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Formulation and *In-Vitro* Evaluation of Colon Specific Polymeric Microspheres of Ornidazole

Prasanta Kumar Choudhury*[1], Padala Narasimha Murthy [1], Niraj Kanti Tripathy[2]

1. Department of Pharmaceutics, Royal College of Pharmacy and Health Sciences, Berhampur-760002, Odisha, India

2. Department of Zoology, Berhampur University, Bhanja Bihar, Berhampur-760007, Odisha, India

ABSTRACT

In the present investigation, pH-dependent and controlled drug release polymeric microspheres of Ornidazole were developed to deliver the active molecule to the colonic region. Microspheres were prepared by emulsion cross-linking method with some modifications, using different proportions of Ornidazole and Polymers (guar gum and gelatin). Gelatin microspheres were coated with Acrycoat L100 to achieve pH sensitive properties and specific biodegradability for colon targeted delivery of Ornidazole. Microspheres were evaluated for size, morphology, sphericity study, % yield, loose surface crystal study, drug content and entrapment efficiency. In vitro drug release study was conducted by buffer change method to mimic GIT environment using buffer solutions of varying pH. The investigations revealed that microspheres prepared with Ornidazole: guar gum ratio (1:2) shows only 10.003±0.885 % drug was released in first 5 hours and 38.849 ± 0.62 % in 12 hours, which proves the potentiality of guar gum for colonic delivery of drugs. While for microspheres prepared with Ornidazole: gelatin (1:2) and coated with Arycoat L100 shows 16.596 ± 3.18 % drug release in first 5 hours and 45.921 ± 3.07 % in 12 hours.

Key Words: colon specific drug delivery, biodegradable polymers, pH-dependent release, In vitro drug release

*Corresponding Author Email: prasant_pharma@yahoo.com

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INTRODUCTION

Colon drug delivery has gained much interest for local as well as systemic delivery due to its prolonged residence time to luminal contents, reduced epithelial enzymatic activity, increased tissue responsiveness to absorption enhancers and natural absorptive characteristics. The delivery systems intended to release drugs in the colon require protection of the drug from hostile environment of stomach and small intestine. This target specific release is required for the topical treatment of diseases associated with the colon such as ulcerative colitis, Crohn's disease, colon cancer and amebiasis. In addition, it has tremendous potentials for the oral delivery of therapeutic proteins and peptides due to the presence of favorable environment in colon in comparison to upper gastrointestinal tract. Various approaches available for colon targeted drug release include coating with pH-sensitive polymers, coating with biodegradable polymers, fabrication of pro-drugs, timed-release systems and embedding in biodegradable matrices and hydrogels[1]. The approach, which is based on pH-sensitive polymers, has not shown promising results due to poor site-specificity. This problem can be partially rectified by targeting multiple unit dosage forms[2]. Microbially degradable polymers especially azo-crosslinked polymers have been investigated for use in targeting drugs to colon[3]. Formulations coated with azo polymers remain intact in the upper gastrointestinal tract and upon arrival, in the colon; they are reduced by azo reductase enzymes present in the colon. Some polysaccharides such as pectin, guar gum, chitosan and amylose have been used as drug carriers for colon targeted release[4].

Colonic diseases are important causes of death by protozoal infections in the developing world even in advanced countries. Ornidazole has extremely broad spectrum of protozoal and antimicrobial activity. It is clinically effective in colonic diseases, both locally and systemically[5]. Based on the observations, an attempt has been made to develop polymeric microspheres using biodegradable, pH sensitive polymers for colon specific delivery of Ornidazole for the treatment of colonic diseases. A colonic polymeric coating (Acrycoat L 100) was also applied on the Gelatin microspheres.

MATERIALS AND METHODS

M/s Diamond Drugs Pvt Ltd, Howrah, WB, India, generously supplied Ornidazole as a gift sample. Guar Gum and Gelatin were procured from S.D. fine-chem Ltd., Mumbai, India. Light Liquid paraffin was obtained from Loba Chemie Ltd, Mumbai. Acrycoat L-100 procured from Corel Pharma-Chem, Gujarat, India. Span 80, Tween 80 was procured from S.D. fine-chem Ltd., Mumbai, India. All other solvents and reagents were of analytical grade.

Preparation of Microsphere formulations

The microspheres of Guar gum and Gelatin were prepared by emulsion cross linking method[6,7] in a dispersing medium consisting of liquid paraffin utilizing Glutaraldehyde as a cross-linker (Formulation compositions of microspheres were revealed in Table 1.). Aqueous solutions of Guar gum and Gelatin in double distilled water was prepared by mixing with Tween 80 (0.2% w/w) and heating was done at 45- 60°C as per necessity followed by addition of the drug (Ornidazole) to the aqueous solution with constant stirring. Concentrated sulphuric acid (0.2 ml) was added and the aqueous phase and the resultant mixture was poured into liquid paraffin (previously heated at 55°C to 70°C) containing Span 80 (1.5% w/v). The system was kept under agitation using mechanical stirrer for 10 min at 1000 rpm and then rapid cooling to 5ºC using an ice bath to hardened the droplets (in case of gelatin microspheres). After 20 minutes of stirring glutaraldehyde (5ml) chilled at 5ºC was added under stirring and stirring was continued for 1hr. The microspheres were collected by centrifugation and filtration followed by washing several times with n-hexane, methanol and acetone or isopropanol to remove traces of oil and dried under vacuum desiccator. By using this method, different batches of microspheres of varying drug: polymer ratio (2:1, 1:1, 1:2), were prepared and the batches were coded as GGM (for guar gum) and GLM (for gelatin).

Table 1: Composition of Guar Gum and Gelatin Microsphere Formulations

Formulation	Drug (mg)	Guar gum (mg)	Gelatin (mg)	Drug: polymer ratio	Quantity of distilled water (ml)	Quantity of liquid paraffin (ml)	Quantity of rigidizing agent (ml)
GGM1	1000	500	--	2:1	25	150	5
GGM2	1000	1000	--	1:1	25	150	5
GGM3	1000	2000	--	1:2	25	150	5
GLM1	1000	--	500	2:1	25	150	5
GLM2	1000	--	1000	1:1	25	150	5
GLM3	1000	--	2000	1:2	25	150	5

Coating of Gelatin microspheres

Coating of gelatin microspheres containing Ornidazole was performed using emulsion solvent evaporation technique[8]. Gelatin microspheres were suspended in 15 ml of an organic solvent (1:1, acetone: methanol) in which Acrycoat L-100 was previously dissolved to give 1:5 core/coat ratio. This organic phase was emulsified into 100ml of liquid paraffin containing span 80 (1% w/v). The system was stirred at 1000 rpm with mechanical stirred for 4 hr at room temperature. The Acrycoat L 100 coated microspheres were collected and rinsed with n-hexane and dried in vacuum desiccator for 48 hr.

IN VITRO CHARACTERIZATION OF MICROSPHERES

Drug-Polymer Interaction analysis:

To eliminate the possibility of polymers interfering with the analysis of drug, Infra-red spectrum was taken by using the Shimdzu-840–os, Japan FTIR model by scanning the sample in potassium bromide (KBr) discs. Before taking the spectrum of the sample, a blank spectrum of air background was taken. The sample of pure drug, pure polymer and the formulations containing both the drug and polymer were scanned.

Particle Size Analysis

Particle size distribution of the microspheres was determined by optical microscopy using calibrated ocular eyepiece[9]. Fifty microspheres were evaluated and the experiment was performed. Geometric mean diameter was then calculated using the equation:

$X_g = 10 X [(n_i * \log X_i) / N]$ ---------- (Equation-1)

Where Xg is geometric mean diameter, n_i is no of particles in the range, X_i is the midpoint of range, N is total no of particles analyzed.

Determination of Shape and Sphericity

Morphological appearance and surface characteristics of the microspheres were studied by dispersing the microspheres in liquid paraffin and observed under 250X magnification using an optical microscope[9].

The particle shape was measured by computing circularity factor (S)[10]. The tracing obtained from optical microscopy were used to calculate area (A) and perimeter (P), which are used to calculate the circularity factor (S) by using the equation:

$S = P^2 / (12.56 * A)$ ---------- (Equation-2)

Determination of drug content [11]

The drug content in Guar gum, Gelatin, Sodium alginate microspheres was determined by taking accurately weighed 100mg of microspheres in a glass mortar and powdered by a glass pastel and treated with 100ml of Phosphate buffer of pH 7.4 in a closed volumetric flask and left over night. Then it was transferred into a 250ml beaker and stirred by magnetic stirrer using Teflon coated magnetic bead, the temperature was maintained at 37ºC. At the end of 1 hour, it was centrifuged and supernatant was filtered, the filtrate was analyzed spectrophotometrically at 319nm (UV 1700, Shimadzu, Japan). Dilution was done whenever required using Phosphate buffer pH 7.4.

Drug Entrapment efficiency (DEE %)

Entrapment efficiency of the microspheres was calculated using the formula

DEE % = Practical Drug Loading/ Theoretical Drug Loading X 100---------- (Equation-3)

Loose surface crystal study [12]

Loose surface crystal study was done to observe the excess drug present on the surface of microspheres. From each batch 11mg of microspheres were shaken in 100 ml of phosphate buffer pH 7.4 for 5 minutes and then filtered through whatman filter paper 41. The amount of drug in the filtrate was determined spectrophotometrically at 319 nm and calculated as a percent of total drug content. This estimates the surface entrapment of the drug by the microspheres.

Measurement of the Yield of the Microencapsulation Process

The weight of the obtained microspheres after drying was divided by to the total weight of polymers and drug used.

Calculation was done as per the equation mentioned below---------- (Equation 4)

Yield of microencapsulation (%) = Practical Yield/ Theoretical Yield

= Produced microspheres (mg)/ Drug (mg) + Polymer (mg) X 100

In Vitro Drug Release from Microspheres

In vitro drug release studies were carried out using USP dissolution rate test apparatus (basket apparatus, 100 rpm, 37 ± 0.1°C) by buffer change technique[8]. Microspheres bearing Ornidazole were suspended in simulated gastric fluid (SGF), pH 1.2, for 1 hr. The dissolution media was then replaced with mixture of simulated gastric fluid and simulated intestinal fluid (SIF), pH 4.5 for next two hours, then for next two hours simulated intestinal fluid (SIF) pH 6.8 and the release study was carried out for a further in simulated intestinal fluid pH 7.4.

Samples were withdrawn periodically and compensated with an equal amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance at 319nm using UV spectrophotometer (UV 1700, shimadzu, Japan).

Drug release mechanism and kinetics

In order to establish the mechanism and kinetics of drug release from the microspheres, the experimental data obtained from the in vitro dissolution study was fitted with different kinetic models like zero order, first order, Higuchi's model, Korsmeyer and peppas model etc.

Korsmeyer's model[13] is widely used; when the release mechanism is not well known or when more than one type of release phenomena could be involved.

Korsmeyer and Peppas equation:

$$Mt/M_\infty = Kt^n,$$

Where

Mt/M_∞ = the fractional drug release in time't',

K=constant incorporating of structural and geometric characteristics of controlled release device.

n = diffusional release exponent indicative of release mechanism.

The best-fit model was determined statistically employing comparison of correlation coefficients. The preparation of graphs and statistical calculations were carried out with the help of Microsoft Excel® software.

RESULTS AND DISCUSSION

To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target site in the optimal amount for a right period of time, thereby causing little toxicity and minimal side effects. In the present investigation attempts have been made to achieve colon specific drug delivery of Ornidazole by preparing Guar gum and Acrycoat L100 coated gelatin microspheres.

After selection of Ornidazole as model drug, thorough analytical study and characterization was done. The UV spectral analysis showed the maxima as per the monograph for Ornidazole (as mentioned in Figure 1 and Table 2). The calibration curves of Ornidazole were prepared in different standard solutions. The linearity was observed between the concentration of Ornidazole and its corresponding absorbance values, at determined λmax.

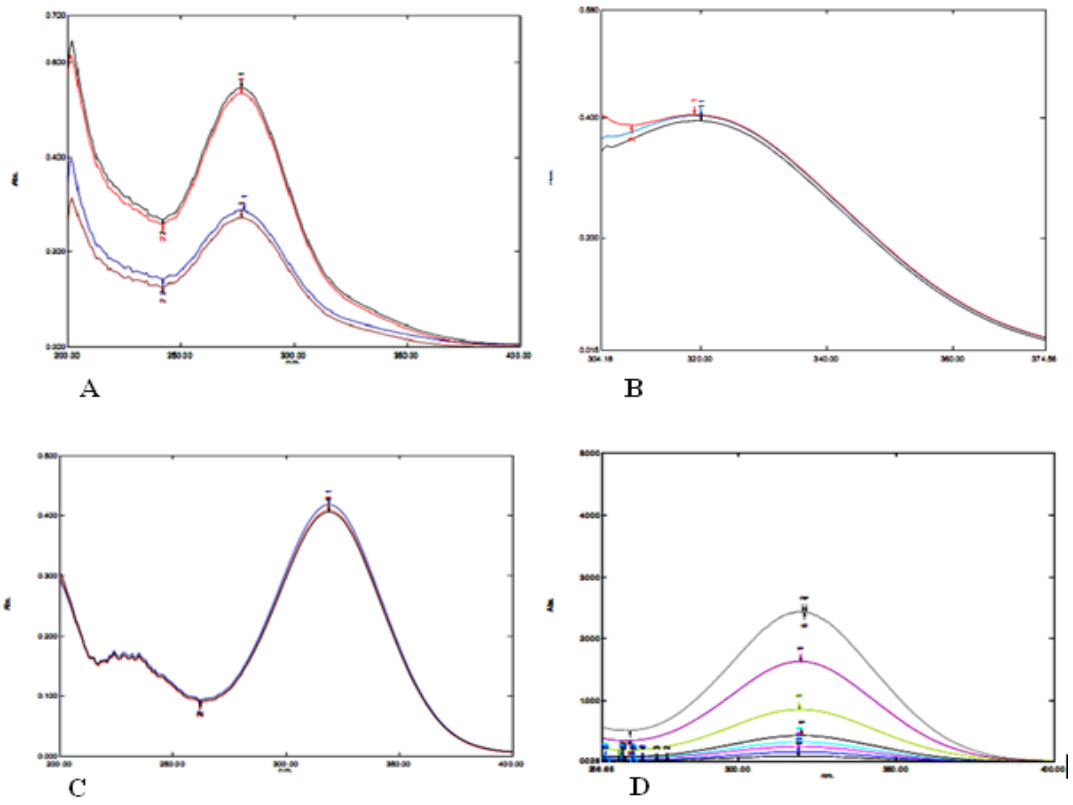

Figure 1: Overlay Spectra of Ornidazole (A) In Hydrochloric Acid Buffer pH 1.2, (B) In Acetate Buffer pH 4.5, (C) In Phosphate Buffer pH 6.8, (D) In Phosphate Buffer pH 7.4

Table 2: UV Spectral analysis of Ornidazole in different buffer solutions of varying pH conditions and determination of λmax

Sl. no.	Solvents	Max. Wavelength (λmax)	Absorbance* at corresponding λmax
1.	HCl Acid Buffer pH 1.2	277 nm	0.272 ± 0.003
2.	PBS pH 4.5	320 nm	0.405 ± 0.004
3.	PBS pH 6.8	319 nm	0.404 ± 0.005
4.	PBS pH 7.4	319 nm	0.433 ± 0.008

*Value expressed as mean ± SD (n=3), PBS- Phosphate buffer solution

Drug-polymer interaction study was done by FTIR analysis of pure drug (as disclosed in Figure 2), pure polymer and the physical mixtures/ formulations containing the respective polymers, which confirmed that there is no such chemical interaction between the drug and polymer (data displayed in Table 3 and Figure 3 to Figure 5).

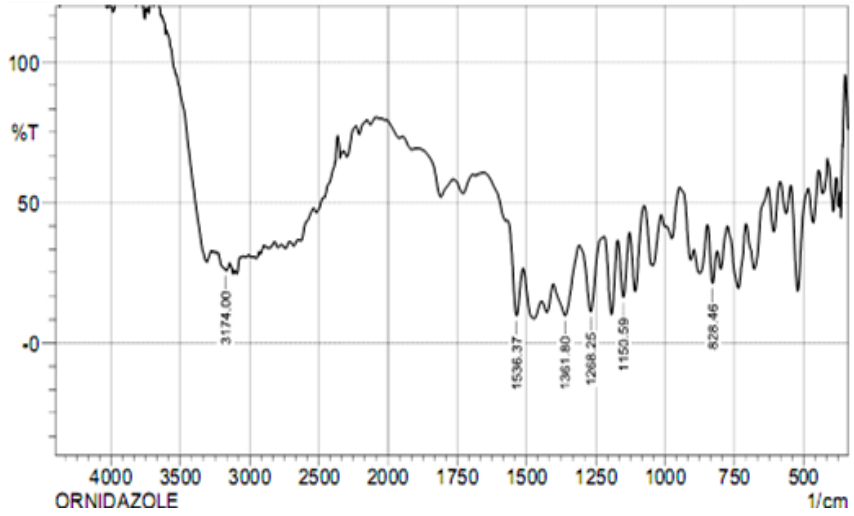

Figure 2: FTIR spectra of pure Ornidazole

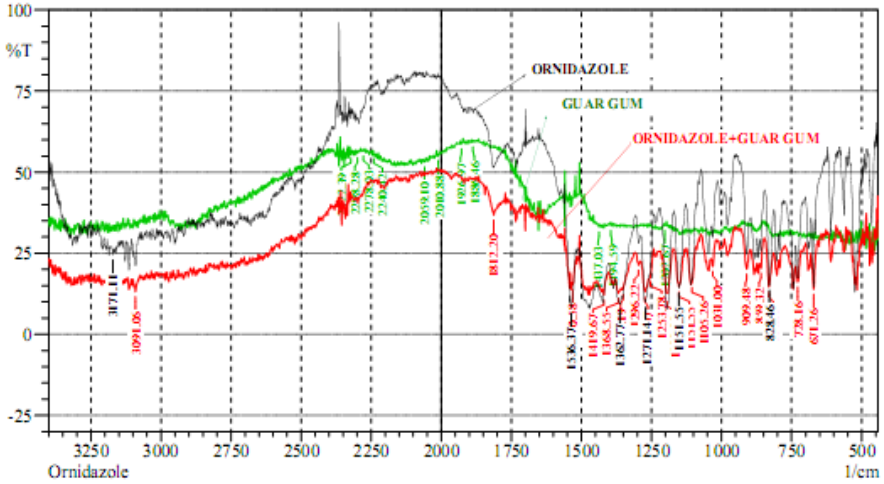

Figure 3: Overlay FTIR spectra of Formulation containing guar gum and Ornidazole, Pure guar gum, Pure Ornidazole

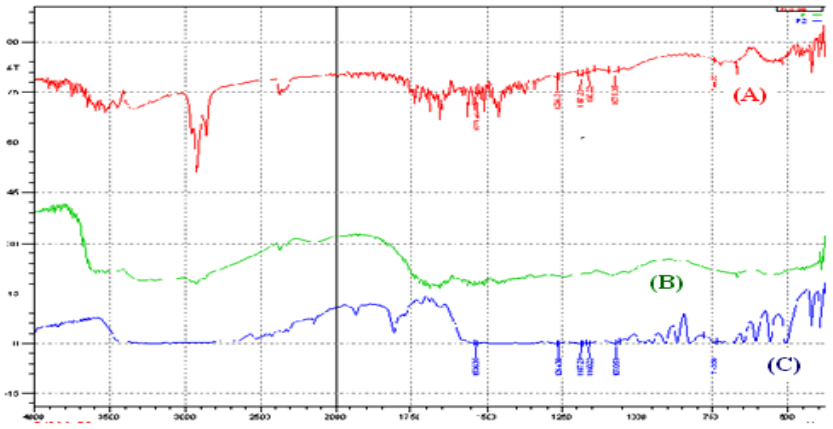

Figure 4: Overlay FTIR spectra of (A) Formulation containing gelatin and Ornidazole, (B) Pure gelatin, (C) Pure Ornidazole

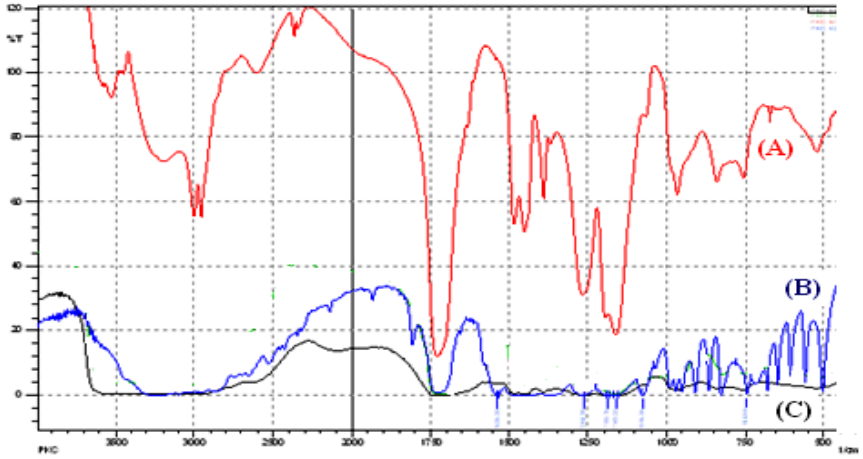

Figure 5: Overlay FTIR spectra of (A) Pure Acrycoat L100 (B) combination mixtures of Acrycoat L100 & Ornidazole, (C) Acrycoat L100 & Gelatin

Table 3: FTIR peak interpretation of Ornidazole

Sl. No.	Assignment	Reported Peak (cm^{-1})	Observed Peak (cm^{-1})
1.	O-H stretching mode asymmetric	3174.1	3174.00
2.	NO_2 stretching mode	1536.9	1536.37
3.	NO_2 stretching mode symmetric	1361, 1269.5	1361.80, 1268.25
4.	C-O stretching mode	1149	1150.59
5.	C-N, N_2 stretching mode	828	828.46

Cross-linked microspheres of guar gum and Gelatin loaded with Ornidazole were successfully prepared by the emulsification technique using light liquid paraffin in the external phase. Rigidity of the guar gum and gelatin microspheres was induced by chemical cross-linking method utilizing glutaraldehyde as cross-linker. The acidic medium required for the process of crosslinking was imparted by the addition of concentrated sulphuric acid.

The effect of different drug and polymer ratio was analyzed in order to optimize the formulation. It was observed that by changing drug: polymer ratio the shape, size as well as the entrapment efficiency of formulations considerably influenced. The microspheres were discrete and fairly spherical in shape while the surface roughness was slightly increased with the incorporation of the drug. Tween 80 was used for the purpose of wetting of guar gum and gelatin. Excellent microspheres were produced when the process was carried out with Drug: guar gum ratio 1:2 while the shape of the microspheres was distorted and some of them fused to each other when guar gum ratio was decreased. It was due to the presence of higher amount of water, which slowly evaporated on stirring, causing the particles to come in contact with each other. The drug particles appeared on the surface of the microspheres when they were prepared with drug: polymer ratio 2:1.

Particle size of the cross-linked guar gum microspheres was determined using optical microscopic method. Mean particle size was found to be 30.2±2.30 µm in case of microspheres having drug: guar gum ratio 2:1 while it was significantly increased to 34.8±2.45µm with drug: guar gum ratio (1:2) (as in Table 5). The size of the microspheres is controlled by the size of the dispersed droplets of guar gum in liquid paraffin. When the concentration of the guar gum in the formulation was increased, there was increment in the size of dispersed droplets that resulted in the formation of microspheres having bigger particle size. With increase in the polymer ratio in the formulations the mean particle size of all the formulations were increased (as mentioned in Table 5). After coating the Gelatin microspheres with Acrycoat L100 the increase in mean particle size was remarkable (as in Table 4). All the microsphere formulations have the circularity factor nearest to "1" which proves that they are almost spherical in shape (as per Table 5). The microspheres showed better Entrapment efficiency with increase in polymer ratio. In case of Guar gum microsphere formulations highest entrapment efficiency was observed for the formulations GGM3 83.519± 4.68.

Table 4: Average particle size, Entrapment efficiency, and circularity factor of Acrylcoat L 100 coated Gelatin Microspheres.

Batch code	Drug : polymer ratio	Particle size(µm)	Circularity Factor (S)
CGLM1	2:1	75.7 ± 4.34	1.01 ± 0.048
CGLM2	1:1	83.8 ± 6.34	1.04 ± 0.062
CGLM3	1:2	95.9 ± 5.21	1.13 ± 0.058

Values are expressed as Mean average ± SD (n=3)

Table 5: Evaluation Parameters of Guar Gum and Gelatin microspheres

Batch code	Drug : polymer ratio	Yield of production %	Particle size (µm)	Circularity Factor (S)	Drug loading (mg)/ Entrapment Efficiency %	Surface Entrapment % (Loose surface crystal study)
GGM1	2:1	81.85 ± 3.74	30.2 ± 2.30	1.06 ± 0.030	74.127±4.88	25.471 ± 5.13
GGM2	1:1	85.76 ± 2.48	32.3 ± 2.43	1.05 ± 0.005	76.499±2.49	22.513 ± 4.22
GGM3	1:2	89.33 ± 4.71	34.8 ± 2.45	1.06 ±0.025	83.519±4.68	15.470 ± 1.57
GLM1	2:1	95.13 ± 1.18	28.30±1.65	1.040 ± 011	64.449±2.89	32.347 ± 4.10
GLM2	1:1	93.40 ± 2.55	29.83± 1.02	1.06 ± 0.024	70.237±3.47	24.236 ± 4.08
GLM3	1:2	95.66 ± 1.74	31.64±3.37	1.04 ± 0.026	67.390±5.60	29.442 ± 4.01

Values are expressed as Mean average ± SD (n=3)

But in case of Gelatin microspheres with increase in drug: polymer ratio from 2:1 to 1:1 entrapment efficiency was increased (i.e. from 64.44±2.89 (GLM1) to 70.237±3.47 (GLM2) but it was decreased when the polymer amount was increased to 1: 2 ratio (as mentioned in Table 4). As the drug : polymer ratio was increased the surface entrapment of the drug on the microsphere surfaces was decreased which is suitable for the colonic delivery of the drugs and the surface entrapment of drug shows a less amount of drug lose due to the process variables.

The cross-linked microspheres were subjected to in vitro drug release rate studies in SGF (pH 1.2) for 1 h and in mixture of SGF and SIF (pH 4.5) for next 2hrs in order to investigate the capability of the formulation to withstand the physiological environment of the stomach and small intestine. The amount of Ornidazole released during first 5 h studies was found to be 16.342 ± 1.13 %, 14.495 ± 0.77 %, 10.003 ± 0.88 %, for GGM1, GGM2, GGM3, (as shown in Figure 6) which attests the ability of the guar gum to remain intact in the physiological environment of stomach and small intestine. The little amount of the drug, which is released during 5 h release rate studies, is due to the presence of un-entrapped drug on the surface of the microspheres. It is a well established fact that as the guar gum comes in contact with the dissolution medium it creates viscous gel layer around it which controls the release of the entrapped drug. The initial release of the drug present on the surface was higher during the 5 h study, which could be due to the fact that there was no viscous gel layer around the particles and it might have formed after 2 or 3 h which controlled the further release of drug. The release of drug from cross-linked guar gum microspheres was supposed to take place after swelling which resulted in the formation of gel followed by the dissolution of Ornidazole and diffusion through the gel. The gel strength of the guar gum microspheres swelled in the dissolution media might be too high and prevented the release of drug from formulation.

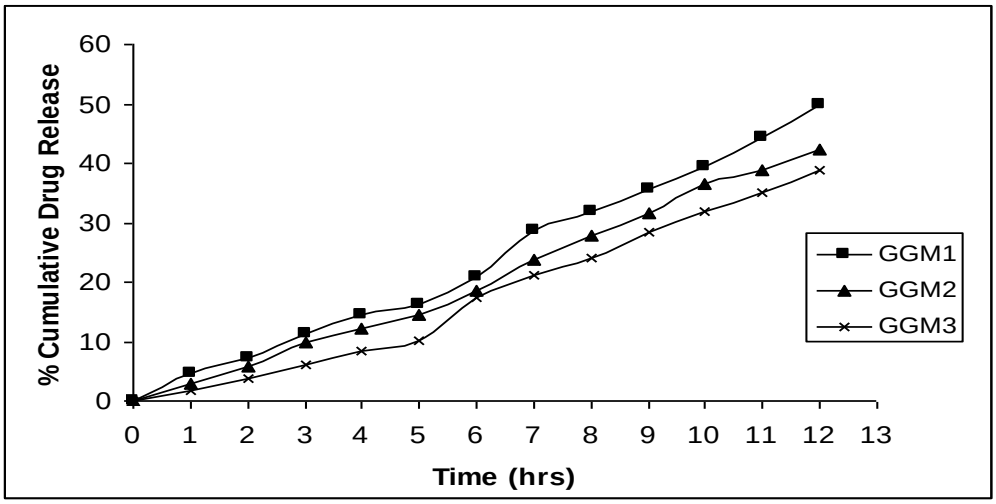

Figure 6: Percentage Cumulative drug release of Guar gum microspheres.

But for GLM1, GLM3 it was found to be 44.779 ± 3.92 %, 50.819 ± 3.06 % and 52.459 ± 2.07 %, (as in Figure 7) which is not suitable for the drugs which are intended for the colonic delivery. Hence needs to be protected by an extra coating formulation which was done by applying Acrycoat L 100 coat around the gelatin microsphere formulations. After coating with Arycoat L 100 (core coat: ratio-1:5) the percent drug release was decreased remarkably in the initial 5 hours of the dissolution study which proves that Acrycoat L 100 can withstand the upper GI environment and can release the drug at the targeted site, colon.

The release of the drug was much faster during the 6-12 hour study period. It is due to the fact that during the initial period (0-5 h) the strength of the barrier was too high to be broken and during 6-12 hour period the network was somewhat loosened which facilitated the release of drug (as in Figure 7 and 8.).

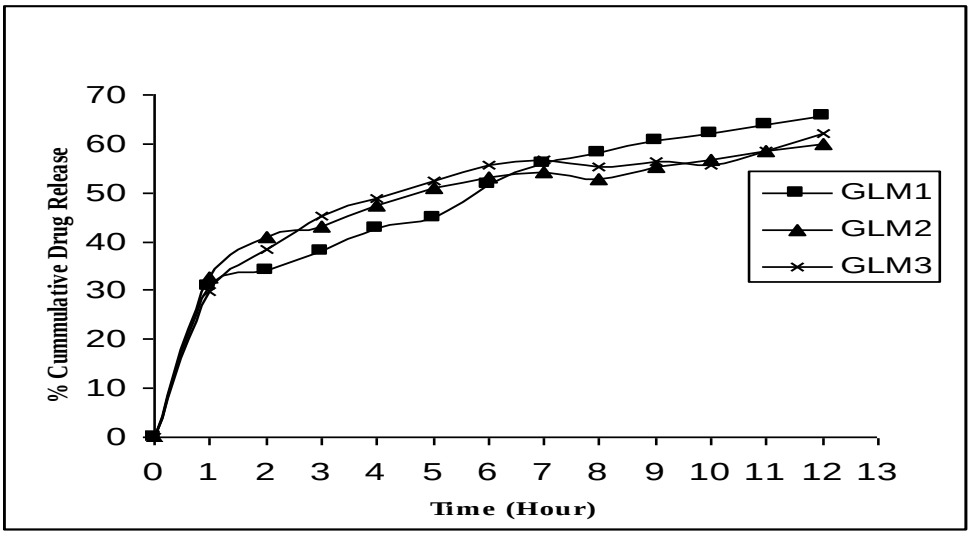

Figure 7: Percentage Cumulative drug release of uncoated Gelatin microspheres.

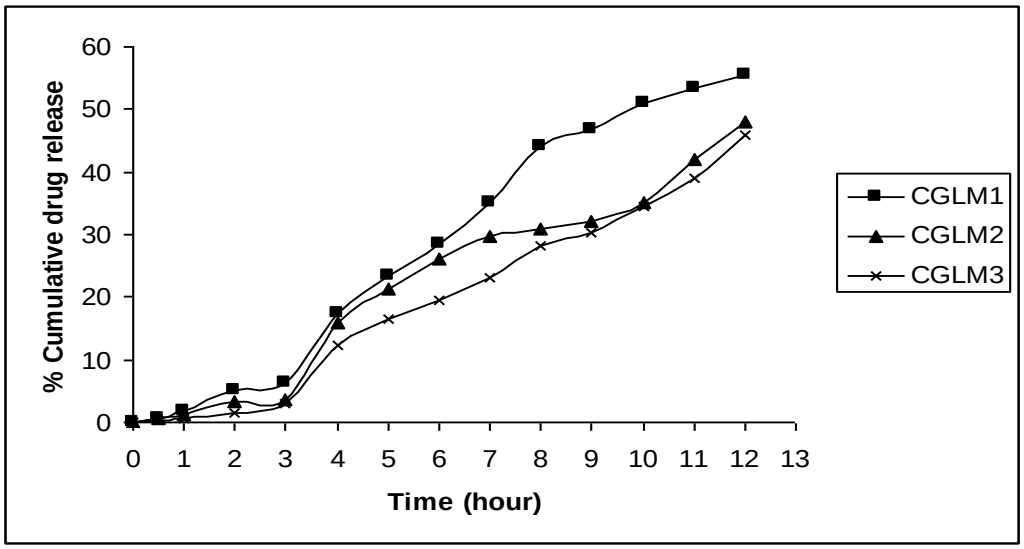

Figure 8: Percentage Cumulative drug release of Acrycoat L100 Coated Gelatin microspheres.

The experimental data was fitted to different kinetic models like zero-order, first-order etc in order to establish the release pattern of the drug from the microspheres. The experimental data was also fitted to Higuchi's model and Korsmeyer's model to ascertain the mechanism of drug release from the microsphere formulations. The correlation coefficient of the slopes of these matrices showed an adequate fit to the zero-order kinetics (as in Table 6).

Table 6: Dissolution kinetics for microsphere formulations

Formulation code	Zero – Order (R^2)	First – Order (R^2)	Higuchi (R^2)	Korsmeyer's Plot (R^2)	Korsmeyer's exponent "n"
GGM1	0.9917	0.9401	0.8892	0.9854	0.9959
GGM2	0.9941	0.9086	0.8910	0.9964	1.0966
GGM3	0.9834	0.9076	0.8547	0.9920	1.3136
GLM1	0.8357	0.9427	0.9717	0.9628	0.3372
GLM2	0.6504	0.8264	0.8767	0.9773	0.2298
GLM3	0.6593	0.7317	0.8848	0.9438	0.2695
CGLM1	0.9795	0.8124	0.8975	0.9852	1.4605
CGLM2	0.9687	0.8072	0.8930	0.9588	1.4456
CGLM3	0.9809	0.8267	0.8690	0.9635	1.6896

All the formulations followed Higuchi's equation proving that the release is by diffusion mechanism. The 'n' values obtained for uncoated microsphere formulations after fitting into Korsmeyer and Peppas equation are closely approximate with n =0.5, indicating Fickian diffusion (Table 7). But for the guar gum microspheres and Acrycoat L 100 coated gelatin microspheres the 'n' value closely equal to 1, indicating case II transport (zero order) release mechanism.

Table 7: Characterization of Drug Release Mechanisms

"n" values	Mechanism
0.5	Fickian Diffusion (Higuchi matrix)
0.5 <n< 1.0	Anomalous transport
1	Case II transport (Zero order release)
n>1	Super case II transport

CONCLUSION

The efficacy of the guar gum was evaluated for colon targeted drug delivery by fabricating it into microspheres. The microspheres of guar gum prepared by emulsification-cross linking method, which is capable of providing protection to the drug in the hostile environment of upper gastrointestinal tract and released the drug at the target site. While the gelatin microspheres prepared by same method showed higher in vitro drug release in the SGF (pH 1.2) and in mixture of SGF and SIF (pH 4.5), hence these were coated with Acrycoat L100 for colonic delivery of Ornidazole. The in vitro drug release studies of guar gum and Acrycoat L100 coated gelatin microspheres revealed that very less amount of the drug was released in the physiological environment of stomach and small intestine. Hence these data attests the potentiality of guar gum and Acrycoat L100 for colon-specific delivery of the drugs.

REFERENCES

1. Rubinstein A. Approaches and opportunities in colon-specific drug delivery. CritRev Ther Drug Carrier Syst 1995; 12(2-3): 101-49.
2. Chourasia MK, Jain SK.Design and Development of Multiparticulate System for Targeted Drug Delivery to Colon, Drug Delivery 2004; (11):201–207
3. Chourasia MK, Jain SK. Pharmaceutical approaches to colon targeted drug delivery systems, J Pharm Pharma Sci, 2003; 6(1): 33-66
4. Ashford M, Fell JT, Attwood D, Sharma H, Wood head PJ. An in vivo investigation into the suitability of pH-dependent polymers for colonic targeting. Int J Pharm 1993; 95: 193-199
5. Mishra B. *et al,* Development of chitosan-alginate microcapsules for colon specific delivery of Ornidazole, Indian Drugs, 2003; 40 (12): 695-700.
6. Chourasia MK, Jain SK. Potential of Guar Gum Microspheres for Target Specific Drug Release to Colon. J Drug Targeting 2004; 12 (7): 435–442
7. Gohel MC, Parikh RK, Amin AF, Surati AK. Preparation and Formulation Optimization of Sugar Crosslinked gelatin Microspheres of Diclofenac Sodium. Indian J Pharm Sci 2005, 67(5): 575-581

8. Chourasia MK, Jain SK. Design and Development of Multiparticulate System for Targeted Drug Delivery to Colon. Drug Delivery 2004; 11: 201–207
9. Shabaraya AR, Narayanacharyulu R. Design and evaluation of chitosan microspheres of metoprolol tatarate for sustained release. Indian J Pharm Sci 2003, 65(3): 250-252.
10. Abu-Izza K, Garcia LC, Robert D. Preparation and evaluation of zidovudine loaded sustained release microspheres: optimization of multiple response variables. J Pharm Sci 1996; 85 (6): 572-574
11. Bhumkar DR, Maheswari M, Patil VB, Pokharkar VB. Studies on effect of variability by response surface methodology for Naproxane microspheres, Indian drugs, 2003; 40 (8): 455-461
12. Mishra B, Jayanta B, Sankar C. Development of chitosan-alginate microcapsules for colon specific delivery of Ornidazole. Indian drugs 2003;40 (12): 695-700
13. Nicolas Grattard, Marc Pernin, Bernard Marty, Gaelle Roudaut, Dominique Champion, Martine Le Meste., Study of release kinetics of small and high molecular weight substances dispersed into spray-dried ethyl cellulose microspheres. J Controlled Release 2002; 84: 125–135