Scholars Academic Journal of Pharmacy (SAJP)
Abbreviated Key Title: Sch. Acad. J. Pharm.
ISSN 2347-9531 (Print)
ISSN 2320-4206 (Online)
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A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com
Pharmaceutics

Evaluation of Mucoadhesive Microspheres of Metronidazole Formulated Using Ionic Gelation Technique

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Original Research Article

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Article History
Received: 15.12.2018
Accepted: 25.12.2018
Published:30.12.2018

DOI:
10.21276/sajp.2018.7.12.4

Abstract: Gastrointestinal drug delivery is a technique to prolong gastric residence time for site-specific targeted drug release. Mucoadhesive microsphere is an approach to achieve gastric retention for improved bioavailability. This study was aimed at preparing and evaluating metronidazole mucoadhesive microspheres using the ionic gelation technique. The microspheres were prepared by dispersing the polymers in distilled water followed by incorporation of the drug and extrusion into aqueous anhydrous calcium chloride solution. Different drug-polymer ratios were used for the process. The microspheres were evaluated for yield, compatibility studies using thin layer chromatography (TLC) and differential scanning calorimetry (DSC), swelling index, mucoadhesion, entrapment efficiency and drug release studies. Microspheres formulated exhibited no possible drug-polymer interactions. Retention factors of the microspheres were approximately the same with a deviation of ± 0.2 and this was corroborated by the information obtained from the DSC thermograms. Microspheres formulated using combinations of alginate and carbopol had higher swelling capacity up to 3 - 4 times their original weight. Percentage mucoadhesion was significant ranging from 48.50% - 95% with combinations of alginate and carbopol adhering more. Entrapment efficiency was excellent ranging from 78.96% - 92.35%. Drug release studies show sustained release of metronidazole and first order release with diffusion and erosion as the predominant release mechanism. Mucoadhesive microspheres of metronidazole can possibly be formulated using ionic gelation technique for site-specific targeted release. Metronidazole mucoadhesive microspheres may be used as an adjunct in the treatment of gastric ulcer, caused by *Helicobacter pylori* which reside mainly in the gastric mucosa.
Keywords: Mucoadhesive, Microspheres, Metronidazole, ionic gelation, *Helicobacter pylori*.

INTRODUCTION

Controlled-release drug delivery systems provide drug release at a predetermined, predictable and controlled rate. The ultimate aim of oral controlled drug delivery system is to prolong the drug release to achieve better bioavailability. A major constraint in oral controlled drug delivery is that not all drug candidates are absorbed uniformly throughout the gastrointestinal tract [1]. These drug delivery systems suffer from mainly two adversities: short gastric retention time and unpredictable short gastric emptying time, which can result in incomplete drug release from the dosage form in the absorption zone (stomach or upper part of small intestine) leading to diminished efficacy of administered dose [2,3].

Gastro-retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Gastro-retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients [4-7].

Muco-adhesive system is an approach to achieve gastroretention, by the use of bioadhesive polymers that can adhere to the epithelial surface in the stomach. Thus, they prolong gastric retention and increase bioavailability [8, 9].

Mucoadhesive microspheres include microparticles and microcapsules having a core of drug of 1-1000 µm in diameter and consisting of a mucoadhesive polymer or having an outer coating of it, respectively. Due to high surface area and more contact with the mucous and absorbing membrane, utilizing the bioadhesive properties of polymers to form bioadhesive microspheres will provide, more intimate contact with the mucus layer, controlled and sustained release of drug from dosage form, specific carriers to target drugs to the absorption site, efficient absorption and increased bioavailability of the drugs [10-12].

Different methods exist for the preparation of mucoadhesive microspheres, one of which is ionic gelation technique that is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogel beads also called gelispheres [13].

Metronidazole is a nitroimidazole antimicrobial which is effective against a variety of anaerobic bacteria and protozoa. Mechanism of action involves the penetration of bacterial cells in the process of passive diffusion and generation of active product in the reduction of the nitro group, which causes damage of cellular DNA [14].

It is actively used as an adjunct in the treatment of infection, caused by *Helicobacter pylori* which reside mainly in the gastric mucosa. *Helicobacter pylori* are implicated as an etiologic factor in the development of the gastritis, gastric ulcer and gastric carcinoma [15, 16].

Treatment of local gastric infection with conventional formulations becomes ineffective due to their short gastric residence time and non-targeted drug release. The high variability of the Gastric emptying process, leads to transfer of the conventional formulation quickly to the intestine without significant release of drug to the mucous site. Thus frequent dosing is required [17, 18]

Therefore the objective of this study was to prepare metronidazole loaded mucoadhesive microspheres by the ionic gelation technique.

MATERIALS AND METHODS

Preparation of Microspheres

The microspheres were prepared by measuring 400 ml of distilled water into a 1000 ml beaker and stirred under a magnetic stirrer at 15^{o}C. Thereafter, sodium alginate equivalent to 15 g was weighed and dispersed in the distilled water. The solution was stirred continuously and maintained at 100 rpm until a uniform dispersion was obtained. 100 ml of distilled water was used to prepare HPMC solution. Both polymer solutions were then allowed to stand for about 15 minutes to remove air bubbles and then mixed together gently. Thereafter, 15 g of metronidazole was transferred into the resulting polymer solution under the influence of the stirrer and the dispersion extruded dropwise into 10 % anhydrous calcium chloride solution using a syringe. The solution was left for 24 hours and the resulting microspheres decanted, filtered and dried at 40^{o}C for 12 hours. This procedure was repeated for other batches with different drug: polymer ratios [19].

Table-1: Composition of Formulated Microspheres

Batches	Ratio of Drug to Polymer
A1	1:1 (Alginate)
A2	1:2 (Alginate)
A3	1:3 (Alginate)
B6	1:1 (3:1 – Alginate : Carbopol)
B7	1:2 (3:1 – Alginate : Carbopol)
C6	1:1(3:1 – Alginate : HPMC)
C7	1:2(3:1 – Alginate : HPMC)
C8	1:1(1:3 – Alginate : HPMC)

Evaluation of Microspheres

Interaction Studies

Thin Layer Chromatography

A quantity of sample equivalent to 0.5 g was weighed and dissolved in 10ml methanol. The mobile phase was prepared using methanol and dichloromethane (9:1). The tank was saturated with the solvent vapour and the developed plates were placed in the tank. Upon elution, the chromatogram was collected and sun-dried. Spots formed were marked and the retention factor of each samples were calculated.

Differential Scanning Colorimetry

A 5 mg quantity of the sample was weighed and deposited in the indent at the bottom of the pan. The pan was thereafter sealed using a press designed for that purpose. The cell was precooled to the desired starting temperature before opening to deposit the pan. The reference and sample pans were placed into each corresponding platform. Both substances were heated at a rate of 10^{o}C/min until complete melting was achieved. The thermal history as well as the melting profile of the reference and sample was determined.

Swelling Index

The swelling index was carried out by weighing microspheres equivalent to 100 mg and allowed to swell in 1ml of distilled water in a petri dish for 24 hours. The excess surface adhered liquid drops were removed by blotting and adsorption on a filter paper. The swollen microspheres were weighed using the analytical balance. The hydrogel microspheres were then dried in an oven at 60^{o}C for 5 hours until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated using the formula below:

$$Swelling\ index = \frac{W_s - W_o}{W_o} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 1$$

Where W_o is weight of dried microsphere and W_s is weight of swollen microsphere

In vitro Drug Release/Dissolution Test

The release studies of the drug-loaded microspheres was carried out in 0.1N HCl. Exactly 100 mg of the microspheres was weighed accurately and gently spread over the surface of 900 ml of dissolution medium (0.1N HCl). The content was rotated at 100 rpm at 37 ± 0.5^{0}C. A 5 ml sample was withdrawn from the dissolution medium at various time intervals and analyzed using a UV–Visible spectrophotometer at 350 nm. The receptor volume was maintained constant by replacing with equivalent volume of dissolution medium after each withdrawal. The concentration of drug in the samples was calculated using the regression equation of the calibration curve [20].

Mucoadhesion Test

The mucoadhesion test was carried out using the wash off technique by applying difference in weight measurements. The weight of a plastic platform was recorded as W_1. A piece of the stomach mucosa was obtained from a female 30g rat and pinned onto the platform and the weight of platform and tissue was taken as W_2. Exactly 0.2g of the microsphere was weighed and spread on the exposed mucosa surface and the weight of platform, tissue and microsphere taken as W_3. The exposed tissue on the platform was then mounted to a retort stand via a clamp and washed off using normal saline from a height of 18cm (0.18m) for two (2) hours at the rate of 10 ml/min. After washing, the weight of the platform, tissue and microspheres was taken and recorded as W_4. The weight adhered was calculated by subtracting the weight after washing from the weight before washing and expressing as a percentage of the amount of microspheres applied using the equation below:

$$\%\ Mucoadhesion = \frac{W_3 - W_4}{0.2} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eqn\ 2$$

Drug Entrapment Efficiency

Exactly 100 mg of microspheres was taken and triturated with 0.1N HCl and transferred to a 10 ml measuring cylinder. The volume was made up to 10 ml and mixed well. The solution was then kept aside for 12 hrs. It was then filtered through membrane filter (0.45µm) and estimated for drug content by measuring the absorbance at 350 nm. The drug entrapment efficiency was calculated using the following formula [21].

$$Entrapment\ Efficiency = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} * 100 \ldots\ldots\ldots\ldots\ldots Eqn\ 3$$

RESULTS AND DISCUSSION

Percentage Yield

The percentage yield indicates the feasibility of this method of microencapsulation for industrial purposes as well as its economic implications. Complete and perfect yield was never possible due to losses resulting from filtration, extrusion and drying. However, percentage yield was greater than 15% irrespective of the loading averaging about 25.13%. Whichever the case, it is also important that the formed microspheres should remain economically competitive.

Table-2: Percentage Yield

Batches	Theoretical Loading	% Yield
A1	50%	38.03%
A2	33.33%	23.62%
A3	25%	16.72%
B6	50%	31.44%
B7	33.33%	19.66%
C6	50%	26.94%
C7	33.33%	21.16%
C8	50%	23.49%

Interaction Studies

The mobile phase comprises of methanol and dichloromethane at a ratio of 9:1. Choice of solvent was based on the solubility of the drug and its tendency to be eluted. The single yellow spots indicate the purity of the samples. The Retention factor is indicative of the absence of interactions between the drug and the polymers. All samples analyzed gave approximately similar retention factor ranging from 0.7-0.8 indicating that the integrity of the drug was not compromised; hence there were no drug-polymer interactions. This implies that the drug remains stable even after encapsulation.

Similarly, to buttress the claim as established by thin layer chromatography, the thermograms of representative batches A-D showed that the integrity of the drug was not compromised indicating compatibility as shown in the Figures 1- 4 below

Table-3: Retention factor (R_f) values

Batches	R_f value (mean + SD)
Pure drug	0.83±0.01
A1	0.83±0.01
A2	0.81±0.01
A3	0.70±0.01
B6	0.66±0.03
B7	0.86±0.01
C6	0.83±0.01
C7	0.83±0.00
C8	0.78±0.03

The peak onset of the pure drug was 191.25^{o}C and that of representative batches of the formed microspheres was between 190.62-191.32^{o}C. Thus, the physicochemical properties of the drug was unaffected by the polymer and this implies that they are compatible.

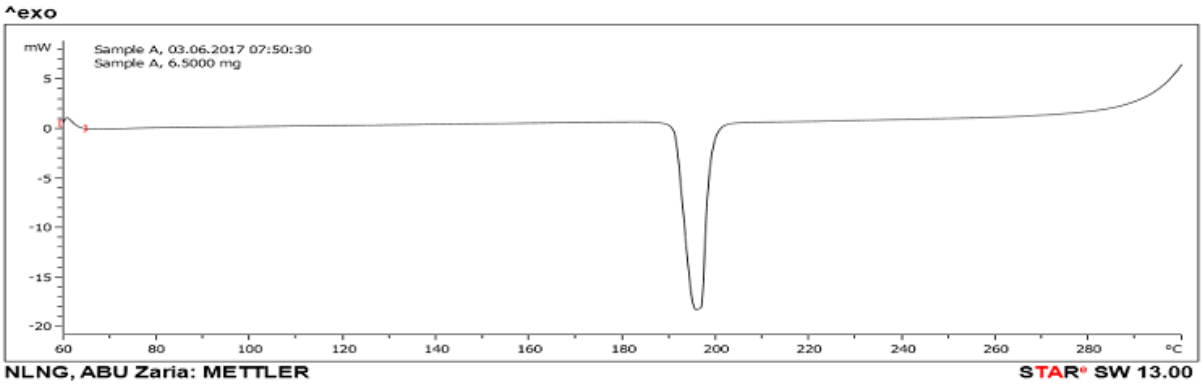

Fig-1: DSC thermogram for pure sample of metronidazole

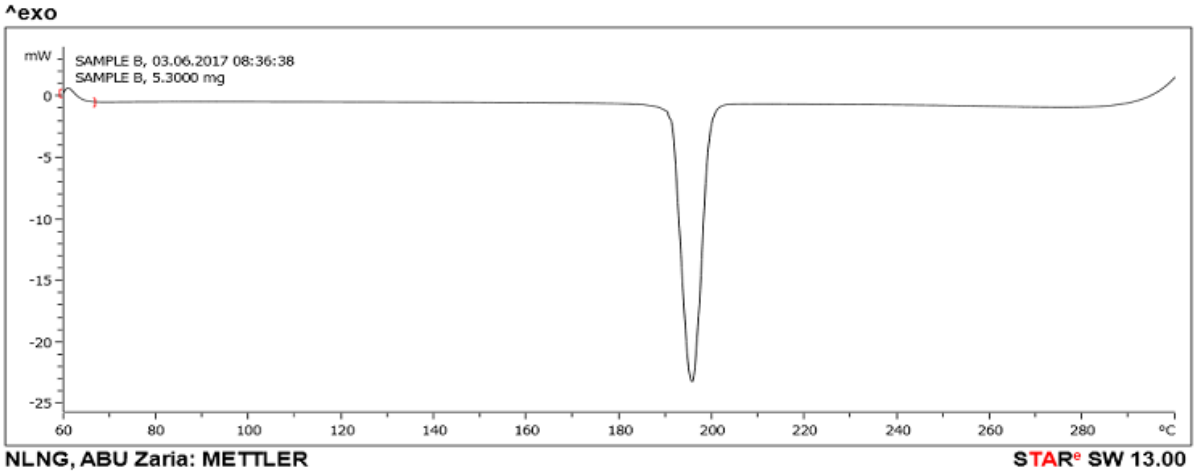

Fig-2: DSC thermogram for batch A3 (Alginate and HPMC combination)

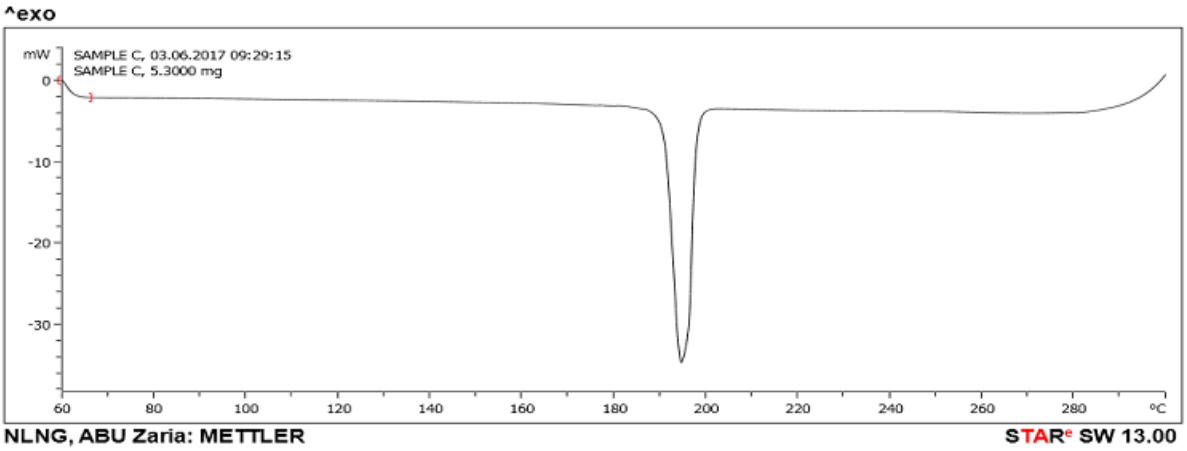

Fig-3: DSC thermogram for batch B7 (Alginate only)

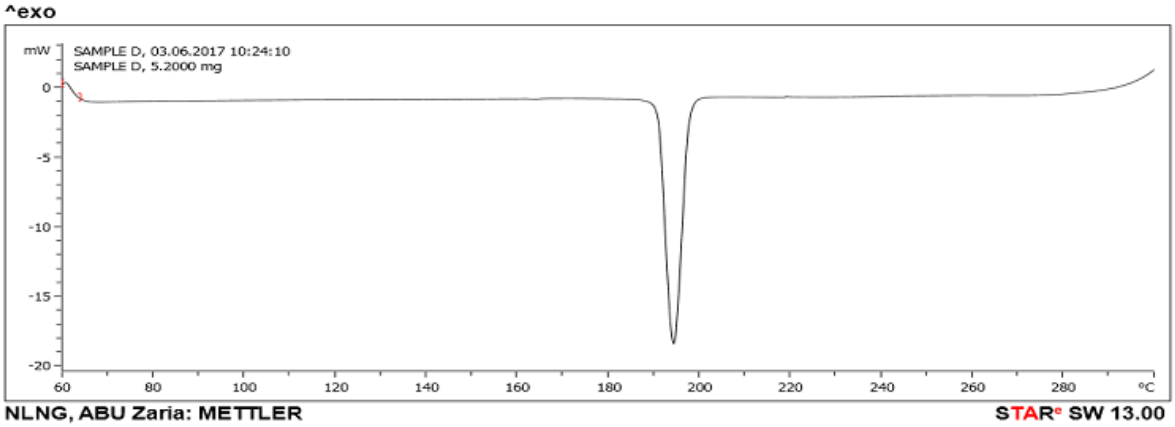

Fig-4: DSC thermogram for batch C8 (Alginate and carbopol combination)

Percentage Entrapment Efficiency

The entrapment efficiency describes the amount of the drug in a given sample of prepared microspheres or as the name implies connotes the percentage of drug entrapped within the polymer coat. Formulations with alginate and carbopol as well as those of alginate and HPMC had comparable entrapment efficiency as evident in batches B6, B7, C6 and C7 with A2 having higher entrapment efficiency. From all formulations, entrapment efficiency ranges from approximately 79 - 92% averaging about 83.72%. These results indicated that more drugs were entrapped in these formulations which in turn would improve their therapeutic performance.

Table-4: Entrapment Efficiency of Formulated Microspheres

Batches	% Loading	%Entrapment Efficiency
A1	50%	82.43%
A2	33.33%	92.35%
A3	25%	81.04%
B6	50%	82.78%
B7	33.33%	85.04%
C6	50%	82.43%
C7	33.33%	84.70%
C8	50%	78.96%

Swelling Index and Percentage Mucoadhesion

The swelling index carried out in 1ml distilled water showed that microspheres formulated with alginate and carbopol could swell four to five times their original weight as evident in the swelling index of batches B6 and B7. This will buttress evidence existing in literature regarding the ability of certain polymers e.g carbopol to swell while adhering to the mucosal surface as in mucoadhesion. Formulations with alginate, batches A1, A3; and alginate and HPMC, C8 also had significant swelling capacity with batches B6 and B7 swelling even greater as shown in table 6.

Table-5: Swelling Index and Percentage Mucoadhesion of Formulated Microspheres

Batches	Swelling Index	Percentage Mucoadhesion (%)
A1	70.00%	48.50%
A2	49.18%	52.50%
A3	66.67%	65.20%
B6	295.06%	85.00%
B7	455.64%	95.00%
C6	36.10%	55.60%
C7	16.67%	52.25%
C8	79.68%	63.36%

The percentage mucoadhesion presented in table 6 showed that all formulations had substantial degree of mucoadhesion after washing for 2 hours averaging about 64.68% in all. However, formulations of alginate and carbopol exhibited higher degree of mucoadhesion. This implies that carbopol could greatly improve the mucoadhesion properties of alginate. With HPMC, mucoadhesion was also improved as in C8 but less than that with carbopol. This property depends on a lot of factors including the molecular characteristics of these polymers.

Dissolution Profile

Fig 1 shows the dissolution profile of the various batches of metronidazole microspheres. All batches showed sustained drug release. Furthermore, batch B6 showed a more controlled release pattern in that 40% of the drug was released after 60 minutes. However, batch A1 had a higher drug release after 60 minutes (> 60%).

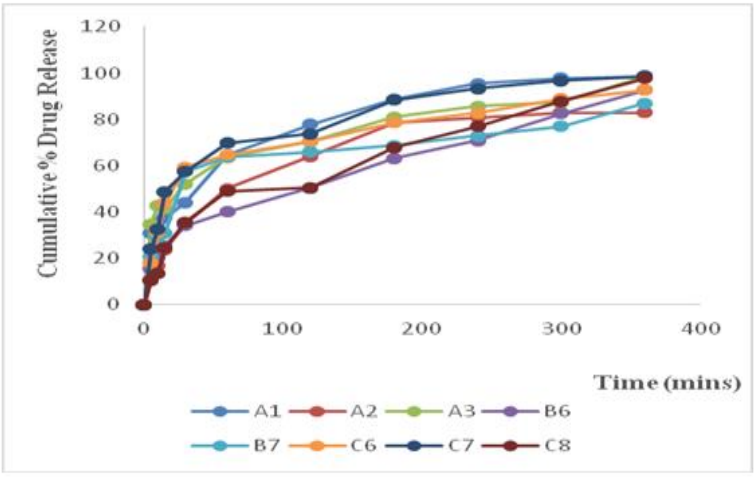

Fig. 5: Drug release profiles of formed microspheres

A1 – 1:1 (Drug : Alginate); A2 – 1:2 (Drug : Alginate); A3 – 1:3 (Drug: Alginate); B6{1:1(3:1 – Alginate : Carbopol)}, B7{1:2(3: 1 – Alginate : Carbopol)}, C6{1:1(3:1 – Alginate : HPMC)},C7{1:2(3:1 – Alginate : HPMC)}, C8{1:1(1:3 – Alginate : HPMC)}.

***In vitro* Release Kinetics**

The *in vitro release* kinetics is indicative of the pattern and order of drug release from the polymer matrix. The various release kinetics of the formulated microspheres is shown below

Table-6: K and R^2 Values for Different Release Models

Batches	Zero Order		First Order		Higuchi		Korsemeyer-Peppas		
	K_o	R^2	K	R^2	K	R^2	K	R^2	N
A1	0.3630	0.2128	0.0050	0.9934	4.9122	0.9426	4.5457	0.7471	0.5952
A2	0.3063	0.5610	0.0023	0.9174	4.7263	0.9549	2.5527	0.8981	0.6624
A3	0.3404	-0.166	0.0036	0.8730	4.1823	0.9070	5.2845	0.6838	0.5586
B6	0.2941	0.6973	0.0026	0.9472	4.4567	0.9895	3.0479	0.8522	0.6131
B7	0.2999	-0.045	0.0018	0.8036	3.8639	0.8557	4.1343	0.7545	0.5816
C6	0.3222	0.0096	0.0027	0.9381	4.3335	0.8812	4.1581	0.7624	0.6013
C7	0.3611	0.0380	0.0046	0.9812	4.7259	0.9028	4.5238	0.7479	0.6007
C8	0.7478	0.7478	0.0036	0.8879	4.9582	0.9823	2.4188	0.9071	0.6672

A critical review of the dissolution profile and the release kinetics show that batch B6 gave the best dissolution profile compared to other batches. The drug release from batches A1, C6 and C7 reveal that all batches possess first order release pattern as the dominant kinetic of drug release. Hence, the release of drug from these batches is concentration dependent. All other batches possess first order release with contributions from Higuchi's kinetics (R^2>0.9). The korsemeyer-Peppas plot of release of metronidazole microsphere using 'n' – the release exponent indicative of the mechanism of drug release, reveals n values between 0.45 and 0.89 indicating anomalous (non-Fikian diffusion).

CONCLUSIONS

The study has established the possibility of formulating mucoadhesive multiparticulate system of metronidazole using ionic gelation technique. Metronidazole mucoadhesive microspheres can help to prolong the gastric retention time in the gastrointestinal tract as well as sustain the release of metronidazole.

Furthermore, carbopol and HPMC can be used to prolong the residence time of metronidazole encapsulated with alginate. In terms of mucoadhesion and swelling capacity, a combination of alginate and carbopol or alginate and HPMC can produce microspheres which would stay longer at the absorbing membrane providing targeted and sustained release of metronidazole. This delivery system may be explored in localizing the release of metronidazole for the management of *Helicobacter pylori* induced peptic ulcer disease.

ACKNOWLEDGEMENT

The authors wish to acknowledge Mr. Benedict Iwetan, Mr. Felix Oboh, Nicholas David, Dr. Edwin Omeje, Dr. Emmanuel Ikpefan, for their technical assistance.

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