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# DEVELOPMENT AND EVALUATION OF GUARGUM-BASED MEBEVERINE MICROSPHERES FOR COLON-TARGETED DELIVERY: OPTIMIZATION, COATING AND RELEASE KINETICS ANALYSIS

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## Abstract

Mebeverine hydrochloride is known to suffer from extensive first pass effect. In an attempt to improve its oral bioavailability and possibility to restrict its absorption only to the colon, mebeverine microspheres of guargum were prepared by emulsion dehydration method. Four formulations were prepared with varying drug and polymer ratio. These formulations were subjected to various evaluation parameters like percent practical yield, entrapment efficiency, particle size, *in vitro* drug release. Practical yield of the microspheres GG3 was up to 85.06 ± 0.97% with encapsulation efficiency up to 86.53 ± 2.21%. Scanning electron microscopy confirmed that the microsphere structures were smooth, spherical, and discrete and the particles were of the size range 74.7 ± 3.46-149.7 ± 3.4 µm. On the basis of drug content, particle size and *in vitro* release studies, formulation GG3 was found to be optimal. Optimized formulation was subjected to double coat to control the drug release in stomach and to achieve controlled drug release in the colon. Formulation GG17 coated with chitosan and carrageenan was found to elicit the desired results in a controlled manner.

**Keywords:** Mebeverine, guargum, controlled release, microspheres, emulsion dehydration.

## INTRODUCTION

Irritable bowel syndrome is a persistent gastrointestinal disorder that is distinguished by abdominal pain and unpredictable changes in bowel movements.[1,2] Mebeverine hydrochloride (MBH), an antispasmodic, has a direct effect on the smooth muscles of the gastrointestinal system, significantly the colon.[3] MBH has long been considered the ideal treatment for IBS.[4,5] According to the drug's pharmacokinetic profile, mebeverine has high hepatic metabolism and is rapidly absorbed from the stomach.[6,7] This impedes the delivery of MBH at the appropriate concentrations to the colon, the site of action. As a result, it is vital to develop drug delivery methods that can deliver MBH to the diseased organ (colon) in ample concentrations to improve overall treatment efficacy

and reduce the occurrence of undesirable side effects.[8] Because the majority of orally administered drugs are absorbed earlier than they reach the colon, oral drug delivery system is unable to successfully treat colon-specific illnesses. Consequently, the development of a drug delivery system that can specifically target the colon, bypassing the upper gastrointestinal tract, holds significant promise. It improves patient compliance with the therapeutic efficacy of the treatment. The natural polysaccharides pectin, inulin, chitosan, alginate, and guar gum are preferentially hydrolyzed by intestinal microorganisms, making these polymers the most promising and investigated carriers for CDDS. Regulating drug release from natural polysaccharides is especially a challenge due to their high hydrophilic properties.[9] The micro-carrier drug delivery systems are accepted and endorsed as a trustworthy way to passage the medication to the particular site with specificity and to maintain the appropriate concentration at the site of interest without undesired belongings. Guargum was preset for the drug distribution system because it is a natural polymer with swelling characteristics and the ability to load large volumes of medication into the system. Using an alkaline deactivation method, chitin gets converted into chitosan, a biocompatible and biodegradable polysaccharide. Nonetheless, in light of the applied concerns, it should make sure that the degree of swelling might also be handled at higher pH, which is typical for the small intestine and colon. Because of its limited solubility pattern at higher pH, chitosan works effectively as a polymer to cover colon-specific drug delivery systems.[9] Double layered coated mebeverine loaded guargum microparticulates have been developed for controlled release of drug with chitosan and carrageenan respectively. Tomida *et al*., have proposed that a carrageenan/chitosan membrane could be useful in following zero-order kinetics in a model drug.[10]

The proposed research work is based on the fabrication of guargum-chitosan-carrageenan micro particulate system for controlled drug delivery of mebeverine. The coating of chitosan on micro particulates reduced the amount of medication released in the jejunum and ileum of the gut. Carrageenan was chosen as the ingredient because of its potential to act as an external barrier to protect internal layers from stomach acid. Due to the ester sulfate's presence, it also exhibits good water solubility and high charge density. Utilizing diltiazem hydrochloride as a model drug, Tapia *et al.*, looked into the viability of using mixtures and/or polyelectrolyte complexes of chitosan and carrageenan as a delayed drug release mechanism.[11] Carrageenan's ability to encourage the entry of water into the matrix was used to control the drug's release.[9,10] In the procedure, firstly mebeverine loaded micro-particles were prepared and followed with the respective coatings, chitosan followed by carrageenan. This enabled the investigation of how the inner and outer layers of the drug delivery system influence the release profile profiles in pH conditions that stimulate body fluids, specifically the stomach and intestine. Considering the fact that the relative amounts of chitosan and carrageenan present on the micro particles are extremely low which do not alter the bulk properties of the guargum matrix.

## MATERIALS AND METHODS

### Material

Piramal Healthcare Ltd. (Chennai, India) provided mebeverine pure drug as a gift sample. Guargum, carrageenan, and castor oil were obtained from CDH Chemicals. Remaining chemicals were of analytical grade.

### Fabrication of Guargum microparticles

Guargum microspheres were prepared by emulsion dehydration method as reported in Kunjwani and Sakarkar's report with some minor modifications.[12] Guargum was immersed in distillate water for three hours in order to figure out a soothing, textured dispersion. 40 ml of 2.5% guargum dispersion with 5 ml of 2% mebeverine was mixed with 35 ml of castor oil containing span 80 (1.0% w/v). The mixture was stirred for 20 minutes at 500 rpm to create a stable w/o emulsion. For dehydrating the guargum droplets, 50 ml of acetone was added. The mixture was stirred for 30 minutes at 30 ºC until solvent evaporation. The hardened microspheres were filtered and washed multiple times with acetone. The microspheres were dried overnight and stored in desiccators. Formulations and process variables were identified to create small, uniform, smooth surfaced, and spherical microspheres. Formulation GG1-GG4 was prepared using different guargum ratios, while Formulation GG13-GG18 was prepared using the same ratio.

### Fabrication of chitosan coated guargum microparticles

Prepared microspheres were firstly coated with chitosan. In 2% v/v acetic acid, chitosan was dissolved. In the 2% w/v chitosan solution 5 ml of 5% NaOH was added and then the solutions were filtered. The microparticles obtained from the procedure described above were introduced in 10 ml of 2% w/v chitosan solution in 2% v/v acetic acid for 1 h and the temperature was maintained approx. 10ºC to harden the microspheres.[12] After that, the microspheres were filtered and rinsed in cold deionized water to get rid of extra NaOH ions. Finally, the microspheres were freeze-dried at −42 ºC for 24 h.[12,13]

### Fabrication of carrageenan-chitosan coated guargum microparticles

Chitosan coated microspheres were again coated with carrageenan to protect the chitosan layer in stomach pH 1.2 as the literature showed that chitosan is soluble in acidic pH. Carrageenan dissolved in deionized water at 70 ºC ± 5 ºC and makes a solution of 2.5% wt/v with the addition of 0.3M KCl solution. To yield the subsequent layer, the previously chitosan layered microspheres were acquaint with the solution of the carrageenan and assorted for about 1 h at 1 ºC temperature to harden the microspheres. After that, the microspheres were filtered and rinsed in cold deionized water to get rid of extra KCl ions. Finally, the microspheres were freeze-dried at −42 ºC for 24 h.[10,11,14,15,16]

### Compatibility study by FTIR

Studies on drug polymers are extremely important for developing a formulation. It is important to evaluate the possible interactions between the drug and the polymers, as the choice of the polymers performed a key role in the drug delivery, compatibility with the drug and polymer and in the stability of the final product. The Infrared spectroscopy studies were carried out for pure drug with

guargum, chitosan and carrageenan. The IR spectra of pure drug and polymers showed their characteristic peaks and these peaks were compared with drug plus polymers composition mixtures.[17]

**Particle Size Assessment**

Particle size assessment was performed using optical microscopy. A minimum of 100 microspheres from each formulation were meticulously examined. Mean particle size was determined from obtained sizes.[18]

**Surface Morphology Analysis (SEM)**

Scanning electron microscopy (SEM) was used to analyze the microspheres' morphology and surface properties. Briefly, samples were prepared using aluminum stubs and double-sided carbon tape. On the surface of the tape holding stub, formulations were dispersed for sampling. Under argon atmosphere and high vacuum, platinum sample coating material was sputter-coated onto the specimen stub to create a thin layer. These samples underwent SEM analysis, and photomicrographs of them at various magnifications were taken.[19]

**Zeta potential study**

Malvern Zeta seizer (Malvern Instrument, UK) was used to study the zeta potential. The analysis was carried out by converting the electrophoretic mobility to the zeta potential. Microsphere samples were placed in an electrophoretic cell after being diluted with distilled water to assess the zeta potential. Every measurement was made in triplicate.[20,21]

**%Encapsulation Efficiency and drug content**:

Encapsulation efficiency refers to the percentage of the active ingredient that is successfully encapsulated within a carrier system. Encapsulation efficiency is calculated by comparing the amount of the active substance that is successfully encapsulated to the total amount of the active substance used in the formulation.[22,23]

% Encapsulation efficiency (EE) was calculated according to the following equation:

$$\% EE = \text{Actual active drug content/Theoretical drug content} \times 100$$

To determine the drug content, the following procedure was employed: initially, 50 mg of crushed microparticles was placed in a 100 ml volumetric flask containing pH 7.4 phosphate buffers. The mixture was stirred for a period of 12 h to ensure complete dissolution of the drug. After the stirring process, the solution was filtered through Whatman filter paper to remove any insoluble particles. Drug content of the filtrate was measured using a Shimadzu UV spectrophotometer at absorbance λmax of 263 nm.

**Percentage Yield**

Practical yield was estimated by taking the total weight of the produced microspheres. Theoretical yield was determined by using the combined weight of the drug, polymer, and all other non-volatile

ingredients employed in the manufacture of the microspheres.[24] The percentage yield was determined using the following formula:

$$\text{Percentage yield of microspheres} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

**Study of Micromeritic Properties**

***Angle of repose***

The flow rate of the developed microspheres was assessed using the fixed funnel technique to measure the angle of repose. The funnel was filled with fixed amount of microspheres. The microspheres were allowed to flow through a funnel, forming a conical heap on a sheet of paper. A circle was drawn around the heap formed on the paper. Height of the conical heap and Radius of the circle was measured using appropriate instrument.[25,26] With the help of the equation angle of repose was calculated.

$$\tan \theta = h/r. \quad \ldots\ldots\ldots\ldots\ldots\ldots .1$$

***Compressibility index (CI)***

The Bulk density (ρb) and the tapped density (ρt) of the microspheres were measured using a10 ml graduated cylinder. 1 g of prepared microspheres was filled in the cylinder and the initial volume was recorded. Then the cylinder was then tapped 100 times on a wooden surface from a height of one inch. After tapping, the final volume of the microspheres in graduated cylinder was measured. This process was repeated three times to obtain triplicate measurements. Every measurement was made in triplicate, and the densities were estimated using the average of the three measurements. The mass-to-volume ratio was used to calculate density. Compressibility index was calculated with the following equation 2 and Hausner's ratio by applying equation 3.

$$CI = \{(\rho t - \rho b)/\rho t 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots .2$$

$$HR = (\rho t/\rho b)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3$$

***In vitro* release study**

The dissolution behavior of guargum microparticles was studied using a USP dissolution apparatus XXI (Electrolab, TDT-06 T, Mumbai, India) with the paddle method. The experiments were conducted at 100 rpm, 37 ºC ± 2 ºC, using 900 ml dissolution medium. A tea bag containing guargum microspheres equivalent to 100 mg of drug was tied to the paddle and immersed in the dissolution medium. The microspheres were initially tested in pH 1.2 (gastric pH) for 2 h, as the average gastric emptying time is about 2 h. Then, the dissolution medium was changed to pH 6.8 (duodenum pH) and maintained for 3 h. Finally the medium was replaced with pH 7.4 (ileum pH) and dissolution continued for further analysis. At regular time intervals, samples were filtered and analyzed using a UV spectrophotometer at a wavelength of 263 nm in pH 1.2 and the respective phosphate buffer media for pH 6.8 and 7.4. The cumulative percentage release of the drug over time was calculated using Beer Lambert's curve. The same procedure was followed for coated microparticles to study the drug release over a 24 h period. These experiments aimed to assess the

dissolution behavior and release profile of the guargum microparticles in different pH conditions simulating the gastric and small intestine environments.[9]

**Kinetics of Drug Release**

To characterize the release kinetics of drugs in various formulations, models were applied to the dissolution data of optimized formulations using linear regression analysis.[14]

***Zero order kinetics***:

zero order kinetics describes the dissolution of drugs from pharmaceutical dosage forms that do not disintegrate rapidly and release the drug slowly, assuming that the surface remains constant and equilibrium conditions are not met. The following equation represents the process:

$$Qt = Q_0 + K_0 t$$

Here, $Q_t$ represents the amount of drug dissolved at time t, $Q_0$ is the initial amount of drug in the solution, and $K_0$ is the zero order release constant.

***First order kinetics:***

First-order kinetics is commonly used to describe the absorption and/or elimination of drugs in dissolution studies. The following equation represents the process:

$$\text{Log } Q_t = \text{Log } Q_0 + K_1 t / 2.303$$

In this equation, $Q_t$ denotes the amount of drug released at time t, $Q_0$ represents the initial amount of drug in the solution, and $K_1$ is the first order release constant.

***Higuchi model***:

Higuchi developed theoretical models to study the release of water soluble and poorly soluble drugs incorporated in semi -solid and solid matrices. These models provide mathematical expressions for dug particles dispersed in a uniform matrix, acting as the diffusion medium. The equation governing the Higuchi model is:

$$Q_t = K_H t_{1/2}$$

In this equation, $Q_t$ signifies the amount of drug released at time t, and $K_H$ represents the Higuchi dissolution constant.

**Korsmeyer and Peppas Model**:

The Korsmeyer and Peppas model is employed to analyze the release of drugs from polymeric dosage forms when the release mechanism is not well-known or when multiple release phenomena are involved. The equation used in this model is:

$$Mt / M = Ktn$$

Here, Mt / M denotes the fraction of drug release, K is the release constant, t is the release time, and n is the diffusion exponent for drug release , which depends in the shape of the matrix dosage form.

During the in vitro drug release study, the obtained results were plotted using four different mathematical models for data analysis:

1. Cumulative percent drug release vs. Time (Zero-order rate kinetics)
2. Log cumulative percent drug retained vs. Time (First-order rate kinetics)

3. Cumulative percent drug release vs. square root of Time (Higuchi model)
4. Log cumulative percent drug release vs. Time (Korsmeyer and Peppas Model)

Significance of differences was evaluated by ANOVA, with $p < 0.05$ being considered statistically significant. All experimental data was calculated using Graph Pad prism 5.02 version.

## RESULT AND DISCUSSION

Guargum microspheres were prepared by emulsion dehydration method in which acetone was used as a hardening agent. Chemical cross-linking agents were avoided due to their toxic and undesirable effects.

### Optimization of guargum formulation

Guargum concentrations of GG1-GG4 (1-4%) were used to prepare blank microspheres. The findings showed that microspheres had discrete nature and agglomeration at lower guargum concentrations. According to Chourasia and Jain (2004), this may be because there is a lot of water in the less concentrated solution, which evaporates slowly and brings the particles into contact with one another.[27] In order to produce spherical microspheres, it was found that a 3% (w/v) solution was required. A solution with a concentration of more than 3% (w/v) was too viscous to handle and could not be used to generate microspheres.

### Compatibility study with FT-IR

The FTIR spectra of the pure drug and polymers were showed in Figure 1. It was observed that mebeverine showed characteristic peak at 3045.39 $cm^{-1}$ for C-H, aromatic stretching, 2979.82 $cm^{-1}$ for C-H stretch, 1714.60 $cm^{-1}$ for C=O stretching, 1608.59-1514.02 $cm^{-1}$ for C=C Ring stretch and C-N Stretching 1399.62, guargum exhibits the characteristic absorption band at 3394.48 $cm^{-1}$ and 2970.17 $cm^{-1}$ due to O-H stretching vibrations of the polymer associated with C-H stretching vibrations, chitosan revealed the presence of peaks at 1029 $cm^{-1}$ and 1050 $cm^{-1}$, which represented the CO stretching at the C-3 location. The peak at 1151 $cm^{-1}$ represented the asymmetric stretching of the COC bridge, although the peak at 1336.58 $cm^{-1}$ represented the CN stretching of amide III, the peak at 3417.63 represented N-H stretching, peak at 3263 represented O-H stretch and peak at 2850.69 showed C-H stretching while the study of carrageenan's by FTIR spectroscopy shows the presence of very strong absorption bands in the 1428.15$cm^{-1}$ region (due the S = O of sulfate esters) and 991.34-1068.49 $cm^{-1}$ region (ascribed to the glycosidic linkage) for carrageenan 3116.5 $cm^{-1}$ for OH strechging, and 2884.05 $cm^{-1}$ for CH strech respectively indicating that there is no interaction between the drug and excipients used in the study.

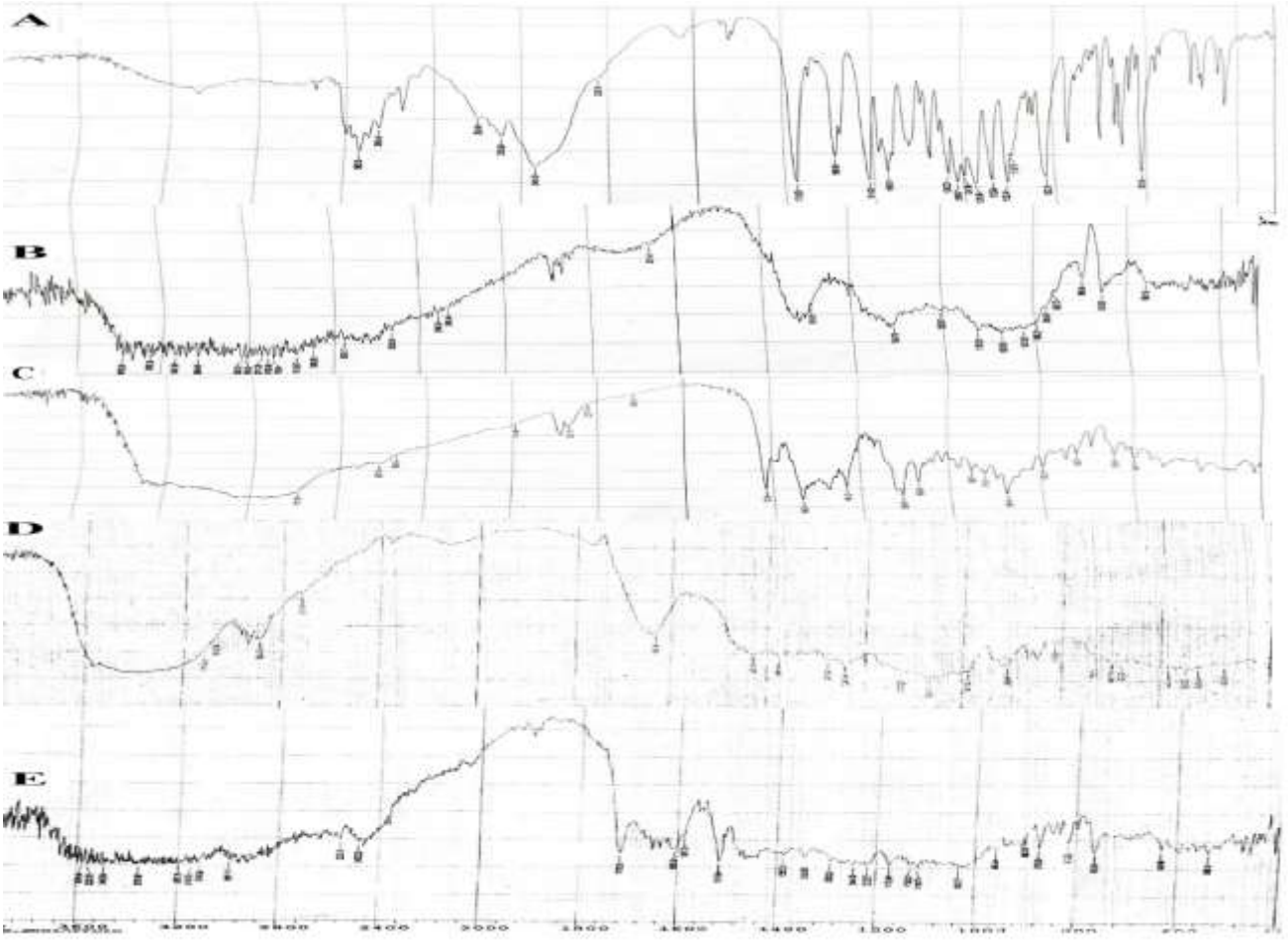


**Figure1:** FTIR spectra of the formulations compared with physical mixture, **(A)** Mebeverine **(B)** Guargum **(C)** Chitosan **(D)** Carrageenan **(E)** mixture of drug and polymers.

**Particle Size, shape and surface morphology study**

Particle size analysis by optical microscopy indicated the presence of almost uniform sized microspheres. The findings of particle size of microspheres ranged from 74.7±3.46 -149.7±3.40 µm, with variations in polymer compositions (1:1, 1:2, 1:3 and 1:4) shown in Table 1. The average mean particle size of the microspheres increased significantly with increase in polymer concentration due to high viscosity of medium at a higher polymer concentration resulting in enhanced interfacial tension and diminished shearing efficiency. The purpose of SEM study is to obtain a topographical characterization of microspheres. SEM micrographs of optimized formulation's guargum microspheres, chitosan coated guargum microspheres and again coated with carrageenan were shown in Figure 2, Figure 3 and Figure 4, respectively. Guargum microspheres were observed to be spherical in shape with slight rough surface. Chitosan and carrageenan microspheres were also observed to be spherical with slight rough surface due to shrinkage during freeze-drying.

**Table 1:** Encapsulation efficiency, drug content and % yield of mebeverine and mean particle size of mebeverine loaded uncoated guargum microspheres.

| Formulation | Actual drug content (± SD) | Encapsulation efficiency (% ± SD) | Production yield (% ± SD) | Mean Particle size |
|---|---|---|---|---|
| **GG1** | 47.55 ± 0.39 | 66.05 ± 1.61 | 72.08 ±1.32 | 74.7 ± 3.46 |
| **GG2** | 78.93 ± 4.25 | 70.16 ± 3.78 | 75.04 ± 1.60 | 94.7 ± 2.80 |
| **GG3** | 146.71 ± 1.16 | 86.53 ± 2.21 | 85.06 ± 0.97 | 125.0 ± 3.65 |
| **GG4** | 179.56 ± 2.32 | 86.30 ± 1.65 | 83.08 ± 1.04 | 149.7 ± 3.40 |

**Encapsulation Efficiency, drug content and % yield of mebeverine**

The drug content was found to be high in all the cases. Polymer loss was found due to adherence of the polymer to container as a result of viscous nature of slurry as it was seen in formulation GG4 with less production yield 83.08 ± 1.04%. It was observed that entrapment efficiency increased from 66.05 ± 1.61%, 70.16 ± 3.78%, 86.53 ± 2.21% and 86.30 ± 1.65% with increase in polymer ratio. Same findings were investigated with production yield 72.08 ± 1.32%, 75.04 ± 1.6%, 85.06 ± 97% and 83.08 ± 1.04% gradually (Table 1). The drug content of the chitosan coated microparticles were found in the decreasing order 147.64 ± 4.08 , 148.94 ± 0.72, and 145.45 ± 0.11 with decrease in % yield 85.81 ± 0.38, 85.83 ± 0.94 and 86.33 ± 0.38%. The outcome of encapsulation efficiency (GG13-GG15) was 85.99 ± 1.45, 86.76 ± 0.23 and 84.24 ± 0.41% respectively. All the results were relevant to each other and revealed that decrease particle size increases the drug retaining property. Formulations GG16-GG18 results for drug content, encapsulation efficiency and % yield was 147.92 ± 3.84, 149.45 ± 1.60 and 147.96 ± 4.01; 86.24 ± 1.83, 86.67 ± 4.25 and 85.35 ± 1.75; 86.33 ± 3.66, 86.66 ± 0.62, and 85.75 ± 0.50%. Observations stated that the different coat concentrations of the carrageenan on the microparticles not affected significantly the physical properties. Particle size was found modified due to coating concentrations of carrageenan (Table 2).

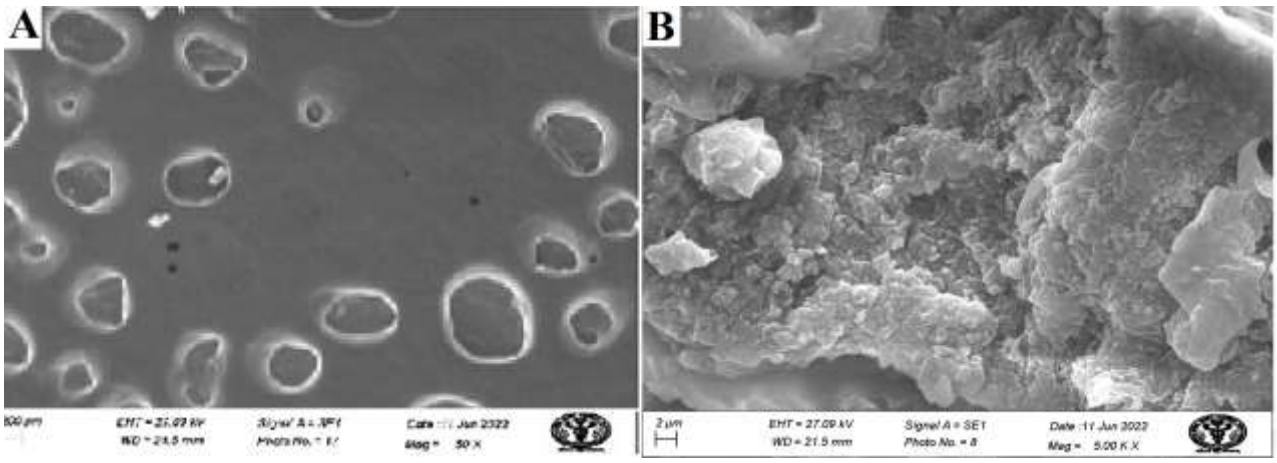

**Figure 2:** SEM photograph of uncoated guargum microspheres**:** The photograph coded 'A' represents whole image; 'B' represents surface photograph.

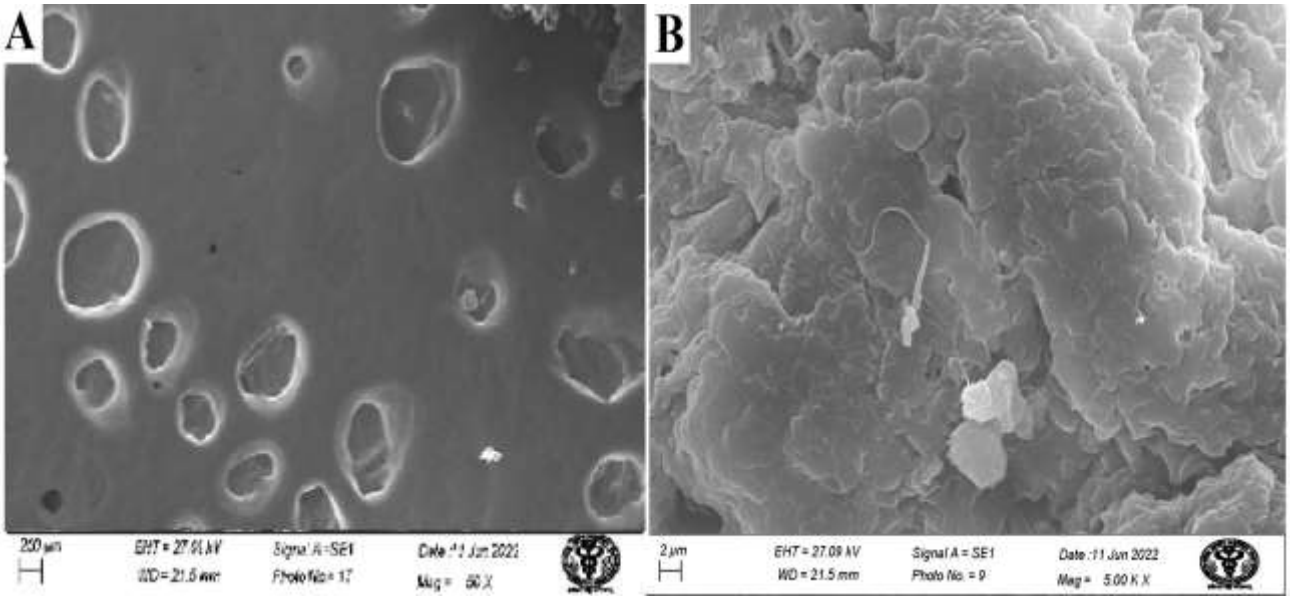


**Figure 3:** SEM photograph of guar gum microspheres coated with chitosan**:** The photograph coded 'A' represents whole image; 'B' represents surface photograph.

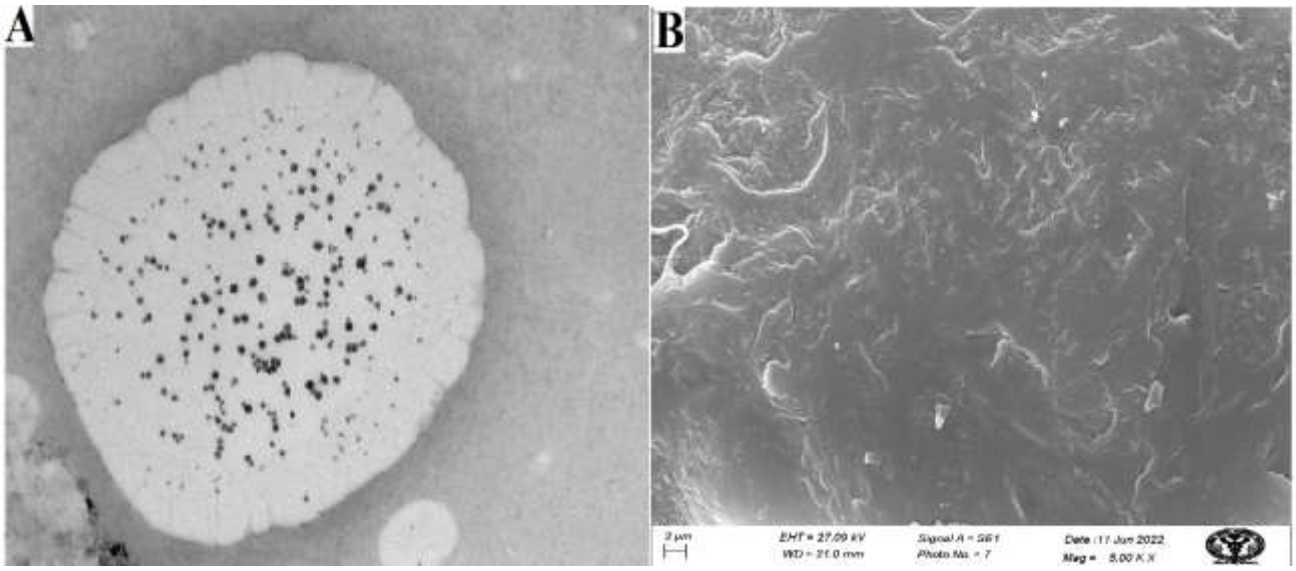


**Figure 4:** SEM photograph of double coated guar gum microspheres with chitosan and carrageenan: The photograph coded 'A' represents whole image; 'B' represents surface photograph.

**Table 2:** Encapsulation Efficiency, drug content and % yield of mebeverine and mean particle size of mebeverine loaded coated guargum microspheres.

| Formulation | Drug :Polymer ratio | Actual drug Content ( ± SD) | Encapsulation efficiency (% ± SD) | Production yield (% ± SD) | Mean Particle size (µm± SD) |
|---|---|---|---|---|---|
| **GG13** (1% chitosan) | 1:3 | 147.64 ± 4.08 | 85.99 ± 1.45 | 85.81 ± 0.38 | 134.80 ± 4.87 |
| **GG14** (2% chitosan) | 1:3 | 148.94 ± 0.72 | 86.76 ± 0.23 | 85.83 ± 0.94 | 155.90 ± 3.84 |
| **GG15** (3% chitosan) | 1:3 | 145.45 ± 0.11 | 84.24 ± 0.41 | 86.33 ± 0.38 | 162.10 ± 4.20 |
| **GG16** (0.5% carrageenan) | 1:3 | 147.92 ± 3.84 | 86.24 ± 1.83 | 86.33 ± 3.66 | 190.40 ± 3.20 |
| **GG17** (1% carrageenan) | 1:3 | 149.45 ± 1.60 | 86.67 ± 4.25 | 86.66 ± 0.62 | 212.57 ± 3.15 |
| **GG18** (2% carrageenan) | 1:3 | 147.96 ± 4.01 | 85.35 ± 1.75 | 85.75 ± 0.50 | 232.98 ± 3.37 |

**Zeta Potential**

To verify the presence of the coating chitosan and then carrageenan, zeta potential measurements were performed. Results after coating of each layer on the micro particles have shown in table 3. Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion or attraction between particles and is one of the fundamental parameters known to affect stability. In the neutral pH uncoated micro particles showed negative zeta potential -22.6 mV. Zeta potential of chitosan-coated microspheres is low -7.47 mV, its absolute value is lower than that for uncoated micro particles. Introduction of the outer carrageenan layer decreases zeta potential -13.7 mV because carrageenan sulfonate groups are ionized regardless of the pH. The observed difference results from the compensation of guargum–chitosan charges. The fact that both layers have limited effect on the zeta potential of the microspheres because both of them are very thin and polymeric chains of the polysaccharides used to form the layer can easily interpenetrate the outer part of the micro particles.

**Table 3:** Zeta potential for uncoated and coated guargum microspheres.

| Coating type | Zeta Potential$\zeta$ (mV) |
|---|---|
| None | -22.6 |
| Chitosan | -7.47 |
| Chitosan and carrageenan | -13.7 |

**Micromeritic properties**

The study focused on evaluating physical parameters, such as bulk density, tapping density, compressibility index, and angle of repose, to optimize the flow properties of microspheres (Table 4). The results showed that all formulations of the microspheres exhibited favorable flow ability, as indicated by an angle of repose ranging from 24.45° ± 0.08 to 26.11° ± 0.12, which was below the desired threshold of 40°. Additionally, the bulk density measurements provided insights into the packaging properties of the microspheres. These findings highlight the successful optimization of the microspheres' flow behavior, indicating their suitability for various applications.

**Table 4:** Micromeritic properties of mebeverine loaded uncoated and coated microspheres (mean ± SD, n = 3).

| Formulation | Evaluation Parameters | | | | |
|---|---|---|---|---|---|
| | **Bulk Density (gm/cm3)** | **Tapped Density (gm/cm3)** | **Carr's Index (%)** | **Hausners Ratio** | **Angle of Repose (θ)** |
| **GG1** | 0.885 ± 0.02 | 0.952 ± 0.02 | 7.07 ± 0.05 | 1.186 ± 0.03 | 26.11 ± 0.12 |
| **GG2** | 0.892 ± 0.03 | 0.943 ± 0.04 | 5.36 ± 0.03 | 1.187 ± 0.05 | 25.82 ± 0.08 |
| **GG3** | 0.884 ± 0.04 | 0.934 ± 0.03 | 5.31 ± 0.06 | 1.21 ± 0.04 | 25.29 ± 0.13 |
| **GG4** | 0.877 ± 0.02 | 0.952 ± 0.06 | 7.89 ± 0.05 | 1.197 ± 0.06 | 25.76 ± 0.10 |
| **GG13** | 0.888 ± 0.03 | 0.943 ± 0.02 | 5.86 ± 0.07 | 1.193 ± 0.03 | 25.62 ± 0.12 |
| **GG14** | 0.885 ± 0.05 | 0.934 ± 0.03 | 5.22 ± 0.05 | 1.208 ± 0.03 | 25.42 ± 0.13 |
| **GG15** | 0.884 ± 0.02 | 0.935 ± 0.02 | 5.30 ± 0.09 | 1.209 ± 0.06 | 25.28 ± 0.11 |
| **GG16** | 0.889 ± 0.07 | 0.943 ± 0.04 | 5.77 ± 0.06 | 1.192 ± 0.03 | 25.15 ± 0.09 |
| **GG17** | 0.885 ± 0.09 | 0.934 ± 0.07 | 5.31 ± 0.08 | 1.209 ± 0.05 | 24.45 ± 0.08 |
| **GG18** | 0.884 ± 0.08 | 0.943 ± 0.09 | 6.19 ± 0.05 | 1.197 ± 0.03 | 24.53 ± 0.11 |

***In vitro* release**

From the results of 12 h *in vitro* study, it was observed that on increasing the polymer ratio the release of drug also decreased. After 12 h, the release investigations was found 95.96 ± 0.89, 75.47 ± 0.55, 74.81 ± 1.2 and 57.91 ± 0.99% for GG1, GG2, GG3 and GG4 respectively (Figure 5B). *In vitro* dissolution study revealed that uncoated guargum microspheres released the drug at pH 1.2 at higher rate in comparison to coated microspheres; GG3 > GG15 > GG17 (Figure 6A). It might be due to the fact that they were not able to maintain their integrity in upper part of GIT and showed maximum release. Double coated guar gum microspheres maintain their integrity in upper part of GIT hence drug release was slow in comparison to uncoated microspheres. A colon-targeted drug delivery system desired that it should not only protect the release of drug in the physiological environment of the stomach and the small intestine but also deliver the maximum drug into the colon. Figure 5(B) depicts that the drug to polymer ratio had a significant effect on the *in vitro* drug release characteristics of the microspheres. GG1, GG2, GG3 and GG4 showed 67.94 ± 1.73, 42.56 ± 0.43, 29.07 ± 0.6 and 36.06 ± 0.3% of drug release respectively at the end of $5^{th}$ h (which is the

expected time for the arrival of dosage form in the colon) as depicted in figure 5(A). The decrease in the drug release with increase in amount of polymer was attributed to the swelling properties of guar gum. The release of the drug from polymer matrix takes place after complete swelling of the polymer. Microspheres externally coated with chitosan observed as the possibility of partial dissolution of the chitosan layer in acidic media as it was seen in formulation GG14 where the drug release was 19.08 ± 0.04% at the end of $5^{th}$ h. In the environment of the colon chitosan layer is more compact and cannot be easily penetrated by water. As a result, it slowed down the drug release as it was exhibited in GG14 formulation 64.94 ± 0.03% in up to 24 h studies. Upon addition of the second layer of carrageenan, the release rate decreased in both environments due to stability of the polymer in the acidic media and as well as high gelling property of carrageenan in colon environment. At the end of 5 h the release of double coated microspheres was only 1.29 ± 0.01% of optimized formulation GG17 which was negligible in comparison to uncoated GG3 and single coated GG14 formulations 29.16 ± 0.6% and 19.08 ± 0.04% respectively.[28] As the amount of the swellable polymer increases in the formulation, the thickness of gel layer formed surrounding the microspheres increases. This increases the diffusion path length for penetration of intestinal fluid and hence decreases the drug release from the microspheres. However, the substantial differences in the drug release profiles from the uncoated, single and double coated micro particles were observed in figure 6(B). This suggested that the significant modification of the micro particles surface has been occurred. A *p* value of <0.05 for *in vitro* drug release in analysis of variance (ANOVA) indicates significant effect in between uncoated, chitosan coated and chitosan-carrageenan coated microspheres

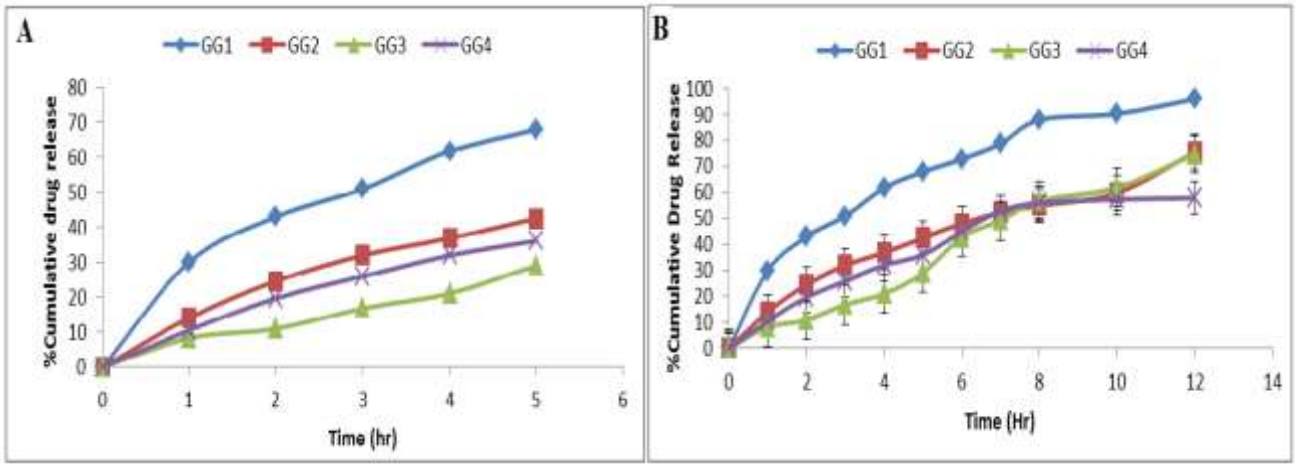


**Figure 5**: *In vitro* release graph of mebeverine loaded guargum microspheres on the basis of drug polymer ratio (**A**) Profiles for the first 5 h (**B**) Full profiles.

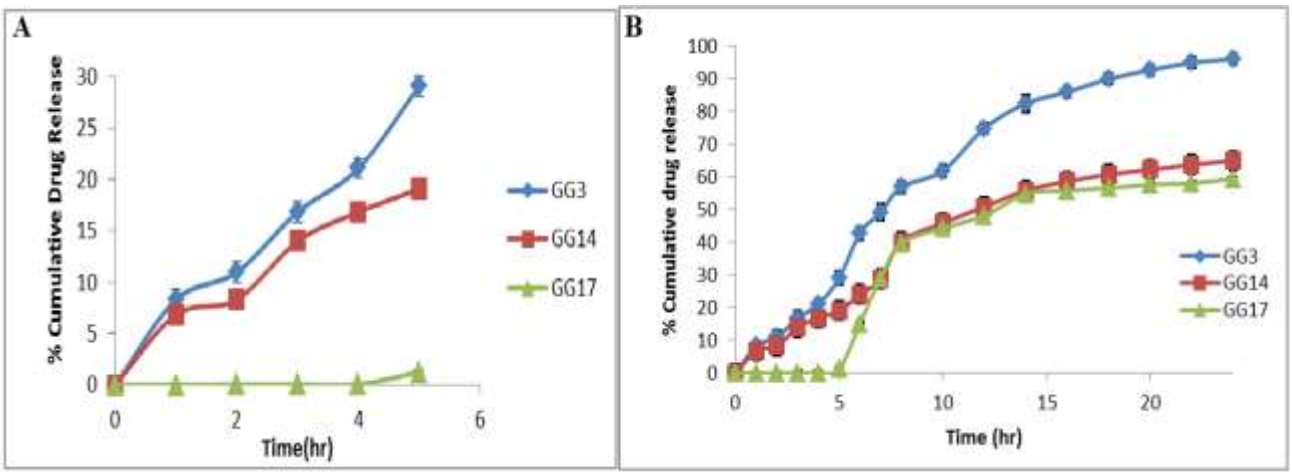


**Figure 6:** Release graph for uncoated and coated microspheres; (**A**) Profiles for the first 5 h; uncoated (diamond), chitosan-coated (square) and chitosan-carrageenan-coated (triangle) microspheres (**B**) Full profiles.

**Kinetic data analysis of *in vitro* drug release**

The formulations based on drug polymer ratio (GG1-GG4) and optimized coated formulations (GG14 and GG17) were explored for zero, first, Higuchi and Korsmeyer -Peppas models, the values were shown in table 6. The graphical representations of different kinetic models of *in vitro* drug release profile of optimized formulations GG3 and GG17 are specified in figures 7 and 8. These values were compared with each other for model fitting equation. According to the highest regression (*r*) values, the best fit model was applied on GG3 for zero order ($R^2$ = 0.9804). Formulation GG2 and GG4 Korsmeyer and Peppas equation showed non-fickian diffusion (n<0.89). Formulation GG3 (*n* > 0.89) higher value of *n* followed the Super case-II transport release. GG14 and GG17 followed the first order reaction as data revealed from table 7. Drug release is dependent on the remaining concentration of drug in the microparticles in first order system. Formulation GG14 containing MB: Guuargum ratio in 1:3, coated with 2% chitosan exhibited n<0.89, indicated non Fickian diffusion behaviour for the drug release. This indicated that drug release is governed by swelling, diffusion and erosion of the microparticles. GG17 containing MB:Guargum ratio in 1:3, double layered 2% chitosan and 1% carrageenan coat exhibited n>1, indicated that drug release followed the super case II system. This indicated that several modifications ensue in microparticles during the drug release.

**Table 6:** Pharmacokinetic parameters of uncoated formulations GG1-GG4.

| **Formulation** | **Zero Order** | | **First order** | | **Higuchi model** | | **Korsmeyer Peppas model** | |
|---|---|---|---|---|---|---|---|---|
| | **$R^2$** | **K** | **$R^2$** | **K** | **$R^2$** | **K** | **$R^2$** | ***N*** |
| **GG1** | 0.8689 | 7.1714 | 0.9819 | -0.1070 | 0.9915 | 28.614 | 0.9926 | 0.4804 |
| **GG2** | 0.9469 | 5.5856 | 0.9672 | -0.0443 | 0.9830 | 21.257 | 0.9823 | 0.6020 |
| **GG3** | 0.9804 | 6.4877 | 0.9791 | -0.0358 | 0.8925 | 23.120 | 0.9646 | 0.9773 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **GG4** | 0.9017 | 5.0548 | 0.9346 | -0.0346 | 0.9591 | 19.472 | 0.9749 | 0.7206 |
| **GG14** | 0.9212 | 2.8602 | 0.9614 | -0.0208 | 0.9520 | 16.070 | 0.8705 | 0.5551 |
| **GG17** | 0.8505 | 3.0854 | 0.8941 | -0.0203 | 0.8407 | 15.764 | 0.8368 | 3.1500 |

**Table** 7: Pharmacokinetic parameters of chitosan and chitosan-carrageenan coated formulations.

| **Formulation** | **Zero Order** | | **First Order** | | **Higuchi Model** | | **Korsmeyer Peppas model** | |
|---|---|---|---|---|---|---|---|---|
| | **$R^2$** | **K** | **$R^2$** | **K** | **$R^2$** | **K** | **$R^2$** | **N** |
| **GG14** | 0.9212 | 2.8602 | 0.9614 | -0.0208 | 0.9520 | 16.070 | 0.8705 | 0.5551 |
| **GG17** | 0.8505 | 3.0854 | 0.8941 | -0.0203 | 0.8407 | 15.764 | 0.8368 | 3.1500 |

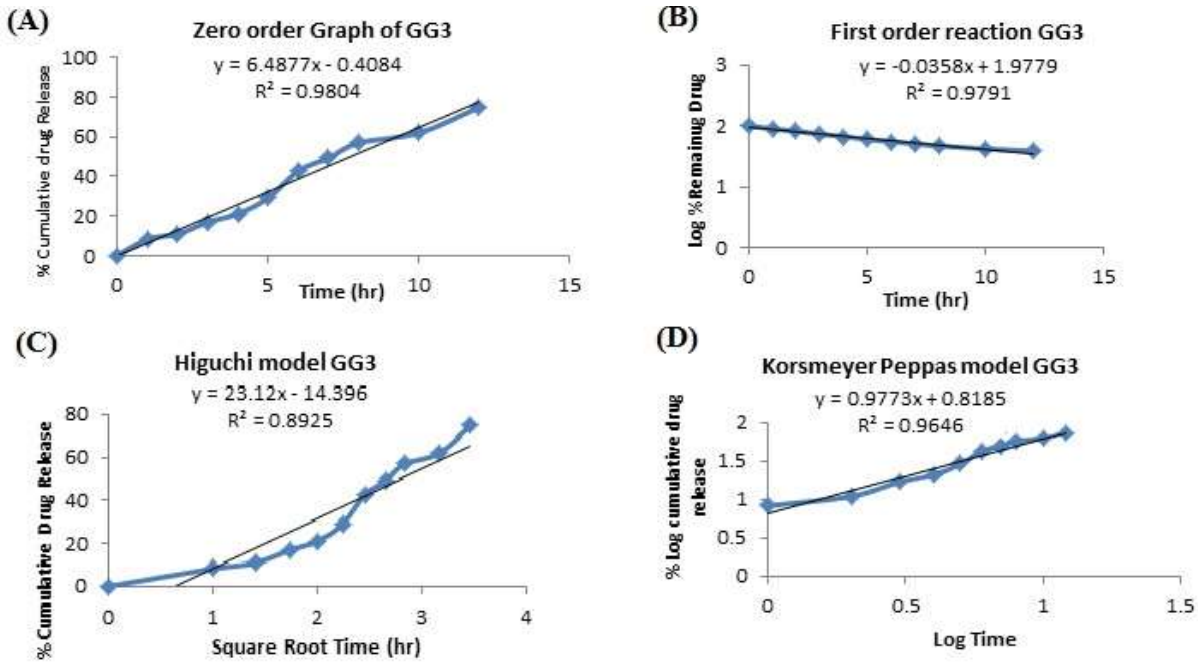


**Figure 7**: Graphical representations of pharmacokinetic models of optimized formulation GG3 **(A)** Zero order **(B)** First order **(C)** Higuchi model **(D)** Korsmeyer Peppas model.

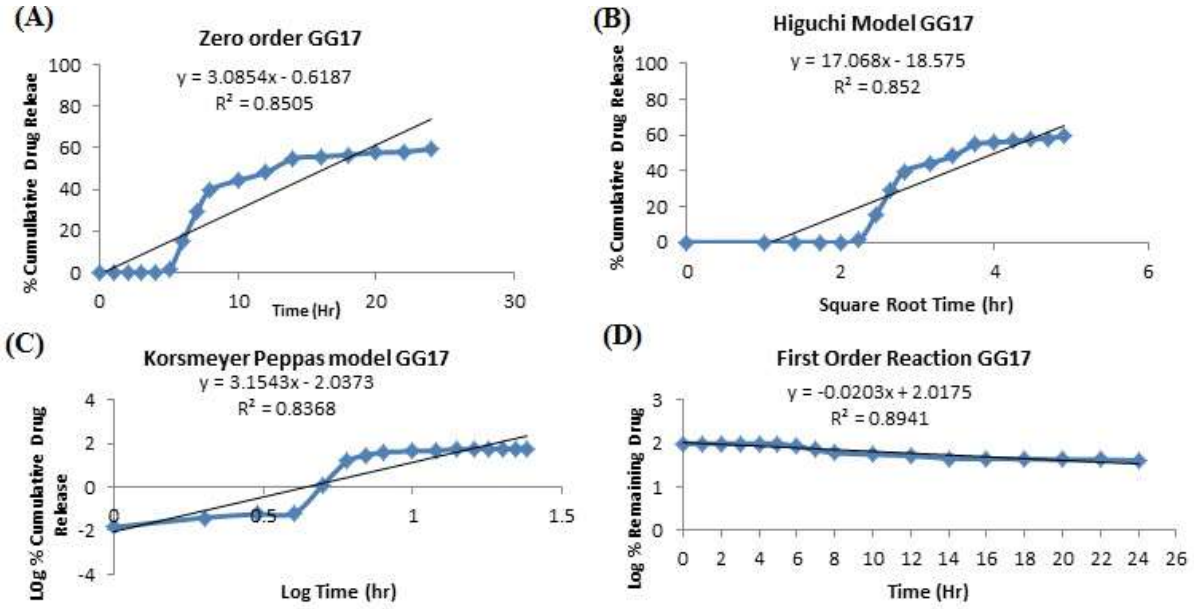


**Figure 8**: Graphical representation of pharmacokinetic models of optimized formulation GG17 **(A)** Zero order **(B)** Higuchi model **(C)** Korsmeyer Peppas model **(D)** First order.

## CONCLUSION

In the present work, colon specific drug delivery systems have been selected to deliver the drug to colon for longer period of time. Mebeverine loaded microspheres(1:3) were successfully prepared by the emulsion dehydration method with high encapsulation efficiency. The particles were discrete spherical shape with near to smooth surface and uniform sizes. Coating effect on the release of mebeverine from guargum microsphere was studied. The results showed that the release behavior depended on the pH of dissolution media and its release site. At pH 1.2 the presence of chitosan coating promoted slow drug release in upper intestine and lower intestine from microspheres. To avoid the drug release in upper intestine an additional coating was prerequisite for the microparticles. Results revealed that coating of carrageenan on chitosan coated microparticles (GG17) subsequently minimize the drug release in stomach in contrast to high release of drug in colon. Drug release from double layered guargum microparticles confirmed that the additional protection against acidic conditions of stomach and the drug release in the intestine can be better controlled .The kinetic data analysis investigation revealed that guargum ratio (1:3) subjected with coating of chitosan (2%) and carrageenan (1%) subjected to controlled drug delivery system.

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## CONFLICT OF INTEREST

The authors have no conflict of financial and personal interests with other people or organization.

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