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FORMULATION AND IN VITRO EVALUATION OF NATURAL POLYMERS BASED MICROSPHERES FOR COLONIC DRUG DELIVERY

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ABSTRACT

The present study describes development and in-vitro evaluation of natural polymers based MTZ microspheres prepared by ionotropic gelation technique for delivering the drug to colon. The current work aimed to evaluate the novel potential of ionotropic gelation technique to produce natural polysaccharides based microspheres bearing MTZ, a sparingly water soluble antiamoebic drug, with increasing entrapment efficiency by gluteraldehyde which increasing crossinking as well as reduced swelling ability of formulations with given prolonged colon targeting. The prepared microspheres were characterized by entrapment efficiency, particle size, micromaritic properties, in vitro release behavior, scanning electron microscopy (SEM), FTIR etc. MTZ loaded microspheres shows high entrapment efficiency (75.2%). The in vitro drug release study was done using U.S.P.dissolution rate test basket type apparatus in different P^H media, which was found to be affected by change in guar gum-alginate and glutaraldehyde concentration. The percentage of drug released after 12 hr was increased up to 85.73%. The rate of drug release followed by first order kinetics and numerical data fitted in peppas model. It is concluded that Metronidazole loaded guar gum- alginate based microsphere can be used effectively for the colon targeting.

Key Words: MTZ-Metronidazole, Guargum-Alginate microspheres, Glutaraldehyde, Ionotropic gelation technique.

INTRODUCTION

The site-specific delivery of the drugs to the target sites has the potential to reduce the side effects and improved the pharmacological response. The colon drug targeting is also exploited for systemic delivery of active drugs. For colonic drug delivery, many physiological barriers must be overcome, the major one being absorption or degradation of the active drugs in the upper part of the GIT-Tract. Most of the peptide and protein drugs are unstable in the stomach and upper part of the intestine. Colon specific drug delivery system protect peptide drug form hydrolysis and enzymatic degradation in duodenum, jejunum and eventually release in the ileum or colon, which leads to greater systemic bioavailability[1]. To achieve successful colonic delivery, a drug needs to be protected from absorption and \ or the environment of the upper gastrointestinal tract (GIT) and then be released into the proximal colon, which is considered the optimum site for colon targeted drug delivery [2].Colon targeting is naturally of for the topical treatment of diseases of colon such as chron's diseases, ulcerative colitis, colorectal cancer and amebiasis [3].

In ionic cross-linking technique, dropping or spraying a sodium alginate solution into a calcium chloride or barium chloride solution produces microcapsules[4]. The divalent calcium or barium ions cross-link the alginate formed gelled droplets. Variations on this method with different polymers have been developed. Chitosan is a preferred polymer, because it has a better biocompatibility than alginate. However, the droplets were relatively

large, because the drops do not fall until they reach a critical mass. Smaller droplets can be formed by using a pump to force the alginate through the pipette a vibration system to help remove the drops from the end of the pipette and an air atomization method.

Natural polymers [5], particularly in the form of microspheres, have an important role in the before they will have widespread use in clinical situations. Among these issues are better understanding of the kinetics of drug release; more effective ways to control burst phenomena; greater understanding of drug-polymer interactions and their effect on shelf life stability; additional animal studies to determine local tissue response, biodegradation rates, and metabolic rate; and, most importantly, as it relates to cancer chemotherapy, well-designed Clinical studies to assess efficacy in relation to current therapies. In the area of drug targeting, there needs to be continuing emphasis on understanding the gocytes and cell receptors. Guar gum is a natural non ionic polysaccharide being used as drug carrier for colonic delivery system due to its release retarding property and susceptibility to microbial degradation. Guar gum is derived from the seeds of Cyamopsis tetragonolobus. Guar gum is hydrophilic in nature and swells in cold water forming viscous colloidal dispersions or sols. This gelling property retards release of the drug from the dosage form as well as it is susceptible to degradation in the colonic environment. Sodium alginate (NaAlg) [6], a water-soluble salt of alginic acid, is a natural polysaccharide extracted from marine brown algae. It contains two uronic acids, β-D-mannuronic acid (M) and α-L-glucouronic acid (G), and it is composed of homopolymeric blocks MM or GG.NaAlg has been used as a matrix for entrapment of drugs and macromolecules. Alginates are biopolymers produced by seaweeds as well as by some bacteria like pseudomonas aeruginosa or Azobacter vinelandi.

Metronidazole is selectively toxic to anaerobic microorganisms. After entering the cell by diffusion its nitro group is reduced by certain redox proteins, operative only in anaerobic microbes to highly reactive nitro radical which exerts cytotoxicity by damaging DNA.Metonidazole is a sparingly soluble drug in water and it is also slightly soluble in acetone and in dichloromethane. Half-life of metronidazole is about 6-7 hours. The objective of present work is to formulate and in vitro evaluation studies of natural polymer based microspheres of MTZ, which targeted to colon and given 12hr. invitro drug release profile. The microspheres were studied for different pH range and the drug release profile match with the possible release mechanism.

MATERIALS AND METHODS

Materials: Metronidazole was obtained as a gift sample from Albert Devid LTD., Kolkata.Guargum and Sodium alginate were purchased form Merck Specialities Private Limited Mumbai. All other chemicals were used as analytical grade.

Methods:

Method of preparation of microspheres

Microspheres are prepared by ionotropic gelatination technique. Here, required amount of guar gum was dispersed in a specified volume of cold water containing the drug and allowed to swell for 2 hours. In another beaker suitable amount of sodium alginate was taken and mixed well with 10 ml of water. The guar gum solution containing the drug was added to sodium alginate solution with stirring to produce a

viscous form. After complete mixing 1.0 ml of glutaraldehyde[7] were added to the dispersion, followed by stirring at a constant speed. Then polymer drug solution was added drop wise by using syringe of 22 G in diameter from a height of about 5 cms into a beaker containing 4% w/v solution of calcium chloride with continuous stirring by magnetic stirrer. Then the solution containing the gel formed microspheres was filtered by using Whatman filter paper no-1. The microspheres were allowed to dry at about 30to 40°C and stored in well-closed container for further use.

Process variables [8]

The process variables were investigated (Bore diameter of the needle, concentration of sodium alginate; concentration of guar gum; concentration of calcium chloride; height of dropping stirring speed and stirring time) and the different batches thus produced were analyzed for size, shape, ease of preparation, drug content and drug release.

Particle Size Analysis

Microspheres were separated into different size fraction by sieving for 10 minuets using a mechanical shaker (Geologists Syndicate pvt Ltd, India) containing standard sieves having mesh size of ≠16, ≠18, and SS≠25. The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula**.** Mean particle size = ∑(mean particle size of the fraction × weight fraction) / ∑ (weight fraction) [9].

Surface morphology:

The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of a adhesive stub. Then the microspheres were coated with gold before microscopy. Finally the morphology and size of the microspheres were observed with the scanning electron microscope (FEI Quanta-200 MK2, Netherlands).

Drug entrapment efficiency, drug loading

The amount of MTZ present in the Guar Gum microspheres was determined by taking the known amount of microspheres in which 200 mg of drug should be present theoretically. Then the microspheres ware crushed and the powdered microspheres was taken and dissolved in 100 ml of phosphate buffer (pH7.4) solution and stirred for 15 minutes with an interval of 5 minutes and allowed to keep for 24 hours. Then the solution was filtered through Whatman No.1 filter paper. Then the absorbance was measured spectrophotometrically at 320 nm against phosphate buffer (pH7.4) solution as blank with the help of UV double beam spectrophotometer and concentrations were determined by employing simultaneous equation: Y= mx+c

DEE (%) = [Experimental drug Content / Initial Drug Content into the Formulation] ×100

Drug Loading (%) = [Q_m / Wm] × 100 [10], Where, W_m = weight of the microspheres; Q_m = quantity of the drug present in the microspheres

FTIR studies

The infrared (IR) spectra were recorded using an FTIR spectrophotometer (Perkin Elmer Spectrum GX) by the KBr pellet method in the wavelength region between 4000 and 400 cm^{-1}. The spectra obtained for metronidazole and physical mixtures of metronidazole with polymers were compared to check compatibility of drug with polymers.

In vitro drug release study

Drug release was performed using USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37±0.5°C) for first 2h in 0.1 N HCL (900ml). Then, 1.7g of KH_2PO_4 and 2.225g of $Na_2HPO_4.2H_2O$ were added, adjusting the pH to 4.5 by adding 1.0 M NaOH. A release study was continued for another 2h. at predetermined time intervals 2 ml samples were withdrawn and replaced by an equal volume of fresh medium. After 4h, the pH of the dissolution medium was adjusted to 7.4 and maintained for 24 h[11]. Samples were filtered, diluted and assayed at each interval for metronidazole content released at λ_{max} of 277 nm using double beam UV- spectrophotometer. Three trials were carried out for all formulations. From this percentage drug release was calculated and plotted against the function

In vitro Drug Release Kinetics

Drug release data were fitted to kinetic model including the zero order [Equation 1], first order [Equation 2], Higuchi matrix [Equation 3], and Korsmeyer-Peppas [Equation4] release equations to find the equation with the best fit.

$$R = k_0 t \quad [1]$$

$$\text{Log UR} = k_1 t / 2.303 \quad [2]$$

$$R = k_h \sqrt{t} \quad [3]$$

$$R = k_{kp} t^{n^*} \quad [4]$$

Where R and UR are the released and unreleased percentages respectively, at time [t]; k_0, k_1, k_h, k_{kp} are the rate constants of zero order, first order, Higuchi matrix and Korsmeyer-Peppas respectively.

Swelling study (degree of swelling)

Microspheres (100 mg) were placed in little excess of distilled water, 0.1N HCl and PBS (pH 7.4) and allowed to swell to constant weight. The microspheres were removed, blotted with filter paper and their changes in weight were measured at an interval period of 10 minutes and recorded. The degree of swelling (a) was then calculated from the formula:

$$a = W_G - W_O / W_O$$

Where, Wo is the initial weight of the beads and Wg is the weight of the beads at equilibrium swelling in the medium.

RESULTS AND DISCUSSIONS

Evaluation of preparation method

In this project attempts have been made to prepare the guar gum alginate microspheres bearing MTZ by ionotropic gelation technique. Here, microspheres were performed with glutaraldehyde which increased the entrapment efficiency, drug loading of microsphere and also increased the cross linking ability as well as reduced the swellability of formulation. The drug- polymer ratio of the formulation described by table no. 1.

Table 1: Formulations of microspheres

Formulation Code	Drug	Guar Gum	Sodium Alginate	Total Polymer	Ratio	Glutaraldehyde (ml)
F1	400	80	320	400	1:1	----
F2	400	240	560	800	1:2	.....
F3	400	400	800	1200	1:3	.....
F4	400	600	1000	1600	1:4	.....
FG1	400	80	320	400	1:1	1
FG2	400	240	560	800	1:2	1
FG3	400	400	800	1200	1:3	1
FG4	400	600	1000	1600	1:4	1

Process optimization

The process variables were investigated and the different batches thus produced were analyzed for size, shape, ease of preparation, drug content and drug release. Optimized process variables are described by Table No.2.

Particle size analysis

Particle size can be determined by sieve analysis method. The mean diameter of Guar gum cross-linked with Glutaraldehyde microspheres increased from 680 ± 0.68 µm to 768±0.38 µm. The average particle size of microspheres increased with increasing polymer as well as cross linking agent, which can describe by Table No.3 and Fig. No.7.

Surface morphology

Scanning electron microscopy (FEI Quanta-200 MK2, Netherlands) was used to observe the surface morphology of Guar gum alginate microspheres without drug, drug before dissolution, and after dissolution study described by Fig. No. 2, 3 and 4.

The results of drug loading increased from 14.59 ± 0.73 % to 24.74 ± 0.63% of microsphere with increasing the amount of polymer as well as cross linking agent.

Table 2: Optimized process variables data

Process Variable Parameters	Optimized Data
Bore diameter of the needle	22G
Height of dropping	5 cm from the level of $Cacl_2$ solution
Drying time and temperature	30^0 to 40^0c for 4 hrs.
Guar gum concentration	30% w\v dispersion.
Sodium alginate concentration	10% w\v dispersion.
Calcium chloride concentration	4% w\v solution
Concentration of glutaraldehyde	1.0 ml

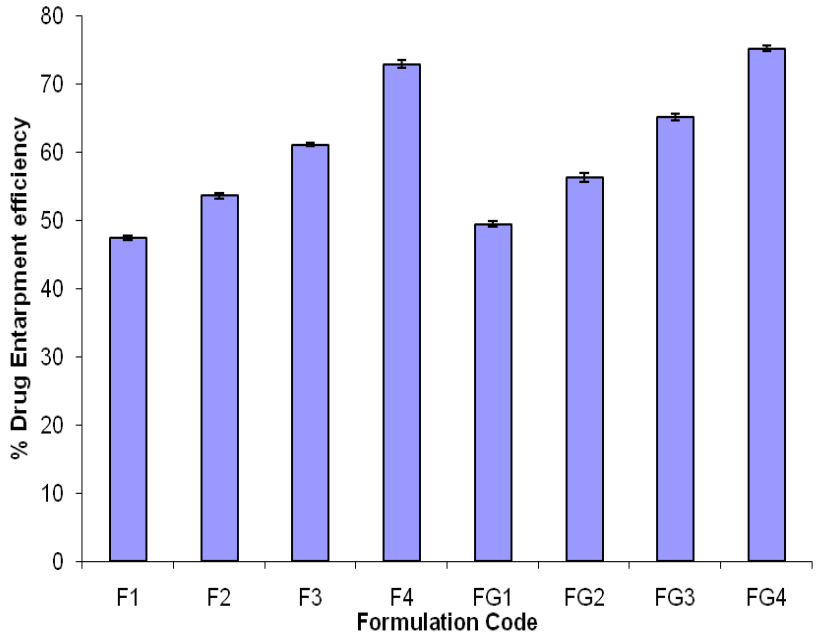

Fig. 1: Histogram diagram of drug entrapment efficiency

Table 3: Particle size analysis, drug entrapment efficiency and drug loading

Sl. No.	Formulation Code	Mean Particle Size µm (±S.D)*	DEE %*	DL %*
1	F1	680± 0.68	47.45 ± 0.34	23.72 ± 0.48
2	F2	733± 0.57	53.65 ± 0.42	17.88 ± 0.64
3	F3	692± 0.61	61.15 ± 0.28	15.28 ± 0.84
4	F4	723±0.29	72.95 ± 0.56	14.59 ± 0.73
5	FG1	710± 0.64	49.48 ± 0.47	24.74 ± 0.63
6	FG2	768±0.38	56.34 ± 0.69	8.78 ± 0.68
7	FG3	708±0.52	65.18 ± 0.48	16.29 ± 0.54
8	FG4	758±0.34	75.21 ± 0.41	15.04 ± 0.39

*Mean **±** S.D (n=3)

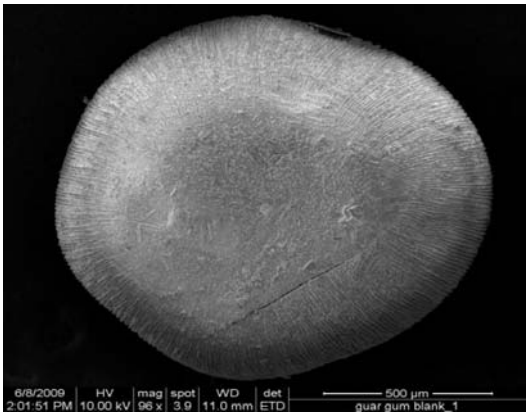

Fig.2: SEM of blank microsphere

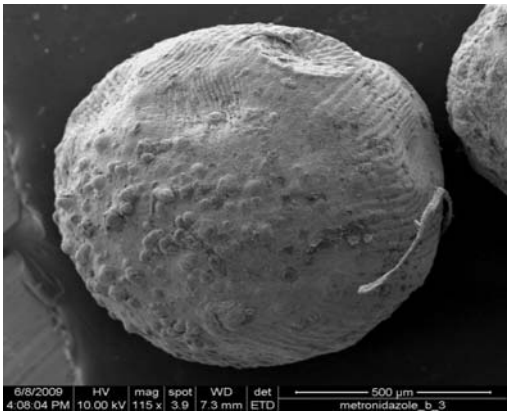

Fig. 3: SEM of MTZ microsphere before dissolution study

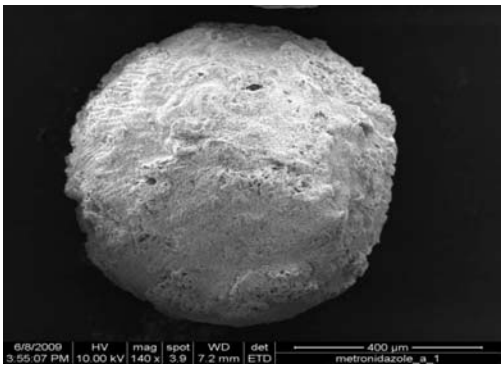

Fig. 4: SEM of MTZ microsphere after dissolution study

Drug loading and drug entrapment efficiency

The percent encapsulation efficiency was increased upto 75.21 ± 0.41% with increasing polymer concentration of Guar gum 30% w/v with Sodium Alginate 50% w/v. Amount of cross linking agent (Glutaraldehyde) also affect the encapsulation efficiency and the amount of Glutaraldehyde increasing up to 1.0 ml. The drug loading and percentage of encapsulation efficiency are described by Table No.3 and Fig. No.1 and 8.

FTIR studies

The FT-IR spectra analysis of metronidazole and the physical mixtures shows that there was no significant interaction between drug and polymers as shown in Fig No.9, 10, 11 and 12.

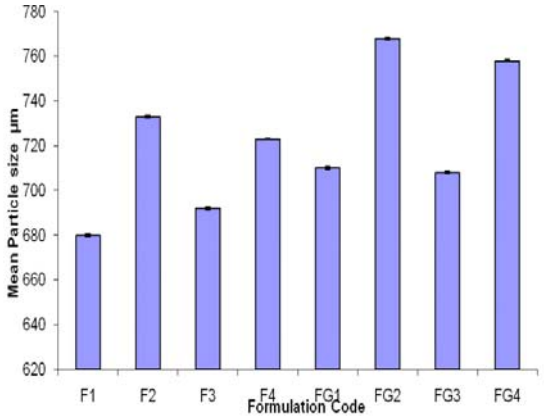

Fig. 7: Histogram diagram of mean particle size

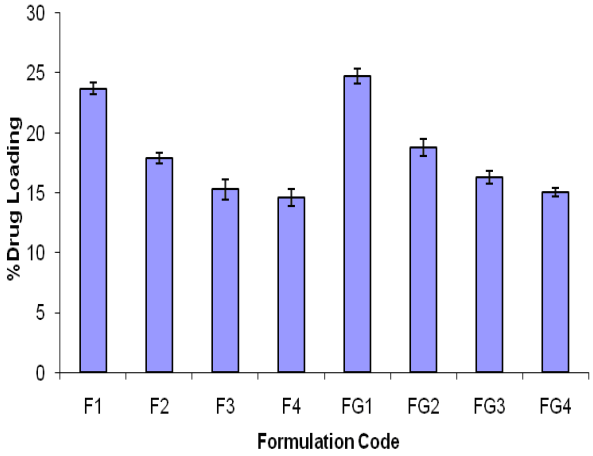

Fig. 8: Histogram diagram of drug loading

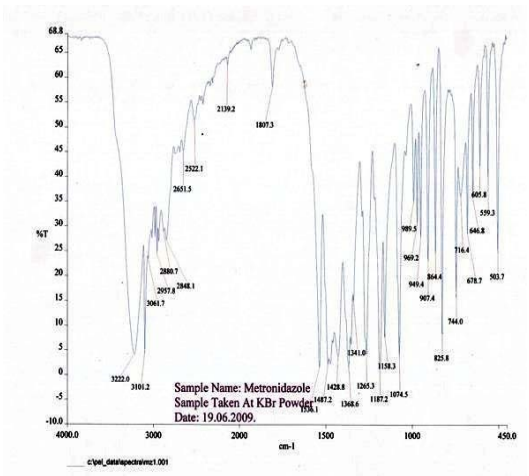

Fig. 9: FTIR spectra of metronidazole

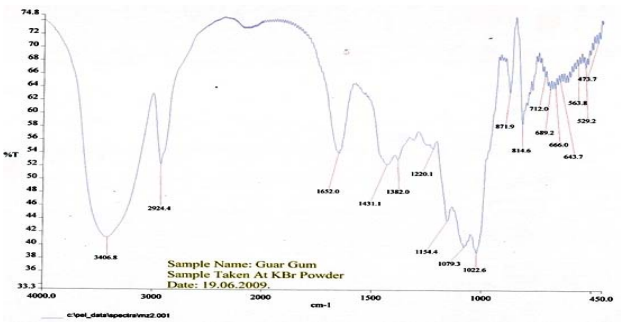

Fig. 10: FTIR spectra of guar gum

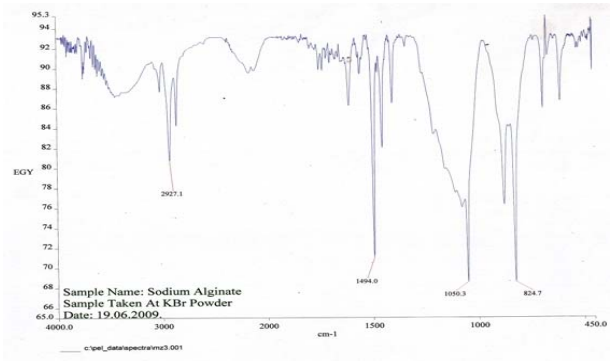

Fig. 11: FTIR spectra of sodium alginate.

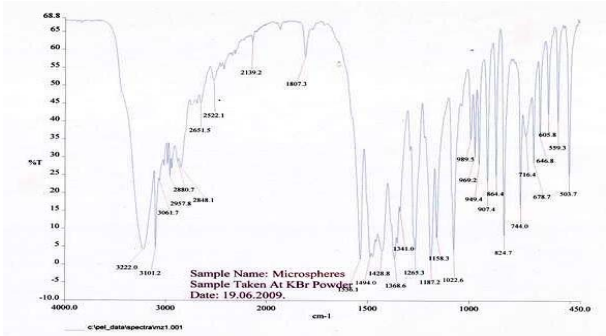

Fig. 12: FTIR spectra of metronidazole microspheres with guar gum and sodium alginate.

In vitro drug release study

Drug release were performed using USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37±0.5°C) for first 2h in 0.1 N HCL (900ml). Then drug release is found 85.73% at the end of 12 h. In-vitro drug release study of metronidazole are shown in Fig. No.13.

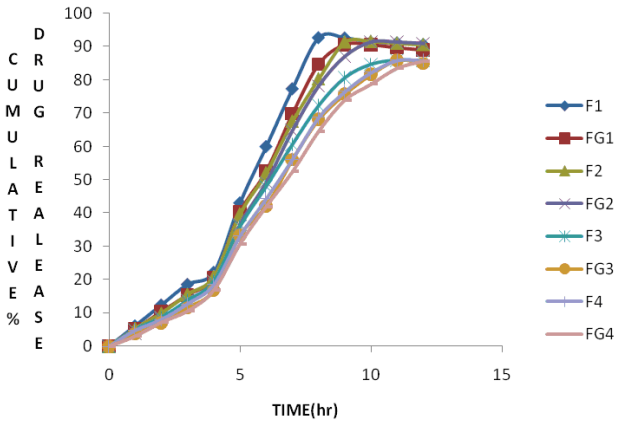

Fig. 13: In-vitro drug release study of metronidazole microsphere

Table 4: In-vitro release kinetic parameters for MTZ microspheres

Formulation Code	Zero order Model		First order Model		Higuchi Model		Korsmeyer Pappas Model	
	r^2	k_0	r^2	k_1	r^2	k_h	r^2	n
F1	0.914	9.23	0.865	0.114	0.861	34.61	0.959	1.221
F2	0.933	9.11	0.899	0.104	0.861	33.83	0.966	1.294
F3	0.946	9.19	0.902	0.109	0.865	33.96	0.970	1.291
F4	0.953	9.25	0.917	0.107	0.858	33.92	0.974	1.410
FG1	0.960	8.53	0.942	0.084	0.871	31.42	0.972	1.298
FG2	0.967	8.45	0.937	0.080	0.859	30.77	0.976	1.412
FG3	0.970	8.41	0.940	0.081	0.866	30.74	0.974	1.320
FG4	0.976	8.26	0.939	0.076	0.860	29.96	0.985	1.456

Drug release kinetics

The rate of drug release followed by zero order kinetics and numerical data fitted into peppas model where the value of n reaches above 1.This represents the case II and super case II transport, which indicates that the release is following zero order. The release kinetic parameters are shown in the Table No.4.

Swellability study

Guar gum- alginate microspheres swell in water, 0.1N HCl and phosphate buffer 7.4.the result of swellability index can be described by table no 6. As a result of cross-linking with glutaraldehyde the overall swelling of polymer decreased significantly. Cross-linking with free access of water to the guar gum hydroxyl group, which is turn reduces the swelling properties of the cross linked polymer. Swellability studies of microspheres are described by table no.5.

Table 5: Swellability study of microsphere

Sl.No.	Swelling percentage of Formulations								
	Nature of solvent	F1	F2	F3	F4	FG1	FG2	FG3	FG4
1.	Distilled water	110	160	310	390	92	120	220	360
2.	0.1N HCL	80	110	200	320	65	90	175	280
3.	Phosphate buffer 7.4	120	200	350	450	100	170	300	400

Micromeritic properties

The value of Angle of repose of formulation within the range of 25°, indicating very good flow properties for the microspheres.The tapped density values ranged between 0.758 to 0.796g/ cm^3. The result of Carr's Index range from 9.305 to 10.402%, suggests excellent flow characteristics of the microspheres. Hausner Ratio range from 1.102 to 1.113%, which indicates good flow property of microspheres were found.

CONCLUSION

In the present study a formulation method was developed and in- vitro characterization of Guar Gum- alginate based microspheres were prepared for colon specific drug delivery, where Guar gum act as a natural Polymer based carrier which is inexpensive and naturally occurring and also having hydrophilic and swelling properties. These properties and the viscous nature of the Guar gum retards release of the drug from the dosage form, making it more likely that degradation will occur in the colon. The Polysaccharides remain intact in the physiological environment of stomach and small intestine, but degrade in colon by enzymatic degradation.

This natural polymer is also appealing for use in drug delivery for a wide range of molecular weights, varying chemical compositions, low toxicity and biodegradability. Moreover, they are selectively degraded in the colon. Therefore, more research is necessary to be focused on the specificity of drug uptake at the colon site.

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