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Original Article

Novel Biopolymeric Microspheres for Colon-Targeted Peptide Drug Delivery: An *in vitro* Investigation

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ABSTRACT

Background: Vancomycin (VAN) is used as a model peptide. It has been used extensively to treat severe infections brought on by Gram-positive bacteria. Vancomycin in colon-targeting studies has its therapeutic relevance in treating colonic infections, particularly antibiotic-associated colitis caused by *Clostridioides difficile*. The use of polymers like Eudragit S100 enables pH-dependent release, protecting the drug in the small intestine and stomach while promoting release in the area of the colon. Similarly, biodegradable carriers such as Gelatin can enhance encapsulation and controlled drug release. **Objectives:** Because of the drugs physicochemical characteristics, poor upper GI absorption, peptide-like behavior, and clinical relevance in colonic diseases, vancomycin is a great model medication while developing and testing colon-targeted drug delivery systems**.** An oral formulation with sustain release targeted to the colon would serve as an ideal formulation. **Materials and Method:** The emulsification method was used to develop microparticles. VAN was enteric coated with Eudragit S-100 after being trapped in a gelatine microsphere. The morphology, particle size, encapsulation effectiveness, and drug release of the microspheres were all evaluated. **Results and Conclusion:** According to the initial research, gelatine and Eudragit S-100 were compatible with VAN. The peptide can be trapped by these microspheres at high concentrations, where the drug's inner gelatin core would enable kinetic control of release and coating guarantees the digestive tract's medication core localisation. For the oral administration of peptide medications like vancomycin, enteric coated cross-linked gelatin microspheres show promising potential.

Keywords: Colon-targeted drug delivery, Gelatin, Microsphere, Peptides, Vancomycin.

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INTRODUCTION

When administering patients' medication, the oral route is thought to be most feasible option and the most important course of administering drugs for most of the systemic effects. Oral route of administration has number of advantages such as greatest dose accuracy and least content variability, the cost of product is low, they have chemical, mechanical and microbiological stability (Lachman *et al*., 1986). Depending on the physicochemical characteristics of the medication, oral administration of traditional dosage forms often dissolves in the stomach or intestinal fluid and absorbs from these parts of the GIT. It is a significant disadvantage in circumstances where a medication must be delivered locally in the colon or when it must be shielded from the upper gastrointestinal tract environment (Chourasia and Jain, 2003; Patel *et al*., 2008). There are several benefits to dosage forms that transport medications into the colon as opposed to the upper gastrointestinal tract, whereby it is possible to attain a high local concentration while reducing adverse effects that arise due to medication release in the upper gastrointestinal tract or unwanted systemic absorption (Rubinstein, 2005). The colon is gaining attention as a location where poorly absorbed medicinal molecules, like proteins and peptides, may have better bioavailability. Colonic delivery is appropriate for formulations that are polar and/or subject to chemical and enzymatic breakdown in the upper GI tract, such therapeutic proteins and peptides. Through intestinal absorption, proteins and peptides including insulin, calcitonin, and vasopressin can be administered systemically (Morishita and Peppas, 2006; Borchardt *et al*., 1997). Oral administration of proteins and peptides has low bioavailability because of the high molecular weight, highly hydrophilic, hydrolysis, poor intestinal membrane penetration and pre-systemic GI tract degradation by enzymes (Prasad *et al*., 2003; Cholewinski *et al*., 1996). The three-dimensional molecular structure of proteins and peptides

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determines their activity. Proteins and peptides may be subjected to severe circumstances during dosage form formation, which could change their structure.

This will have an impact on the effectiveness and immunogenic reaction to peptides and proteins. Proteins and peptides may undergo chemical and physical deterioration during the development of dosage forms.

Physical deterioration entails changing the parent compound's original structure to a higher order structure, which could be brought about via precipitation, unfolding, aggregation, or adsorption. Bond cleavage is typically involved in chemical degradation, which results in the development of a new product. Physical processes like unfolding, which expose the concealed residues to chemical reactions, come before chemical breakdown. The procedures used in deamidation, oxidation, disulphide exchange, and hydrolysis are examples of chemical degradation. After formulation processing, proteins and peptides must be evaluated for changes in conformation, size, shape, surface characteristics, and biological activity (Capelle *et al.*, 2007). Many pharmacological approaches have been put forth to increase the bioavailability of proteins and peptides such as co- administration of protease inhibitors, the utilization of multifunctional polymer, use of penetration enhancers and micro-/nanoparticulate drug delivery systems (Maroni *et al.*, 2012; Bernkop-Schnürch, 1998; Maher and Brayden, 2012).

A glycopeptide antibiotic identified as Vancomycin is used to treat severe, potentially fatal infections caused by Gram-positive bacteria that fail to respond to conventional antibiotics. When penicillin resistance occurs, Vancomycin is especially helpful in treating pseudo membranous colitis and needs to be administered orally in order to go to the infection site (Vasir *et al.*, 2003; Arhewoha and Okhamafe, 2004). The challenges in the development of oral formulations are acid catalysed degradation in the stomach and proteolytic breakdown in the gastro-intestinal tract. To overcome these problems enteric coating of microsphere is useful. In the stomach, the release of drug does not take place due to enteric coating. As these microspheres passes in the colon, due to increased pH the drug release takes place (Mehta *et al.*, 2011; Philip and Philip, 2010).

MATERIALS AND METHODS

Materials

Vancomycin (MW 14.5 kDa) was supplied as a gift by Alkem lab Mumbai. Eudragit S-100 was supplied as a gift by BASF Mumbai. The peptide assay employed analytical-grade reagents. The store department of Gurunanak College of Pharmacy, Nagpur, provided all additional chemicals and glassware utilised in the study.

Preparation of Vancomycin loaded microsphere

9 g of gelatin was added to 30 mL of warm water. The mixture was gently swirled until it dissolved uniformly. The two phases were then emulsified for 10 min using a stirrer spinning at roughly 1000 rpm after 60 mL of cotton seed oil that had been heated to the same temperature was added. After the emulsion droplets were formed, they were quickly chilled to 5°C in an ice bath while being constantly stirred for at least 10 min. They were then dehydrated by adding 90 mL of acetone that had been precooled to 5°C. For an additional 15 min, the stirring was maintained. After filtering and washing with 25 mL of acetone, the resulting microspheres were dried. After that, an aliquot of 50 mg of the microparticles was added to 25 mL of an aqueous vancomycin solution (1 mg/mL) and stirred for a whole day. The microparticles that were loaded were isolated from the filtering and centrifugation of the residual solution. After that, the microparticles were cleaned with water and filtered once more.

Coating

A beaker holding 250 mL of an organic solvent mixture comprising Methanol (ME), acetone, and Dichloromethane (DCM) solution containing 250 mg of Eudragit S-100 was filled with 300 mg of microparticles. The resulting suspension was stirred until the organic solvent evaporated. The enteric coated microspheres were subsequently cleaned, filtered, and dried.

Calibration curve of Vancomycin HCl

The standard solution (100 µg/mL) of pure drug was prepared in buffer solution (phosphate) of pH 7.4. The standard mixtures were diluted with buffer to prepare different concentration solutions (5, 10, 15, 20, 25 µg/mL). The solution shows an absorption maxima at 281 nm. A linear correlation was seen between 5 and 25 µg/mL. The linear plot value obtained is y= 0.004x - 0.002 and R^2 value of 0.999.

Characterization of the Microparticles

Encapsulation efficiency, drug content and Production yield

An accurately weighed 20 mg of microparticles were used. Volume of 5 mL ethanol was added. Following the complete dissolution of the microspheres, 5 mL of phosphate buffer (pH 7.4) were added to the mixture and thoroughly stirred. Vancomycin content was determined by UV-spectrophotometer (Shimadzu UV A-1700, Pharmaspec, Tokyo, Japan) at 281nm, using combination of ethanol mixture (1:1) and phosphate buffer (pH 7.4) as blank. The mean (+SD) of the three experiments was used to express the results. The following formula was used to determine encapsulation efficiency (Bodhankar *et al.*, 2011).

$$\text{Actual loading} = \frac{\text{Actual vancomycin content}}{\text{Weight of microspheres}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual vancomycin content}}{\text{Theoretical vancomycin content}} \times 100$$

Particle size analysis

Vancomycin-loaded Eudragit S-100 microspheres were subjected to particle size analysis using optical microscopy and a motic microscope. A minute quantity of the dried microsphere was suspended in 10 mL of pure water. 5 sec of ultrasonication were applied to the suspension. On the glass slide, a little drop of suspension was established. 300 particles were measured using a calibrated ocular micrometre after this slide containing microspheres was placed on the microscope's stage (Bodhankar *et al*., 2011).

Surface Morphology

Microparticles were mounted on the standard specimen-mounting stubs and were fixed onto the Scanning Electron Microscope (SEM) adhesion pads and plated with gold in a high vacuum evaporator using a gold sputter module. A SEM (Leo, VP-435, Cambridge, UK) was used to examine the specimens at 10 kV and working distance of 19 mm was maintained to give data of the surface features, shape and morphology of the microparticles (Alhnan and Basit, 2011).

FTIR Characterization of drug with excipients

Excipients can undergo phase transition during different stage of formulation and development. It is crucial to comprehend and keep an eye on developments in order to create a strong and stable formulation. FTIR serve as tool for interaction studies between peptide and excipients. The powder spectrum was measured with 2 mg of KBr as pellet press (Bodhankar *et al*., 2011).

In vitro drug release studies

A pH 7.4 alkaline phosphate buffer was employed as the releasing media in the USP method II, also known as the paddle dissolution test. The temperature of the release medium was maintained at 37±0.5°C while stirring at 100 rpm. A comparable quantity of microspheres were added to the dissolving media along with 250 mg of Vancomycin. After that, samples were taken out at predetermined intervals and replaced with new buffer that had been heated to 37±0.5°C. Following appropriate dilution, the samples were filtered through a Whatman filter (0.45µm) and spectrophotometrically measured at 281 nm. The quantity of Vancomycin was computed using the calibration curve. The mean of three experiments was utilised for data analysis, and the cumulative percentage of medication released was computed (United States Pharmacopeial Convention, 1980).

Stability studies

The optimised formulation was subjected to a stability investigation at 40°C±2°C and 75%±5% relative humidity for 90 days and were analyzed for the drug content and drug release after 0, 30, 60 and 90 days. The microspheres were put in an amber-colored screw-capped bottle, wrapped in aluminium foil, and incubated for three months at the conditions mentioned above (International Conference on Harmonisation [ICH], 2005).

RESULTS

Encapsulation efficiency, drug content and Production yield

Three formulations (F1-F3) of vancomycin-loaded gelatin microparticles were successfully prepared with satisfactory production yields ranging from 72.22±1.39% to 83.33±1.26% (Table 1). The highest production yield was observed for formulation F1 (83.33±1.26%), whereas F3 exhibited the lowest yield.

The actual drug content ranged between 32.25±0.63% and 39.62±0.55%, with formulation F3 showing the highest drug loading.

Drug entrapment efficiency varied from 64.50±1.76% to 79.25±1.48%. Among all formulations, F3 demonstrated the highest entrapment efficiency (79.25±1.48%), while F2 exhibited the lowest value (64.50±1.76%). A comparative representation of production yield, drug content and entrapment efficiency is shown in Figure 1.

Particle size analysis

Particle size analysis demonstrated that the prepared microparticles possessed a relatively narrow size distribution. The majority of particles (32%) were distributed within the size range of 140-150 µM, followed by 150-160 µM (13%) (Table 2). Very few particles were observed below 110 µM or above 180 µM, indicating successful preparation of uniformly sized microparticles. The particle size distribution histogram is presented in Figure 2.

Surface Morphology

Scanning Electron Microscopy (SEM) images revealed that the prepared microparticles were predominantly spherical with smooth and well-defined surfaces (Figure 3). The particles appeared discrete with minimal aggregation, suggesting successful formation of gelatin-based microparticles during the emulsification process.

FTIR characterization of drug with excipients

The FTIR spectra of pure vancomycin, gelatin, Eudragit S100 and the drug-loaded microparticles are presented in Figures 4-7. The characteristic absorption peaks of vancomycin remained identifiable in the drug-loaded formulation without the appearance of additional peaks or disappearance of characteristic functional group vibrations, indicating the absence of significant chemical interaction between vancomycin and the selected excipients.

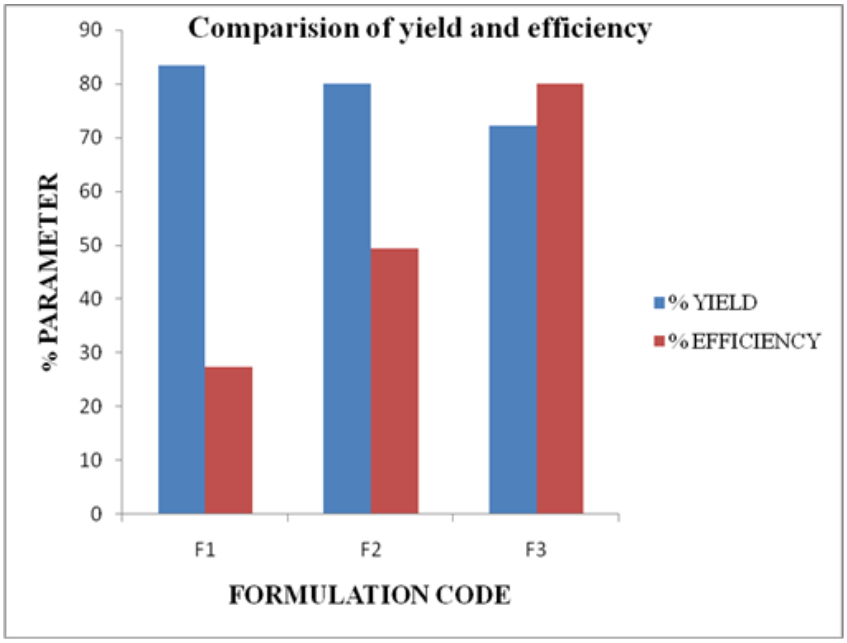

Figure 1: Comparative evaluation of yield and efficiency.

Table 1: Microparticles formulation parameters.

Batch code	Production Yield %	Actual Drug Content%	Drug Entrapment Efficiency %
F1	83.33+1.26	37.50+0.28	75.00+1.13
F2	80.00+1.65	32.25+0.63	64.50+1.76
F3	72.22+1.39	39.62+0.55	79.25+1.48

In vitro drug release studies

The *in vitro* release profile demonstrated an initial lag phase followed by a sustained release of vancomycin over approximately 8 hr (Figure 8). Drug release was minimal during the simulated gastric and intestinal conditions, whereas a marked increase in release was observed under colonic pH conditions. The release profile exhibited characteristics consistent with diffusion-controlled drug release.

Stability study

The optimised formulation remained physically stable throughout the three-month stability study. No significant changes were observed in drug content, entrapment efficiency or *in vitro* drug release profile, indicating satisfactory stability under the storage conditions employed.

DISCUSSION

The present study successfully developed vancomycin-loaded gelatin microparticles with satisfactory production yield, drug loading and encapsulation efficiency. The observed variation in encapsulation efficiency among formulations can be attributed primarily to differences in the composition of the organic solvent system used during microparticle preparation. Vancomycin is a highly hydrophilic glycopeptide antibiotic and therefore exhibits a strong tendency to diffuse into the external aqueous phase during emulsification, resulting in reduced drug entrapment. Previous studies have similarly reported that solvent composition and the rate of solvent evaporation are critical factors governing the encapsulation efficiency of hydrophilic drugs in polymeric particulate systems. Rapid polymer solidification minimises drug diffusion into the continuous phase, thereby improving drug retention within the polymer matrix (Loveymi *et al.*, 2012).

The increase in entrapment efficiency and drug content observed with the optimised DCM:methanol:acetone ratio suggests that the selected solvent combination promoted faster formation of the gelatin matrix while reducing drug leakage during emulsification. Similar findings have been reported for polymeric microspheres and nanoparticles encapsulating hydrophilic drugs, where optimisation of the organic phase significantly improved drug loading and encapsulation efficiency (Loveymi *et al.*, 2012).

Particle size analysis demonstrated that most microparticles were distributed between 140 and 150 µm, indicating relatively uniform particle formation. The decrease in particle size with increasing stirring speed is consistent with emulsion-based microparticle preparation, where higher shear forces generate smaller emulsion droplets before polymer solidification. Uniform particle size is

advantageous because it contributes to reproducible drug release, better flow properties and consistent gastrointestinal transit. Similar relationships between stirring speed and particle size have been reported in polymeric microsphere formulations intended for controlled drug delivery (Ahmadi *et al.*, 2018).

SEM examination confirmed that the microparticles possessed a predominantly spherical morphology with smooth surfaces and minimal aggregation. Spherical particles generally provide better flowability, improved packing characteristics and more predictable diffusion pathways than irregularly shaped particles.

Table 2: Frequency distribution of microparticles.

Sl. No.	Diameter (µM)	Frequency	Sl. No.	Diameter (µM)	Frequency
1.	100-110	03	6.	150-160	13
2.	110-120	10	7.	160-170	07
3.	120-130	10	8.	170-180	06
4.	130-140	15	9.	180-190	01
5.	140-150	32	10.	190-200	03

*µM- micrometer.

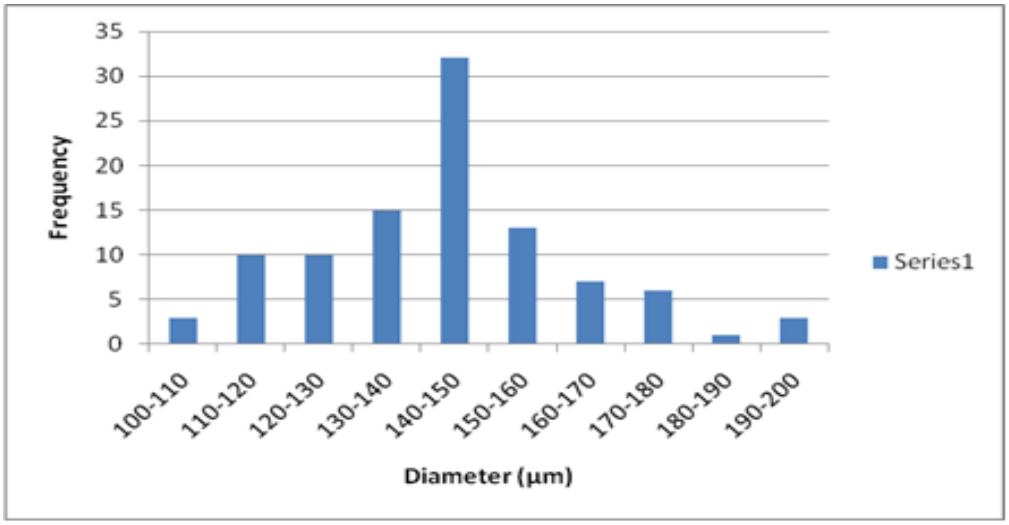

Figure 2: Particle size distribution of microparticles.

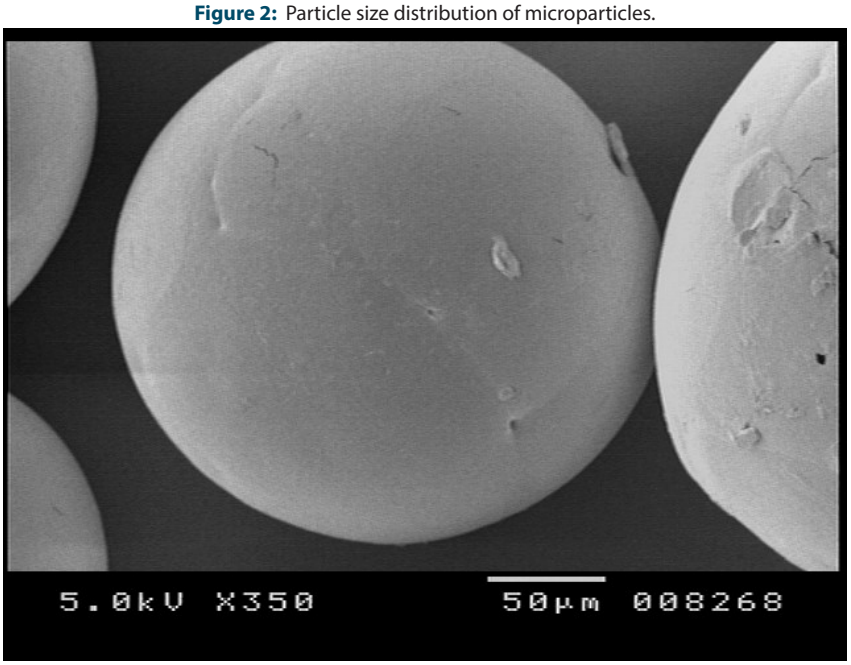

Figure 3: Scanning electron micrograph of microparticle.

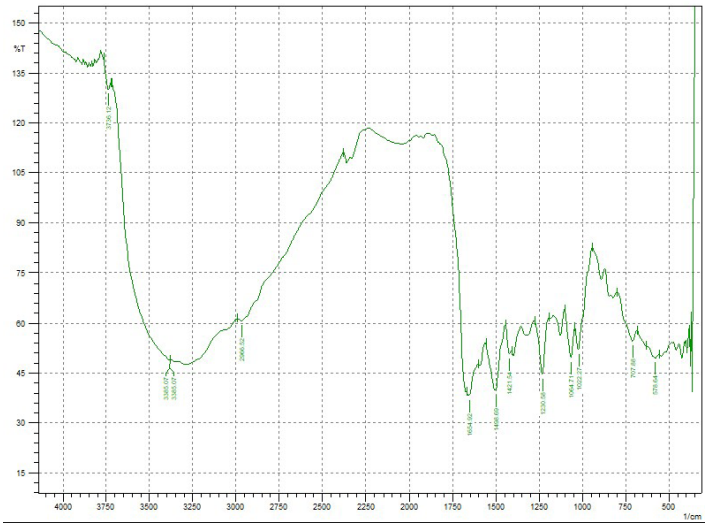

Figure 4: FTIR of Vancomycin.

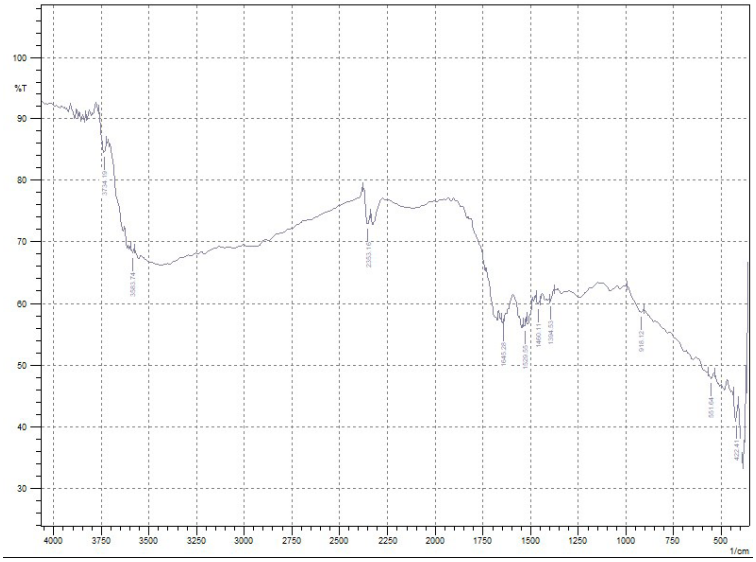

Figure 5: FTIR of gelatin.

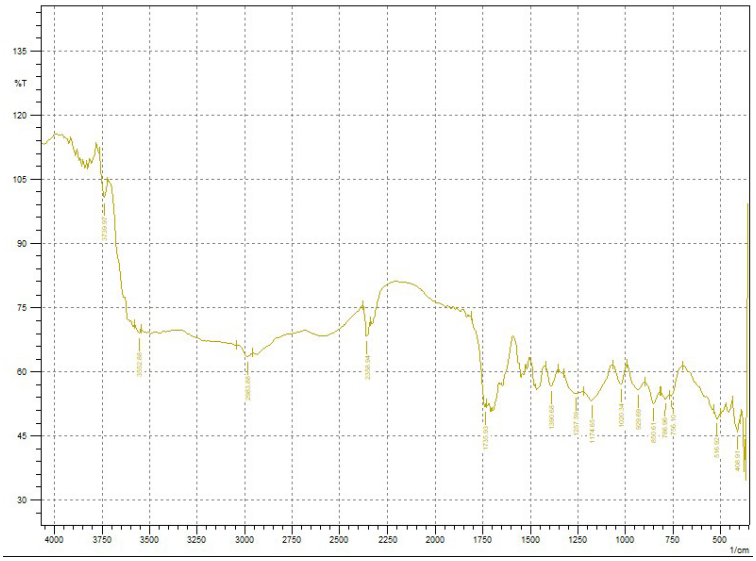

Figure 6: FTIR of Eudragit S100.

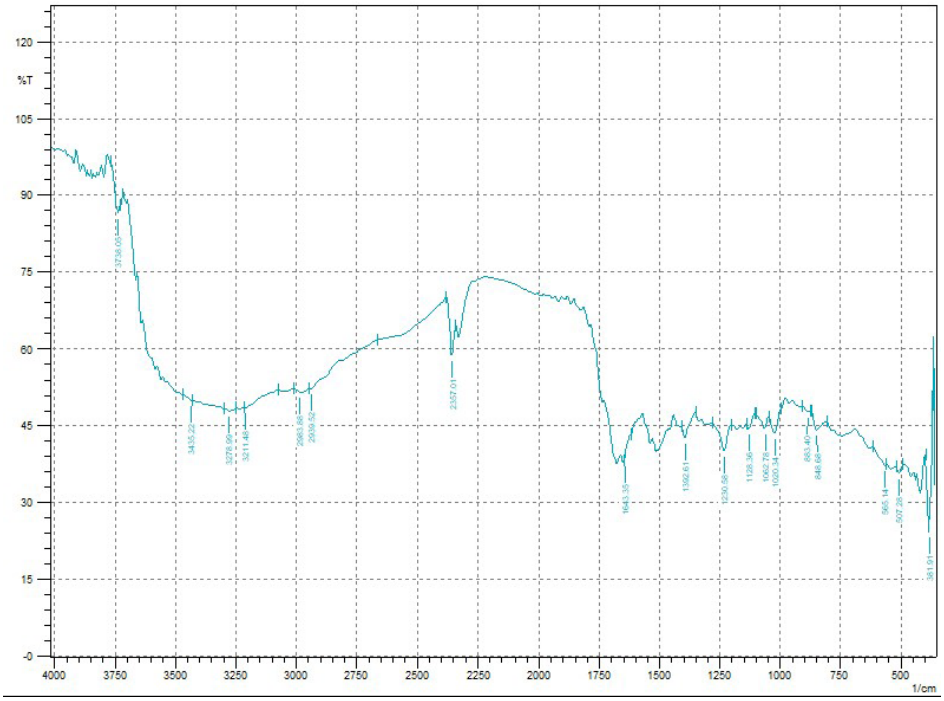

Figure 7: FTIR of Vancomycin + Gelatin + Eudragit S100.

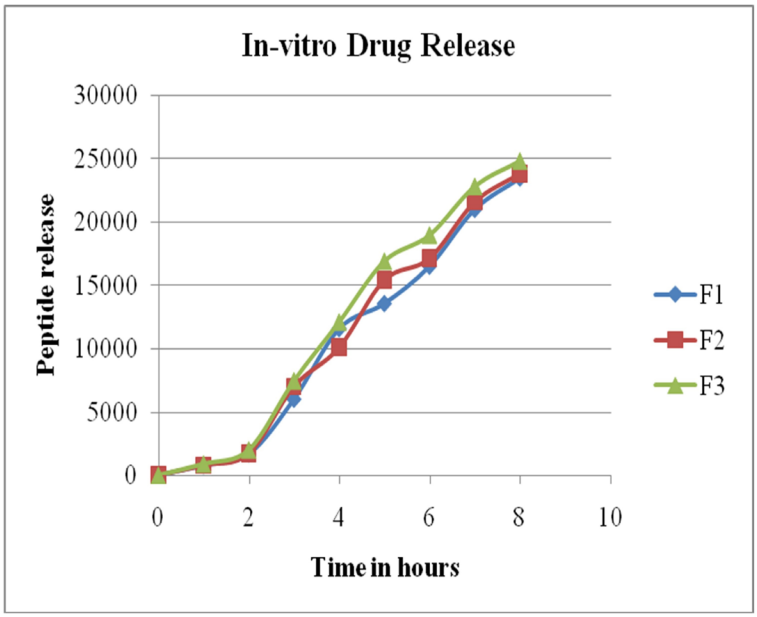

Figure 8: *In vitro* release of peptide formulation.

Comparable spherical morphology has been described for Eudragit-based colon-targeted microspheres and microsponges, where uniform particle architecture contributed to sustained drug release and improved formulation stability (Kumari *et al.*, 2018).

FTIR analysis demonstrated that the characteristic absorption bands of vancomycin remained unchanged after encapsulation, indicating the absence of significant chemical interaction between the drug and the excipients. Preservation of the principal functional group peaks suggests that vancomycin remained chemically stable throughout the formulation process. Similar observations have been reported for vancomycin-loaded polymeric delivery systems and other colon-targeted formulations, confirming the suitability of FTIR spectroscopy for evaluating drug-excipient compatibility (Loveymi *et al.*, 2012).

The *in vitro* drug release profile demonstrated an initial lag phase followed by sustained release over approximately 8 hr, with release kinetics closely resembling the Higuchi diffusion model. This release behaviour is desirable for colon-targeted drug delivery because it minimises premature drug release in the upper gastrointestinal tract while allowing gradual drug diffusion after reaching the colon. The delayed release can be attributed to the combined barrier properties of the gelatin matrix and the pH-dependent characteristics of Eudragit S100, which remains intact under acidic conditions but dissolves at colonic pH. Comparable release behaviour has been reported in several colon-targeted drug delivery systems employing Eudragit S100, where diffusion-controlled release following polymer swelling was identified as the predominant release mechanism (Khan *et al.*, 2010).

The present findings are also consistent with reports published in the Journal of Young Pharmacists, which describe polymeric colon-targeted formulations exhibiting satisfactory drug encapsulation, compatibility between drug and excipients, and Higuchi diffusion-controlled drug release. Further it has been highlighted that pH-sensitive polymers such as Eudragit S100 effectively prevent premature drug release in the stomach and small intestine while facilitating targeted drug delivery to the colon.

Finally, the stability study demonstrated that the optimised formulation maintained its drug content and release characteristics over three months without significant changes. This indicates that the formulation possesses adequate physical and chemical stability, suggesting that the selected polymer combination is capable of protecting vancomycin during storage. These findings support the potential of the developed gelatin-Eudragit microparticles as a promising carrier for colon-targeted delivery of peptide drugs, although further *in vivo* pharmacokinetic and therapeutic evaluation will be necessary to establish their clinical applicability**.**

CONCLUSION

Microspheres that have been cross linked using gelatin and are enteric coated can be regarded as an appealing method of delivering peptide medications such as Vancomycin. These microspheres have the capacity to trap the peptide at high concentrations, where the coating guarantees the localisation of the drug core in the intestinal system and the inner gelatin core carrying the drug would enable kinetic control of release. Therefore, formulations containing microsphere with a balance of protein strength, degradation rate, and porosity can lead to controllable and predictable release of proteins. Research is required to ascertain this kind of distribution system's dependability. It is also important to critically assess how nutrition and illness affect these systems' performance. However, it is likely that peptide oral delivery systems will be developed effectively.

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ABBREVIATIONS

VAN: Vancomycin; **GIT:** Gastrointestinal tract; **GI:** Gastrointestinal; **MW:** Molecular weight; **kDa:** Kilodaltons; **HCl:** Hydrochloride; **µg/mL:** Micrograms per milliliter; **nm:** Nanometers; **UV:** Ultraviolet; **SD:** Standard deviation; **µM:** Micrometers; **SEM:** Scanning electron microscope; **kV:** Kilovolts; **mm:** Millimeters; **FTIR:** Fourier-transform infrared spectroscopy; **KBr:** Potassium bromide; **USP:** United States Pharmacopeia; **rpm:** Revolutions per minute; **°C:** Degrees Celsius; **ICH:** International Conference on Harmonisation; **ME:** Methanol; **DCM**: Dichloromethane; **R^2:** Coefficient of determination.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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