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ORGANO MONTMORILLONITE AS DRUG DELIVERY VEHICLE FOR THE EXTENDED RELEASE OF AN ANTIBIOTIC DRUG

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ABSTRACT

The aim of the present work was to develop organo Montmorillonite (OMt) as a drug delivery vehicle for the extended release of an antibiotic drug, Metronidazole (MTZ). OMt was synthesised by the modification of the reported procedure.[01] Interaction of MTZ with OMt was studied as a function of pH of the aqueous solution of the drug, contact time of batch extraction and initial concentration of MTZ in the solution. A maximum loading of MTZ was found to be about 1150 µg/50mg of OMt. The adsorption isotherm was well fitted by the Langmuir model and was observed to follow pseudo-second-order kinetics. The synthesized OMt-MTZ complex has been characterized using various appropriate analytical techniques. The *in-vitro* release behaviour of the drug from pure MTZ, the commercially available tablets (Metrogyl and Flagyl) and the synthesized OMt-MTZ complex was investigated in the simulated gastric and intestinal fluids. In the present case possible bioavailability for pure MTZ, the commercially available tablets and the OMt-MTZ complex has been calculated from the *in- vitro* drug release data and it has been observed that, compared to the pure MTZ and the commercially available tablets performance of the synthesised OMt-MTZ complex has not only been found superior in terms of probable % bioavailability but also in terms of availability of the drug in the system for a longer period of time.

KEYWORDS: Montmorillonite, PF68, Adsorption, Isotherm, Kinetics and extended release.

INTRODUCTION

The adsorptive study of antibiotics and many drugs onto clays and clay mineral has been studied since 1950s. In recent years, pharmaceutical grade Mt have more utilised by the researchers[2] as delivery vehicle for drug and it found that Mt has much higher affinity for

antibiotics compared to other excipient including kaolinite and oxide minerals.[3] Mt is used in pharmaceutical field due to their high specific area, higher cation exchange capacity, absorption capacity,[4] suitable for medicinal products[05] and also has intercalation capacity of organic compounds.[6,07] The advantageous characteristic physiochemical properties of Mt provides it all the properties of an ideal drug delivery vehicle.[8,9] After modifications of Mt it change in the form of OMt allows the creation of new materials and its applications in drug delivery vehicle because these materials are not widely explored in the area of drug delivery.

Interlayer space of OMt is high as compare to pristine Mt and has more cation exchange capacity (CEC).[10] In the present study FDA approved Mt and a non-ionic surfactant Pluronic F68 has been selected because of their structural, biological and industrial importance for the synthesis of OMt. OMt is being further explored as delivery vehicle for extended release of antibiotic drug MTZ.

The use of antibiotics in human and veterinary medicine grew very fast since their discovery. Their use in preventive medicine and in the treatment of several infections turned these compounds into some of the most widely popular ones in the human and animal health care.[11] MTZ is an antibacterial agent used in treatment of anaerobic infection.[12,13] It also has antiprotozoal action,[14, 15] antibacterial,[16] and antiameobic agent.[17,18] It is used in the treatment of *trichomoniasis* of the genitourinary tract in male and female. In amoebiasis, it is effective at all sites of infections and also used in the treatment of giardiaasis and of Vincent's infection. It constitutes a family of antibiotics have clinically effective in a variety of infections caused by obligate anaerobic bacteria and microaerophilic bacteria.

The usual dose of MTZ for adult and children over 10 years is three times daily after food for 7 to 10 days. In elderly women it used to clear vaginitis, in such case is given as vaginal pessaries in a dosage of daily for 10 to 20 days and it also used for eradication of cysts in symptom less carries, treatment with MTZ three times daily for 5 to 10 days,**[19]** due to this reason its needs as an extended formulation of this drug.

MATERIALS AND METHODS

Materials

Metronidazole, PF-68 and Montmorillonite KSF was obtained from Sigma Aldrich USA. Analytical grade, Ortho-phosphoric acid, HCl, KCl, NaOH, KH_2PO_4 was procured from MERCK (Germany). HPLC grade methanol, acetonitrile and water were used for drug

estimation by HPLC technique. Water used in the experiments was deionized and filtered by Millipore (HV 0.45µm and GV 0.22 µm from Millipore (India) Pvt Ltd Bangalore).

Instruments

Absorbance of the supernatant solutions was measured by spectrophotometer (UV– Visible spectrophotometer Analytic Jena) equipped with a quartz cell having a path length of 1 cm. 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). TEM images were recorded using TECNAI G2T30 FEI Instrument operated with an accelerating voltage of 300 KV.

EXPERIMENTAL

Synthesis of Organo Montmorillonite

A non-ionic triblock copolymer PF68 was selected as surfactant for the synthesis of OMt. OMt was synthesised by the modification of the reported procedure.[01] In brief an aqueous Mt dispersion was allowed to swell up to 24 hours at constant magnetic stirring of 100 rpm to form a stable Mt dispersion. PF68 solution (>CMC of PF68 surfactant) was prepared separately and added gradually drop wise to the Mt dispersion and stirring up to 8 hours at normal room temperature. The resulted hydrophobic reaction media was centrifuged at 24,500 rpm followed by several washings to remove the excess or unreacted PF68. The obtained residue was lyophilized thus obtained organo Mt was further used as delivery vehicle for the MTZ.

Quantitative estimation of Metronidazole

MTZ in aqueous solution shows pH dependent absorption behaviour in the range of 200 nm to 400 nm. At pH 1, λ_{max} corresponds to 278 nm from pH 4.0 to pH 10; λ_{max} is unchanged and corresponds to 320 nm.[20] The drug is in its stable form at pH 1.0 and pH 4.0 to pH 10 therefore, calibration plot was prepared at pH 1.0. The Beer – Lambert's law was valid in the range of 1 ppm to 18 ppm with slope 0.0436 and correlation coefficient 0.9981, Fig 1A. Hence for quantitative estimation of drug from samples containing unknown amount of drug, absorbance of each solution was measured at 278 nm, after maintaining the solutions at the same pH value of 1.0.

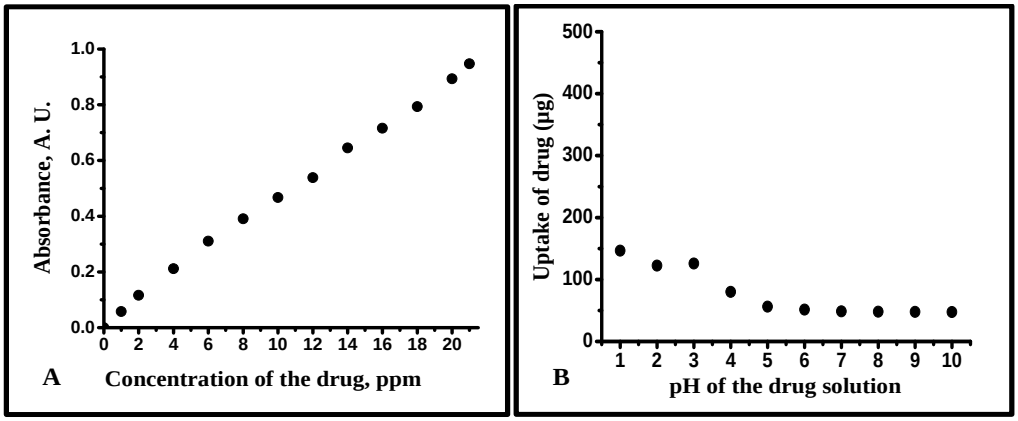


Fig. 1: (A) Absorbance as a function of concentration of the MTZ
(B) Uptake of MTZ as function of pH of aqueous drug solution

Batch extraction studies of Metronidazole

The effect of initial pH of drug solution, contact time period during batch extraction and initial concentration of drug in the solution on the efficiency of drug uptake by OMt has been evaluated. Adsorption isotherms and kinetic studies were also used to ascertain the nature of interaction between the drug and the OMt. OMt-complex thus obtained under optimized synthetic conditions was further used for the evaluation of release behavior (*in-vitro*) in the simulated gastric and intestinal fluids. The free MTZ concentration in the supernatant was determined using UV–visible spectrometry and HPLC.

RESULTS AND DISCUSSION

Extraction of drug as a function of the pH, contact time and initial concentration

In the pH study, the uptake efficiency of OMt for each drug was performed using 50 mg of OMt and 25 mL of double distilled water containing 1000µg of the drug for 15 minutes duration at a temperature of 25 ± 1°C using orbital shaker. The maximum and minimum uptake efficiency of OMt for MTZ was observed to be 147µg at pH 1.0 and 48 µg at pH 10.0 respectively, Fig 1B. Therefore, the time and the concentration dependent studies were carried out at pH1.0. The quantitative estimation of MTZ was also performed at pH 1.0. In the contact time, during the initial 10 minutes the retention of MTZ on OMt was very slow and about 12% (120µg) was retained. After 120 minutes 32.8% (328µg) was retained and saturation was reached, Fig 2A. Therefore, for further experiments, contact time period was set to 120 minutes. In the initial concentration experiment loading of MTZ on OMt increased with increasing initial drug amount up to 10000µg of MTZ. Beyond this concentration, loading capacity remained constant, which suggests saturation of all the active sites. The highest uptake was found to be 1146 µg at 10000µg of MTZ, Fig 2B. This synthesised OMt-MTZ

complex is further used for characterisation with appropriate analytical technique and also used for *in-vitro* drug release studies.

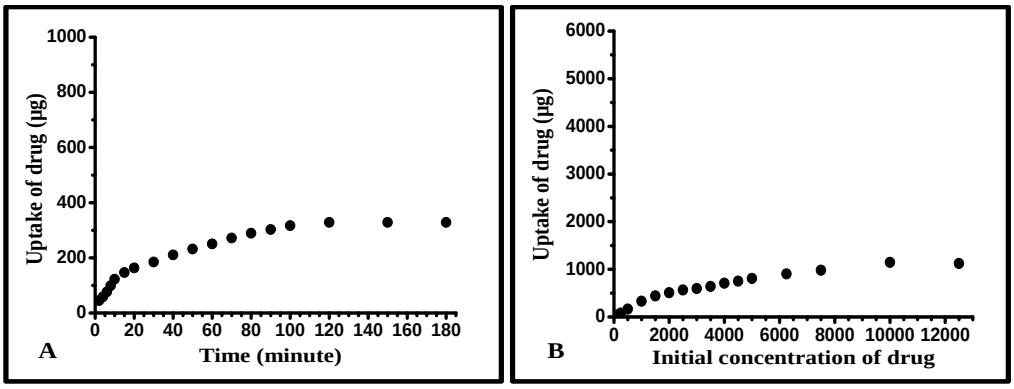


Fig. 2: (A) Uptake of MTZ as function of contact time
(B) Uptake of MTZ as function of initial concentration

Isotherms model

Adsorption isotherms are mathematical models that describe the distribution of adsorbate species between liquid and solid phase based on a set of assumptions that are mainly related to the heterogeneity/homogeneity of adsorbents. Adsorption data are usually described by adsorption isotherms, such as Langmuir and Freundlich isotherms. The Freundlich and Langmuir isotherms shows the correlation coefficient (R^2) values 0·9531 and 0.9843 respectively, suggested that the linear form of Langmuir isotherms produces a better fit in comparison with linear form of Freundlich isotherm, Fig. 3. Parameter of Langmuir and Freundlich isotherm are listed in Table-1.

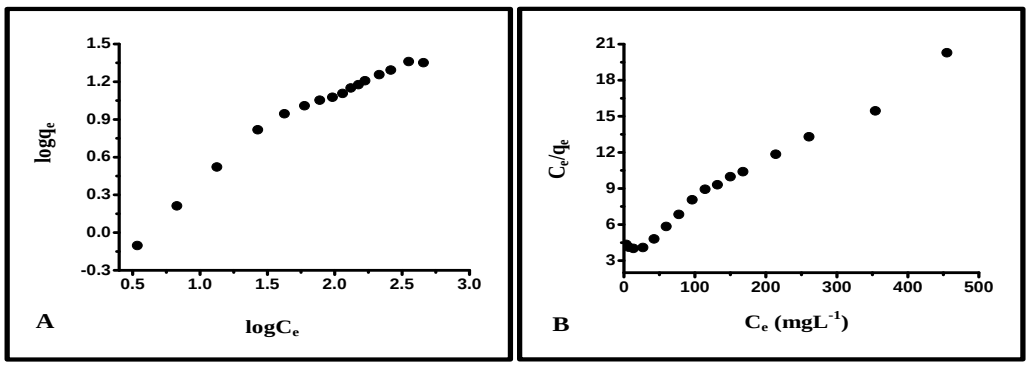


Fig. 3: Adsorption behavior of MTZ on OMt: (A) linear form of Freundlich
(B) linear form of Langmuir

Table: 1 Parameter of Freundlich and Langmuir isotherm model

Freundlich isotherm constants			Langmuir isotherm constants		
1/n	K_f	R^2	q_{max}	K_L	R^2
0.6654	0.5353	0.9531	28.169	0.00883	0.9843

Kinetics studies

The kinetic studies were carried out to measure the rates of reaction and to determine the influence of time and concentration on the rates of reaction. The pseudo-first order model was presented by Lagergren[21] which is the earliest known equation describing the adsorption rate based on the adsorption capacity. The adsorption data was also analysed in terms of pseudo second order model, described by Ho and McKay.[22]

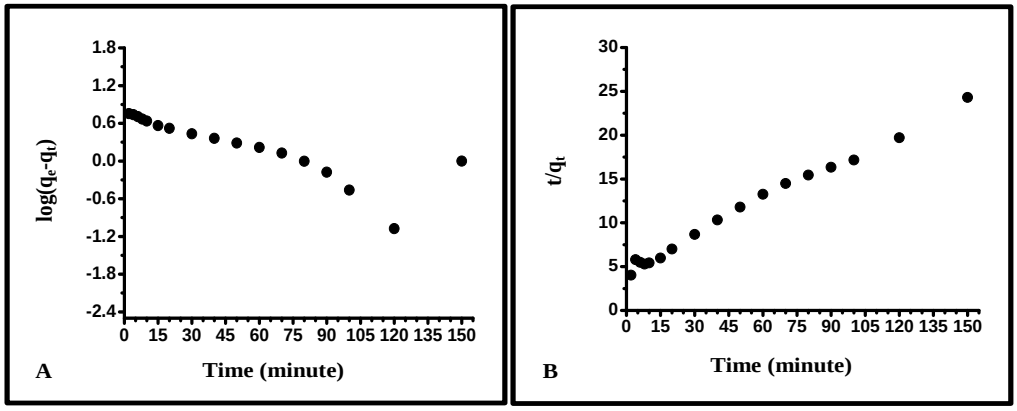


Fig. 4: Kinetic model for the adsorption of MTZ on OMt: (A) Pseudo-first order (B) Pseudo-second order

In case of pseudo–first order reaction model the correlation coefficient was found to be 0.5755 suggesting that the adsorption process is not favourable for pseudo first order kinetic model,. Pseudo-second order plot shows a linear behaviour with correlation coefficient, R^2 (0.9881), therefore, the kinetic parameters suggest that adsorption process follows pseudo-second order kinetic model. Parameter of pseudo-first and pseudo-second order was listed in Table-2, Fig 4.

Table 2: Kinetic parameters of pseudo first-order and pseudo second-order kinetic model

Pseudo-first order model			Pseudo-second order model		
K_1	q_e	R^2	K_2	q_e	R^2
0.0283	5.9375	0.5755	0.00527	7.6161	0.9881

XRD studies

The XRD pattern of pristine Mt shows characteristic diffraction peak of 001 plane at 2θ value of 6.04° corresponding to basal spacing (d) of 14.62 Å. In case of OMt, this peak is shifted to 2θ value of 4.76° and corresponding basal spacing (d) of 18.69 Å. The increase in basal spacing case of OMt is due to the intercalation of surfactant within the Mt layers. In case of

OMt-MTZ complex the characteristic peak of Mt seems to be disappearing suggesting exfoliation of Mt layers, Fig 5.

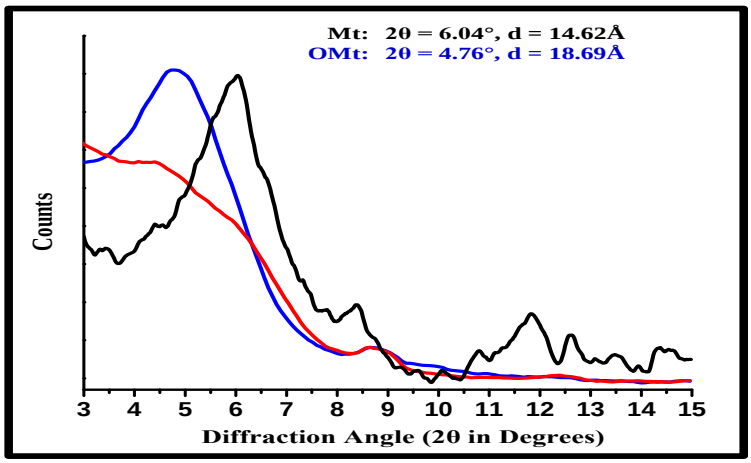
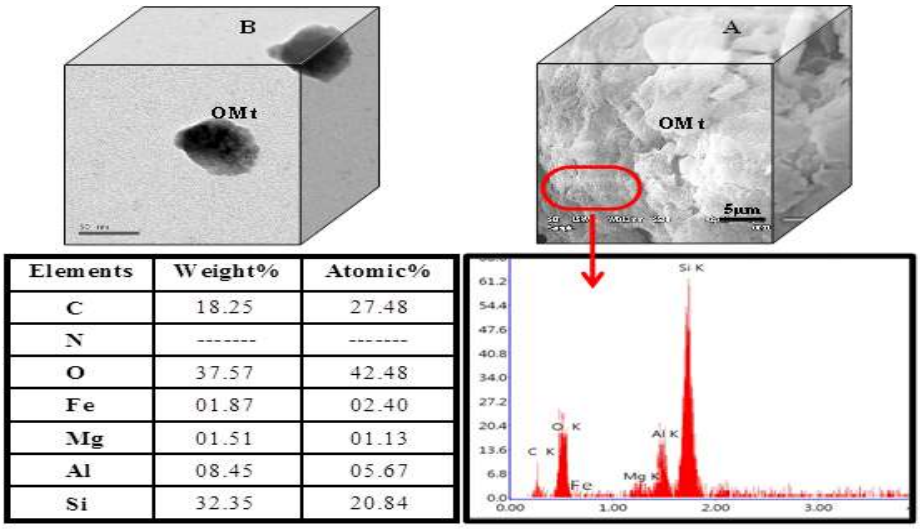
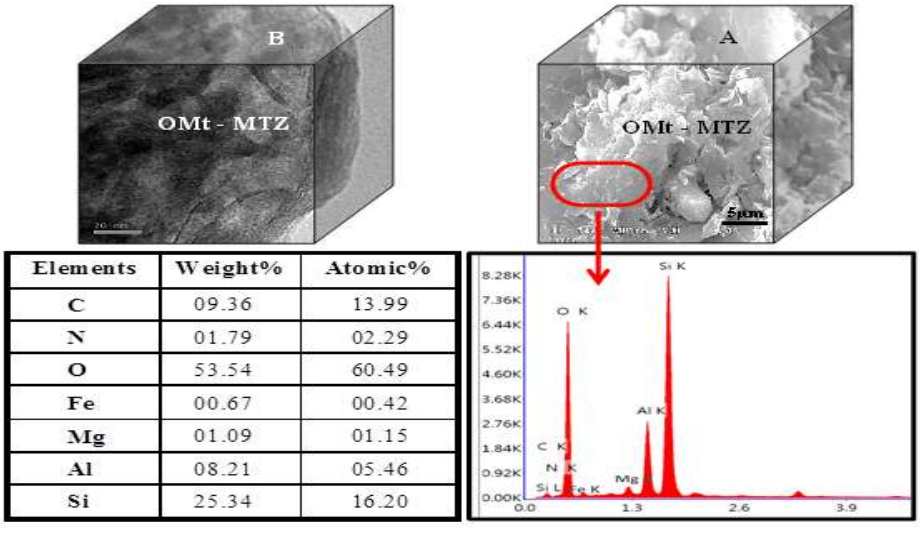


Fig. 5: XRD patterns of Mt, OMt and OMt-MTZ complex



Elements	Weight%	Atomic%
C	18.25	27.48
N	-------	-------
O	37.57	42.48
Fe	01.87	02.40
Mg	01.51	01.13
Al	08.45	05.67
Si	32.35	20.84

Fig. 6: SEM (A) and TEM (B) image of OMt



Elements	Weight%	Atomic%
C	09.36	13.99
N	01.79	02.29
O	53.54	60.49
Fe	00.67	00.42
Mg	01.09	01.15
Al	08.21	05.46
Si	25.34	16.20

Fig. 7: SEM (A) and TEM (B) image of OMt-MTZ complex with EDX data

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) with energy dispersive X-ray spectrometric (EDX) analysis

SEM and TEM provides detailed high resolution images of the sample by rastering a focussed electron beam across the surface and detecting/counting the backscattered electron and transmitted electron respectively. An energy dispersive X-ray analyser (EDX or EDA) is also used to provide elemental identification and quantitative compositional information.

SEM image of OMt indicates layered structure, Fig. 6A. The presence of carbon peak and absence of sodium peak (observed in case of pristine Mt) in the EDX data can be taken as an evidence for the presence of surfactant on the surface of Mt and/or in the interlayer region of Mt. The nearly spherical particle of about 70nm size was observed in the corresponding image of TEM. SEM image of the OMt-MTZ complex indicates destruction of layered structure, Fig. 7A. The TEM image more clearly indicates exfoliation of Mt layers, Fig. 7B. As indicate by EDX analysis, the presence of additional peaks of nitrogen in OMt-MTZ complex along with oxygen, silicon, aluminium, iron and magnesium can be taken as an evidence for the presence of drug on the OMt-MTZ complex.

***In-vitro* drug release behaviour**

In-vitro release behaviour of pure MTZ and OMt-MTZ complex was carried out in the simulated gastric and intestinal fluid using dialysis bag method.[23,24]

A known amount of the pure drug and the synthesized OMt- MTZ complex was kept in the dialysis bag along with 5.0 mL of appropriate buffer solution. The dialysis bag was then dipped into 500mL of simulated gastric/intestinal fluids contained in 6 bowls of the dissolution apparatus (LABINDIA, DISSO 8000, equipped with USP paddle) maintained at 37±0.5ºC.

In the simulated gastric fluid (pH 1.2) minimum cumulative release of 21% of the drug was observed in the initial 2 hours period in case of OMt-MTZ complex. In the simulated intestinal fluid (pH 7.4) maximum cumulative release of 73.45% of the drug was observed over a period of 12 hours in case of OMt-MTZ complex, Fig 8A and B, Table 3. The commercially available tablets and the pure MTZ show higher release in the simulated gastric fluid, which is not desirable. Since the gastric emptying time of drug is highly variable; the normal gastric residence times usually range between 5 minutes to 2 hours.[25] Hence, for a particular drug if stomach is not the absorption

site, one has to look for the amount of drug available in the intestinal fluid, after passing through stomach, for absorption in the systemic circulation. Therefore, in the present case possible bioavailability for pure MTZ, the commercially available tablets and the OMt-MTZ complex has been calculated from the *in- vitro* drug release data (Fig. 9).

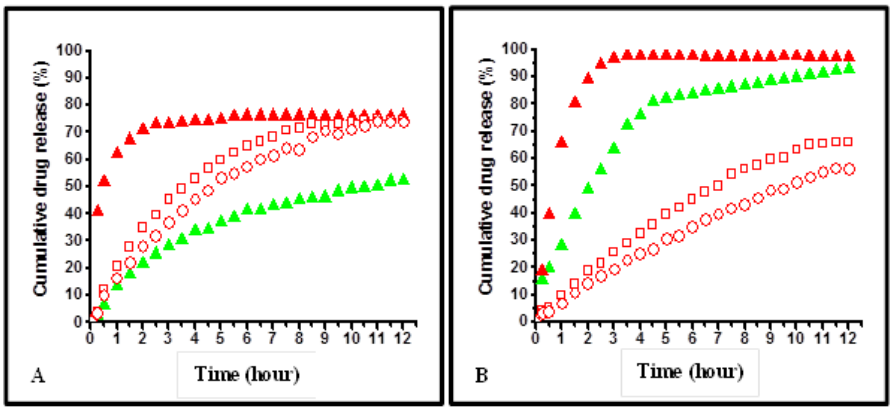


Fig. 8: Drug release behavior
(A) in simulated gastric
(B) in simulated intestinal fluid
MTZ (▲), Metrogyl tablet (□), Flagyl tablet (○) and OMt-MTZ complex (▲)

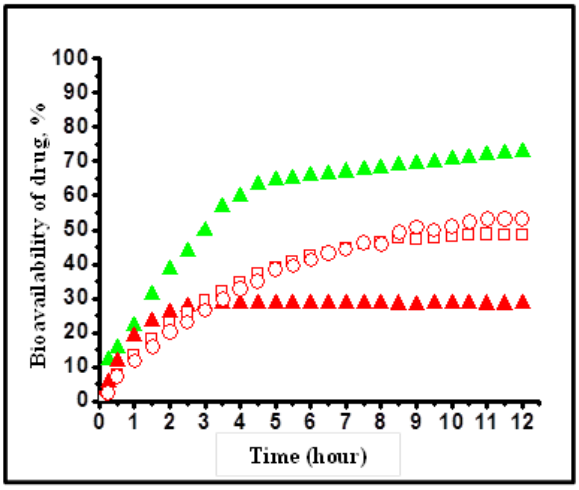


Fig. 9: Bioavailability of MTZ in various compositions
Pure MTZ (▲), Metrogyl tablet (□), Flagyl tablet (○) and OMt-MTZ complex (▲)

Table 3: Cumulative drug release as a function of time

SAMPLE TYPE	TOTAL CUMULATIVE RELEASE OF MTZ IN THE SIMULATED GASTRO INTESTINAL FLUID, %		BIOAVAILABILITY IN THE INTESTINAL FLUID, %
	GASTRIC FLUID (In 2 hrs.)	INTESTINAL FLUID (In 12 hrs.)	
PURE MTZ	71	28.40	28.4
METROGYL	35	42.90	42.9
FLAGYL	28	40.30	40.3
OMt – MTZ COMPLEX	21	73.45	73.5

It has been observed that , compared to the pure MTZ and the commercially available tablets (Flagyl and Metrogyl) performance of the synthesised OMt-MTZ complex has not only been found superior in terms of probable % bioavailability but also in terms of availability of the drug in the system for a longer period of time.

Kinetic models of *in-vitro* drug release

Two kinetic models (first order model and Korsmeyer-Peppas model) were applied to show kinetics of drug release behaviour[26] given in, Fig 10. The most fitted parameter are listed in table 4.

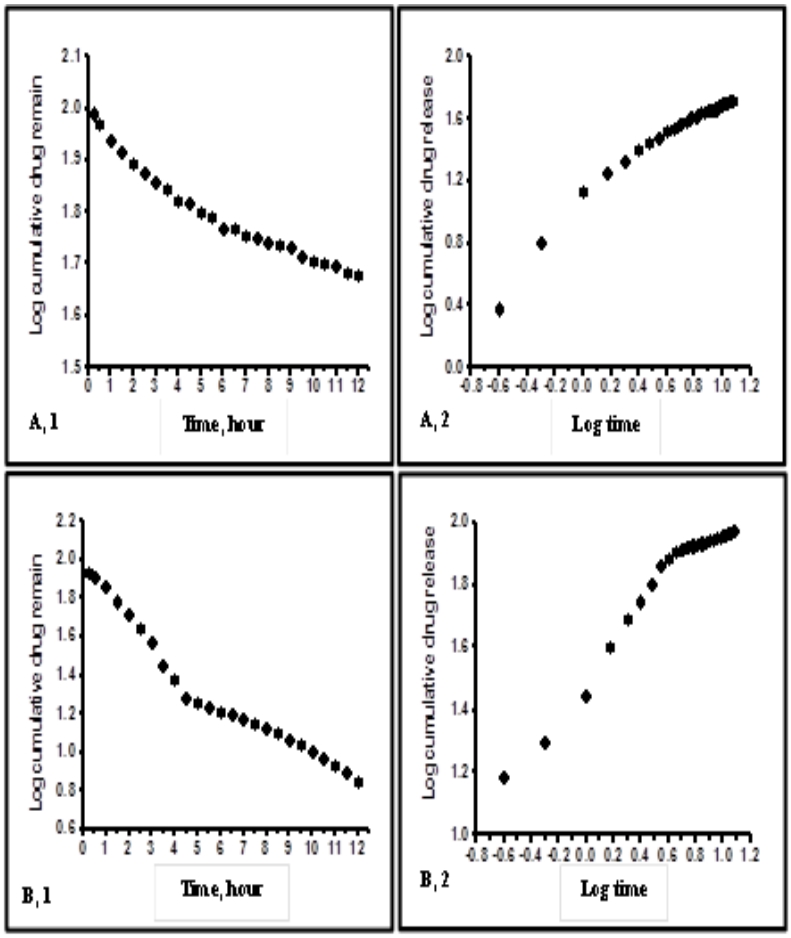


Fig 10: Kinetic models for explaining *in-vitro* drug release from OMt-MTZ complex:
(A) In simulated gastric fluid, 1-first order, 2- Korsmeyer - Peppas
(B) In simulated intestinal fluid, 1- first order, 2- Korsmeyer – Peppas

Table 4: Release kinetics constants of OMt-MTZ complex

Buffer media	First order		Korsmeyer-Peppas	
	R^2	k_1, h^{-1}	R^2	k_{kp}, h^{-n}
Simulated gastric fluid	0.9481	0.0560	0.9437	0.6983
Simulated intestinal fluid	0.9385	0.2027	0.9455	0.4827

CONCLUSION

The OMt-MTZ complex was successfully synthesised and *in-vitro* drug release behaviour and kinetics of the process in the simulated gastric and intestinal fluid was studied. Release profile of the drug from pure MTZ, the commercially available tablets (Metrogyl and Flagyl) and OMt-MTZ complex were compared. It was observed that the OMt-MTZ complex can provide 73.5% bioavailability of the drug which is 2.6, 1.7 and 1.8 fold increase as compared to the pure MTZ, Metrogyl and Flagyl respectively. The OMt-MTZ complex also has the potential to extend the release of drug up to 12 hours (~3.5 times more extended as compared to the pure MTZ). Although, the commercially available tablets (Metrogyl and Flagyl) also show extended release behaviour up to 12 hours but at much lower bioavailability (~ 40%), pure MTZ not only has the poor bioavailability (28.4%) but also show fast release, 95% release in 2.5hours. Because of higher bioavailability of drug in OMt-MTZ complex lesser amount of drug intake would be required. Thus, OMt-MTZ complex has the potential to minimize the dosing frequency and may result in better patient compliance.

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