Journal of Applied Pharmaceutical Research
Volume 12 Issue 1, Year of Publication 2024, Page 93 – 99
DOI: 10.18231/j.joapr.2024.12.1.93.99

Check for updates

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | *JOAPR*
www.japtronline.com *ISSN: 2348 – 0335*

FORMULATION AND EVALUATION OF MUCOADHESIVE MICROSPHERES OF METRONIDAZOLE

Dheeraj D. Chechare[1]*, M. Siddaiah[2]

Article Information
Received: 2nd November 2023
Revised: 28th January 2024
Accepted: 15th February 2024
Published: 29th February 2024

Keywords
Metronidazole, mucoadhesive microspheres, Chitosan, Sodium alginate, Sodium carboxymethyl cellulose, drug delivery

ABSTRACT
Objective: Develop and evaluate mucoadhesive microspheres using Chitosan, Sodium alginate, and Sodium carboxymethyl cellulose (NaCMC) for sustained oral delivery of Metronidazole, aiming to improve bioavailability. **Methods:** Metronidazole-loaded microspheres were prepared via ionotropic gelation method with varying polymer ratios. Particle size, entrapment efficiency, swelling index, mucoadhesion (sheep mucosae), morphology (SEM), in-vitro wash-off test, drug release profile, and stability (6 months) were evaluated. **Results:** Chitosan content positively correlated with microsphere size. Entrapment efficiency ranged from 51.43% to 94.15%. Chitosan-based formulations, especially MTZ-7 (Chitosan:NaCMC, 3:1), displayed the highest mucoadhesion. SEM analysis revealed rough, spherical microspheres with a continuous polymeric coat. In-vitro wash-off test demonstrated prolonged residence time for Chitosan formulations. Sustained drug release was observed throughout the study, with MTZ-7 exhibiting the most desirable release profile. Stability studies showed no significant changes in drug release for selected formulations after 6 months. **Conclusions:** Chitosan-based microspheres, particularly MTZ-7, demonstrated superior mucoadhesive properties, sustained and controlled drug release, and desirable stability. These findings suggest the potential of Chitosan-based microspheres as a promising oral drug delivery system for Metronidazole, potentially addressing bioavailability concerns and improving therapeutic efficacy.

INTRODUCTION

Oral drug delivery remains the most preferred route due to its convenience and patient compliance. However, a significant challenge is overcoming poor bioavailability, especially for drugs absorbed in the upper gastrointestinal tract. This limitation hinders the development of efficient controlled-release formulations. Researchers have explored various strategies to extend drug residence time within the gastrointestinal tract. One promising approach involves microspheres made from natural, biodegradable polymers. These systems offer sustained drug release and have garnered significant attention in recent years. Additionally, advancements in dosage forms capable of

[1]*Department of Pharmaceutical Sciences, Bhagwant University, Rajasthan, India*
[2]*Jawaharlal Nehru Technological University Anantapur, Anantapuram, Andhra Pradesh, India*

For Correspondence:** **dheerajchechare7@gmail.com

precisely controlling release rates and targeting specific body sites have revolutionized the development of novel drug delivery systems [1]. One critical issue with controlled-release formulations is their limited residence time in the stomach and proximal small intestine, the primary absorption sites. This rapid transit can significantly reduce drug absorption efficiency. Therefore, developing sustained-release formulations that prolong retention at the optimal absorption site holds the potential for substantial therapeutic improvements [2]. Chitosan, Sodium alginate, and Sodium carboxymethyl cellulose (NaCMC) are attractive options for microsphere preparation due to their excellent mucoadhesive properties. These readily available and non-toxic polymers offer several advantages, including Superior mucoadhesive properties, Biocompatibility and safety, and High viscosity at low concentrations [3]. This study focuses on developing and evaluating mucoadhesive microspheres containing Metronidazole, an antiprotozoal drug. Metronidazole exhibits fluctuating plasma levels with conventional dosage forms despite its good oral bioavailability (around 80%). By employing various concentrations of Chitosan, Sodium alginate, and NaCMC as mucoadhesive polymers, this research aims to investigate their effects on Metronidazole's drug delivery and absorption characteristics.

MATERIALS AND METHODS

Materials

Metronidazole was purchased from Orbiton Pharma (Gujarat, India). Chitosan was obtained from the Central Institute of Fisheries Technology, Cochin, India. Sodium alginate, sodium carboxymethyl cellulose, calcium carbonate,and other chemicals were obtained from S.D. Fine Chem Ltd, Mumbai, India.

Methods

An accurately weighed quantity of Metronidazole was gradually added to a 100 ml alginate solution, creating a uniform dispersion. To formulate modified alginate microspheres, varying quantities of chitosan were introduced to the homogeneous dispersion and thoroughly mixed. Formulations containing sodium carboxymethyl cellulose were uniformly blended with the alginate solution before the drug was added. Each 20 ml dispersion was carefully dropped through a 16G syringe needle into a gently agitated 100 ml solution of 1.5% (w/v) calcium chloride. The microspheres were produced under the same processing conditions as the initial trials. After a single wash with distilled water, the resulting microspheres were transferred to a 100 ml solution of 1% w/v chitosan in 1% w/v acetic acid for 10 minutes and subsequently dried at room temperature for 24 hours [3,4]. Table 1 shows various ingredients and their proportions used in preparing the formulations.

Evaluation of metronidazole microspheres:

Particle size analysis and swelling studies: Particle size analysis and swelling studies were conducted to characterize the developed metronidazole-loaded microspheres. These evaluations provided crucial information regarding the size distribution and water uptake capacity, significantly impacting drug release and in-vivo behavior.

Particle size analysis: The size distribution of the microspheres was determined using a microscopic method. The obtained data were used to calculate the average particle sizes, providing insights into the uniformity of the microsphere population [4].

Swelling studies: To assess the water uptake capacity, a weighed amount of microspheres was immersed in a specific volume of simulated physiological fluid at 37±2℃ temperature. The microspheres were carefully retrieved at predetermined intervals, gently blotted to remove excess surface liquid, and reweighed. The swelling ratio was then calculated using a formula considering the initial dry weight and the weight after immersion [5, 6].

Entrapment efficiency (EE): To assess the proportion of Metronidazole effectively encapsulated within the microspheres, the entrapment efficiency was determined. This involved incubating accurately weighed microspheres in a suitable buffer solution for a specific time, allowing for the dissolution of the cross-linked structure and the release of the entrapped drug. The theoretical amount of drug present was calculated based on the initial quantity added relative to the total weight of all formulation ingredients. The entrapment efficiency was then calculated using the formula [7]:

$$\text{EE}\ (\%) = \frac{Practical\ drug\ \ in\ microspheres}{Theoretical\ drug\ in\ microspheres} \times 100$$

Determination of Morphology by Scanning Electron Microscopy:

SEM creates a magnified image using electrons instead of light, yielding topographic images and elemental information when

used with energy-dispersive X-ray analysis or wavelength-dispersive X-ray spectrometry. SEM produces a higher resolution than possible using a light microscope, and the images obtained are three-dimensional. The maximum resolution for SEM (the minimum distance by which the two objects can be separated and observed as distinct objects) is 10–20 nanometers compared to 200– 300 nanometers for light microscopy [8].

***In-Vitro* wash-off test:** The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test, which is a simple and quick method reported by Lehr et al. as follows: pieces of tissue (pig stomach, about 2 x 5 cm, and small intestine, about 2x15 cm, obtained from a slaughterhouse and stored in Tyrodes solution) were tied onto a plastic slide (about 2 x 15 cm) using rubber bands. Microspheres were spread (~25) onto each wet, rinsed tissue specimen and counted. Immediately after that, the prepared two slides were connected with suitable support onto one of the groves of a USP tablet disintegrating test apparatus, permitting a slow, regular up and down movement (~30min-1) in a test fluid (0.1N HCl, pH 1.2 and phosphate buffer pH 6.8) kept at 37℃. The motor was stopped at given intervals, and the number of microspheres still adhering to the tissue was counted. The results obtained can be used to measure bioadhesion [8].

Table No. 1 Composition of formulations used for preparation of Metronidazole microspheres

Formulation	Amount of Na-alginate (gm)	Amount of drug (gm)	Polymers	Amount of additive Polymer (gm)	Drug/Na-Alginate ratio	Coagulation Medium $CaCl_2$ solution
MZ-1	2	5	-	0	2.5:1	1.5%
MZ-2	2	5	Sodium CMC	1	2.5:1	1.5%
MZ-3	2	5	Sodium CMC	2	2.5:1	1.5%
MZ-4	2	5	Sodium CMC	3	2.5:1	1.5%
MZ-5	2	5	Chitosan	1	2.5:1	1.5%
MZ-6	2	5	Chitosan	2	2.5:1	1.5%
MZ-7	2	5	Chitosan	3	2.5:1	1.5%
MZ-8	2	5	HPMC K4M	1	2.5:1	1.5%
MZ-9	2	5	HPMC K4M	2	2.5:1	1.5%

In-vitro drug release studies: In-vitro dissolution studies were conducted to evaluate the drug release behavior of the Metronidazole-loaded microspheres. Simulated gastric and intestinal fluids were used in a USP dissolution apparatus II at 50 rpm, mimicking the gastrointestinal environment. Accurately weighed microspheres, equivalent to a specific dose, were placed in the dissolution vessel. Samples were withdrawn at predetermined intervals, filtered, and analyzed using UV-visible spectrophotometry to quantify drug release. Cumulative release percentage was plotted to generate dissolution profiles and compared to a reference standard. This approach aimed to assess the sustained release potential of the microspheres [10,11]

Stability studies: To assess the long-term stability of the developed microspheres, accelerated stability testing was conducted following the ICH Q1A guidelines [8]. The selected formulations (MTZ-3 and MTZ-6) were stored in a humidity chamber for 3 months under controlled conditions (40°C ± 2°C, 75% ± 5% RH) that mimic exaggerated storage environments. The formulations were visually inspected for any physical changes throughout the storage period. Additionally, the stored formulations' remaining drug content and in vitro drug release profiles were compared to freshly prepared ones after storage. These evaluations aimed to predict the shelf life and identify potential stability concerns under stressful conditions, informing their long-term storage and performance [12,13].

RESULTS

The study demonstrates a direct correlation between chitosan content and particle size, signifying the ability of chitosan to influence microsphere formation. Further, the observed swelling behavior in different media suggests the microspheres' potential to adapt and respond to the varying physiological conditions of the gastrointestinal tract, data presented in Table 2. This characteristic could be crucial for controlled drug release throughout the digestive system. The variations in entrapment efficiency across formulations suggest further optimization of the formulation process to achieve a consistent and high drug loading. The superior bioadhesion observed in chitosan-containing formulations (MTZ-7 and MTZ-9) can be attributed to the inherent adhesive properties of chitosan. The rough

surface topography depicted in the SEM images for MTZ-3 and MTZ-6 confirms the complete coverage of the microspheres with the polymeric matrix. This observation aligns with the microspheres' desired functionality and supports the drug's encapsulation within the polymeric shell shown in Figure 2.

Table 2: Determination of Particle Size and Swelling Behaviour Analysis

Formulations	Particle size (mm)		Relative Swelling (at 3 h)		% EE
	Length	Breadth	pH1.2	pH6.8	
	Mean (±) SD	Mean (±) SD	Mean (±) SD	Mean (±) SD	
MTZ- 1	0.76±0.38	0.75±0.27	17.60±0.25	22.10±0.24	94.15±0.36
MTZ- 2	0.73±0.83	0.73±0.03	16.20±0.30	21.84±0.10	74.60±0.13
MTZ- 3	0.73±0.93	0.74±0.34	15.52±0.29	21.45±0.18	62.30±0.41
MTZ- 4	0.75±0.29	0.74±0.74	23.10±0.20	22.10±0.72	51.43±0.50
MTZ- 5	0.75±0.39	0.73±0.33	16.10±0.15	27.77±0.13	81.78±0.27
MTZ- 6	0.74±0.43	0.74±0.05	21.5±0.10	19.04±0.26	73.20±0.06
MTZ- 7	0.74±0.29	0.74±0.38	25.18±0.28	26.06±0.10	75.10±0.55
MTZ- 8	0.76±0.53	0.73±0.37	22.10±1.65	21.42±0.07	87.20±1.06
MTZ- 9	0.73±0.30	0.74±0.29	23.10±0.22	24.80±0.30	78.54±0.67

RESULTS

The study demonstrates a direct correlation between chitosan content and particle size, signifying the ability of chitosan to influence microsphere formation. Further, the observed swelling behavior in different media suggests the microspheres' potential to adapt and respond to the varying physiological conditions of the gastrointestinal tract, data presented in Table 2. This characteristic could be crucial for controlled drug release throughout the digestive system. The variations in entrapment efficiency across formulations suggest further optimization of the formulation process to achieve a consistent and high drug loading. The superior bioadhesion observed in chitosan-containing formulations (MTZ-7 and MTZ-9) can be attributed to the inherent adhesive properties of chitosan. The rough surface topography depicted in the SEM images for MTZ-3 and MTZ-6 confirms the complete coverage of the microspheres with the polymeric matrix. This observation aligns with the microspheres' desired functionality and supports the drug's encapsulation within the polymeric shell shown in Figure 2.

The sustained release profiles exhibited by all formulations compared to the Flagyl® tablet highlight the potential of the developed microspheres for improved drug delivery. This characteristic could lead to reduced dosing frequency and potentially better patient compliance. Selecting the most suitable kinetic model (Zero Order, First Order, Korsmeyer-Peppas, Hixson-Crowell, and Matrix) for each formulation provides valuable insights into the underlying drug release mechanism. This information is crucial for optimizing the formulation and achieving the desired release profile shown in Figures 3 to 11.

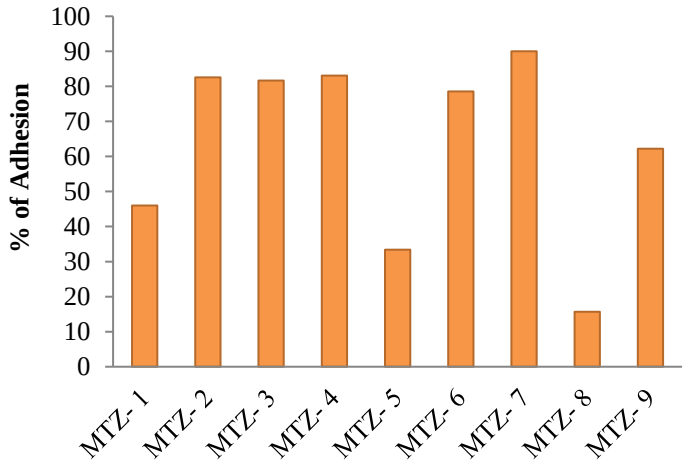

Figure 1: Mucoadhesion of formulating Microspheres

Batch code	% Mucoadhesion of Microspheres	
	Stomach (Mean±SD)	Intestine (Mean±SD)
MTZ- 1	46.00±81	62.33±14.43
MTZ- 2	82.57±0.67	82.05±0.57
MTZ- 3	81.67±5.11	88.23±7.22
MTZ- 4	83.10±4.64	92.00±4.76
MTZ- 5	33.40±4.83	63.00±4.4
MTZ- 6	78.57±4.30	92.67±6.11
MTZ- 7	90.00±4.36	83.57±6.15
MTZ- 8	15.67±4.19	24.47±9.25
MTZ- 9	62.23±16.07	39.67±9.10

The stability of MTZ-3 and MTZ-6 over 3 months demonstrates their suitability for long-term storage without compromising their drug release characteristics. This finding is essential for ensuring consistent product quality and therapeutic efficacy data presented in Figure 12.

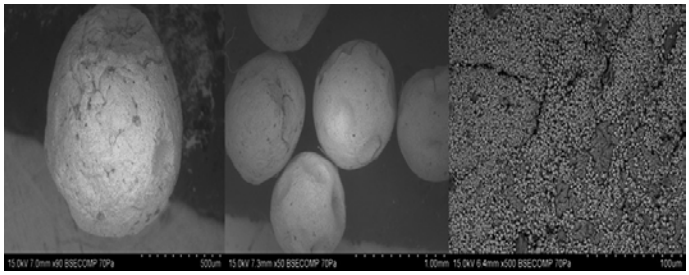

Figure 2: SEM of prepared microspheres

The observed direct correlation between chitosan content and particle size can be attributed to the polycationic nature of chitosan, which promotes inter-particle aggregation as its concentration increases. The variations in entrapment efficiency might be due to factors like the drug-to-polymer ratio, preparation method, and processing parameters. Further optimization could involve exploring different types of chitosan or modifying the preparation techniques. The superior bioadhesion of chitosan-containing formulations is likely due to the formation of hydrogen bonds between the positively charged amino groups of chitosan and the mucus layer in the gastrointestinal tract. The sustained drug release profiles can be explained by the diffusion-controlled release of metronidazole from the hydrolytically-degradable polymeric matrix. The chitosan content and other formulation components might also influence the polymer degradation rate, impacting the drug release kinetics.

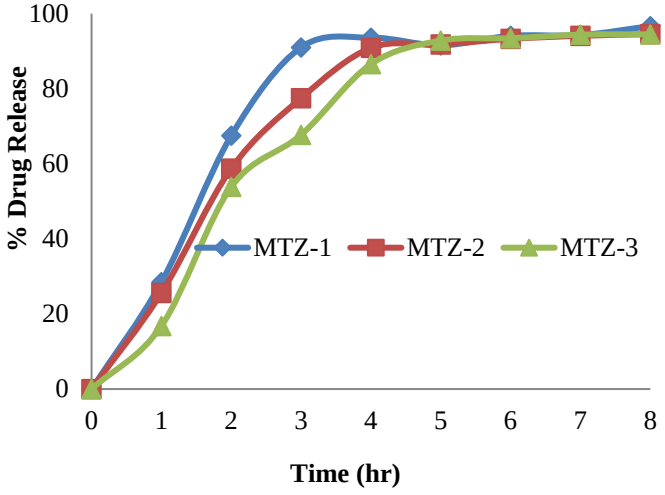

Figure 3: Drug Release of Formulation MTZ-1 to MTZ-3

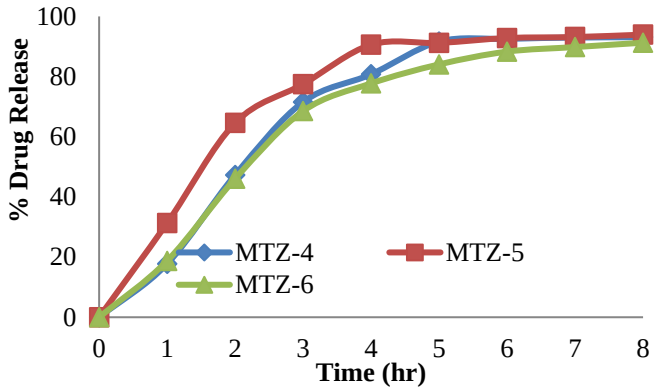

Figure 4: Drug Release of Formulation MTZ-4 to MTZ-6

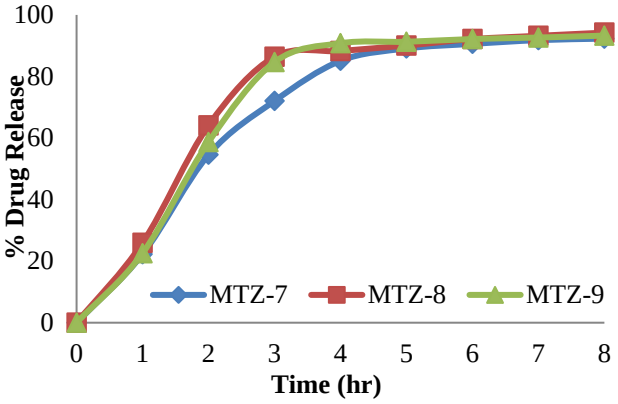

Figure 5: Drug Release of Formulation MTZ-7 to MTZ-9

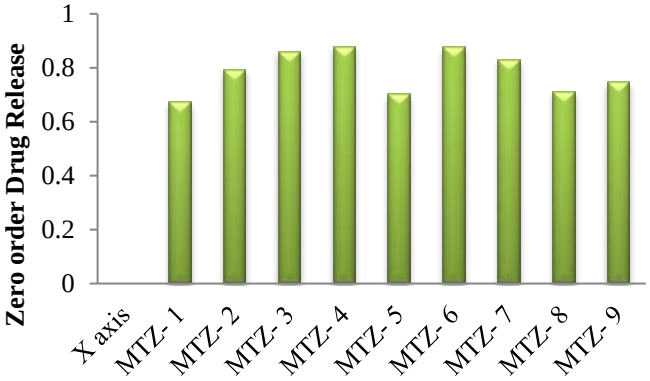

Figure 6: Zero-order Kinetics Drug Release

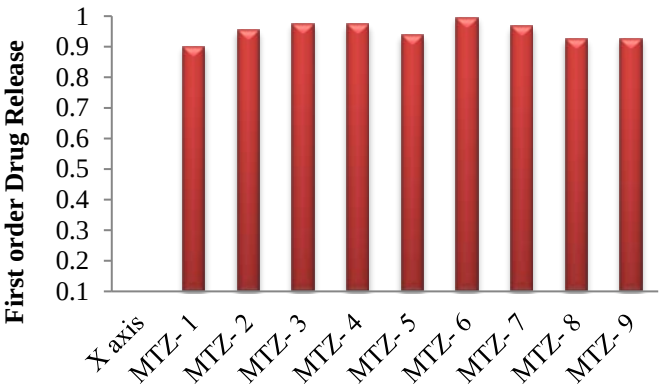

Figure 7: First order kinetics drug release

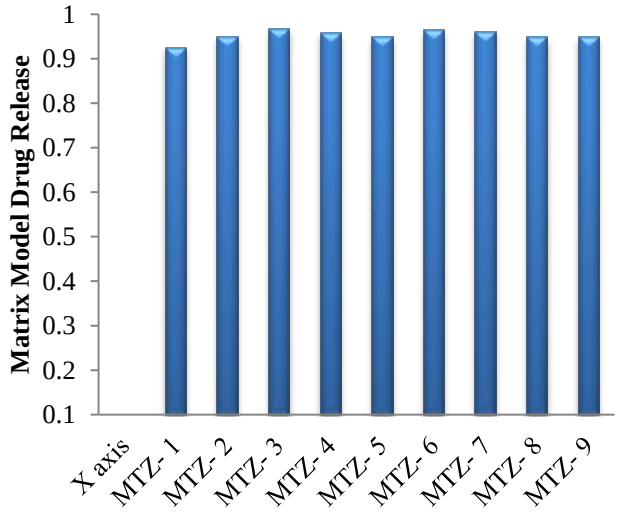

Figure 8: Matrix Model Drug Release

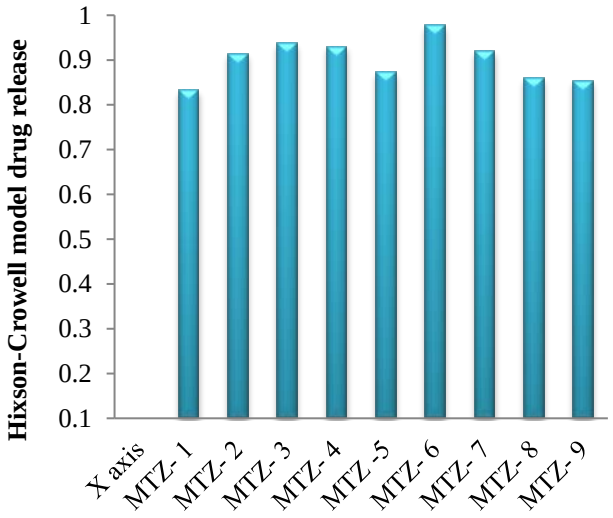

Figure 9: Hixson-Crowell Model Drug Release

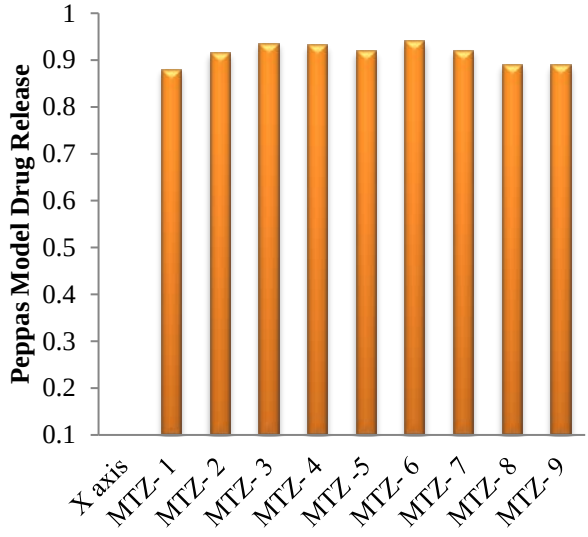

Figure 10: Peppas Model Drug Release

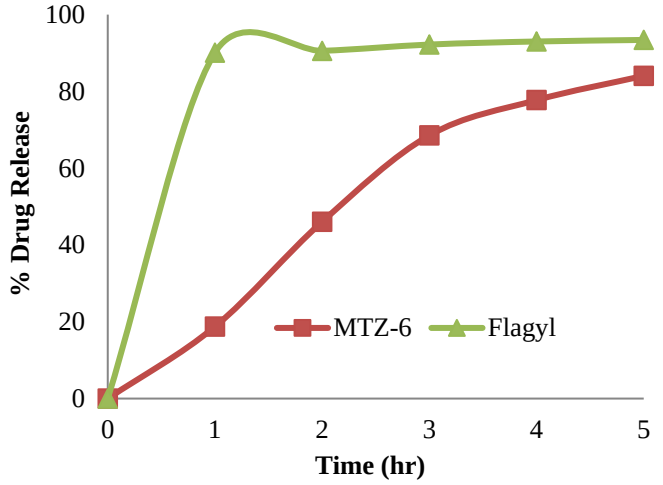

Figure 11: % drug release of MTZ-6 and Flagyl® in pH 6.8

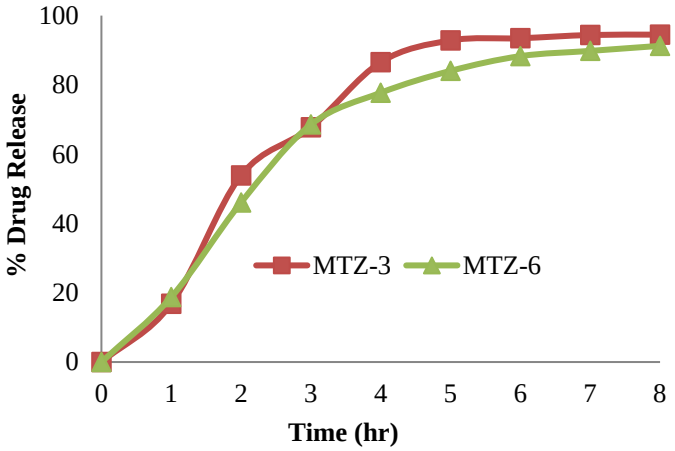

Figure 12: % of Metronidazole released from MTZ-3 and MTZ-6 formulations after storage for 6 months.

This study successfully developed and characterized Metronidazole-loaded mucoadhesive microspheres with promising properties for improved drug delivery of metronidazole. Further optimization and in vivo studies are warranted to translate these findings into a clinically viable drug delivery system.

CONCLUSION

This study successfully developed and characterized mucoadhesive microspheres loaded with Metronidazole for oral administration. These microspheres were designed to overcome the limitations of rapid gastrointestinal transit and improve the drug's bioavailability. It has been observed that increasing chitosan content resulted in a proportional increase in microsphere size, allowing for potential control over drug release kinetics. Further, Formulations containing Chitosan exhibited the strongest mucoadhesive properties, potentially

leading to extended residence time in the gastrointestinal tract and improved drug absorption. In vitro studies demonstrated sustained release profiles, suggesting prolonged drug delivery compared to conventional dosage forms. Notably, the stability study suggests no significant changes in drug release. These results support the potential of Chitosan-based mucoadhesive microspheres as a promising approach for the sustained and controlled release of Metronidazole. This approach can potentially address the challenges associated with conventional dosage forms by improving drug bioavailability and therapeutic efficacy.

FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHOR CONTRIBUTION
This study was done under the guidance of M. Siddaiah. Dheeraj Chechare planned and designed the experiments and performed them, collected data, and prepared the draft of the manuscript. All authors read and approved the final manuscript.

REFERENCES
[1] Wagh RT, Jadhav SL, Banerjee SK, Gaikwad DD. Mucoadhesive Microsphere: A Review. *International Journal of Pharmaceutical Excellence*, **1(1)**, 26-44 (2014).

[2] Carvalho FC, Bruschi ML, Evangelista RC, Gremio MP. Mucoadhesive drug delivery system. *Brazilian Journal of Pharmaceutical Sciences*, **46(1)**, 1-17 (2010).

[3] Huang Y, Li X, Tong Y, Li J. Mucoadhesive microspheres of chitosan/carboxymethyl chitosan for oral delivery of tetracycline hydrochloride: Preparation, characterization and in vitro evaluation. *Carbohydrate Polymers*, **98(2)**, 1422-1430 (2013).

[4] Kaur P, Kaur S, Singal G. Chitosan-based nanoparticles for targeted delivery of raloxifene: design, development, and characterization. *Journal of Drug Delivery Science and Technology*, **53**, 101257 (2019).

[5] Abdelrahman SH, Abdel-Aziz RE, Abdelbary AA, Eissa HS. Design and in vitro evaluation of mucoadhesive microspheres for nasal delivery of loratadine. *Journal of Drug Delivery Science and Technology*, **44**, 74-83 (2018).

[6] Learoyd TP, Burrows JL, French E, Seville PC. Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. *European Journal of Pharmaceutics and Biopharmaceutics*, **68**, 224-34 (2008).

[7] Lynne W, John HC, John TF. Amoxycillin release from a floating dosage form based on alginates. *International Journal of Pharmaceutics*, **210 (1)**,45-49 (2000).

[8] Joshi VC, Khan IA, Maged HM. Use of scanning electron microscopy in the authentication of botanicals. *Pharmacopeial Forum*, **34**, 1075-1078 (2008).

[9] Liu Z, Zhang Z, Huo M, Li J, He H. Mucoadhesive thiolated chitosan nanoparticles for oral delivery of insulin: In vitro and ex vivo evaluation. *International Journal of Pharmaceutics*, **520(1-2)**, 22-32 (2017).

[10] Kumar P, Pandit, JK. Development and in vitro evaluation of chitosan-alginate based mucoadhesive microspheres for the sustained and targeted delivery of Metronidazole. *International Journal of Pharmaceutics*, **471(1-2)**, 221-230 (2014).

[11] Feng S, Yu Y, Wang Y, Zhu D. Preparation and evaluation of pH-sensitive chitosan/hydroxypropyl methylcellulose phthalate (HPMCP) nanoparticles for oral delivery of Metronidazole. *Colloids and Surfaces B: Biointerfaces***,** **144**, 306-313 (2016).

[12] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). (2019). ICH Q1A: Stability testing of new drug substances and products. https://www.ema.europa.eu/en/ich-q1a-r2-stability-testing-new-drug-substances-and-drug-products-scientific-guideline

[13] Mathews BR. Regulatory aspects of stability testing in Europe. *Drug Development Industrial Pharmacy*, **25 (7)**, 831-856 (1999).