Original Article

Formulation And Characterization Of Chitosan Based Mucoadhesive Microspheres For Treatment Of Intestinal Infection

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Abstract

The mucoadhesive properties of chitosan microspheres prepared by different method were evaluated by studying the interaction between mucin and microspheres at targeted site. The aim of this study was to investigate the capabilities of chitosan microspheres as drug carrier system to improve the site specific drug delivery via mucoadhesion approach. Different formulations were developed and morphological studies of the optimized formulas showed that the size range of spherical shaped particulate matters. It was planned to develop naturally occurring biodegradable polymer based colon-targeted drug delivery systems of selected drug or drug combination for the prevention of intestinal round worm. Such site-specific drug delivery systems are expected to provide majority of their drug load to colon without being released in stomach and small intestine. As a result, it may be possible to provide an effective and safe therapy for the prevention of intestinal infection with a lower dose of drug. The in vivo mucoadhesive study was further selected by the radiological technique for justified the increased residency time in the stomach. Thus chitosan microspheres appear, technically, promising mucoadhesive drug delivery systems for delivering metronidazole to treatment stomach worms at site specific intestine.

Keywords: Colon targeted, Mucoadhesion, Metronidazole, Chitosan, Microspheres

Introduction: Oral system is the most desirable and preferred method of administering therapeutic agents for their systemic effects. In addition, the oral medication is generally considered as the first avenue investigated in the discovery and development of new drug entities and pharmaceutical formulations, mainly because of patient acceptance, convenience in administration, and cost-effective manufacturing process. Targeted-release dosage form that releases drug at or near the intended physiologic site of action. Targeted-release dosage forms may have either immediate- or extended-release characteristics **[1]**. Site-specific oral drug delivery requires exact placement of a drug delivery device at a desired site within the gastro intestinal tract. Although it is virtually possible to localize a device within each part of gastro intestinal tract, the attainment of site-specific delivery in the oral cavity and the rectum is relatively easier than in the stomach and the small and large intestines. The latter requires consideration of both longitudinal and transverse aspects of gastro intestine constraints **[2].** The controlled-release formulations for oral drug delivery are diffusion-controlled systems; solvent activated systems, and chemically controlled systems. Diffusion-controlled systems include monolithic and reservoir devices in which diffusion of the drug is the rate-limiting step, respectively, through a polymer matrix or a polymeric membrane. Chemically controlled systems release

drugs via polymeric degradation (surface or bulk matrix erosion) or cleavage of drug from a polymer chain **[3].** Colon specific drug delivery systems are designed to obtain targeted drug delivery to the large intestine (colon). They provide local delivery for the treatment of colonic diseases like inflammatory bowel disease (ulcerative colitis and crohn's disease) and colon cancer, where it is necessary to attain high concentration of the drug. These systems are also useful for delivery of therapeutic peptides and proteins, which are other wise degraded and / or poorly, absorbed in the stomach and small intestine but may be better absorbed from the colon **[4].** The colon is divided into the caecum, ascending colon, transverse colon, descending colon, sigmoid colon, rectum and anal canal. A dosage form that allows at least a twofold reduction in dosage frequency as compared to that drug presented as an immediate-release (conventional) dosage form. Examples of extended-release dosage forms include controlled-release, sustained-release, and long-acting drug products **[5].** The oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic circulation of body. However oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastro-intestinal tract. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 μm range in diameter having a core of drug and entirely outer layers of polymer as coating material. However, the success of these microspheres is limited due to their short residence time at site of absorption. It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. This can be achieved by coupling bioadhesion characteristics to microspheres and developing "mucoadhesive microspheres". Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site **[6-7]**. Mucoadhesion is defined as the interaction between a mucin surface and a synthetic or natural polymer. Mucoadhesion has been widely promoted as a way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulations such as "microspheres" along with the active pharmaceutical ingredient (API). Mucoadhesive microspheres include microparticles and microcapsules (having a core of drug) of 1- 1000μm in diameter and consisting either entirely of a Mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery; but coupling of bioadhesive properties to microspheres has additional advantages e.g. efficient absorption and bioavailability of the drugs due to high surface to volume ratio, a much more intimate contact with the mucous layer, specific targeting of drugs to the absorption site. Bioadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in eye, nasal cavity **[8-9]**. Metronidazole is a prodrug; it requires reductive activation of the nitro group by susceptible organisms. Metronidazole has been used widely for the treatment of the microaerophilic organism Helicobacter pylori, the major cause of ulcer disease and gastritis worldwide. Metronidazole is readily and almost completely absorbed after oral administration and is widely distributed in the body. Metronidazole is readily absorbed from the gastro intestinal tract and from the rectal mucosa and widely distributed in body tissues. The objective of the present investigation was to develop targeted drug delivery system for treatment of intestinal round worm using metronidazole as model drug in a controlled manner. It was planned to develop naturally occurring biodegradable polymer based colon- targeted drug delivery systems of selected drug or drug combination for the prevention of intestinal round worm. Such site-specific drug delivery systems are expected to provide majority of their drug load to colon without being released in stomach and small intestine. As a result, it may be possible to provide an effective and safe therapy for the prevention of intestinal infection with a lower dose of drug. Thus the broad objective of the investigation is to develop site specific mucoadhesive drug delivery systems of drug for the prevention of intestinal round worm infection.

Material and Methods

Determination of absorption maxima (λ_{max}): The determination of absorption maxima was determined by UV scanning of drug solution under ultraviolet spectrophotometer between 200 to 400 nm wavelengths offer drug sample i.e. metronidazole. The resulting solution was having 10 µg / ml concentration. The solution was run within the range of 200 – 400 nm range in double beam UV spectrophotometer (Shimadzu, UV-1800, Shimadzu Corporation, Japan).

Preparation of calibration curve of drug sample: The calibration curve was determined in various solvents i.e. simulated gastric fluid (pH 1.2).

Preformulation Study: Preformulation studies are needed to ensure the development of a stable as well as therapeutically effective and safe dosage form. The various parameters i.e. physical appearance, melting point, solubility study, partition coefficient and drug excipients incompatibility study.

Preparation of Chitosan Microspheres: The chitosan microspheres of metronidazole drug were prepared by modified emulsion cross-linking method.

Factorial Design: The present work was employed to develop the optimized formulation by utilizing the 3^2 factorial designs with fitted in interactive and polynomial provisions to assess the response (Equation 1):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 \quad (1)$$

where Y is the dependent variable, b_0 is the arithmetic mean response of all runs, and bi (b_1,b_2, b_{12},b_{11}, and b_{22}) is the estimated coefficient for the factor Xi (X_1,X_2,X_{12},X_{11}, and X_{22}). X_1 and X_2 signified the average data of each factor at a time from low–medium–high values. The interaction terms, X_1 and X_2, show response changes when two factors are changed simultaneously, whereas polynomial terms (X_{12} and X_{22}) are included to investigate nonlinearity. Where, b1 is the estimated coefficient for the factor X1, while Y1 is the calculated response. The coefficients corresponding linear effects (b1 and b2), interaction (b12) and the quadratic effects (b11 and b22) were determined from the outcomes of the experiments. The model work on comparison established between the experimental and predicted values of the responses and presented in terms of percent accuracy.

Method: The drug metronidazole chitosan containing microspheres were prepared by modified emulsion cross-linking method. The microspheres were prepared by using two different phases. One of the aqueous phase prepared by dissolving chitosan as polymer in distilled water at 50°C. The drug 100 mg was subsequently added to above prepared solution upto completely dissolving. Other was organic phase in the ratio of 50:50 w/w of petroleum ether and light liquid paraffin with required quantity of emulsifier span 80 as surfactant. Now, the prepared aqueous phase was again added to an organic phase, with constant stirring using a mechanical stirrer to form w/o type of emulsion. The cross-linking agent gluteraldehyde (1 ml) was added as to this solution after 10 min, at 40°C, with required stirring speed upto 3 h (Table 1) for individual formulation. The resulting microspheres were washed and filtered with n-hexane, dried under vacuum at 40°C for 1 h and stored in air tight container **[10]**.

Characterization of microspheres: The prepared chitosan mucoadhesive microspheres evaluated by such parameters i.e. Particle size analysis, Flow properties, Shape and Surface Characterization of Microspheres by Scanning Electron Microscopy, Percentage Yield, Drug Entrapment, In vitro swelling, In-Vitro drug release studies.

Particle size analysis: Particle size analysis plays an important role in determining the release characteristics of drug. The sizes of microspheres were measured by using an optical microscope, and the mean particle size was calculated by measuring nearly 100 particles with the help of a calculated ocular micrometer.

Flow properties: The flow properties of prepared microspheres were characterized for identification of flow character of powder in terms of carr's index, hausner's ratio and angle of repose. The Carr's index ((IC)) and Hausner's ratio (HR) of drug powders were calculating according to following equation:

Carr's Index (IC) = ρTapped - ρBulk / ρTapped

Hausner's ratio (HR) = ρTapped / ρBulk

The angle of repose (θ) was measured by fixed height method. This was calculated by following equation: Angle of repose (θ) = $\tan^{-1}$ 2 H / D

Where H is the surface area of the free standing height of the powder pile and D is diameter of pile that formed after powder flow from the glass funnel **[11]**

Scanning Electron Microscopy analysis for Shape and Surface Characterization: The shape and surface characteristics of the microspheres were observed by scanning electron microscopy. The freeze-dried microspheres were coated with gold using a sputter coater under high vacuum microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology.

Percentage Yield: The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} * 100$$

Drug Entrapment: The various formulations of the chitosan microspheres were subjected for drug content. 100 mg of microspheres from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved with 10ml ethanol in 100ml volumetric flask and makeup the volume with phosphate buffer pH 7.4 buffer. This resulting solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 10 ml was taken out and diluted up to 100 ml with phosphate buffer pH 7.4 buffer. Again from this solution 1 ml was taken out and diluted up to 10 m1 with phosphate buffer pH 7.4 buffer and the absorbance was measured at 320 nm against phosphate buffer pH 7.4 buffer as a blank **[12]**. The percentage drug entrapment was calculated as follows.

$$\text{Percent Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} * 100$$

Swelling Index (%): The Swelling index of prepared chitosan drug loaded coated microspheres was determined by placing 100 mg of microspheres and in a cellophane membrane dialysis bag containing phosphate buffer (pH 7.4) dissolution medium. Then microspheres were allowed to swell for a period upto 12 h. The changes in weight were measured by removal of the samples and blotted with a filter paper for 10 sec to absorb excess solvent on surface. The degree of swelling was determined using the following equation:

Si = Wt – W0 / W0

where Si represents the degree of swelling, Wt and W0 represent weights of the sample at equilibrium swelling and the original dry weight, respectively

In-Vitro drug release studies: The dissolution study of prepared coated microspheres was kept in a USP paddle apparatus in different pH condition according to optimization of various coating layer at 50 rpm at 37 ± 0.5 °C. The polymeric matrix system was tested using 0.1N HCL pH 1.2 for 2 h. The dissolution

of optimized formulation was carried out at pH 1.2 for 2 h followed by phosphate buffer pH 6.8 for 4 h and further continued in phosphate buffer pH 7.4 for 2 h. The samples were withdrawn at various time intervals and replaced with an equivalent amount of fresh dissolution medium. Dissolution samples were filtered through a whatmann filter paper and analyzed using a validated UV spectroscopy method. The absorbance of all samples were measured at 272 nm, 320 nm and 319 nm for 0.1 N HCl solution, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 respectively for drug metronidazole.

Kinetic study models:

Zero order release kinetics: Zero order release kinetics refers to the process of constant drug release from a drug delivery device such as oral osmotic tablets, transdermal systems, matrix tablets with low-soluble drugs and other delivery systems. In its simplest form, zero order release can be represented as:

$$Q = Q0 + K0\ t \qquad \text{Eq. 1}$$

where Q is the amount of drug released or dissolved (assuming that release occurs rapidly after the drug dissolves), Q0 is the initial amount of drug in solution (it is usually zero), and K0 is the zero order release constant. The plot made was cumulative % drug release vs time (zero order kinetic models).

First order release kinetics: The rate laws predicted by the different mechanisms of dissolution both alone and in combination, have been discussed by Higuchi.

$$\text{Log } C = \text{Log } C0 - kt / 2.303 \qquad \text{Eq. 2}$$

where, C0 is the initial concentration of drug and K is first order constant. The equation in resemblance to the other rate law equations, predicts a first order dependence on the concentration gradient (i.e. Cs - Ct) between the static liquid layer next to the solid surface and the bulk liquid **[13]**.

Results and Discussion

An acidic solution of metronidazole was scanned in the U.V. range of 200-400 nm using Shimadzu 1800 UV Visible spectrophotometer as prescribed in I.P. 1996. The spectrophotometric method of analysis of metronidazole at λ_{max} 272.0 nm was found to be reproducible and highly sensitive. The standard curves of metoprolol were prepared in simulated gastric fluid (pH 1.2) at λ_{max} 272.0, phosphate buffer solution (pH 7.4) at λ_{max} 320.0 and simulated intestinal fluid (pH 6.8) at λ_{max} 319.0 and,. The data were regressed to obtain the straight line. The correlation coefficient greater than 0.99 was observed in all the cases, which indicated that, the drug follows Beer-Lambert's law in the concentration range of 2-20 µg/ml. The organoleptic property of drug metronidazole was white to pale yellow, Bitter slightly saline taste, odourless, crystalline and hygroscopic powder. The melting point of drug was 160°C. In the present study, polymers were selected on the basis of their solubility's and non-interference in the estimation of drug. The absorbance data of both drug and different additives were noted. The absorbance data had shown no appreciable change in the absorbance of drug solution at 272.0 nm indicating no interference of polymers in the estimation of metronidazole. Solubility study in different solvents at room temperature revealed that it is soluble in distilled water and insoluble in chloroform, benzene etc. Partition coefficient value of metronidazole also revealed its hydrophilic nature for n-Octanol/PBS (pH 7.4) and 10.57×10^{-2}. The particle size was calculated by measuring nearly 200 particles with the help of a calculated ocular micrometer. The result was varied mean particle size 138.07 – 261.18 µm. The shape and surface characteristics of the microspheres were observed by scanning electron microscopy. Microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology. The microspheres were shown rough surface structure and observe balloon like structure. The flow properties of prepared microspheres were characterized for identification carr's index, hausner's ratio and angle of repose. Flow properties of different batches of microsphere were have good to fair flow in characterstics because of rough surface structure of prepared microspheres. Percentage yield of different batches of microsphere have varied from 77.1 % - 97.7 %. The result was

optimized that the coated microspheres have more percentage yield properties than the other ones. The various formulations of the chitosan microspheres were subjected for drug content. The percentage drug entrapment was calculated of different batches of microsphere were showed more drug loading capacity for coated microspheres varied from 78.38 % - 93.73 %. The swelling index was determined by allowing swelling for a period of 12 h. The changes in weight were measured and degree of swelling of different batches of microsphere was depending on the higher percentage of chitosanpolymer during the formulation and was varied from 11.11 % - 37.21 %. The formulation release more than 99 % of the drug in gastric environment in controlled and sustained manner upto 12 h. Regression analysis was performed and the r^2 values suggested that the curves were fairly linear and slope values were computed from the graph. The release exponent "n" values were in the range of 1.1312 to 1.3209 for MCM1 to MCM8. For all of the batches the value of release exponent "n" was > 0.89 indicating Super-case II transport mechanism. The in-vitro drug release studies of chitosan microspheres characterized for release percentage and release rate **k**. Release data within the linear range were selected and fitted to various mathematical model: The linear equation is based on regression of at least three release data, and only correlation coefficient of over 0.99 is acceptable.

Summary and Conclusion: In the present study chitosan microspheres were prepared by chemical-cross linking method. Various variables such as the drug: polymer ratio with variation in concentration of surfactant and stirring speed and the cross-linking time were optimized by the factorial design. A 3^2 experimental design was employed to identify optimal formulation parameters for a microsphere preparation with the minimum value of particle size and maximum value of in vitro mucoadhesion. Results flow properties study indicated there is no aggregation between drug–polymer in the microspheres. Hence, the results of the present study clearly indicated promising potentials of chitosan microspheres for delivering metronidazole at site specific alternative approach. The mucoadhesion capacity of dried mucoadhesive microspheres was evaluated in simulated gastric fluid, MTM1 containing exhibiting good response. The in-vitro Release profile of microspheres was characterized for release percentage and release rate **k**. Release data within the linear range were selected and fitted to various mathematical model: The linear equation is based on regression of at least three release data, and only correlation coefficient of over 0.99 is acceptable. The in vivo mucoadhesive study was further selected by the radiological technique for justified the increased residency time in the stomach. The value of release exponent "n" was > 0.89 indicating Super-case II transport mechanism.

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Table 1: Formulation of drug loaded microspheres

F. Code	Drug (mg)	Chitosan (mg)	Emulsifier (Span 80) %	Stirring Rate (rpm)
MCM1	100	500	5	500
MCM2	100	1000	5	500
MCM3	100	500	10	500
MCM4	100	1000	10	500
MCM5	100	500	5	1000
MCM6	100	1000	5	1000
MCM7	100	500	10	1000
MCM8	100	1000	10	1000

Table 2: Important band frequencies in IR spectrum of metronidazole

S. No.	Frequency cm^{-1}	Group assigned
1.	3875.56, 3789.89, 3455.11	NH stretch

2.	3147.51	O-H Stretch
3.	2937.22, 2980.62, 2902.11	CH stretch
4.	1709.02, 1746.59, 1698.13	C=O stretch
5.	1639.48, 1602.95	C=C stretch
6.	1544.65	S=O stretch
7.	1452.45, 1508.42	N-H out of plane
8.	1357.47, 1251.12	C-H bend
9.	1166.47, 1152.66	C-N stretch
10.	993.26, 912.63, 850.48	N-H bend, O-H bend
11.	1413.52	CH2,CH3, Stretch

Table 3: Physical characteristics of different batches of chitosan microspheres

F. Code	Mean particle size[b] (µm)	Bulk density[a] (g/cm^3)	Tapped density[a] (g/cm^3)	Carr's index[a] (%)	Angle of repose[a] (Θ°)
MCM1	246.12±2.11	0.37±0.021	0.44±0.021	15.90±1.07	25.12±1.60°
MCM2	244.42±3.25	0.34±0.012	0.41±0.015	12.48±0.01	30.58±2.58°
MCM3	250.34±3.67	0.42±0.011	0.47± 0.012	10.63±0.13	24.25±1.23°
MCM4	248.14±2.47	0.41±0.014	0.46±0.014	14.23±1.25	28.11±1.21°
MCM5	142.42±3.25	0.38±0.015	0.44± 0.018	13.63±0.85	22.98±1.01°
MCM6	142.56±2.12	0.49±0.012	0.50±0.014	14.48± 0.89	26.79±1.43°
MCM7	148.93±3.92	0.41±0.028	0.46± 0.026	10.86±0.95	22.16±1.67°
MCM8	148.14±2.47	0.41±0.01	0.44± 0.014	12.66±0.33	26.45±1.43°

Table 4: in-vitro Dissolution data of chitosan microspheres of metronidazole (MCTM1 – MCTM8)

Time (h)	MCM1	MCM2	MCM3	MCM4	MCM5	MCM6	MCM7	MCM8
0	0	0	0	0	0	0	0	0
1	0.421	0.482	0.781	0.991	1.102	1.206	1.38	1.459
2	3.47	4.06	2.92	11.36	7.32	5.72	14.23	14.34
3	7.34	9.21	8.89	13.11	14.23	9.34	18.34	28.43
4	13.11	13.65	13.23	18.76	28.43	11.67	32.12	33.54

5	21.38	19.98	15.34	24.98	32.34	22.23	38.67	39.62
6	28.71	28.46	29.45	31.23	39.01	31.87	42.67	41.41
7	35.02	33.43	32.12	37.12	45.21	39.54	44.23	54.31
8	49.05	39.89	47.23	45.43	61.02	46.31	59.67	55.89
9	71.23	61.89	68.23	53.67	77.61	68.98	75.6	62.32
10	88.01	77.99	84.21	70.76	89.02	91.34	88	69.31
11	99.08	87.21	98.98	81.98	95.01	98.11	94.6	98.23
12	99.89	99.67	99.89	99.88	99.99	99.05	99.98	99.68

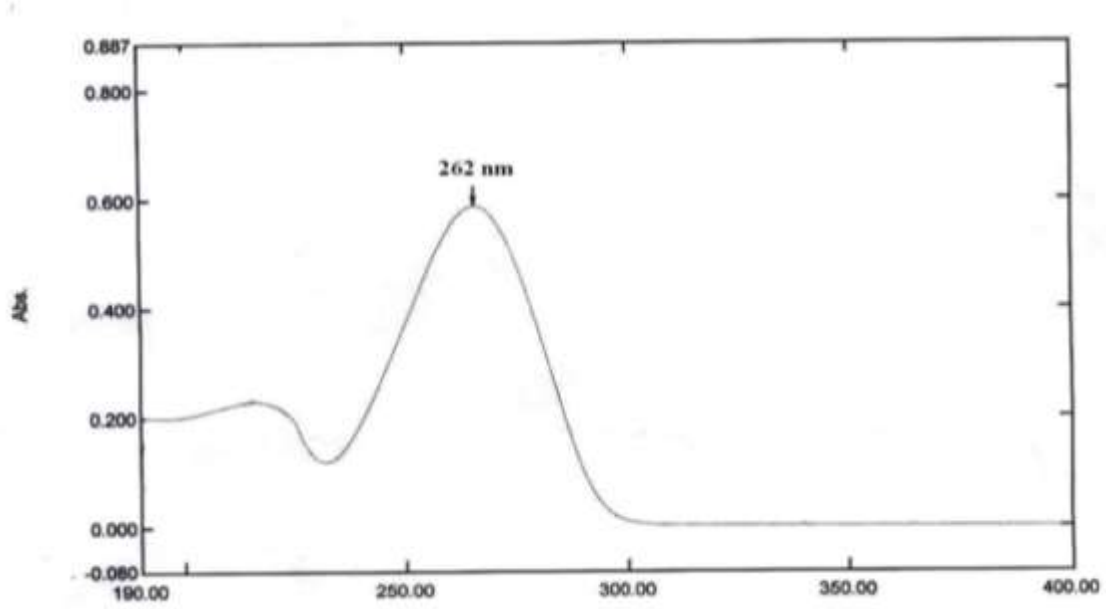

Figure 1: UV absorption maxima of drug in simulated gastric fluid (pH 1.2) at λmax 272.0 nm

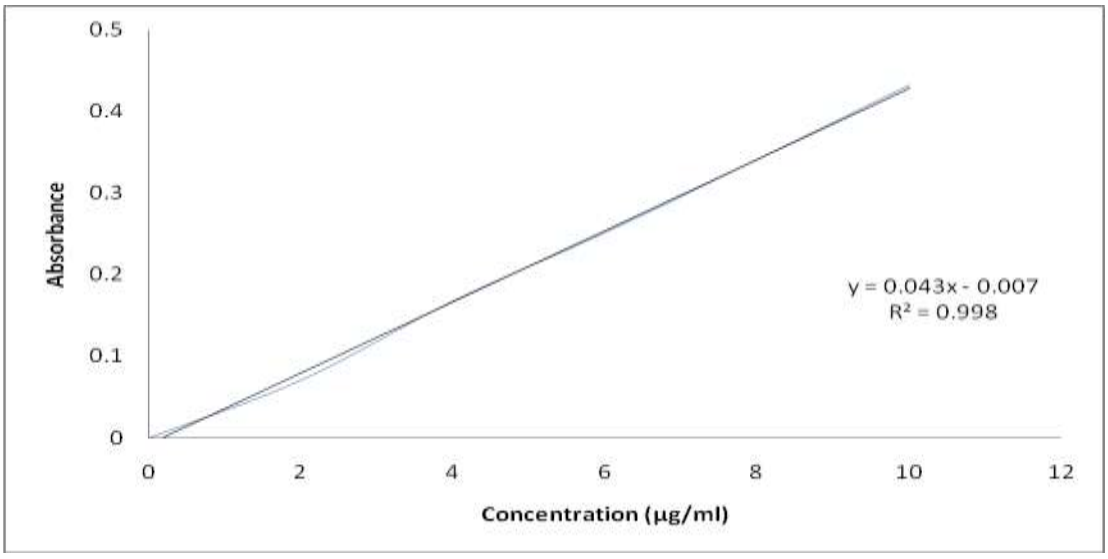

Figure 2: Standard Curve of drug in simulated gastric fluid (pH 1.2) at 272.0 nm

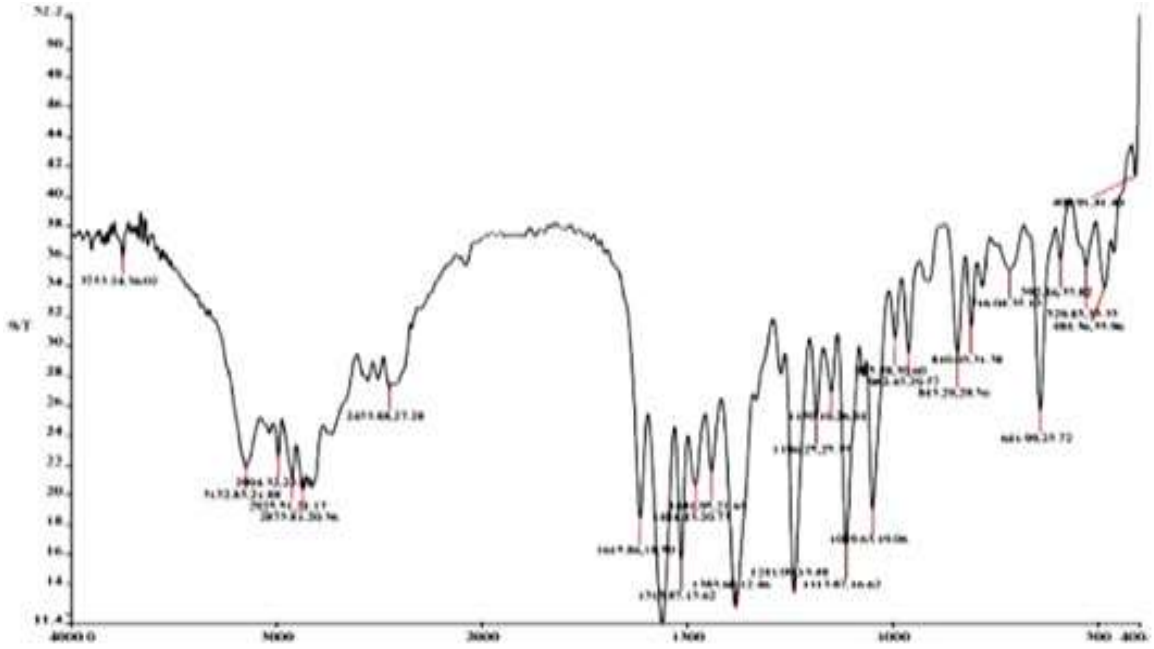

Figure 3: FTIR spectra of drug meronidazole

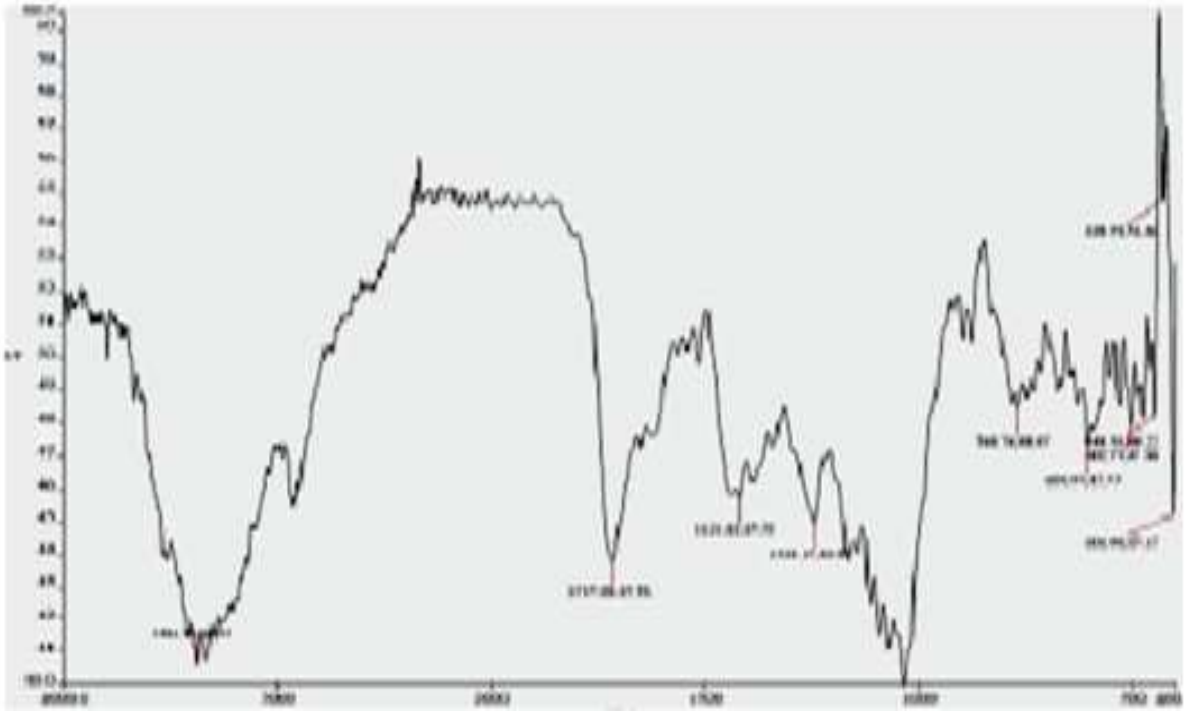

Figure 4: FTIR spectra of metronidazole with excipients

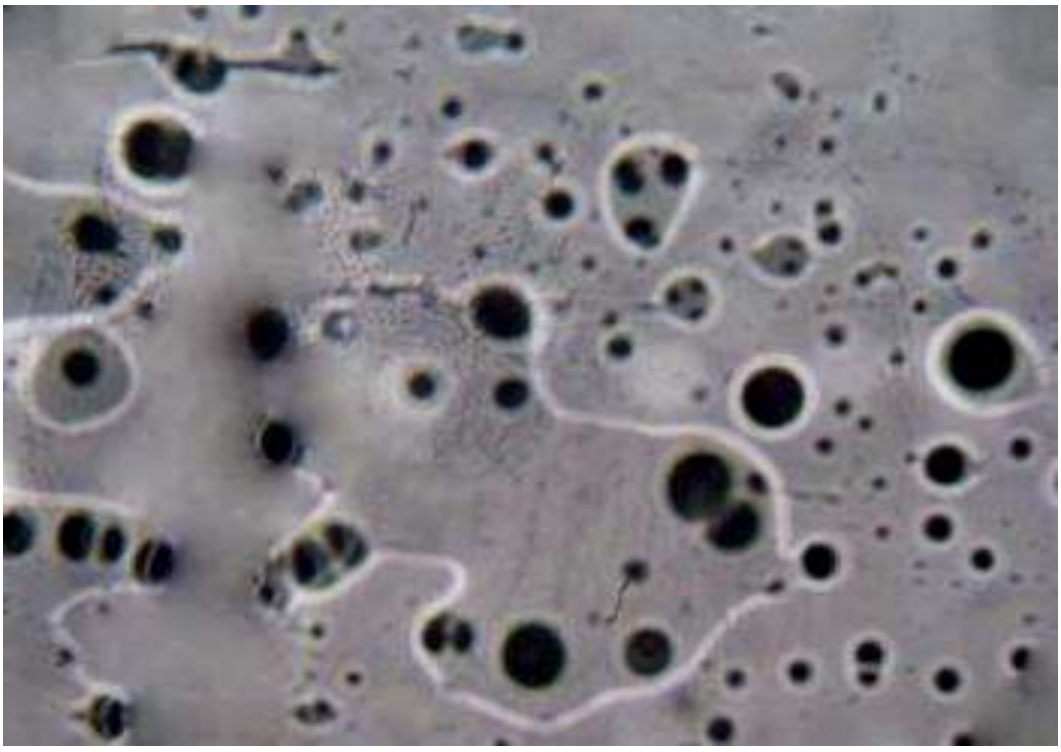

Figure 5: Photomicrograph off colon targeted microspheres (100X) (MCTM30)

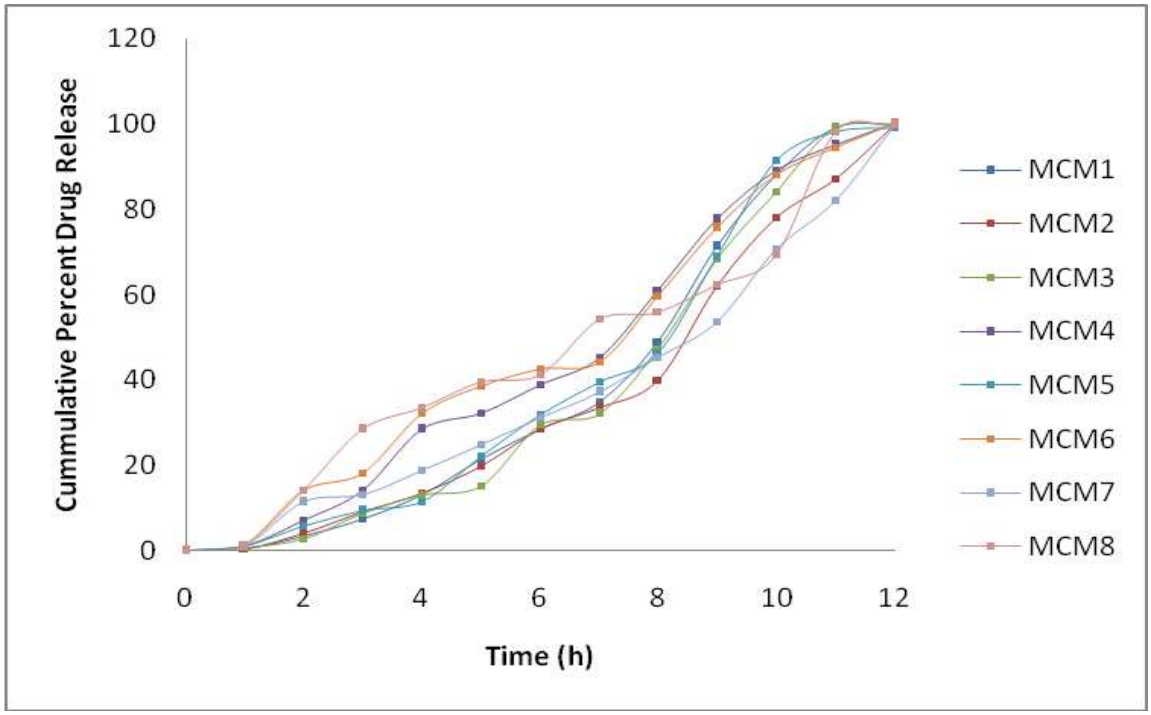

Figure 6: Zero-order plots of chitosan microspheres of metronidazole (MCTM1 – MCTM8)

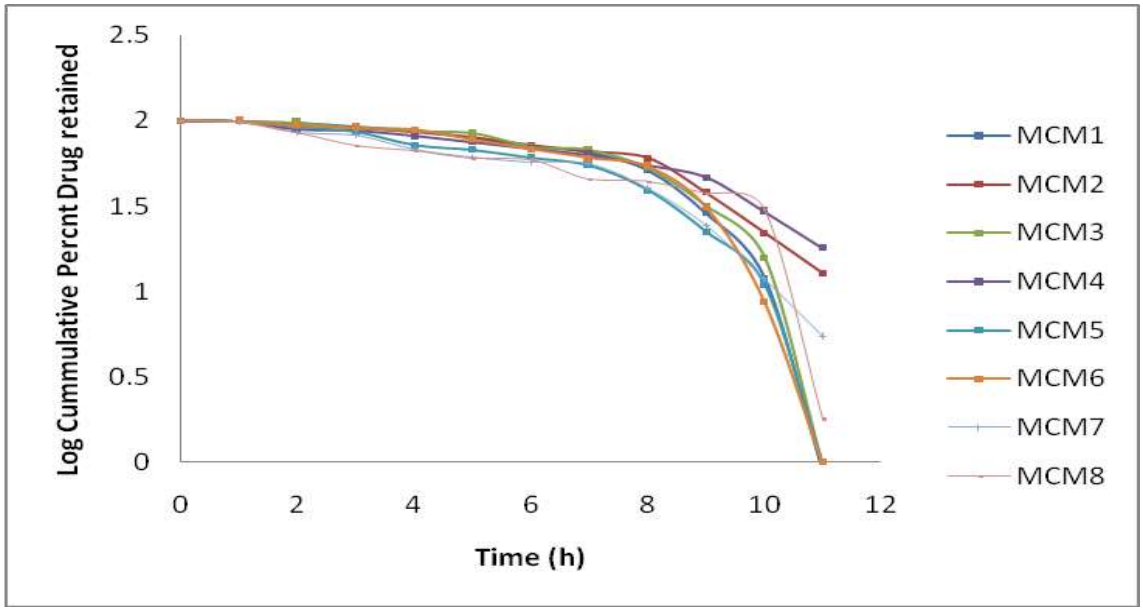

Figure 7: First-order plots of chitosan microspheres of metronidazole (MCTM1 – MCTM8)

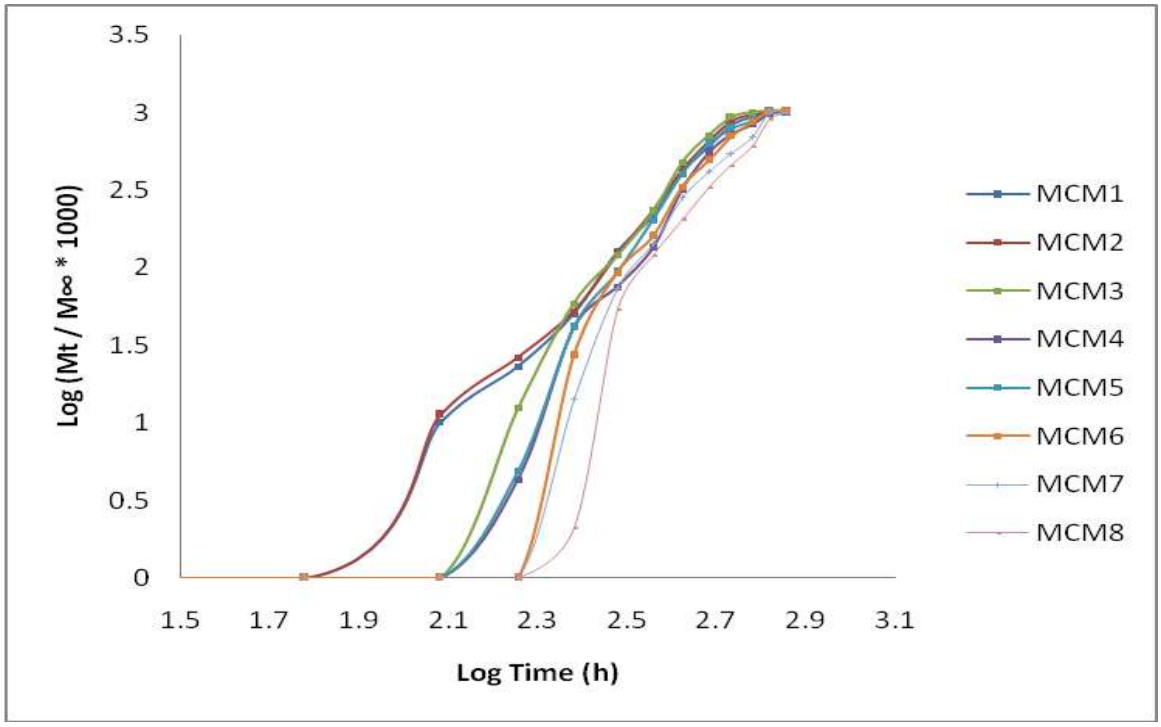

Figure 8: Korsmeyeyr's Peppas plots of chitosan microspheres of metronidazole (MCTM1 – MCTM8)

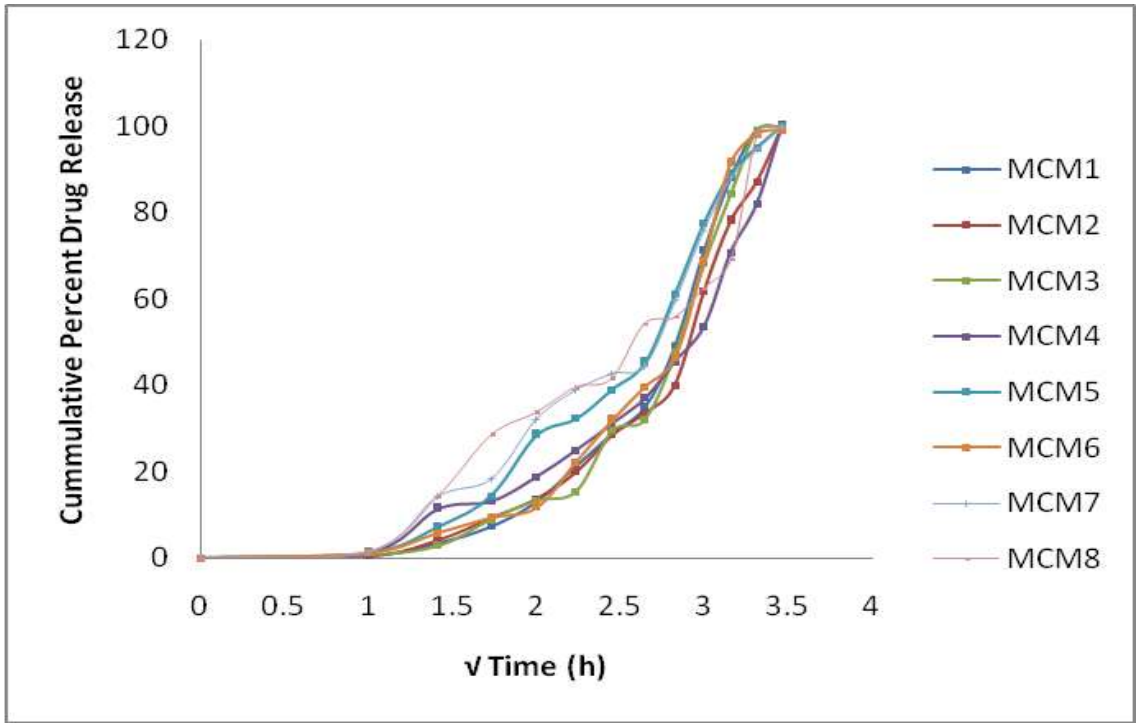

Figure 9: Higuchi plots of chitosan microspheres of metronidazole (MCTM1 – MCTM8)