

DESIGN, DEVELOPMENT AND CHARACTERIZATION OF COLON-SPECIFIC MICROSPHERES CONTAINING VONOPRAZAN FUMARATE

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Abstract

The present study was aimed at the design, development, and characterization of colon-specific microspheres containing Vonoprazan fumarate for targeted drug delivery to the colon. Chitosan microspheres were prepared by the ionotropic gelation method using sodium tripolyphosphate (STPP) as a cross-linking agent. Six formulations (F1–F6) were developed by varying the concentrations of chitosan and STPP while maintaining a constant drug concentration. The prepared microspheres were evaluated for flow properties, percentage yield, drug entrapment efficiency, particle size, zeta potential, in-vitro drug release, release kinetics, and stability. The flow property studies revealed acceptable micromeritic characteristics for all formulations. The percentage yield ranged from $70.32 \pm 0.45\%$ to $82.25 \pm 0.25\%$, while drug entrapment efficiency ranged from $68.15 \pm 0.65\%$ to $79.32 \pm 0.74\%$. Formulation F5 exhibited the highest percentage yield and entrapment efficiency and was selected as the optimized formulation. Particle size and zeta potential analyses of F5 confirmed the formation of stable microspheres with suitable characteristics for controlled drug delivery. To achieve colon-specific targeting, the optimized microspheres were coated with Eudragit S100. In-vitro drug release studies demonstrated minimal drug release in simulated gastric fluid and enhanced release in colonic pH conditions. The coated microspheres released only 0.69% drug in acidic medium after 1 hour, while 93.32% cumulative drug release was achieved after 12 hours at colonic pH. Drug release kinetic analysis indicated that the release mechanism followed the Korsmeyer–Peppas model with an R^2 value of 0.9341, suggesting non-Fickian diffusion. Stability studies conducted for three months showed no significant changes in particle size, entrapment efficiency, or physical appearance under refrigerated conditions. The findings of the present investigation indicate that Eudragit S100-coated chitosan microspheres of Vonoprazan fumarate can serve as a promising colon-targeted drug delivery system with controlled release characteristics, enhanced stability, and potential therapeutic benefits.

1. Introduction

Oral drug delivery remains the most preferred route of administration due to its convenience, patient compliance, and cost-effectiveness. However, conventional oral dosage forms often fail to achieve site-specific drug delivery, leading to reduced therapeutic efficacy and increased systemic side effects [1-2].

Colon-specific drug delivery systems have emerged as an attractive approach for delivering drugs directly to the colon, particularly for the treatment of local

diseases such as inflammatory bowel disease [3], colorectal cancer, and infections, as well as for systemic delivery of proteins and peptides that are susceptible to degradation in the upper gastrointestinal tract [4].

The colon offers several physiological advantages for targeted drug delivery, including a near-neutral pH, prolonged transit time, relatively low enzymatic activity, and a diverse microbial

population capable of degrading specific polysaccharides ^[5].

These characteristics can be exploited to design formulations that protect the drug during transit through the stomach and small intestine and release it specifically in the colon ^[6]. Various approaches have been investigated for colon targeting, including pH-dependent systems, time-dependent systems, pressure-controlled systems, and microflora-activated delivery systems ^[7].

Microspheres are free-flowing spherical particles ranging from 1 to 1000 μm in size and have gained considerable attention as controlled and targeted drug delivery carriers. Microspheres provide several advantages such as enhanced drug stability, controlled drug release, improved bioavailability, reduced dosing frequency, and protection of drugs from harsh gastrointestinal conditions. Their small size and large surface area facilitate uniform distribution throughout the gastrointestinal tract, making them suitable candidates for colon-targeted delivery ^[8].

Vonoprazan fumarate is a novel potassium-competitive acid blocker (P-CAB) that suppresses gastric acid secretion by reversibly inhibiting the H^+ , K^+ -ATPase enzyme in gastric parietal cells ^[9]. Compared to conventional proton pump inhibitors, vonoprazan exhibits rapid

onset of action, prolonged acid suppression, and improved stability in acidic environments. It is widely used in the treatment of gastroesophageal reflux disease (GERD), peptic ulcers, *Helicobacter pylori* eradication therapy, and other acid-related disorders ^[10].

Despite its therapeutic advantages, conventional oral administration of vonoprazan may result in fluctuations in plasma drug concentration and frequent dosing requirements. Encapsulation of vonoprazan fumarate into microspheres can provide controlled release characteristics, improve drug stability, and enhance therapeutic effectiveness. Furthermore, the development of colon-specific microspheres may offer sustained drug release, reduced gastric irritation, and improved patient compliance ^[11].

The design of colon-specific microspheres generally involves the use of biodegradable polymers capable of protecting the drug during transit through the upper gastrointestinal tract and releasing it selectively upon reaching the colon. Natural and synthetic polymers such as chitosan, sodium alginate, pectin, Eudragit polymers, and ethyl cellulose have been extensively investigated for this purpose. These polymers can be formulated into microspheres using techniques such as ionotropic gelation,

solvent evaporation, emulsion cross-linking, and spray drying^[12].

1.1.1 Drawbacks of Oral Drug Delivery System

The oral route is the most widely accepted and convenient route of drug administration; however, it is associated with several physiological and pharmacokinetic limitations that can affect drug stability, absorption, bioavailability, and therapeutic efficacy. One of the major limitations is exposure to the harsh gastrointestinal (GI) environment. The highly acidic gastric environment, generally ranging from pH 1–2.5, can cause degradation or denaturation of acid-labile drugs, particularly proteins, peptides, and other biologically sensitive molecules. In addition, digestive enzymes such as pepsin, trypsin, chymotrypsin, and pancreatic lipases may enzymatically degrade susceptible active pharmaceutical ingredients before they reach the systemic circulation. The mucus layer covering the gastrointestinal tract can also act as a physical and diffusion barrier, particularly for poorly soluble, hydrophobic, or particulate drug delivery systems, thereby limiting their interaction with the absorptive epithelium^[13–16].

Another important limitation of oral drug delivery is associated with pharmacokinetic and metabolic processes.

Drugs absorbed from the gastrointestinal tract generally enter the portal circulation and subsequently pass through the liver, where extensive first-pass metabolism may occur. This can substantially reduce the fraction of the administered dose reaching systemic circulation and may result in low or variable bioavailability. Furthermore, orally administered drugs generally require disintegration, dissolution, gastric emptying, and intestinal absorption before producing a systemic therapeutic effect. Consequently, the onset of action may be slower than that achieved through parenteral or certain other routes of administration. Absorption may also show considerable inter- and intra-subject variability because of differences in gastric emptying, intestinal motility, gastrointestinal pH, food intake, enzymatic activity, and gut microbiota^[17].

The oral route also has important patient-related and clinical limitations. Oral dosage forms may not be suitable for unconscious, uncooperative, severely vomiting, or dysphagic patients. Certain drugs can irritate the gastrointestinal mucosa and may produce undesirable effects such as nausea, abdominal discomfort, vomiting, or gastric ulceration. Moreover, although modified-release and advanced oral formulations are designed to overcome some of these limitations,

formulation failure may occasionally result in an unintended rapid release of a large quantity of drug, commonly referred to as dose dumping. Such uncontrolled release may increase systemic drug exposure and the possibility of adverse effects, particularly with potent or narrow-therapeutic-index drugs [18–19]. These limitations have encouraged the development of site-specific approaches such as colon-targeted drug delivery systems.

1.1.2 Benefits of Colon-Targeted Drug Delivery System

Colon-targeted drug delivery systems are designed to deliver the drug predominantly to the colon, thereby reducing premature drug release in the stomach and upper small intestine. One of the important advantages of colon targeting is the ability to achieve a predictable and reproducible release profile at the desired gastrointestinal site. Appropriate formulation strategies can minimize the influence of gastric residence and provide controlled delivery of the active pharmaceutical ingredient after the dosage form reaches the colon. Such systems may also reduce inter- and intra-subject variability in drug exposure by minimizing the influence of physiological conditions in the upper gastrointestinal tract [20–22].

Colon targeting can improve the local therapeutic concentration and, in suitable cases, overall drug bioavailability, particularly when the drug is unstable or poorly absorbed in the upper gastrointestinal tract. It can also reduce premature exposure of the drug to the stomach and small intestine, thereby potentially decreasing gastrointestinal irritation and improving tolerability. Site-specific delivery is particularly advantageous for drugs intended to treat colonic disorders because the active ingredient can be released close to the site of action, potentially reducing the required dose and systemic exposure [20–22].

Another major advantage is the flexibility in formulation design. Colon-targeted systems can employ different approaches, including pH-dependent coatings, time-dependent systems, microbiologically triggered delivery, pressure-controlled systems, biodegradable polymers, and combinations of these mechanisms. Multiparticulate systems such as pellets can also be combined with different compositions or release characteristics within a single dosage form, allowing the formulation to produce a customized or unique drug-release pattern. Such approaches can improve formulation stability and provide better control over

the spatial and temporal delivery of the drug [20–22].

Colon-targeted delivery may further contribute to improved patient comfort, therapeutic effectiveness, and compliance by reducing dosing frequency and minimizing unwanted systemic or upper-GI effects. Properly designed systems can provide sustained or controlled drug release and maintain therapeutic drug concentrations for a longer duration. Importantly, when the formulation is appropriately optimized, site-specific release can reduce the likelihood of unintended drug release in the upper gastrointestinal tract. The technology also provides opportunities for product differentiation, lifecycle management, and potential extension of intellectual-property protection through novel formulation and delivery approaches, thereby helping pharmaceutical products remain competitive in the global market [23–26].

Characterization of microspheres is an essential step in formulation development and includes evaluation of particle size, surface morphology, percentage yield, drug entrapment efficiency, swelling behavior, flow properties, in vitro drug release, release kinetics, and stability studies. Such characterization helps in understanding the performance of the formulation and ensures reproducibility

and quality of the developed drug delivery system.

Therefore, the present study is focused on the design, development, and characterization of colon-specific microspheres containing vonoprazan fumarate with the objective of achieving controlled and site-specific drug release. The developed microspheres are expected to enhance therapeutic efficacy, minimize systemic side effects, and provide an efficient approach for targeted drug delivery to the colon.

2. Material and Methods

2.1 Material

Vonoprazan fumarate (Sun Pharmaceutical Industries Ltd., India) was used as the model drug for the preparation of colon-specific microspheres. Chitosan (Sigma-Aldrich, Merck, India) served as the primary biodegradable polymer for microsphere formation, while sodium tripolyphosphate (STPP) (Sigma-Aldrich, Merck, India) was employed as the ionic cross-linking agent. Glacial acetic acid (Merck Life Science Pvt. Ltd., India) was used for the preparation of the chitosan solution. Eudragit® S100 (Evonik Industries AG, Germany) was utilized as a pH-dependent coating polymer to achieve colon-targeted drug release. Distilled water was used throughout the formulation process. All chemicals and reagents used

in the study were of analytical grade and were used without further purification.

2.2 Methods

2.2.1 Preparation of colon targeting microspheres of Vonoprazan fumarate

Chitosan microspheres of Vonoprazan fumarate were prepared by the ionotropic gelation method. A 1% w/v chitosan stock solution was prepared by dissolving chitosan in 1% v/v acetic acid at room temperature. The drug (50 mg) was then dispersed in the chitosan solution. Sodium tripolyphosphate (STPP) solution (1% w/v) was prepared separately in distilled water. The STPP solution was added

dropwise into the drug-loaded chitosan solution under continuous magnetic stirring to form microspheres via ionic crosslinking. The compositions of different formulations (F1–F6) were varied as shown in Table 1, with chitosan concentrations of 250 mg and 500 mg and STPP concentrations ranging from 500 mg to 1000 mg, while keeping the drug amount constant at 50 mg ^[27]. The mixture was stirred for 30 minutes, followed by filtration and washing with distilled water. The obtained microspheres were air-dried for 24 hours and further dried in an oven at 40°C for 6 hours.

Table 1: Formulations of chitosan microspheres

Sr. No.	Formulation Code	Vonoprazan fumarate (mg)	Chitosan (mg)	STPP (mg)
1.	F1	50	250	500
2.	F2	50	250	750
3.	F3	50	250	1000
4.	F4	50	500	500
5.	F5	50	500	750
6.	F6	50	500	1000

2.2.2 Coating of chitosan microspheres

Eudragit S-100 coated microspheres of Vonoprazan fumarate were prepared by the solvent evaporation method. Microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolving Eudragit S-100 (500 mg) in a

mixture of ethanol:acetone (2:1), maintaining a coat-to-core ratio of 5:1. This organic phase was then slowly poured into 70 mL of light liquid paraffin containing 1% w/v Span 80 under continuous stirring. The system was maintained at an agitation speed of 1000

rpm at room temperature for 3 hours to allow complete evaporation of the organic solvent and formation of coated microspheres. The resulting microspheres were then filtered, washed with n-hexane to remove residual oil, and dried in a desiccator until further use^[28-29].

2.3 Evaluation of microspheres

There are many formulations and process variables involved in mixing step and all these can affect characteristics of blend produced, bulk density, true density and percent compressibility index have been measured which are given in table.

2.3.1 Bulk density

Bulk density is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup^[30]. A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, V_o , to the nearest graduated unit. Calculate the bulk density, in gm per ml gm/ml, by the formula:

$$\text{Bulk density} = \text{Bulk Mass} / \text{Bulk Volume}$$

2.3.2 Compressibility index (Carr's index)

Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities. Carr's index a material having values of less than

20% to 30% is defined as the free flowing material. It can be calculated as per given formula:

$$\text{C.I.} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

2.3.3 Hausner ratio

It indicates the flow properties of the powder and it can be measured by the ratio of tapped density to bulk density.

$$\text{Hausner ratio} = \text{Tapped density} / \text{Bulk Density}$$

2.3.4 Percentage Yield

The prepared microspheres F1-F6 were collected and weighed from each formulation. The percentage yield (%) was calculated using formula given below:

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 100$$

2.3.5 Entrapment Efficiency

Amount of Vonoprazan fumarate in each formulation was calculated according to procedure given below ^[31]:

10 mg of chitosan microspheres from each batch were accurately weighed. The powder of chitosan microspheres were dissolved in 10 ml 7.4 pH phosphate buffer and centrifuge at 1000 rpm. This supernatant solution is then filtered through whatmann filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.4 pH phosphate buffer. The supernatant was analyzed for drug content by measuring the absorbance at 282.0nm ^[32].

2.3.6 Measurement of mean particle size

The mean particle size of the microspheres was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer (Malvern particle size analyser) at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement ^[33].

2.3.7 Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern particle size analyser) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate ^[34].

2.3.8 In-vitro release studies

The prepared microspheres were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus ^[35]. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows:

- *1st hour*: Simulated gastric fluid (SGF) of pH 1.2.
- *2nd and 3rd hour*: Mixture of simulated gastric and Intestinal fluid of pH 4.5.
- *4th to 5th hour*: Simulated intestinal fluid (SIF) of pH 6.8.
- *6th hour and onward*: SIF pH 7.4

A weighed quantity of formulation (equivalent to 30mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 282.0 nm for percent of release Vonoprazan fumarate using UV visible spectrophotometer. The release of microsphere was calculated with the help of Standard curve of Vonoprazan fumarate ^[36].

2.3.9 Drug release kinetic data analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following three equations are commonly used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsemeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time)^[37].

2.3.10 Stability studies for optimized formulation

The optimized colon-specific Vonoprazan fumarate microspheres (F3) were subjected to stability studies under controlled storage conditions of $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$ for 3 months. Samples were withdrawn at predetermined intervals and evaluated for physical appearance, particle size, percentage entrapment efficiency (% EE), and drug content. The results were compared with the initial values to assess any changes in the formulation during storage. The optimized microspheres showed satisfactory stability at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$, with no significant

changes in their physical appearance, particle size, entrapment efficiency, or drug content. Greater changes were observed under accelerated conditions, indicating the influence of elevated temperature and humidity on formulation stability. Formulation F5 demonstrated satisfactory stability under the recommended storage condition^[37].

3. Results and Discussion

3.1 Result of flow properties of prepared microspheres

The flow properties of the prepared Vonoprazan fumarate microspheres (F1–F6) were evaluated using bulk density, tapped density, compressibility index, and Hausner ratio Table 2. These parameters provide an indication of the packing characteristics and flowability of the prepared microspheres, which are important for their handling, processing, filling, and further formulation development.

The bulk density of the prepared microspheres ranged from 0.315 to 0.348 g/cm³, while the tapped density ranged from 0.429 to 0.459 g/cm³. Formulation F5 exhibited the highest bulk density (0.348 g/cm³) and tapped density (0.459 g/cm³), indicating relatively better packing characteristics among the prepared batches. In contrast, F6 showed the lowest

bulk density (0.315 g/cm^3), suggesting comparatively lower packing efficiency.

The compressibility index of the formulations ranged from 24.12% to 27.08%. F3 showed the lowest compressibility index (24.12%), followed closely by F5 (24.18%), whereas F6 showed the highest value (27.08%). Lower compressibility index values generally indicate better flow and reduced interparticle cohesiveness. Based on the obtained values, F3 and F5 demonstrated comparatively better flow characteristics than the other formulations.

The Hausner ratio of the prepared microspheres ranged from 1.318 to 1.371. Formulation F3 exhibited the lowest Hausner ratio (1.318), followed by F5 (1.319), indicating comparatively better flowability. F6 showed the highest Hausner ratio (1.371), suggesting greater interparticle friction and comparatively poorer flow. The microspheres demonstrated fair to passable flow characteristics, with F3 and F5 showing the most favorable flow properties based on their lower compressibility index and Hausner ratio.

Table 2: Result of flow properties of prepared microspheres

F. Code	Bulk density(gm/cm^3)	Tapped density(gm/cm^3)	Compressibility index	Hausner ratio
F1	0.325	0.438	25.80	1.348
F2	0.318	0.429	25.87	1.349
F3	0.346	0.456	24.12	1.318
F4	0.336	0.448	25.00	1.333
F5	0.348	0.459	24.18	1.319
F6	0.315	0.432	27.08	1.371

3.2 Percentage yield for different formulation

The percentage yield of the prepared Vonoprazan fumarate microspheres formulations (F1–F6) was determined to assess the efficiency of the microsphere preparation process. As shown in Table 3,

the percentage yield of the formulations ranged from $70.32 \pm 0.45\%$ to $82.25 \pm 0.25\%$. The variation in percentage yield among the different formulations may be attributed to differences in polymer concentration, cross-linking efficiency, processing conditions, and the extent of

microsphere recovery during formulation and washing procedures.

Formulation F1 showed a percentage yield of $70.32 \pm 0.45\%$, whereas F2 demonstrated a slightly higher yield of $74.65 \pm 0.36\%$. Formulation F3 exhibited a further increase in yield to $78.98 \pm 0.55\%$. F4 showed a percentage yield of $74.65 \pm 0.65\%$, which was comparable to that of F2. The highest percentage yield was observed for F5 ($82.25 \pm 0.25\%$), indicating efficient formation and recovery of microspheres under the conditions employed for this formulation. In contrast, F6 showed a lower yield of $71.65 \pm 0.36\%$.

The higher yield observed for F5 may be related to better polymer-drug interaction and efficient ionic cross-linking, which could have promoted the formation of stable microspheres and minimized material loss during processing. The lower yields observed in F1 and F6 may be associated with incomplete microsphere formation, loss of fine particles during washing or filtration, or less efficient cross-linking. All formulations showed acceptable percentage yields, while F5 demonstrated the highest yield ($82.25 \pm 0.25\%$), indicating efficient formulation processing and good recovery of the prepared microspheres.

Table 3: Percentage yield for different formulation

Formulation code	Percentage Yield*
F1	70.32 ± 0.45
F2	74.65 ± 0.36
F3	78.98 ± 0.55
F4	74.65 ± 0.65
F5	82.25 ± 0.25
F6	71.65 ± 0.36

*Average of three determinations (n=3)

3.3 Entrapment efficiency for different formulation

The percentage entrapment efficiency (% EE) of the prepared Vonoprazan fumarate microspheres was determined to evaluate the ability of the formulation variables to

retain the drug within the polymeric matrix. As presented in the table, the entrapment efficiency of formulations F1–F6 ranged from $68.15 \pm 0.65\%$ to $79.32 \pm 0.74\%$. The observed variation in % EE may be attributed to differences in

polymer concentration, drug-to-polymer ratio, cross-linking efficiency, and the interaction between Vonoprazan fumarate and the polymeric matrix during microsphere formation.

Formulation F1 exhibited an entrapment efficiency of $68.15 \pm 0.65\%$, while F2 showed an increased value of $72.25 \pm 0.25\%$. Formulation F3 demonstrated a further increase to $75.65 \pm 0.44\%$, indicating improved drug incorporation within the microspheres. F4 showed an entrapment efficiency of $72.25 \pm 0.36\%$, which was comparable to F2. The highest entrapment efficiency was observed with F5 ($79.32 \pm 0.74\%$), suggesting that the formulation conditions used for F5 were more favorable for effective drug entrapment. F6 showed a lower entrapment efficiency of $69.12 \pm 0.36\%$.

The higher entrapment efficiency observed for F5 may be associated with efficient polymer-drug interaction and adequate cross-linking of the polymeric network, which could reduce drug diffusion into the external aqueous phase during microsphere preparation. Conversely, the lower % EE observed in F1 and F6 may be due to comparatively inefficient drug incorporation or greater drug loss during the preparation and washing steps. The results indicate that the formulation variables had a noticeable influence on drug entrapment. F5 exhibited the highest entrapment efficiency ($79.32 \pm 0.74\%$), indicating better incorporation and retention of Vonoprazan fumarate within the microspheres and supporting its selection for further optimization and characterization.

Table 4: Entrapment efficiency for different formulation

Formulation code	% Entrapment Efficiency
F1	68.15 ± 0.65
F2	72.25 ± 0.25
F3	75.65 ± 0.44
F4	72.25 ± 0.36
F5	79.32 ± 0.74
F6	69.12 ± 0.36

*Average of three determinations (n=3)

3.4 Results of Particle SIZE AND Zeta potential of optimized formulation F5

The particle size and zeta potential of the optimized Vonoprazan fumarate-loaded chitosan microspheres (F5) were determined to evaluate their size distribution and surface charge characteristics. As shown in Figure 1, the optimized F5 formulation exhibited an average particle size of 157.2 nm. The nanoscale particle size indicates successful formation of the chitosan-based microspheres and suggests a relatively large surface area, which may be beneficial for drug loading and controlled drug release. The particle size of the microspheres can be influenced by several formulation and processing variables, including polymer concentration, drug-to-

polymer ratio, cross-linking agent concentration, stirring speed, and duration of cross-linking.

The zeta potential of the optimized F5 formulation was found to be +32.2 mV, as illustrated in Figure 2. The positive surface charge can be attributed primarily to the presence of protonated amino groups of chitosan on the microsphere surface. The magnitude of the zeta potential indicates good electrostatic repulsion between the particles, which can help minimize particle aggregation and contribute to the physical stability of the dispersion. A zeta potential value of approximately +30 mV or higher is generally considered favorable for maintaining dispersion stability.

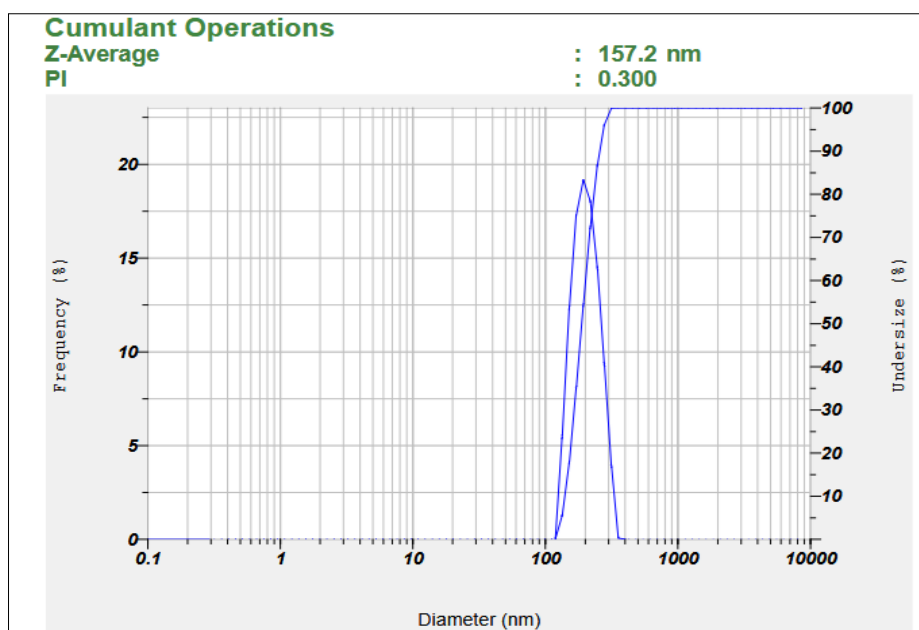


Figure 1: Particle size data of chitosan microspheres (F5) 157.2nm

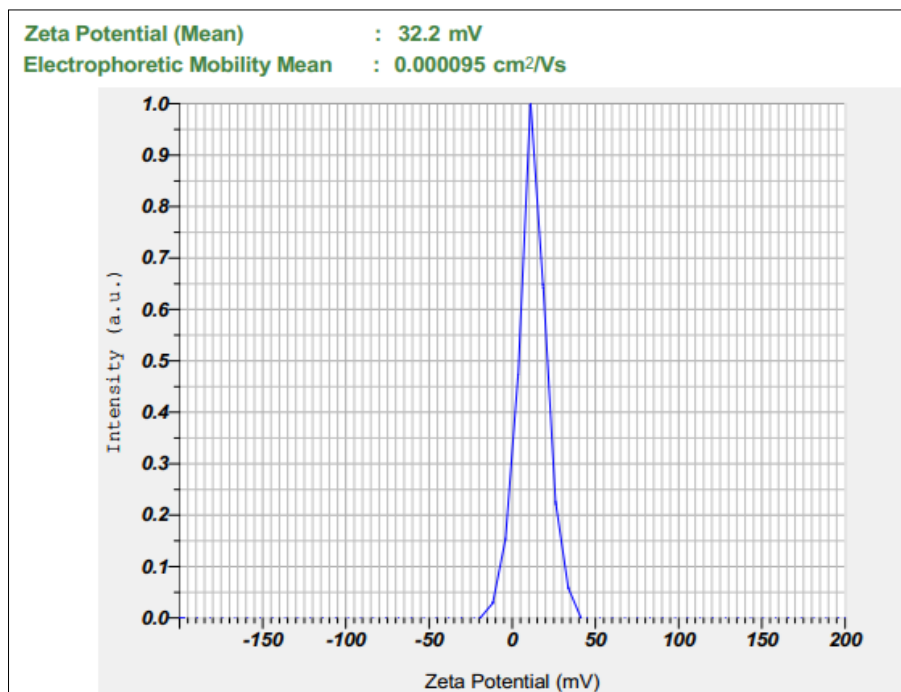


Figure 2: Zeta potential data of chitosan microspheres (F5) 32.2mV

3.5 Cumulative % drug release

The in-vitro drug release study of Vonoprazan fumarate from the optimized chitosan microspheres and Eudragit® S100-coated microspheres was performed under sequential gastrointestinal pH conditions to evaluate the ability of the formulation to protect the drug in the upper gastrointestinal tract and provide controlled release at higher intestinal/colonic pH. The cumulative percentage drug release showed a distinct difference between the uncoated chitosan microspheres and the Eudragit® S100-coated microspheres.

In simulated gastric fluid (SGF, pH 1.2), the uncoated chitosan microspheres

exhibited a cumulative drug release of $14.65 \pm 0.34\%$ after 1 h, which increased to $20.25 \pm 0.45\%$ after 2 h. In contrast, the Eudragit® S100-coated microspheres showed only $0.69 \pm 0.09\%$ and $1.12 \pm 0.14\%$ drug release at 1 and 2 h, respectively. The markedly lower release from the coated microspheres indicates that the Eudragit® S100 coating effectively restricted premature drug release under acidic gastric conditions. This behavior is desirable for a colon-specific delivery system because it minimizes drug loss before the formulation reaches the distal gastrointestinal tract.

When the dissolution medium was changed to SGF + SIF (pH 4.5), the drug release from uncoated microspheres

increased to $38.98 \pm 0.35\%$, $46.65 \pm 0.85\%$, and $59.98 \pm 0.36\%$ at 3, 4, and 5 h, respectively. The Eudragit® S100-coated microspheres, however, showed substantially lower release values of $1.89 \pm 0.32\%$, $3.96 \pm 0.45\%$, and $9.15 \pm 0.036\%$ at the corresponding time points. The limited release from the coated microspheres demonstrates the protective effect of the pH-dependent polymer coating and suggests that the formulation can withstand the relatively acidic conditions encountered before reaching the higher-pH intestinal environment.

In simulated intestinal fluid (SIF, pH 6.8), the uncoated chitosan microspheres exhibited a progressive increase in drug release, reaching $69.74 \pm 0.45\%$ at 6 h and $76.65 \pm 0.65\%$ at 7 h. The Eudragit® S100-coated microspheres showed comparatively controlled release of $18.98 \pm 0.45\%$ and $23.32 \pm 0.36\%$ at 6 and 7 h,

respectively. This relatively limited release at pH 6.8 indicates that the coating remained substantially intact and continued to restrict drug diffusion.

At SIF pH 7.4, a pronounced increase in drug release from the Eudragit® S100-coated microspheres was observed. The cumulative release increased from $50.36 \pm 0.25\%$ at 8 h to $73.32 \pm 0.14\%$ at 9 h and $84.45 \pm 0.32\%$ at 10 h, followed by $93.32 \pm 0.14\%$ at 12 h. This increase in release at the higher pH can be attributed to the pH-dependent dissolution or ionization behavior of Eudragit® S100, which allows the coating to become increasingly permeable or dissolve under intestinal/colonic pH conditions. In comparison, the uncoated chitosan microspheres showed $82.25 \pm 0.85\%$, $92.12 \pm 0.74\%$, $96.65 \pm 0.36\%$, and $98.12 \pm 0.74\%$ release at 8, 9, 10, and 12 h, respectively.

Table 5: Cumulative % drug release of Vonoprazan fumarate from Chitosan Microspheres and Eudragit S100 coated microspheres at different pH

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release	
			Chitosan Microspheres	Eudragit S100 Coated Microspheres
1	SGF (pH 1.2)	1	14.65 ± 0.34	0.69 ± 0.09
2		2	20.25 ± 0.45	1.12 ± 0.14
3	SGF+SIF(pH 4.5)	3	38.98 ± 0.35	1.89 ± 0.32
4		4	46.65 ± 0.85	3.96 ± 0.45
5		5	59.98 ± 0.36	9.15 ± 0.036

6	SIF (pH 6.8)	6	69.74±0.45	18.98±0.45
7		7	76.65±0.65	23.32±0.36
8	SIF (pH 7.4)	8	82.25±0.85	50.36±0.25
9		9	92.12±0.74	73.32±0.14
10		10	96.65±0.36	84.45±0.32
11		12	98.12±0.74	93.32±0.14

*Average of six determinations (n=6)

3.6 Regression analysis data of microspheres formulation (F5)

The drug release data of the optimized Vonoprazan fumarate-loaded chitosan microspheres (F5) were subjected to different kinetic models to determine the probable mechanism and order of drug release. The coefficient of determination (R^2) values obtained for the zero-order, first-order, Higuchi, and Korsmeyer–Peppas models were 0.8872, 0.7921, 0.7897, and 0.9341, respectively.

Among the mathematical models evaluated, the Korsmeyer–Peppas model showed the highest R^2 value (0.9341), indicating the best fit of the experimental drug-release data. The comparatively lower R^2 values obtained for the zero-order

(0.8872), first-order (0.7921), and Higuchi (0.7897) models suggest that these models provided a less satisfactory representation of the release behavior. The higher correlation with the Korsmeyer–Peppas model indicates that drug release from the optimized microspheres was predominantly governed by a combination of drug diffusion and polymeric matrix-related mechanisms.

The relatively good fit to the zero-order model also suggests a reasonably controlled release pattern, while the lower correlation with the Higuchi model indicates that simple diffusion through a homogeneous matrix was not the sole mechanism responsible for drug release.

Table 6: Regression analysis data of microspheres formulation (F5)

Formulation	Zero order	First order	Higuchi plot	Pappas plot
(r^2)	0.8872	0.7921	0.7897	0.9341

3.7 Characterization of stability study of Optimized formulation F5

The stability study of the optimized Vonoprazan fumarate-loaded chitosan

microspheres (F5) was carried out for 3 months under long-term storage conditions of $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ and accelerated storage conditions of $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{ RH}$. The

stability of the optimized microspheres was assessed on the basis of average particle size, percentage entrapment efficiency, physical appearance, and in-vitro drug release. The results indicated that formulation F5 remained comparatively stable throughout the study period, with only minor changes in the evaluated parameters.

The average particle size of F5 under long-term conditions increased slightly from 157.8 nm at 1 month to 160.4 nm at 3 months, whereas under accelerated conditions, it increased from 161.6 nm to 168.9 nm during the same period. The relatively small increase in particle size under accelerated conditions may be attributed to slight particle aggregation during storage at elevated temperature and humidity. However, the microspheres maintained their nanosized range throughout the study, indicating satisfactory physical stability.

The percentage entrapment efficiency showed a gradual but limited decrease during storage. Under long-term conditions, the entrapment efficiency decreased from 85.42% at 1 month to 83.98% at 3 months, while under accelerated conditions it decreased from 84.05% to 80.62%. This minor reduction may be associated with gradual drug diffusion from

the polymeric matrix or slight structural changes in the chitosan microspheres during storage. Nevertheless, the formulation retained a relatively high entrapment efficiency, suggesting that the microspheres were capable of maintaining the incorporated drug during the stability period.

The physical appearance of the optimized formulation remained unchanged at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{ RH}$ throughout the 3-month study. In contrast, slight aggregation was observed under accelerated conditions at 1, 2, and 3 months. The absence of noticeable physical changes under long-term conditions indicates good physical stability, while the slight aggregation observed at elevated temperature and humidity further supports the minor increase in particle size.

The in-vitro drug release also showed only a moderate decrease during storage. Under long-term conditions, the cumulative drug release decreased from 94.82% at 1 month to 93.91% at 3 months. Under accelerated conditions, drug release decreased from 93.76% to 90.86% over the same period. The reduction in drug release under accelerated conditions may be related to particle aggregation and minor changes in the polymeric matrix, which could influence drug diffusion.

Table 7: Characterization of stability study of Optimized formulation F5

Characteristic	1 Month		2 Month		3 Month	
Storage condition	$25 \pm 2^\circ\text{C} / 60 \pm 5\% \text{ RH}$	$40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$	$25 \pm 2^\circ\text{C} / 60 \pm 5\% \text{ RH}$	$40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$	$25 \pm 2^\circ\text{C} / 60 \pm 5\% \text{ RH}$	$40 \pm 2^\circ\text{C} / 75 \pm 5\% \text{ RH}$
Average Particle	157.8	161.6	159.1	164.7	160.4	168.9

Size (nm)						
Entrapment Efficiency (% EE)	85.42	84.05	84.76	82.41	83.98	80.62
Physical Appearance	No change	Slight aggregation	No change	Slight aggregation	No change	Slight aggregation
In-vitro Drug Release (%)	94.82	93.76	94.35	92.41	93.91	90.86

4. Conclusion

The present study successfully developed and characterized colon-specific microspheres of Vonoprazan fumarate using chitosan and sodium tripolyphosphate by the ionotropic gelation method. Among all formulations, F5 exhibited the best performance with high percentage yield, excellent entrapment efficiency, satisfactory flow properties, and controlled drug release behavior. Eudragit S100 coating effectively minimized drug release in the gastric environment and promoted targeted release in the colonic region. Stability studies confirmed the formulation's good physicochemical stability. Therefore, the developed Eudragit S100-coated chitosan microspheres show significant potential as an effective colon-targeted drug delivery system for Vonoprazan fumarate.

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6. Conflict of Interest

The authors declare that there is no conflict of interest associated with the present research work, its authorship or publication.

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8. Author Contributions

All authors contributed to the conception and design of the study. The experimental work, data collection, analysis, interpretation of results, and preparation of the manuscript were carried out with the involvement of the authors. All authors

reviewed and approved the final version of the manuscript.

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