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MONTMORILLONITE-ALGINATE COMPOSITE BEAD AS DRUG DELIVERY VEHICLE FOR THE EXTENDED RELEASE OF AN ANTIBIOTIC DRUG

Arun Kant and Monika Datta*

Analytical Research Laboratory, Department of Chemistry, University of Delhi, Delhi 110007, India.

***Corresponding Author: Prof. Monika Datta**
Analytical Research Laboratory, Department of Chemistry, University of Delhi, Delhi 110007, India.

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ABSTRACT
The present study is aimed at developing Montmorillonite (Mt) Alginate (ALG) composites bead as drug delivery vehicle for the extended release of an antibiotic drug, Metronidazole (MTZ). ALG-MTZ and Mt-ALG-MTZ composite bead were synthesised by the modification of the reported procedure.[1, 2] Interaction of MTZ with ALG and Mt was studied and presented with a proper mechanism. The synthesized ALG-MTZ composite bead and Mt-ALG-MTZ composite bead have been characterized using appropriate analytical techniques. The *in-vitro* release behaviour of MTZ from the synthesized ALG-MTZ and Mt-ALG-MTZ composite bead were investigated in the simulated gastric and intestinal fluids. From the *in-vitro* drug release data possible bioavailability of MTZ in case of for ALG-MTZ and Mt-ALG-MTZ composite bead have been calculated and it has been observed that, compared to the pure MTZ and the commercially available tablets (Metrogyl and Flagyl) performance of the synthesised ALG-MTZ and Mt-ALG-MTZ composite bead has not only been found superior in terms of probable percentage bioavailability but also in terms of availability of the drug in the system for a longer period of time.[3]

KEYWORDS: Montmorillonite, Alginate, Encapsulation, Kinetics and extended release.

INTRODUCTION
Alginates are one of the most versatile biopolymers which have been used as a drug carrier.[4, 5] Alginate is easily gelled in the presence of a divalent cation such as calcium ion. Alginate is isolated from brown seaweed by alkaline extract which solubilizes the alginic acid present in it. Alginic acid can be converted to a salt of sodium alginate and is the major form currently available. Alginic acid is a linear[6-8] polymer consisting of D-mannuronic acid and L-guluronic acid residues that are arranged in the polymer chain in blocks. The distinctive properties of Alginates namely biodegradability, biocompatibility and nontoxicity make it a suitable carrier for drug delivery. The most popular ‘‘egg-box’’ model is usually used to describe the formation of alginate gels in the presence of alkaline earth metals[9] **Fig. 1**.

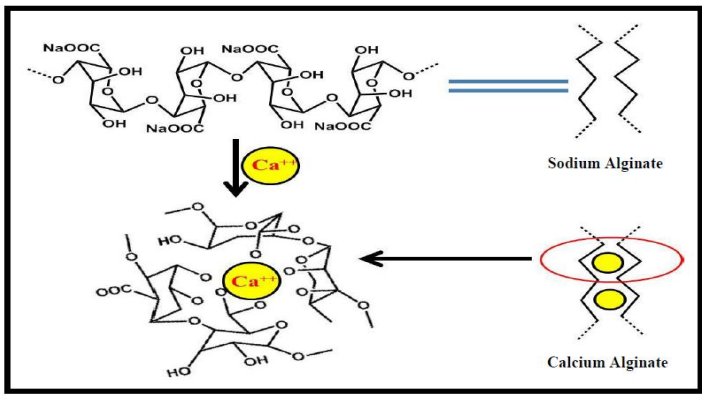


Fig. 1: Schematic representation of sodium alginate blocks and calcium - cross linking with alginate in egg box model

A relatively mild gelation process free of organic solvents enables biomolecules and cells to be incorporated into the matrices with the retention of the three-dimensional structure, porosity of the gel allows for acceptable diffusion rates and binding properties of drugs molecule.[10, 11]

Mt is FDA approved and used in pharmaceutical field due to their high specific area, higher cation exchange capacity, absorption capacity, suitable for medicinal products and also has intercalation capacity of organic compounds.[12-15] The physiochemical properties of Mt has been provides all the properties of an ideal drug delivery vehicle.[16, 17]

Some reports based on combination of Mt-ALG for the controlled release of proteins, vitamins, drugs like Diclofenac sodium, Irinotecan, Venlafaxine hydrochloride[18-22] was available but to the best of our knowledge there is no report involving Mt- alginate composite beads for extended release of MTZ.

The use of antibiotics in human provide preventive and in the treatment of several infections and of the most widely popular ones in the human and animal health care.[23] MTZ is an antibacterial agent used against anaerobic infection[24], antibacterial[25] and antiameobic agent.[26] It is used in the treatment of *trichomoniasis* of the genitourinary tract in male and female. It constitutes a family of antibiotics have clinically effective in a variety of infections caused by obligate anaerobic bacteria and microaerophilic bacteria. It is used in the treatment of amoebic dysentery and amoebic liver abscess as well as for the eradication of *E. histolytica* from patients passing cysts. It is still a standard antiprotozoal in the estimation of the activity of new antiprotozoal.[27, 28] The usual dose of MTZ for adult and children over 10 years is three times daily for 7 to 10 days. It also used for eradication of cysts in symptom less carries, treatment with MTZ three times daily for 5 to 10 days[29], there is no extended formulation available in market, at present it is administrated in the form of multiple dose.

In view of the above facts, the objective of this work was to synthesize Mt-ALG composite beads as host material for extended release of MTZ with possibility of better patient compliance with reduced number of doses.

MATERIALS AND METHODS

Materials: Metronidazole and Montmorillonite KSF was obtained from Sigma Aldrich USA. Analytical grade, HCl, NaOH and $CaCl_2.2H_2O$ was procured from Merck (Germany). Sodium alginate was obtained from Thomas Baker, Mumbai.

Instruments: UV– Visible spectrometer (Jena Analytik Specord 250 spectrophotometer) was used for the quantitative estimation of MTZ. X-ray diffraction patterns were recorded on a Philips X' Pert-PRO PMRD (D8 Discover Bruker AXS, Germany), Electron Microscopic (SEM) images were recorded using a scanning electron microscope (JEOL JSM-6610LV).

EXPERIMENTAL

Synthesis of ALG-MTZ and Mt-ALG-MTZ composite bead: ALG-MTZ and Mt-ALG-MTZ composite bead were synthesized by employed ion gelation process.[1, 2] Quantitative estimation of MTZ in the synthesized ALG-MTZ and Mt-ALG-MTZ composite bead was estimated using UV–visible spectrometry.

A known quantity of ALG was taken in distilled water with agitation on a magnetic stirrer for one hour and then known amount of aqueous MTZ solution was added while maintaining the stirring rate after the complete addition of MTZ solution was stirring for 3 hr. The resulting solutions were then filled in a syringe and added drop wise into a 50 mL of 0.1M $CaCl_2$ solution to form ALG-MTZ composite bead.

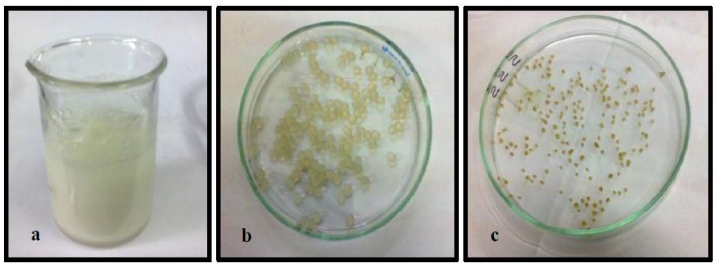


Fig 2: Photographic images of (a) Mt-ALG-MTZ dispersion, (b) Mt-ALG-MTZ composite bead and (c) Air dried Mt-ALG-MTZ composite bead

In case of Mt - ALG – MTZ composite bead, known quantity of ALG was taken in distilled water on a magnetic stirrer for one hour and a known amount of aqueous MTZ solution was added and stirring for 3 hour to form ALG-MTZ dispersion. In this ALG-MTZ dispersion Mt was added with constant stirring to form Mt-ALG-MTZ dispersion. The resulting solutions were added drop wise into a 50 mL of 0.1M $CaCl_2$ solution with the help of syringe to form Mt-ALG-MTZ composite bead. The supernatant and the beads thus obtained were used for the estimation of drug encapsulation efficiency. The beads were air dried, **Fig. 2** and were further characterized using appropriate analytical techniques.

Quantitative estimation of Metronidazole in calcium chloride solution

Stock solution ($100mgL^{-1}$) of MTZ was prepared in 0.1 M aqueous calcium chloride solution. From this stock solution a series of solutions with various known concentrations was prepared in 25 mL standard volumetric flask after maintaining the same concentration of calcium chloride (pH = 6.12).

Absorbance value of each solution was measured at 320 nm and it was observed that the Beer – Lambert's law was valid in the range of 1 ppm to 18 ppm**.** Calibration plot was found to be linear with slope 0.0534 and correlation coefficient 0.9998.

Hence for quantitative estimation of drug in samples containing unknown amount of drug, absorbance of each solution was measured at 320nm, after maintaining the solutions at same concentration of calcium chloride. Concentration of the drug in each sample was evaluated with the help of the calibration plot, **Fig. 3**.

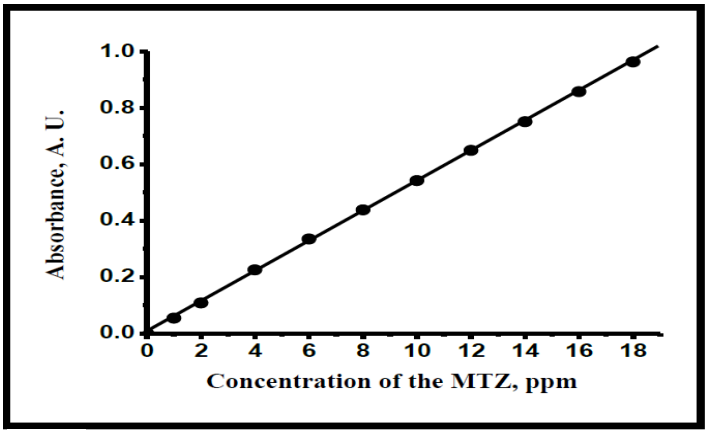


Fig. 3: **Absorbance as a function of concentration of the MTZ**

Optimization of encapsulation efficiency

The encapsulation efficiency for a particular drug is defined as the

$$\text{Encapsulation Efficiency} = \frac{\text{Amount of drug retained by the composite beads}}{\text{Initial Amount of drug taken}}$$

It is generally express as percentage encapsulation efficiency. For the synthesis of drug containing ALG and Mt-ALG composite beads, initially the amount of ALG was kept constant and the amount of the drug was varied AD1-AD5. The maximum encapsulation efficiency of 34% was observed with 20 mg of the drug (sample code AD4).

After optimising the amount of ALG and drug, MTZ, Mt was added to this composition in increasing amount (sample code AMtD1- AMtD6). Maximum encapsulation efficiency of 42% was observed with 200 mg Mt (sample code AMtD5) was mention in **Table -1.** The best optimized condition was observed with composite bead code AD4 and AMtD5. These composites beads were characterized using appropriate analytical techniques and were also used for *in-vitro* drug release studies.

Table -1: Optimization of encapsulation efficiency of MTZ in ALG - MTZ composite bead and Mt- ALG - MTZ composite bead

Code	Clay, Mt (mg)	ALG (mg)	MTZ (mg)	Encapsulation MTZ, %
AD1	------	200	05	15.64
AD2	------	200	10	17.74
AD3	------	200	15	24.72
AD4	**------**	**200**	**20**	**33.80**
AD5	------	200	25	26.82
AMtD1	25	200	20	21.02
AMtD2	50	200	20	26.02
AMtD3	100	200	20	30.07
AMtD4	150	200	20	32.02
AMtD5	**200**	**200**	**20**	**41.49**
AMtD6	250	200	20	35.87

RESULTS AND DISCUSSION

XRD studies: The XRD pattern of pristine Mt shows characteristic diffraction peak of 001 plane at 2θ value of 6.02° corresponding to basal spacing (d) of 14.66 Å. In case of Mt-ALG-MTZ composite bead characteristic diffraction peak with low intensity at 5.15° corresponding to 001 plane with d spacing of 17.04 Å was observed so can say that intercalation of ALG-MTZ moiety in interlayer of Mt but intensity of Mt peak seems to be disappearing suggesting exfoliation of Mt layers. The decreases intensity to become hump like peak of the Mt-ALG-MTZ composite bead suggests it becomes amorphous nature, **Fig. 4**.

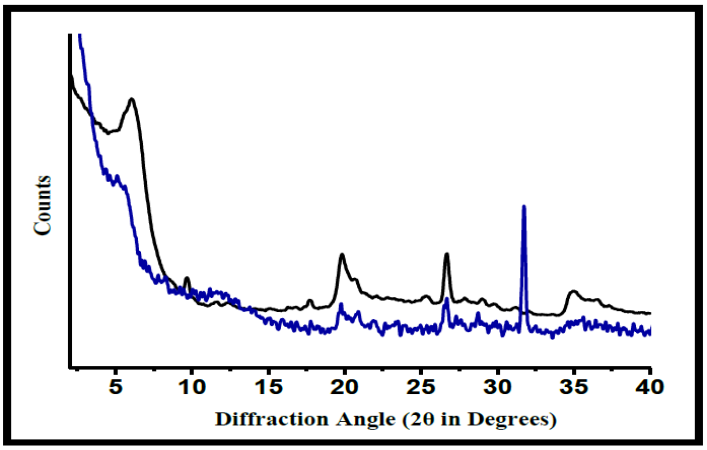


Fig. 4: XRD patterns of Mt and Mt-ALG-MTZ composite bead

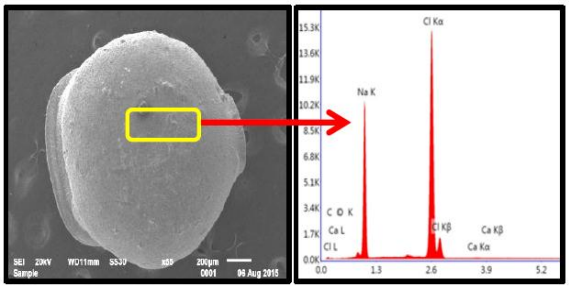
Electron microscopic (SEM) imaging with EDX analysis

Surface morphology of pure ALG, ALG-MTZ composite bead and Mt-ALG-MTZ composite bead was analysed by SEM. An energy dispersive X-ray analyser (EDX or EDA) is also used to provide elemental identification and quantitative compositional information.

The SEM image of ALG bead appears to be spherical rigid structure with smooth surface of 1400µm size. EDX data of pure ALG shows characteristic peak of elements like carbon, oxygen, sodium, chlorine and calcium, **Fig. 5**.

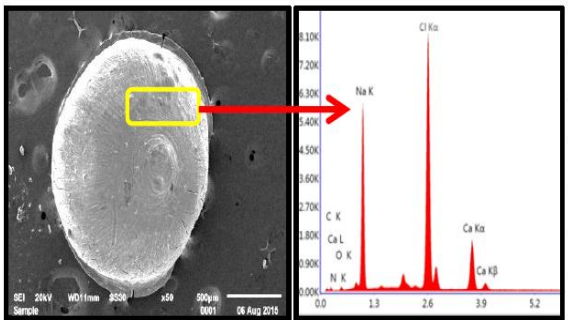
In case of ALG-MTZ composite bead also have smooth surface but some hair line cracks appear may be the presence of MTZ. It is also confirmed by EDX data because of the presence of nitrogen along with carbon, oxygen, sodium, chlorine and calcium which absent in ALG bead, **Fig. 6**.

In case of Mt-ALG-MTZ composite bead a clear change in the surface morphology as compare to ALG bead and ALG-MTZ composite bead is observed. The spherical shape becomes flat on going from ALG-MTZ to Mt-ALG-MTZ composite bead was shown due to presence of Mt as well as MTZ molecule. The presence of additional peaks of nitrogen in Mt-ALG-MTZ composite bead along with oxygen, silicon, sodium, iron and calcium (EDX analysis) can be taken as an evidence for the presence of MTZ in the Mt-ALG-MTZ composite bead **(Fig. 7**).



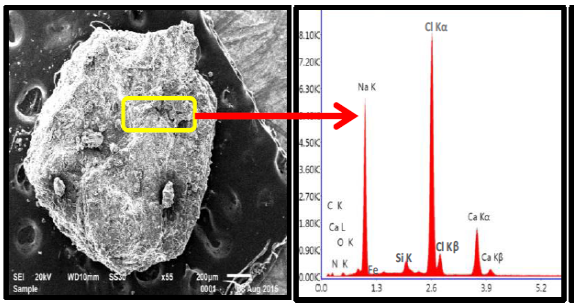
Elements	Weight%	Atomic%
C	1.29	3.05
O	0.54	0.97
Na	39.64	49.04
Cl	58.36	46.82
Ca	0.16	0.12

Fig. 5: SEM image with EDX graph of ALG bead



Elements	Weight%	Atomic%
C	5.83	11.29
N	0.02	0.04
Cl	10.84	8.05
O	2.07	3.20
Na	57.61	61.30
Ca	23.63	16.12

Fig. 6: SEM image with EDX graph of ALG-MTZ composite bead



Elements	Weight%	Atomic%
C	6.91	13.76
N	0.01	0.02
O	2.19	3.27
Si	0.14	0.17
Cl	13.49	8.07
Na	50.43	58.94
Ca	26.21	15.64
Fe	0.62	0.13

Fig. 7: SEM image with EDX graph of Mt-ALG-MTZ composite bead

***In-vitro* drug release behaviour of composite bead**

In-vitro release behaviour of pure MTZ, ALG-MTZ composite bead and Mt-ALG-MTZ composite bead was carried out in the simulated gastric and intestinal fluid using dialysis bag method.[30] A known amount of the pure MTZ and synthesised ALG-MTZ composite bead and Mt-ALG-MTZ composite bead was kept in the dialysis bag along with 5.0 mL of appropriate buffer solution. The dialysis bag was then dipped into 500mL of appropriate buffer solution contained in 6 bowls of the dissolution apparatus (LABINDIA, DISSO 8000, using USP paddle method) maintained at 37±0.5ºC. Gastric emptying of drug is highly variable; the normal gastric residence times usually range between 5 minutes to 2 hours.[31]

In the simulated gastric fluid pure MTZ release of 71% in the initial 2 hr followed by a relatively fast cumulative release of 76% in 6 hr was observed after that it becomes constant. In the simulated intestinal fluid pure MTZ release of 89% of the drug in the initial 2 hr followed by very fast cumulative release approaching to 98% in 3 hour and 30 minute after that it becomes constant. In the simulated gastric fluid ALG-MTZ composite bead release of 34% in the initial 2 hr followed by a relatively slow cumulative release of 66% over a period of 30 hr was observed. In the simulated intestinal fluid ALG-MTZ composite bead release of 67% in the initial 2 hour followed by a relatively fast cumulative release of 98 % over a period of 30 hr.

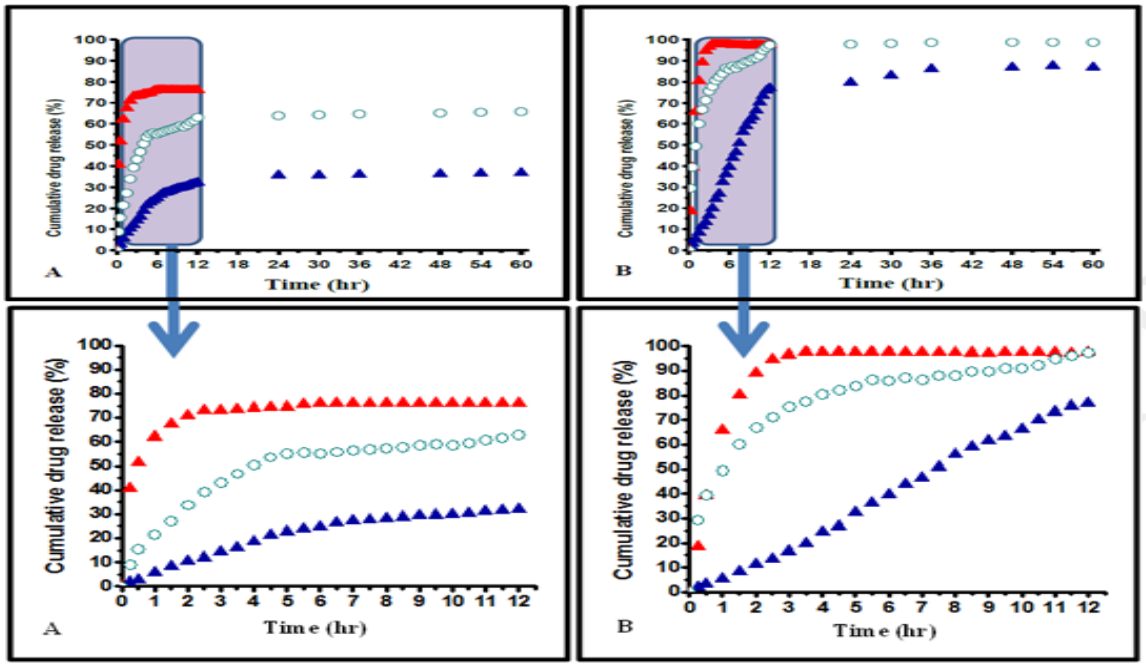


Fig. 8: Drug release behavior

(A) in simulated gastric (B) in simulated intestinal fluid MTZ (▲), Alginate MTZ bead (⬭) and Mt Alginate MTZ bead (▲)

In the simulated gastric fluid Mt-ALG-MTZ composite bead release of 10 % of the MTZ in the initial 2 hr followed by a relatively slow cumulative release of 32 % over a period of 12 hr and it extended 36% up to 36 hr. In the simulated intestinal fluid Mt-ALG-MTZ composite bead release of 77 % of the MTZ in the initial 12 hour followed by a relatively slow cumulative release of 87 % over a period of 36 hr and it sustain up to 60 hr.

Bioavailability of the Synthesised composite bead

Bioavailability for ALG-MTZ composite beads and Mt-ALG-MTZ composite bead been estimated from the *in-*

vitro drug release data obtained in the simulated gastric and intestinal fluids and was compared with the release profile of pure MTZ, **Fig. 9**. The bioavailability of MTZ, in case of pure drug is 28.4%. In case of commercially available tablets (Metrogyl and Flagyl) also show extended release behaviour up to 12 hr but at much lower bioavailability 43% and 40% respectively.[3]

However, in case of ALG-MTZ composite bead and Mt-ALG-MTZ composite bead the bioavailability was observed to be 64.47% and 69.01 respectively, **Table-2**. Thus, it can be concluded that the ALG-MTZ composite bead and Mt-ALG-MTZ composite bead can provide 2.27 and 2.47 fold increased bioavailability as compare to Pure MTZ.

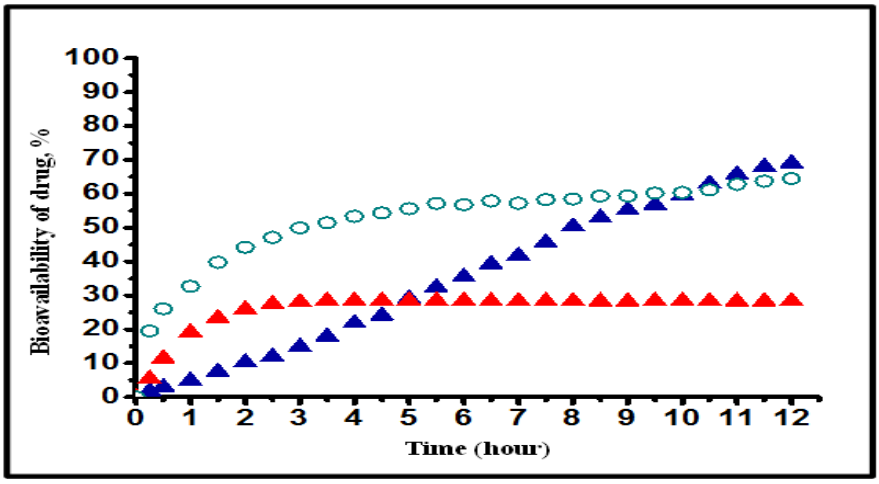


Fig. 9: Drug bioavailability

MTZ (▲), ALG-MTZ composite bead (○), and Mt-ALG-MTZ composite bead (▲)

Table 2: Cumulative drug release as a function of time

Sample Name	Total cumulative release of MTZ in the Simulated gastrointestinal fluid, %		Bioavailability in the Intestinal fluid, %
	Gastric fluid (In 2 hrs.)	Intestinal fluid (In 12 hrs.)	
Pure MTZ	71	28.40	28.40
ALG-MTZ composite bead	34	64.47	64.47
Mt-ALG-MTZ composite bead	10	69.01	69.01

CONCLUSION

The ALG-MTZ composite bead and Mt-ALG-MTZ composite bead was successfully synthesised and *in-vitro* drug release behaviour and kinetics of the process for the antibiotic drug, MTZ, ALG-MTZ composite bead and Mt-ALG-MTZ composite bead was studied in the simulated gastric and intestinal fluid. Release profile of the ALG-MTZ composite bead is sustained and Mt-ALG-MTZ composite bead are extended as compare to pure MTZ. It was observed that the ALG-MTZ composite bead and Mt-ALG-MTZ composite bead can provide much bioavailability of the drug as compared to the pure MTZ and commercially available tablet (Metrogyl and Flagyl). The ALG-MTZ composite bead and Mt-ALG-MTZ composite bead also has the potential to extend the release of drug up to 12 hr (~3.5 times more extended as compared to the pure MTZ. Because of higher bioavailability of drug in ALG-MTZ composite beads and Mt-ALG-MTZ composite bead lesser amount of drug intake would be required.

Thus, ALG-MTZ composite bead and Mt-ALG-MTZ composite bead has the potential to minimize the dosing frequency and may result in better patient compliance.

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