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Research Article

BIODEGRADABLE POLYMER: A NOVEL PHARMACEUTICAL CARRIER FOR SUSTAINED RELEASE OF METRONIDAZOLE

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ABSTRACT

Objective: To optimize and evaluate the formulation of metronidazole (MT)-loaded chitosan microspheres and to investigate the efficiency of biodegradable polymer in developing sustained release formulation of MT to prolong the action of drug.

Methods: MT microspheres were prepared using emulsion cross-linking method. Polymer-drug compatibility study was done using Fourier transform infrared. Physical characteristics were evaluated by particle size,SEM, flow properties etc. *In vitro* studies for evaluating drug release for MT-loaded chitosan microspheres were done by dissolution study.

Results: Particle size of the formulated microspheres was found to be within the range of 110-130 µm. Flow properties of F1-F7 such as angle of repose, bulk density, and tapped density were found to be within limits. Drug entrapment efficiency was found to be better for all the formulations within the range of 74.82-84.32% w/w. Drug loading capacity was found to be in the range of 56-83.2% w/v. *In vitro* drug release was found to be in the range of 81.32-96.23% w/v.

Conclusion: In spite of all the above results, we conclude that F5 formulation was optimized depending on the data obtained from the drug loading capacity and percentage drug release studies. F5 formulation is formulated with drug-polymer ratio 1:2 with 1% of di octyl sodium sulfo succinate and 8 ml of glutaraldehyde as a cross-linking agent.

Keywords: Metronidazole, Chitosan microspheres, Scanning electron microscopy, Cross-linking.

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INTRODUCTION [1,2]

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1-1000 µm). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass microspheres, polymer microspheres, and ceramic microspheres are commercially available. Solid and hollow microspheres vary widely in density and therefore are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are. Polyethylene and polystyrene microspheres are two most common types of polymer.

Metronidazole (MT) is a synthetic, chemotherapeutic drug, which is a derivative of nitroimidazole. MT is one of the most effective drugs in the eradication of *Helicobacter pylori*, which resides mainly in the gastric mucosa and is an etiologic factor in the development of gastritis, gastric ulcer, and gastric carcinoma [3]. The objective of this study was to prepare and evaluate Metronidazole loaded chitosan microspheres by emulsion cross-linking technique [4]. The obtained microspheres were characterized for size, morphology, drug loading, and entrapment efficiency. The particle size was determined by scanning electron microscopy (SEM) [5,14]. The *in vitro* drug release and mathematical modeling of MT release were also evaluated.

METHODS

MT was purchased from Max-Med Laboratories, Chennai. Chitosan and potassium dihydrogen phosphate were purchased from Chenchems, Chennai. Glutaraldehyde, n-hexane, sodium dihydrogen phosphate, and sodium hydroxide were purchased from Microfine, Chennai.

Chitosan microspheres containing MT were prepared by emulsion cross-linking technique (Table 2). The chitosan solution was prepared in 5% aqueous acetic acid, in which the drug was dispersed. The resultant mixture was extruded through a syringe (no. 20) into 100 ml of liquid paraffin (heavy and light, 1:1 ratio) containing 0.2% w/v of di octyl sodium sulfo succinate (DOSS). The stirring was performed using a magnetic stirrer with the speed of 1200 rpm. After 2 minutes, 4 ml of glutaraldehyde saturated toluene was added into the dispersion. Then, after 15 minutes, additional 4 ml of 25% aqueous glutaraldehyde was added drop by drop and stirring was continued for additional 3 hr. The microspheres thus obtained were filtered and washed several times with n-hexane to remove traces of oil. They were then washed thrice with ice cold water to remove acetic acid and glutaraldehyde. The microspheres were then dried in hot air oven at 50°C overnight and stored in desiccators at room temperature.

Process variables

Different formulations were formulated with varying polymer:drug ratio (1:1, 1.5:1, 2:1), di octyl sodium sulfo succinate (DOSS) (0.5%-1%), cross-linking agent volume (8 ml, 9 ml, and 10 ml) and keeping a speed of rotation of 1200 rpm for all the formulations (Table 1).

Evaluation of microspheres [4]

The shape and surface characteristics of chitosan microspheres was assessed by SEM (Fig. 1). The particle diameters of more than 200 microspheres were measured randomly. The average particle size was determined. Drug encapsulation efficiency was quantified using a spectrophotometric method. The microspheres drug content was determined by ultraviolet (UV) spectrophotometry. Flow property was quantified. *In vitro* drug release studies in simulated gastrointestinal fluids (SGFs).

Microsphere morphology [7]

The shape and surface characteristics of chitosan microspheres were assessed by SEM, and size distribution of microspheres was determined by optical microscopy.

Fourier transform infrared (FTIR) of MT-chitosan microspheres [8,9]

The compatibility of the drug and polymer was evaluated by FTIR (Fig. 2 and Table 4).

Particle size distribution of microspheres [7]

The particle diameters of more than 200 microspheres were measured randomly. The average particle size was determined.

Drug encapsulation efficiency of MT microspheres [10]

The microspheres containing drug equivalent to 50 mg of MT were dissolved in 50 ml of HCl (0.1 N). After complete dissolution of chitosan, the amount of drug was quantified using a spectrophotometric method at 320.5 nm against the blank.

Drug loading capacity

The microspheres' drug content was determined by the UV spectrophotometry. A weighed amount of drug-loaded microspheres was dissolved in 1 ml of chloroform, followed by the addition of 9 ml of methanol to precipitate the polymer. The resulting suspension was centrifuged and the supernatant liquid was filtered and analyzed in the UV spectrophotometer (Table 5).

$$\text{Experimental drug loading} = \frac{\text{Weight of detected drug}}{\text{Weight of drug-loaded microspheres}}$$

$$\text{Theoretical drug loading} = \frac{\text{Weight of added drug}}{\text{Weight of added drug} + \text{weight of added polymer}}$$

Flow properties

Angle of repose

The angle of repose is the constant, three-dimensional angle (relative to horizontal base) assumed by a cone-like pile of material formed by any of several different methods. When the angle of repose exceeds 50°, the flow is rarely acceptable for manufacturing purposes (Table 3) [11].

Apparent bulk density

The bulk density was determined by transferring the accurately weighed sample of powder to the graduated cylinder. The initial volume and weight were noted. Ratio of weight of the sample was calculated using the formula [12]:

Density = Mass/Volume

Tapped density

The tapped density was determined by transferring the powder sample to the graduated cylinder. Fixed number of tappings was given(500). The tapped density was determined by the following formula.

Density = Mass/Tapped density

Percentage compressibility

Based on the apparent bulk density and the tapped density, the percentage compressibility of the bulk drug was determined by the formula [13].

Hausner ratio

It indicates the flow properties of powder and is measured by the ratio of tapped density to bulk density [15].

***In vitro* drug release studies in SGFs [9]**

Chitosan microspheres were evaluated for *in vitro* drug release in SGFs [16]. The drug release study of microspheres was performed by the Basket method (Model TDT-08L, Electrolab) specified in USP 1. Microspheres (equivalent to 50 mg of drug) were weighed accurately and filled in gelatin capsule [17,18,19]. Capsule was taken

Table 1: Formulation table

Ratios			
Polymer:Drug	1:1	1.5:1	2:1
Surfactant:DOSS	0.5% w/v	0.75% w/v	1% w/v
Volume of cross-linking agent	8 ml	9 ml	10 ml

DOSS: Di octyl sodium sulfo succinate

Table 2: Formulation composition for metronidazole microspheres

Formulation code	Drug: polymer	Concentrations of DOSS (di octyl sulfo succinate)% (W/W)	Amount of cross-linking agent (ml)
F1	1:1	0.5%	8
F2	1:1.5	0.5%	8
F3	1:2	0.5%	8
F4	1:2	0.75%	8
F5	1:2	1%	8
F6	1:2	0.5%	9
F7	1:2	0.5%	10

DOSS: Di octyl sodium sulfo succinate

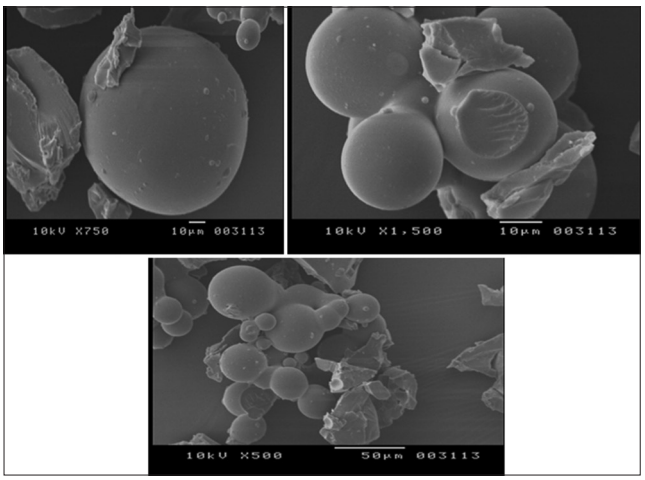

Fig. 1: Particle before drug dissolution study (F5)

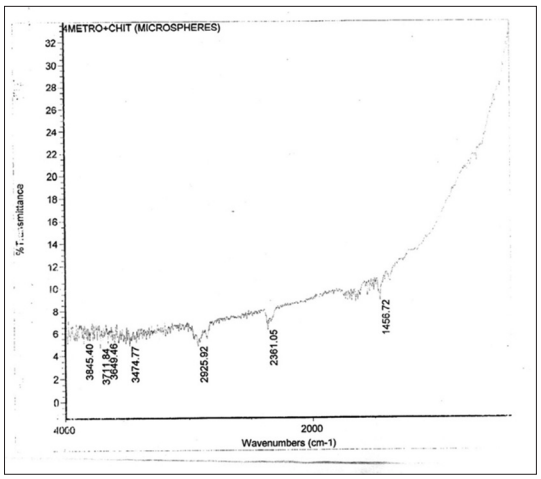

Fig. 2: FTIR Scanning electron microscopy (F5)

Table 3: Flow properties of microspheres

Formulation code	Angle of repose (°)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratio	Percentage compressibility
F1	24	0.467	0.613	1.215	16.900
F2	23	0.447	0.684	1.250	13.200
F3	23	0.547	0.552	1.190	15.430
F4	24	0.432	0.432	1.136	12.765
F5	21	0.533	0.591	1.161	11.430
F6	22	0.432	0.543	1.195	11.999
F7	23.3	0.464	0.439	1.165	15.988

Table 4: FTIR chart of metronidazole-chitosan microspheres

S. No.	Wave number	Bond	Mode
1	2925.92	C-H	Stretching
2	3474.77	C=O	Stretching
3	3649.46	N-H	Stretching
4	2361.05	C≡C	Stretching
5	1456.72	C-H	Bend in plane

FTIR: Fourier transform infrared

Table 5: Drug entrapment efficiency and drug loading capacity

Formulation code	Entrapment efficiency (%)	Percentage drug loading
F1	75.32	56.12
F2	80.12	63.87
F3	82.50	68.71
F4	82.59	74.38
F5	84.32	83.2
F6	76.55	75.24
F7	74.82	72.43

in the basket which was immersed in 500 ml of dissolution medium. The basket was rotated at 100 rpm at 37°C ± 0.5°C. Perfect sink conditions were maintained during the drug dissolution study period. The simulation of G1 transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hrs using 0.1 N HC1. Then, KH_2PO_4 (1.7 g) and $Na_2HPO_42H_2O$ (2.2 g) were added to the dissolution medium, the pH was adjusted to 4.5 with 1.0 M NaOH, and the release rate study was continued for additional 2 hrs. After 4 hrs, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH, and release studies were extended up to 24 hrs. 5 ml samples were withdrawn from the dissolution medium at various time intervals using pipette with a treated cellophane membrane. The samples pipetted out were suitably diluted with 1.2 pH buffer and the rate of MT release was analyzed with the aid of an UV spectrophotometer and absorbance was recorded at 320.5 nm [20,21]. The receptor volume was maintained constant by replacing with an equivalent amount of buffer. The concentrations of MT in the samples were calculated by reference to the calibration curve. All dissolution studies were performed in triplicate.

Microsphere morphology

The obtained microspheres were found to be spherical in shape using SEM [22].

Particle size distribution study

Particle size of the formulated microspheres was found to be within the range of 110-130 µm (Fig. 3) [23-26].

RESULTS

For F1 formulation, percentage yield – 75.3% w/w, particle size – 50-70 µm, angle of repose – 24°, bulk density – 0.467 g/ml, tapped density – 0.613 g/ml, Hausner ratio – 1.215, % compressibility – 16.900%, drug entrapment efficiency – 75.32% w/v, drug loading capacity – 56.12% w/v, and *in vitro* drug release at the end of 24 hrs – 86.49% w/v.

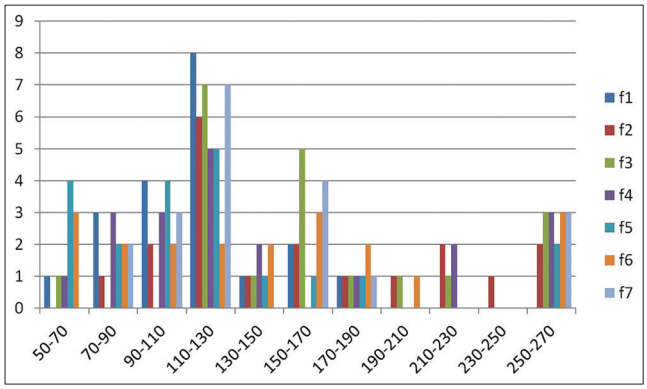

Fig. 3: Size range versus frequency

For F2 formulation, percentage yield – 76.67% w/w, particle size – 70-90 µm, angle of repose – 23°, bulk density – 0.447 g/ml, tapped density – 0.684 g/ml, Hausner ratio – 1.250, % compressibility – 13.200%, drug entrapment efficiency – 80.12% w/v, drug loading capacity – 63.87% w/v, and *in vitro* drug release at the end of 24 hrs – 83.41% w/v.

For F3 formulation, percentage yield – 72% w/w, particle size – 50-70 µm, angle of repose – 23°, bulk density – 0.547 g/ml, tapped density – 0.552 g/ml, Hausner ratio – 1.190, % compressibility – 15.430%, drug entrapment efficiency – 82.50% w/v, drug loading capacity – 68.71% w/v, and *in vitro* drug release at the end of 24 hrs – 81.32% w/v.

For F4 formulation, percentage yield – 72.6% w/w, particle size – 90-110 µm, angle of repose – 24°, bulk density – 0.432 g/ml, tapped density – 0.432 g/ml, Hausner ratio – 1.136, % compressibility – 12.765%, drug entrapment efficiency – 82.59% w/v, drug loading capacity – 74.38% w/v, and *in vitro* drug release at the end of 24 hrs – 84.32% w/v.

For F5 formulation, percentage yield – 88.0% w/w, particle size – 110-130 µm, angle of repose – 21°, bulk density – 0.533 g/ml, tapped density – 0.591 g/ml, Hausner ratio – 1.161, % compressibility – 11.430%, drug entrapment efficiency – 84.32% w/v, drug loading capacity – 83.2% w/v, and *in vitro* drug release at the end of 24 hrs – 96.23% w/v. The morphological examination of the MT-loaded chitosan microspheres was performed by SEM. The MT-loaded chitosan microspheres were spherical in shape and had a smooth surface without pores or cavities, which could affect the release of encapsulated drug. The presence of polymer debris and accumulation of microspheres were observed for few particles that may be caused by the presence of liquid paraffin. Overall, all the microspheres were found to be smooth and spherical in shape.

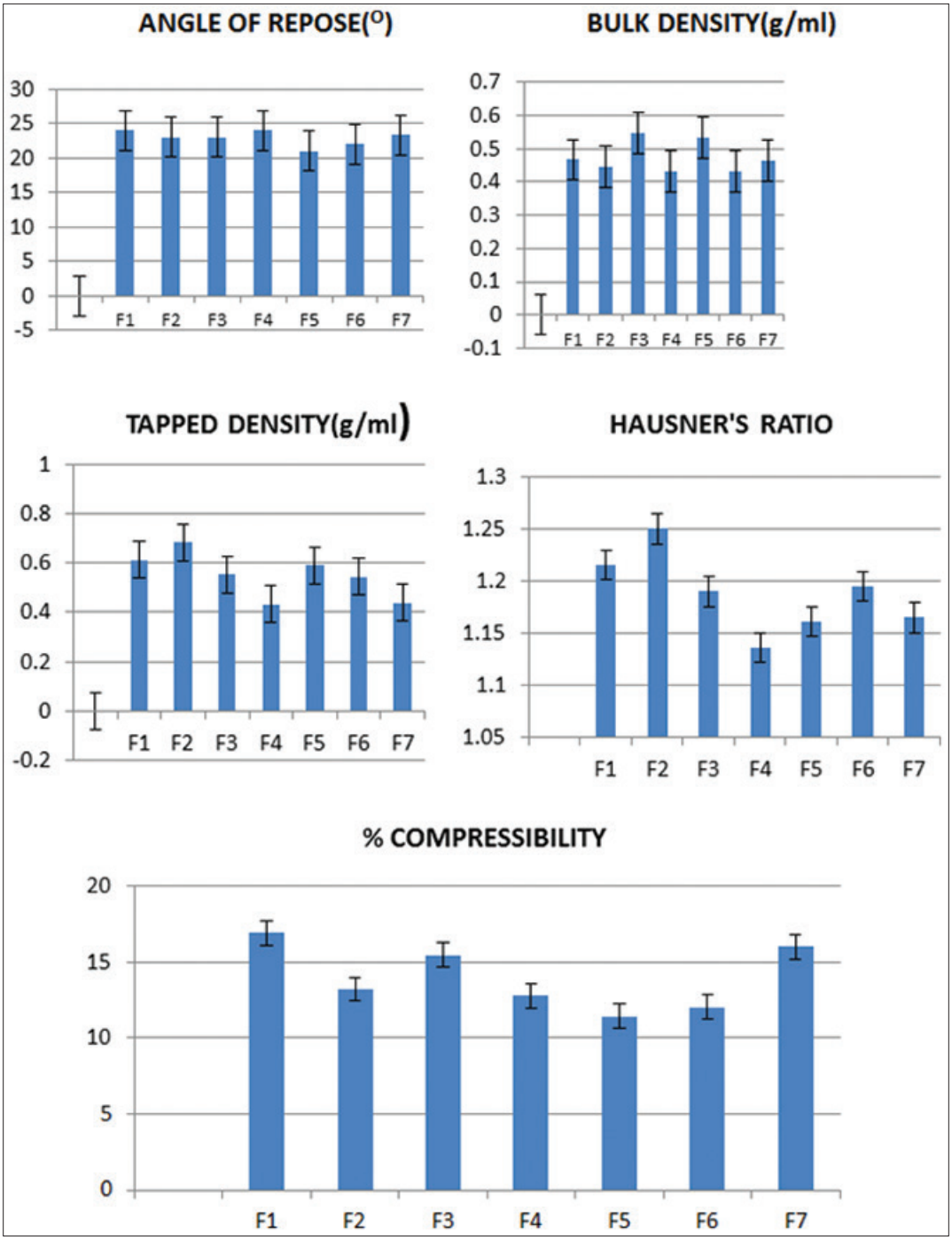

Fig. 4: Flow property

Table 6: *In vitro* drug release

Time	F1	F2	F3	F4	F5	F6	F7
With buffer pH - 1.2							
1	0	0	0	0	0	0	0
2	26.31	29.42	24.42	25.24	22.32	26.42	20.21
With buffer pH - 4.5							
4	38.26	35.37	39.28	32.17	42.83	39.28	35.28
6	45.17	49.26	45.92	38.28	59.43	49.25	44.43
8	54.28	63.42	53.48	49.24	63.48	50.23	53.24
12	63.24	71.23	64.97	55.28	79.28	62.29	65.96
With buffer pH - 7.4							
18	74.91	78.89	73.28	67.92	86.42	73.42	77.49
20	86.49	83.41	78.14	77.77	91.17	85.49	86.42
24	-	-	81.32	84.32	96.23	90.32	91.42

For F6 formulation, percentage yield – 86.4% w/w, particle size – 150-170 µm, angle of repose – 22°, bulk density – 0.432 g/ml, tapped density – 0.543 g/ml, Hausner ratio – 1.195, % compressibility – 11.999%, drug entrapment efficiency – 76.55% w/v, drug loading capacity – 75.24% w/v, and *in vitro* drug release at the end of 24 hrs – 90.32% w/v.

For F7 formulation, percentage yield – 83.2% w/w, particle size – 70-90 µm, angle of repose – 23.3°, bulk density – 0.464 g/ml, tapped density – 0.439 g/ml, Hausner ratio – 1.165, % compressibility – 15.988%, drug entrapment efficiency – 74.82% w/v, drug loading capacity – 72.43% w/v, and *in vitro* drug release at the end of 24 hrs – 91.42% w/v.

Commonly, the FTIR shows no interaction for all the formulations F1-F7.

Hence, in spite of all the data obtained from other formulations, F5 formulation observed to be the best formulation.

DISCUSSION

The range of percentage yield was found to be from 72.6% to 88%. The percentage yield was found to be in the range of 75.3%-88% for F1-F7. It was observed that as the drug to the polymer ratio in the formulation from F1 to F7 increased, the product yield decreased (Table 4). Less percentage yield in some formulations may be due to the loss of microspheres during washing and drying. 100% yield cannot be achieved in the production of microspheres because of the homogenization and change of glass-wares during filtration and drying. The microspheres stick to the filter paper while drying. There may be loss even on aeration.

Particle size of the formulated microspheres was found to be within the range of 110-130 µm. Flow properties of F1-F7 such as angle of repose, bulk density, and tapped density were found to be within limits. The angle of repose of F5 formulation was found to be 21° (Table 3). The bulk density was found to be 0.533 g/ml. The tapped density was found to be 0.591 g/ml. The Hausner ratio was found to be 1.161 (Fig. 4). The % compressibility was found to be 11.43. Drug entrapment efficiency was found to be better for all the formulations (F1-F7) within in the range of 74.82-84.32% w/w (Table 5). Drug loading capacity was found to be in the range of 56-83.2% w/v (Fig. 5). *In vitro* drug release was found to be in the range of 81.32-96.23% w/v (Fig. 6).

The scanning electron photomicrographs of F5 show that the drug-loaded microspheres were round and smooth in appearance. Hence, after comparing all the results of all formulations, F5 was found to be the best formulation, so the SEM studies were done for the same.

The FTIR shows that there is no interaction between the polymer and the drug for the formulations such as MT, chitosan, MT-chitosan microspheres, blank microspheres, and MT and chitosan raw materials.

The stability study was initiated for the optimized formulation F5 at 25°C ± 2°C/60% RH and 40°C ± 2°C/75% RH.

CONCLUSION

The percentage yield of the formulation F5 was in the range of 88.0 as and when compared to other formulations. The loss of microspheres arises in the process of drying and washing. The maximum particle size of the optimized formulation was found to be in the range of 110-130 µm. The flow property of the formulated microspheres was good for formulation F5 as compared to the other formulations. The drug entrapment efficiency of the F5 formulation was found to be 82.50% w/v. Hence, the drug got well entrapped in the formulation. The drug loading capacity of the F5 formulation was found to be 83.2% w/v. The *in vitro* drug release of F5 formulation was found to be 96.23% w/v at the end of 24 hr. The SEM shows that the microspheres are round and smooth in appearance. Hence, by the results of all the formulations, F5 was found to be the best formulation, so the SEM studies were done for the same.

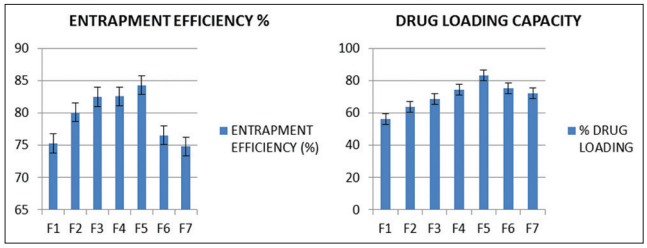

Fig. 5: *In vitro* drug release study (% w/v)

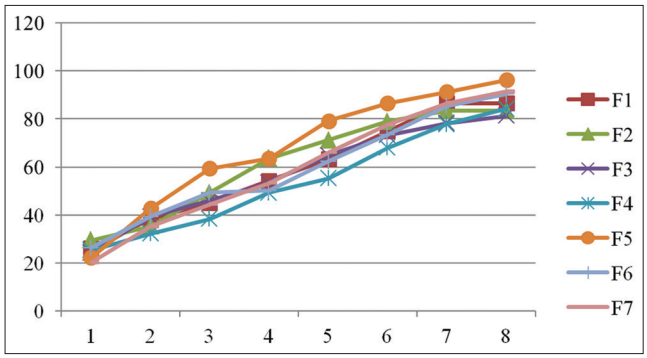

Fig. 6: *In vitro* drug release

In spite of all the above results, we conclude that F5 formulation was optimized depending on the data obtained from the drug loading capacity and percentage drug release studies. F5 formulation is formulated with drug-polymer ratio 1:2 with 1% of DOSS and 8 ml of glutaraldehyde as a cross-linking agent. Hence, biopolymeric carrier formulated with chitosan for sustained release delivery of MT was found to render acceptable drug loading capacity (83.2% w/v) and the percentage drug release rate was found to be 96.23% w/v. Thus, the novel formulation of chitosan-MT gives newer prolongation of action.

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