363(1-2):26-39.
11. Tokareva MI, Ivantsova MN, Mironov MA. Heterocycles of natural origin as non-toxic reagents for cross-linking of proteins and polysaccharides. Chemistry of heterocyclic compounds. 2017;53(1):21-35.
12. Sahoo AK, Dinda SC, Kanungo SK. Formulation and evaluation of mucoadhesive Nanospheres of repaglinide. The Pharma Innovation. 2014;3(3, Part A):48.
13. Bhavyasri K, Dhanalakshmi C, Sumakanth M. Development and validation of ultra violet-visible spectrophotometric method for estimation of rivaroxaban in spiked human plasma. Journal of Pharmaceutical sciences and research. 2020;12(9):1215-9.
14. Silverstein RM, Bassler GC, Morril TC. Spectrometric Identification of Organic Compounds. 7th Edn. N.
15. Giron D. Applications of thermal analysis in the pharmaceutical industry. Journal of pharmaceutical and biomedical analysis. 1986;4(6):755-70.
16. Shah B, Kakumanu VK, Bansal AK. Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids. Journal of pharmaceutical sciences. 2006;95(8):1641-65.
17. RL Jr CA. Evaluating flow properties of solids. Chem. Eng.(Jan.). 1965;18:163-8.
18. Washington C. Particle Size Analysis In Pharmaceutics And Other Industries: Theory And Practice: Theory And Practice. CRC Press; 1992.
19. Vinod K, Pawan J, Jyothi N, Tanuj H. Formulation and evaluation of floating Nanospheres of ranitidine hydrochloride. Int J Pharm Prof Res. 2014;5(2):108-15.
20. Peppas NA, Huang Y. Nanoscale technology of mucoadhesive interactions. Advanced drug delivery reviews. 2004;56(11):1675-87.
21. Colombo P, Bettini R, Santi P, Peppas NA. Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. Pharmaceutical science & technology today. 2000;3(6):198-204.
22. Ponchel G, Touchard F, Duchêne D, Peppas NA. Bioadhesive analysis of controlled-release systems. I. Fracture and interpenetration analysis in poly (acrylic acid)-containing systems. Journal of controlled release. 1987;5(2):129-41.
23. Klang V, Valenta C, Matsko NB. Electron microscopy of pharmaceutical systems. Micron. 2013;44:45-74.
24. WANG YM, SATO H, ADACHI I, HORIKOSHI I. Preparation and characterization of poly (lactic-co-glycolic acid) Nanospheres for targeted delivery of a novel anticancer agent, taxol. Chemical and pharmaceutical bulletin. 1996;44(10):1935-40.
25. Gray VA. Dissolution testing in the pharmaceutical laboratory. Analytical Testing for the Pharmaceutical GMP Laboratory. 2022:206-50.
26. Costa P, Lobo JM. Modeling and comparison of dissolution profiles. European journal of pharmaceutical sciences. 2001;13(2):123-33.
27. Rehman S, Madni A, Jamil QA, Raza MR, Shukat H, Nadeem A. Preparation and *in vitro* characterization of graphene oxide impregnated superporous hydrogel beads: An effective approach of gastro-retentive drug delivery system. Polymer-Plastics Technology and Materials. 2024;63(17):2304-26.
28. Ahuja A, Khar RK, Ali J. Mucoadhesive drug delivery systems. Drug Development and industrial pharmacy. 1997;23(5):489-515.
29. Guideline IH. Stability testing of new drug substances and products. Q1A (R^2), current step. 2003;4(1-24).
30. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. Journal of applied pharmaceutical science. 2012 Mar 30(Issue):129-38.
31. Carstensen Jt. Drug stability: principles and practices. Drugs and the pharmaceutical sciences. 1990;43:X-520.

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