AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Design and evaluation of Coated Microspheres of Antiprotozoal drug for colon specific delivery

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ABSTRACT

Colon-specific drug delivery has gained increased importance in the delivery of drugs for the treatment of local diseases associated with the colon, such as Crohn's disease, ulcerative colitis, colorectal cancer and amoebiasis. Metronidazole, an antiprotozoal drug is clinically effective in colonic diseases, both locally and systemically. The aim of this work was to prepare and evaluate Eudragit coated chitosan microspheres of metronidazole for colon targeting. Chitosan microspheres were prepared by emulsion dehydration method using different ratios of drug and polymer. Eudragit coated chitosan microspheres were performed by solvent evaporation method and were evaluated for percentage yield, flow property, particle size analysis, surface morphology, determination of drug content, drug entrapment efficiency, degree of swelling, *in vitro* drug release and its kinetic profile. The percentage yield of all formulations was more than 80%. The microspheres showed a spherical structure with a smooth surface morphology. The drug entrapment efficiency was found to be in between 68.8%-81.6%. The *in vitro* release was found to be in following order F3>F1>F2>F4>F5>F6. In the case of F3 only 13.03% of the drug was released in 5 hrs, but it showed high and fast increase in drug release from 6^{th} hr in pH 6.8 phosphate buffer. It shows 90.52% drug release after 12hrs.The formulations gave good fit to the Zero order and the mechanism of drug release was diffusion. The results clearly demonstrated that the Eudragit S100 coated metronidazole chitosan microspheres is a potential system for colon-specific drug delivery.

Keywords: Metronidazole; Chitosan; Eudragit S 100; Emulsion dehydration-solvent evaporation technique; Colon targeting.

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Received 30 May 2013, Accepted 06 June 2013

Please cite this article in press as: Behin SR. *et al.*, Design and evaluation of Coated Microspheres of Antiprotozoal drug for colon specific delivery. American Journal of PharmTech Research 2013.

INTRODUCTION

Oral controlled release formulations for the small intestine and colon have received considerable attention in the past 25 years for a variety of reasons including pharmaceutical superiority and clinical benefits derived from the drug - release pattern that are not achieved with traditional immediate or sustained release products. Colon represents an important and challenging target site in the gastrointestinal tract to provide more effective treatment for diseases[1].

Site specific drug delivery has gained an important role in the last decade for formulations. One such specific target is colon targeted where both local and systemic drug delivery can take place. A local means of drug delivery could allow topical treatment of diseases associated with the colon such as ulcerative colitis, amoebiasis, Crohn's disease, and irritable bowel syndrome and systemic means of drug delivery includes delivery of proteins, therapeutic peptides, anti-asthmatic drugs, antihypertensive drugs and anti-diabetic agents[2].

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs[3]. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 micro meters[4].

Colon targeted drug delivery has gained increased importance not just to deliver drugs for the treatment of various colonic diseases but also for its potential for delivery of proteins and therapeutic peptides[5]. For successful colon targeted drug delivery, the drug needs to be protected from absorption and/or the environment of the upper gastrointestinal tract and then be abruptly released into the colon[6]. Hence continuous efforts have been made on designing colon targeted drug delivery systems with improved site specificity and versatile drug release kinetics to fulfill different therapeutic needs[7].

Colonic delivery can be accomplished by oral or rectal administration. Rectal dosage forms such as suppositories and enemas are not always effective since a high variability in the distribution of these forms is observed. Suppositories are only effective in the rectum because of the confined spread and enema solutions can only offer topical treatment to the sigmoid and descending colon. Therefore oral administration is preferred, but for this purpose, many physiologic barriers have to be overcome. Absorption or degradation of the active ingredient in the upper part of the GIT is the major obstacle and must be circumvented for successful colonic drug delivery[8, 9].

The colon is approximately five feet (1.5 meters) in length, begins at the ileo-cecal valve, and ends at the recto sigmoid junction. The colon's role is to transfer nutrients into the bloodstream through the absorbent

walls of the large intestine while pushing waste out of the body. In this process, digestive enzymes are released, water is absorbed by the stool, and a host of muscle groups and beneficial microorganisms work to maintain the digestive system[10, 11].

The various approaches that can be exploited to target the release of drug to colon include prodrug formation, coating with pH sensitive polymers, coating with biodegradable polymers, embedding in biodegradable matrices, hydrogel, timed release systems, osmotic and bioadhesive system. Among the different approaches to achieve targeted drug release to the colon, the use of polymers especially biodegradable by colonic bacteria holds great promise and to deliver proteins and peptides to the colon for their systemic absorption[12, 13].

Colonic diseases are important causes of death by protozoal infections in the developing world and even in advanced countries. Hence, in the present study metronidazole (MTZ) was selected as a model drug which has extremely broad spectrum of protozoal and antimicrobial activity. It is clinically effective in colonic diseases, both locally and systemically[14]. Antiprotozoal drugs are medicines that treat infections caused by protozoa *Entamoeba histolytica*. Antiprotozoal drugs are used to treat a variety of diseases like amoebiasis, giardiasis, pneumocytosis carinii pneumonia (PCP), African sleeping sickness and malaria caused by protozoa[15].

The objective of present work is to investigate the colon specificity of the polymer chitosan in the formulation of microspheres and perform *in vitro* evaluation study, so that the drug can be targeted to colon and provide longer *in vitro* drug release profile.

MATERIALS AND METHODS:

The following chemicals and solvents were used: Metronidazole (yarrow chemicals, Mumbai), Chitosan (Indian sea foods, Cochin), Eudragit S 100 (Yarrow chemicals, Mumbai), Isooctane (Labort fine chem., Surat), Span 80 (Loba chemie, Mumbai), Acetone (Labort fine chem., Surat), Ethanol (Poly pharma laboratories, Gujarat), Liquid Paraffin (Nice chemicals, Cochin), n- hexane (Merck specialties, Mumbai), Hydrochloric acid (Merck specialties, Mumbai), Potassium dihydrogen Phosphate (Merck specialties, Mumbai), Disodium hydrogen Phosphate (Merck specialties, Mumbai).

Standard curve of metronidazole:

100 mg of Metronidazole was accurately weighed and dissolved in 100 ml of PBS pH 1.2 to prepare stock solution. From the above stock solution a series of dilutions 5, 10, 15, 20, 25 and 30µg/ml were prepared respectively. The procedure was repeated using pH 6.8 and 7.4 buffers. Then the absorbance was measured in a UV spectrophotometer at 277nm against pH 1.2 as blank and at 319nm against pH6.8 and pH 7.4 buffers as blank. Calibration curve was constructed and shown in figure 1.

Pre formulation Studies:

Solubility analysis:

Preformulation solubility analysis was done to select a suitable solvent system to dissolve the drug as well as various excipients used for formulation and also to test drug solubility in the dissolution medium, which was to be used.

Drug –excipient interaction study:

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 7800 and 350 cm-[1]. The spectra obtained for Metronidazole and physical mixtures of Metronidazole with other excipients were compared to check compatibility of drug with excipients.

Formulation of microspheres: [16, 17, 18]

The chitosan microspheres were prepared by emulsion dehydration technique. Metronidazole (1g) and Chitosan (3g) were dissolved in 20 mL of distilled water and stirred overnight to solubilize completely. This drug-polymer solution was dispersed in 50 mL isooctane containing 1.25% wt/vol span 80 and stirred at 1000 rpm continuously to obtain stable water/oil (w/o) emulsion. The solution was rapidly cooled to 15°C and then 50 mL of acetone was added in order to dehydrate the chitosan droplets. This system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at 25°C for 30 minutes to allow the complete solvent evaporation. The microspheres were dried overnight and kept in an airtight container for further studies. Chitosan microspheres were prepared using different ratios of MTZ: Chitosan (i.e., 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6) (Table 1).

Table 1.Preparation of Metronidazole Loaded Chitosan Microspheres

Sr. No	Ingredients	F1	F2	F3	F4	F5	F6
1.	Metronidazole	1g	1g	1g	1g	1g	1g
2.	Chitosan	1g	2g	3g	4g	5g	6g
3.	Distilled water	40ml	40ml	40ml	40ml	40ml	40ml
4.	Isooctane	50ml	50ml	50ml	50ml	50ml	50ml
5.	Span80(%w/v)	1.25	1.25	1.25	1.25	1.25	1.25
6.	Acetone	50ml	50ml	50ml	50ml	50ml	50ml

Table 2. Coating of Metronidazole Loaded Chitosan Microspheres using Eudragit S 100

Sr.No	Ingredients	F1	F2	F3	F4	F5	F6
1.	Chitosan microspheres	50mg	50mg	50mg	50mg	50mg	50mg
2.	Eudragit S 100	500mg	500mg	500mg	500mg	500mg	500mg
3.	Ethanol: acetone	2:1	2:1	2:1	2:1	2:1	2:1
4.	liquid paraffin	100ml	100ml	100ml	100ml	100ml	100ml
5.	Span 80	1%w/v	1%w/v	1%w/v	1%w/v	1%w/v	1%w/v
6.	n- hexane	For washing	For washing	For washing	For washing	For washing	For washing

Coating of metronidazole loaded chitosan microspheres using Eudragit S100:

Chitosan microspheres were coated with Eudragit S 100 using oil-in-oil solvent evaporation method. Chitosan microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolution of 500 mg of Eudragit S 100 in ethanol: acetone (2:1) to give 5:1 (coat: core ratio).

This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-hexane, and dried overnight (Table 2).

Evaluation and characterization of microspheres: [19, 20, 21, 22, 23]

A. Percentage yield:

Percentage yield of microspheres was calculated by the equation

$$\text{Yield of microspheres (\%)} = \frac{\text{Weight of the microspheres (mg)}}{\text{Drug (mg) + polymer (mg)}} * 100$$

B. Flow property:

Flow property of microspheres was studied by measuring the angle of repose of the formulation by employing fixed funnel method, which measures the resistance to particle flow. Metronidazole microspheres were passed through the funnel, which was kept at a height of 'h' from the horizontal surface. The passed microspheres formed a pile of a height 'h' above the horizontal surface and the radius 'r' of the pile was measured and the angle of repose was determined by using the formula

$$\tan\theta = h/r$$

θ = angle of repose

h = height of the pile

r = radius of the pile

C. Particle size analysis:

Determination of average particle size of metronidazole was carried out by optical microscopy. A minute quantity of microspheres was spread on a clean glass slide and average size of 100 particles was measured with the help of the eye piece micrometer in each batch.

$$\text{Mean particle size} = \frac{\text{Total sum of particle size}}{\text{Total number of particles}}$$

Actual particle size =Mean particle size* Calibration factor

D. Surface morphology:

The shape and surface morphology of chitosan microspheres and Eudragit-coated chitosan microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminium stub. The stubs were then coated with gold to a thickness of ~300 A^0 under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (Figure 3).

E. Determination of drug content:

Drug loaded microspheres (100mg) were powdered and taken in 100ml standard volumetric flask. To this 75ml of 0.1N Hcl was added and kept overnight. The volume was made up with 0.1 N Hcl. The final

solution was filtered using whatman filter paper, from this 10ml was pipette out into a 100ml standard flask and made up the volume with 0.1N Hcl and the drug content was determined spectrophotometrically at 277nm using a regression equation derived from the standard graph.

$$\text{Drug content} = \frac{\text{Absorbance}}{\text{Slope}} * \text{dilution factor}$$

F. Estimation of Drug entrapment efficiency:

Drug loaded microspheres (100mg) were transferred into glass mortar and powdered and further digested in 100ml of 0.1N Hcl for 10min to dissolve the drug. The microspheres were then centrifuged at 1000rpm for 10min to remove any insoluble solids, the supernatant layer decanted and filtered. The drug content was determined spectrophotometrically at 277nm using a regression equation derived from the standard graph.

$$\% \text{ entrapment efficiency} = \frac{\text{Amount of drug in known amount of spheres}}{\text{Initial drug load}} * 100$$

G. Degree of swelling:

The swelling ability of the microspheres on physiological media was determined by suspending them in the phosphate buffer of PH 7.4. Accurately weighed amount of microspheres were immersed in little excess of phosphate buffer of PH 7.4 and allowed to swell up to constant weight. The swelling of microspheres was calculated by using the following formula

$$\alpha = \frac{Wg - W0}{W0}$$

α=Degree of swelling

Wg=Final weight of microspheres

W0=initial weight of microspheres

H. Stability study:

To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The promising formulation was stored at 40ºC / 75% RH for 3 months and evaluated for their drug content.

I. *In vitro* drug release and kinetics: [24, 25]

100mg of microspheres were weighed accurately and filled into tea bags. The tea bags were tied using thread with paddle and loaded into the basket of the dissolution apparatus. The content was rotated at 100rpm and maintained in pH progression medium at $37^0C \pm 0.5^0C$. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals.

The ability of the various delivery systems, understudy, to protect the drug in the physiological environment of the stomach and small intestine and allow its release into the colon was assessed by carrying out drug release studies in 0.1N Hcl for 2 hours, pH 7.4 buffer for 3 hours and PBS pH 6.8 up to 12 hours. The samples were withdrawn from the dissolution medium at various time intervals. The rate of drug release was analyzed using UV spectrophotometer. The receptor volume was maintained constant by replacing equivalent amount of SGF.

Kinetic Data Analysis:

The methods to investigate the kinetics of drug release from this formulation are as follows:

a. Zero Order [% R = kt]**:**

This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as matrix tablets containing low soluble drugs. Data obtained from *in vitro* drug release studies were plotted as cumulative percentage of drug released versus time.

b. First Order [log (fraction unreleased) = kt/2.303]**:**

The model is applicable to hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices. Data obtained were plotted as log cumulative percentage of drug remaining versus time.

c. Higuchi Matrix [% R = kt 0.5]**:**

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug. Data obtained were plotted as cumulative percentage drug release versus square root of time.

d. Peppas Korsmeyer Equation:

Log Mt/Mα = Log K + n Log t

Where, Mt/Mα is the fraction of drug released at a time‘t’, K is the constant term incorporating the structural and geometrical characteristics of the drug/ polymer system, n is diffusion exponent related to the mechanism of the release. Above equation can be simplified by applying log on both sides**;**

When the data is plotted as log of drug released versus log time, yield a straight line with a slope equal to ‘n’ and the ‘K’ can be obtained from y intercept.

RESULTS AND DISCUSSION:

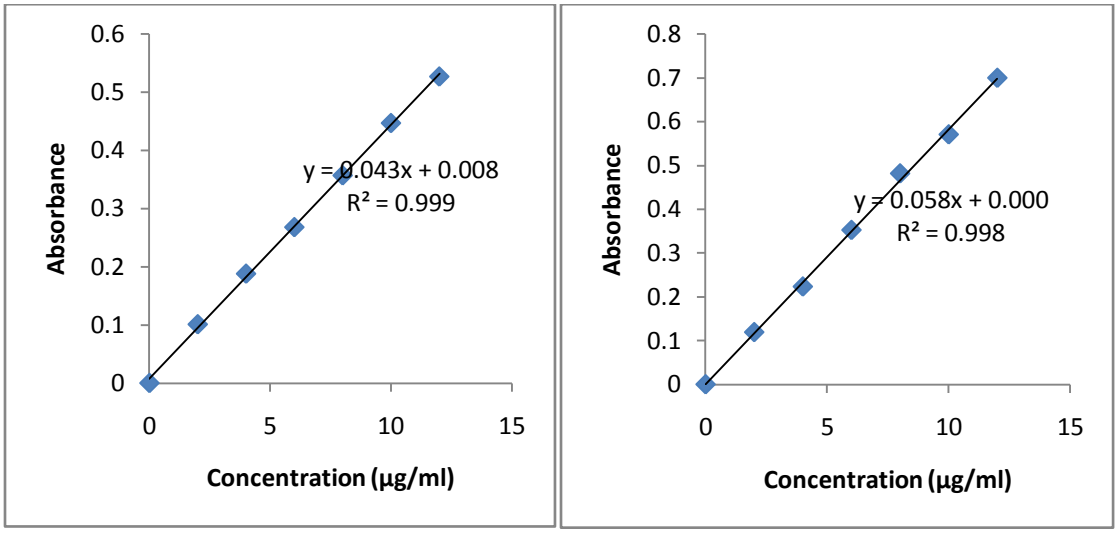
Standard Curve for Metronidazole:

Figure 1 shows the standard calibration curve for pure drug. The curve was found to be linear in the range of 5-30µg/ml, which was thus considered as the beer’s range.

Preformulation studies:

Solubility Analysis:

From the solubility analysis of drug metronidazole (10µg/ml) it was found that it is soluble in water, ethanol, 0.1N Hcl, phosphate buffer pH 6.8 and 7.4. The solubility of excipients like chitosan & Eudragit S100 was analyzed. Chitosan was sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, neutral or alkali solutions at pH above approximately 6.5. Eudragit S100 was soluble in acetone, ethanol, insoluble in water, n-hexane.



A B

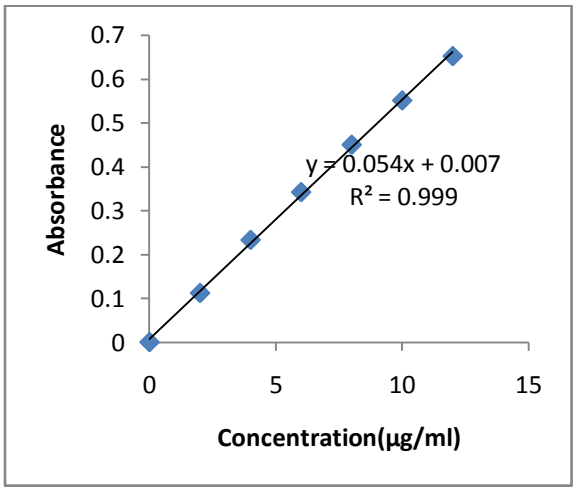


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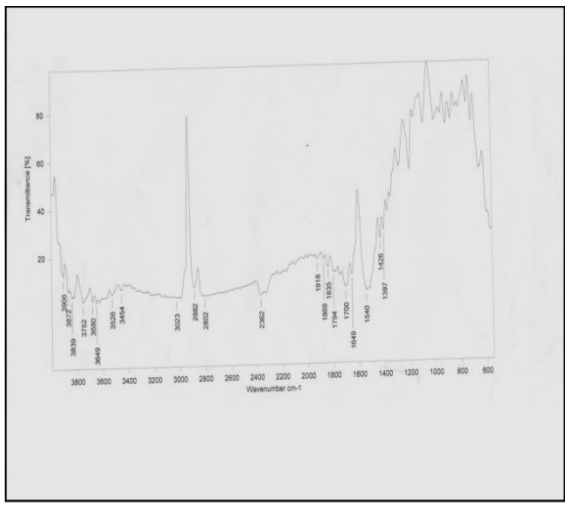
Figure 1: Standard Curve of Metronidazole in A) Acidic buffer pH 1.2, B) Phosphate buffer pH6.8 and C) Phosphate buffer pH 7.4



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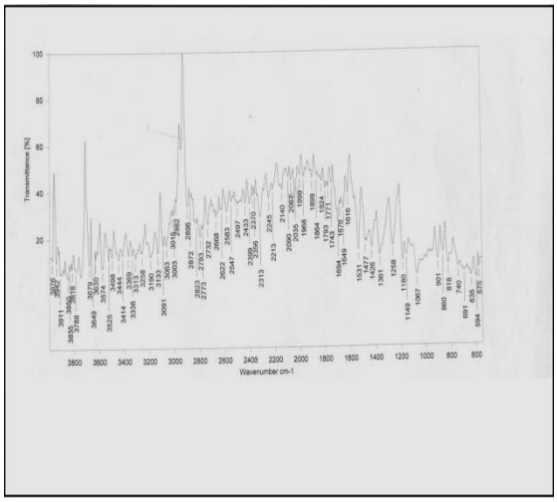

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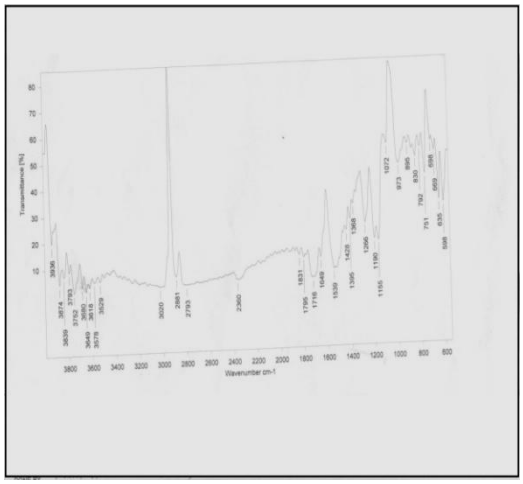

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Figure 2: FTIR spectrum of A) Metronidazole B) Metronidazole and Chitosan C) Metronidazole and Eudragit S100

FTIR Study:

The FTIR spectra of metronidazole, metronidazole-chitosan, metronidazole-Eudragit S100 are shown in Figure. 2 A, 2B, 2C. Infrared spectra of pure drug metronidazole showed sharp peaks at 1076.21, 1477.37, 3217.04, 1431.08 cm-1 that confirmed the presence of C-N stretch, N=O, C-H, and O-H stretch and it was almost similar to drug peaks. So the drug was found to be pure compared to pure drug. There were no changes in the major peaks of Metronidazole in the presence of chitosan, Eudragit S 100. So the drug and excipients are compatible with each other.

Evaluation and characterization of microspheres:

A. Yield of microspheres:

The percentage yield of microsphere formulation F1 to F6 containing different polymer concentration of Chitosan formulations was in the range of 81 to 85% (as shown in Table 3). The effect of polymer concentration on the percentage yield of microspheres was found that the yield of microspheres increased with increasing polymer concentration.

B. Flow property:

Flow property of microspheres was studied by measuring the angle of repose of the formulations. The angle of repose of formulation F1 to F6 containing different polymer concentration of Chitosan formulations was in the range of 23.74^0-34.82^0(as shown in Table 3). The values of angle of repose indicate good-passable flow properties.

C. Particle size analysis:

The mean particle size of the microspheres formulation F1 to F6 containing different polymer ratio of Chitosan was in the range of 90.87µm to 120.51µm respectively (as shown in Table 3). Fig.9 shows the comparison of average particle size of formulated microspheres. The effect of polymer concentration on the particle size of microspheres was determined. It was observed that with increase polymer

concentration in the microspheres from F1 to F6, the particle size of the microspheres increases. This was due to the increase in relative viscosity, which leads to increase in particle size.

Table 3. Evaluation and Characterization of Microspheres

Formula	% Yield*	Angle of repose*	Actual particle size(µm) *	Drug content*	% Drug Entrapment efficiency*	Degree of swelling*
F1	81±0.26	23.74±1.43	90.87±2.25	96.55±0.28	68.8±1.76	0.154±0.15
F2	81.6±0.39	25.17±1.21	93.9±3.12	95.41±0.12	76.5±2.89	0.163±0.12
F3	82.5±0.48	26.56±1.55	101.57±2.47	97.55±0.34	81.6±1.23	0.165±0.23
F4	84±0.36	28.28±1.15	108.94±3.13	96.10±0.26	75±2.14	0.168±0.18
F5	84.3±0.43	31.22±1.37	116.35±1.49	96.26±0.42	72.2±2.38	0.173±0.20
F6	85±0.51	34.82±1.13	120.51±2.56	94.49±0.24	70.02±2.44	0.179±0.16

* All values represented as mean±SD (n=3)

D. Surface morphology:

The surface morphology of microspheres was examined by scanning electron microscopy. The view of the microspheres showed a spherical structure with a smooth surface morphology (Figure 3). The smoothness of the surface increased which may be due to increasing ratio of polymer. Very less particulate matter of the drug were seen on the surface of the microspheres indicating uniform distribution of the drug in the polymer network. The microscopic image of formulation F3 can be seen in figure 4.

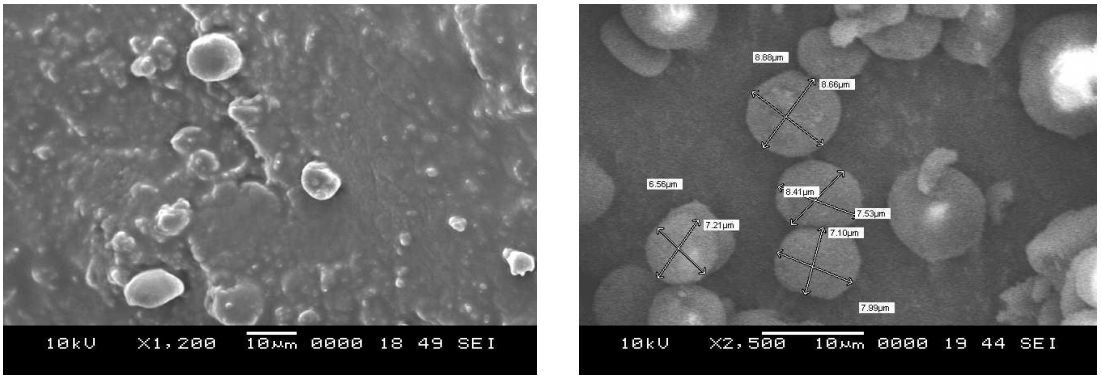

Figure 3: SEM image of Metronidazole loaded chitosan microspheres of F3 (without coating and with coating).

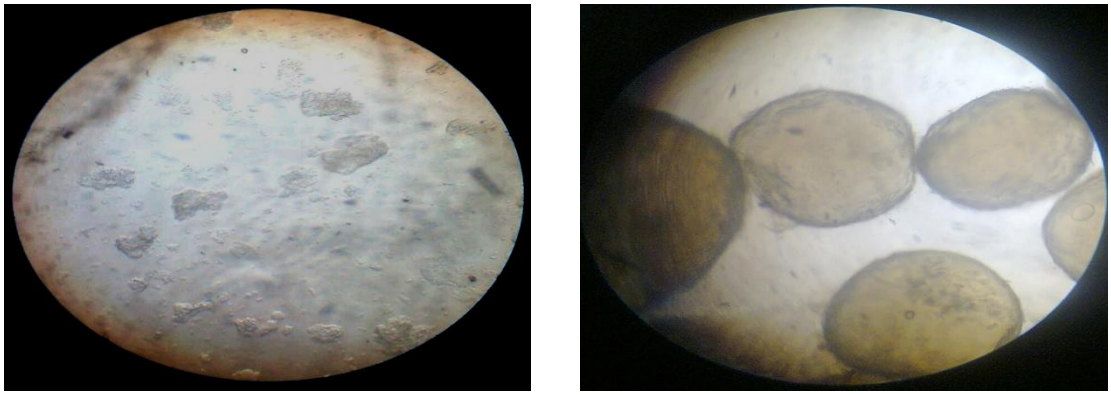

Figure 4: Microscopic image of prepared microspheres of F3 (without coating and with coating)

E. Drug content:

The drug content of formulation F1 to F6 containing different polymer concentration of chitosan formulation was in the range of 94.26-97.55% (as shown in Table 3).

F. Drug entrapment efficiency:

The drug entrapment efficiency of formulation F1 to F6 containing different polymer concentration of chitosan formulation was in the range of 68.8 to 81.60% (as shows in Table 3). Fig. 10 shows the comparison of % entrapment efficiency of formulated microspheres. Results showed that increase in polymer concentration increased the drug entrapment efficiency. Further increase in polymer concentration showed slight reduction in the entrapment efficiency. The drug entrapment efficiency was found to be good in all formulations.

G. Degree of swelling:

The chitosan microspheres were coated with Eudragit S 100 to prevent dissolution of chitosan in the upper part of GIT. The swelling ability of microspheres on buffer pH 6.8 from F1 to F6 was determined and it showed swelling of 0.154 to 0.179 respectively (as shown in Table 3). From the result it was found that no significant swelling was observed with Eudragit S 100 coated microspheres. Thus ensuring better resistance of Eudragit S 100 coated microspheres in upper GIT and prevent the drug release at the non target site.

H. Stability study:

Stability study of the formulation was carried out as per the ICH guidelines. The best formulation F3 was subjected to stability study 40^0C at ambient humidity for a period of 3 months. The physical appearance and the chemical stability were analyzed by change in the drug content. The results showed that there were no significant changes in the drug content. 97.37% drug content was present at the end of the study versus 97.55% drug content at the start of the study, indicating a stable formulation.

I. *In vitro* drug release and kinetics:

In vitro release study of prepared microspheres were carried out for 2 hours in 0.1N Hcl, 3 hours in pH 7.4 phosphate buffer and up to 12hours in pH 6.8 phosphate buffered saline (PBS). Drug release study has shown that polysaccharides protect the drug from being released completely in the physiological environment of stomach and small intestine. All these batches in first 2hours i.e. pH 1.2 showed no significant release, then it showed slight increase in pH 7.4 phosphate buffer. This revealed that the drug was retained comfortably inside the microspheres and only 9.11-18.92% of the drug was released in 5 hours. But it showed high and fast increase in drug release from 6^{th} hour in pH 6.8 phosphate buffer, it showed 50.31%, 50.08%, 50.67%, 49.09%, 47.20%, 46.57% drug release in the case of F1, F2, F3, F4, F5, F6 respectively as it enters in colonic pH. Metronidazole loaded chitosan microspheres in the ratio of 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 showed percentage drug release after 12hours as 88.78%, 84.84%, 90.52%, 82.70%, 79.83%, 78.70% respectively (Table 4). The drug release of metronidazole significantly decreased with increasing chitosan concentration. Metronidazole coated formulations shows no

significant release of drug in stomach and small intestine, but delivered drug to the colon resulting in slow absorption of the drug and making drug available for local action in the colon. Formulation F3 showed the best release data as it released 13.03% of drug after 5hourrs, which was less than that released by other formulations. The complete drug release from formulation F3 was 90.52% (Table 4).

Table 4. *In vitro* Drug release of all Formulations

Time in hr	%CDR F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	1.03±0.11	1.0±0.13	0	0.414±0.08	0.51±0.05	0.41±0.07
2	1.55±0.14	1.13±0.16	0	0.722±0.13	0.72±0.07	0.72±0.09
In phosphate buffer pH 7.4						
3	12.95±0.70	10.45±1.39	10.28±1.0	9.951±1.13	9.12±1.17	9.11±1.01
4	14.1±0.94	12.23±0.98	12.56±0.67	10.39±1.22	10.71±1.23	10.55±1.18
5	18.92 1.28	15.87±1.27	13.03±1.19	13.17±1.16	13.17±1.19	12.83±1.12
In phosphate buffer pH 6.8						
6	50.31±1.06	50.08±1.78	50.67±1.34	49.08±1.45	47.20±1.26	46.57±1.26
7	54.14±1.33	53.75±1.52	54.98±1.25	52.75±1.67	53.90±1.35	53.18±1.37
8	57.07±1.23	55.73±1.24	57.92±1.45	54.88±1.49	55.63±1.44	55±1.29
9	65.65±1.41	63.21±1.32	63.07±1.16	62.97±1.55	62.16±1.19	61.22±1.31
10	73.86±0.89	71.07±0.89	72.96±1.39	70.05±1.60	69.23±1.25	68.59±1.42
11	81.36±1.10	79.08±1.11	80.14±1.27	77.67±1.38	76.84±1.31	75.25±1.25
12	88.78±1.31	84.84±1.21	90.52±1.35	82.70±1.27	79.84±1.39	78.7±1.36

Data obtained from *in vitro* release study was utilized for release kinetics. The values of *in vitro* release were attempted to fit into various mathematical models i.e. Zero order, first order, Korsmeyer Peppas, and Higuchi matrix. These values were compared with each other for model fitting equation. Based on highest regression value(r), formulation gave good fit to the zero order kinetics and the *in vitro* kinetic data subjected to log time log drug release transformation plot(Peppas model) revealed the fact that the drug release follows a super case II transport with diffusion exponent (n) value >1.

The *in vitro* kinetic plots are given in Figure 5A, 5B, 5C, 5D. Kinetic data obtained from *in vitro* release profiles of different formulations of colon targeting Metronidazole loaded chitosan microspheres are given in Table 5.

Table 5. Kinetic Data obtained from *In Vitro* Release Profile for Colon Targeting Metronidazole Loaded Chitosan Microspheres

Formulation	R^2 values				n values
	Zero order	First order	Higuchi matrix	Peppas Exponential equation	
F1	0.9593	0.9046	0.8515	0.9585	1.941
F2	0.9474	0.9186	0.8375	0.9471	1.972
F3	0.9423	0.8741	0.8286	0.9318	2.180
F4	0.9397	0.9225	0.7907	0.9368	2.081
F5	0.9381	0.9309	0.825	0.9338	2.123
F6	0.9376	0.9327	0.7481	0.9336	2.154

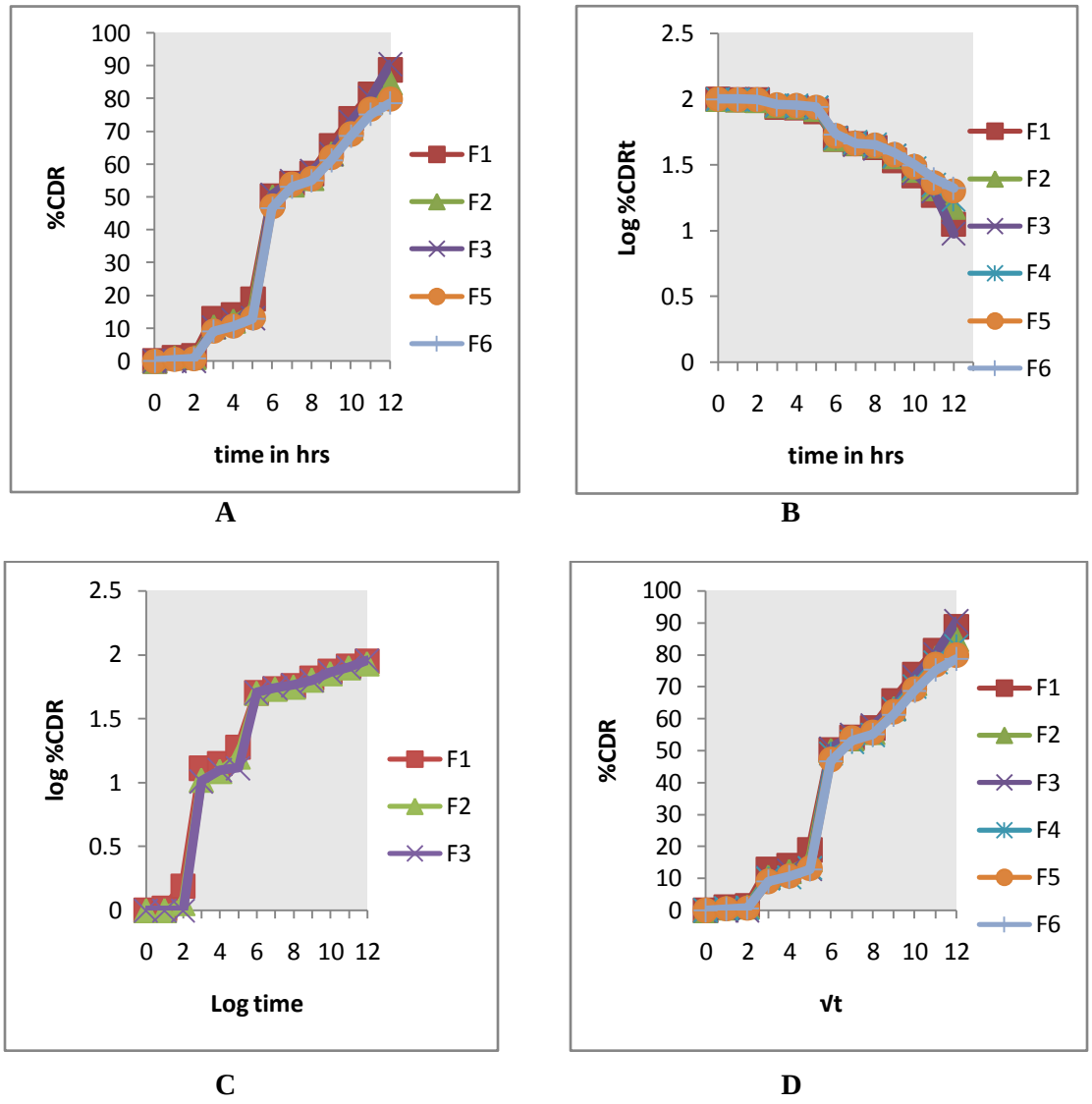

Figure 5: A) Zero order kinetics B) First order kinetics C) Peppas Model D) Higuchi Matrix

CONCLUSION:

Targeting drugs and delivery systems to the colonic region of the gastrointestinal tract has received considerable interest in recent years. Successful colonic delivery could be achieved by protecting the drug from absorption in the environment of the upper GIT and then be abruptly releasing into the proximal colon, which is considered the optimum site for colon targeted delivery of drugs. The view of the microspheres showed a spherical structure with a smooth surface morphology. In the case of entrapment efficiency the results showed that with increase in polymer concentration increased the drug entrapment efficiency. Stability study showed that there were no significant changes in the drug content. The *in vitro* release was found to be in following order F3>F1>F2>F4>F5>F6 and ensuring better resistance of Eudragit S 100 coated microspheres in upper GIT and preventing the drug release at the non target site. The formulations gave good fit to the Zero order and the mechanism of drug release was diffusion. The

experimental results demonstrated that F3 batch of Eudragit S-100 coated chitosan microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

ACKNOWLEDGEMENT:

I express my sincere thanks to the Principal, and management of Shree Devi College of Pharmacy, Mangalore for providing all facilities to complete this work.

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