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Der Pharmacia Lettre, 2010, 2(6): 106-113
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Chitosan/Sodium tripolyphosphate cross linked microspheres for the treatment of gastric ulcer

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ABSTRACT

The aim of the study was to develop microspheres containing Amoxicillin trihydrate and Metronidazole for the treatment of gastric ulcer using chitosan as mucoadhesive polymer. Microspheres were prepared by cross linking it with sodium tripolyphosphate. The influence of formulation parameters were studied on particle size, content uniformity, swelling studies and mucoadhesive studies. In vitro release of chitosan microspheres were studied in 0.1N HCl at $37^0 \pm 0.5^0$C. The prepared microspheres were characterized by DSC. The morphology of microspheres was investigated by scanning electron microscopy(SEM). It was found that chitosan microspheres showed mucoadhesive property and the sustained drug release over a period of 10 hours. SEM photograph confirmed the spherical shape of microspheres. It was observed from the DSC that the decomposition temperatures of pure drugs and formulations remained the same indicating there is no interaction between the drugs and polymers used. It can be concluded that the prepared mucoadhesive microspheres can be used as carriers for the treatment of gastric ulcer caused by H.Pylori since they increase the residence time of the drugs inside stomach and give better therapeutic action.

key words: Chitosan, Microspheres, Mucoadhesive, Ionic Crosslinking.

INTRODUCTION

Chitosan is a polycationic polymer with numerous applications in the food, agriculture and cosmetic industries. Advantages of this polymer include high availability, low cost, high biocompatibility, biodegradable and ease of chemical modification. Pharmaceutical applications include use as a tablet binder and a disintegrant. Chitosan has gel forming properties in the low pH range and is used as a drug carrier in hydrocolloids and gel formulations. Chitosan is also used as a constituent in polymeric matrix systems, microspheres and microcapsules for the sustained release of water soluble drugs. The mucoadhesive properties of chitosan and ability to act as an absorption enhancer has lead to its use as coating material for multilamellarr liposomes

and use in formulation aimed at controlled drug delivery at specific sections in the gastro-intestinal tract and across other mucosal surfaces such as the nasal, buccal and vaginal epithelia [1-2].

Since the discovery of *Helicobacter pylori* by Marshall and Warren , *H. pylori* is believed as a main microorganism causing gastric or peptic ulcers. Therefore, treatment for *H. pylori* is a prerequisite for curing a gastric or peptic ulcer and preventing a recurrence. Mucoadhesive controlled release dosage formulations have gained considerable attention due to their ability to adhere to the mucus layer and release the loaded drug in a sustained manner. Mucoadhesive dosage forms were also reported to improve the absorption and systemic bioavailability of the drugs that were normally poorly absorbed. Amoxicillin trihydrate and Metronidazole are effective in treating H.pylori. when used under *in vitro* conditions, they score poorly to treat infections in *in vivo* situation. The failure of these drugs has been proposed to be an outcome of sub-effective bactericidal concentrations available at the site and their instability following oral adminstration. Microspheres are polymeric particles ranging in size from 1-1000µm containing dispersed drug in either solution or microcrystalline form [3-4].

The use of polymers as bioadhesives (adhering to epithelium) and mucoadhesives (adhering to mucus) offer significant potential for oral drug delivery. Cargos formulated with mucoadhesive polymers may increase gastrointestinal tract residence time leading to improved oral drug bioavailability, as the formulation should allow more opportunity to contact the epithelium. Adhesive polymeric systems may also be useful for topically coating the damaged intestinal wall in inflammatory bowel disease, or for facilitating healing in the oral cavity. For these applications, polymers need to be inert, nonabsorbable, stable, and easy to process. A requirement for any adhesive polymer is quantifiable and reproducible adhesion to tissues and/or supramucosal gels. Changes in indices of intestinal barrier structure and function may be caused by adhesion of some polymers. Examples include the opening of tight junctions by chitosan and reduction of bacterial access to the epithelial surface by polyethylene glycol [5, 10].

In the present study the microspheres were prepared by using cross linking. Chitosan is made to react with sodium tripolyphosphate (cross–linking agent) by ionic linkage. The amount of chitosan and STPP was varied to see effect on swelling and drug release. The objective of varying the concentration of cross linking agent was to achieve an optimized formulation, which would give a sustained release of drug over a period of time. Amoxicillin trihydrate and Metronidazole were chosen as the model drugs and were loaded into the complex microspheres. This choice was made because a combination of two antimicrobial agents such as Amoxicillin trihydrate and Metronidazole is known to have enhanced therapeutic efficacy. This study further characterized the complex microspheres by examining the release rate of the active ingredient, the dissolution rate of the microspheres and their morphologies.

MATERIALS AND METHODS

2.1. Materials

Chitosan was purchased from Vishu Aquatech (Madras, India) with a deacetylation degree of approximately 85%. Sodium tripolyphosphate was purchased from Loba chemi pvt Ltd (Mumbai, India). Acetic acid was purchased from Ranbaxy fine chemicals Ltd. Amoxicillin trihydrate and Metronidazole was provided by KAPL (Bangalore, India) as a gift sample. All other chemicals were of reagent grade and used without further purification.

2.2. Methods

2.2.1. Preparation of mucoadhesive microspheres

Chitosan was weighed and dissolved in 0.1M acetic acid using homogenizer (Remi motors, Mumbai) at 5000rpm for about 30 minutes, then drug was added to chitosan solution. Sodium tripolyphosphate (STPP) was dissolved in water under stirring for 30 min. Chitosan solution was poured into STPP solution under stirring at 5000 rpm for 45 min. microspheres were precipitated out. Microspheres were filtered and vacuum dried. Prepared microspheres were taken for further studies [6].

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Amoxicillin trihydrate (mg)	500	500	500	500	-	-	-	-	250	250	250	250
Metronidazole (mg)	-	-	-	-	500	500	500	500	250	250	250	250
Chitosan (% w/v)	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
STPP % w/v	4	4	2	2	4	4	2	2	4	4	2	2

Table-1. Formulation of microspheres containing Amoxicillin trihydrate and Metronidazole

2.2.2. Drug content

An amount of microspheres containing 20 mg of drug was taken using a digital balance (Shinko sansui, Japan) in 100 ml standard volumetric flask. To this 75 ml of 0.1N HCl was added and kept overnight. The volume was made up with 0.1NHCl. The final solution was filtered using Whatman filter paper, From this 10ml was pipetted out into a 100ml standard volumetric flask and made upto volume with 0.1N HCl and estimated for drug content using UV-Visible spectrophotometer (Shimadzu-1701, Japan).

2.2.3. Particle size analysis

Microspheres were studied for their size. Particle size was studied using optical microscope, consisting of eye piece and stage micrometer. Calibration factor was calculated then microspheres were placed on the slide using glycerin and size was observed [17].

2.2.4. Morphology

The prepared microspheres were subjected to SEM analysis. SEM analysis was done using Joel SEM analysis instrument Japan. It was done to study surface morphology (shape) of microspheres.

2.2.5.. *In vitro* drug release of microspheres

In vitro drug release from the microspheres was carried out in triplicate at 37°C ± 0.5°C using a Six basket dissolution tester-USP XXII, TDT-08L, with auto sampler (Electro lab, Mumbai) at a rotation speed of 50 rpm. Drug release from microspheres was studied in 900 ml of 0.1 N HCl for 10 hours. An aliquot of the release medium (3 ml) was withdrawn at the predetermined time interval and an equivalent amount of fresh medium was added to the release medium and analyzed for the drug using a UV visible spectrophotometer (Shimadzu-1701, Japan) [10,12]. The release data obtained were fitted into various mathematical models using PCP – Disso-V2.08 software to know which mathematical model is best fitting the obtained release profile.

2.2.6 Swelling studies

The swelling property of microspheres was studied in 0.1N HCl. 200 mg of microspheres were placed in 20 ml of 0.1N HCl solution for 8 hrs. At every hour interval, the microspheres were removed and excess surface liquid was removed by blotting and their weights were recorded [10,12].

The percentage swelling (S) was determined by the following equation :

$$S = \frac{\text{weight of swollen microspheres} - \text{weight of dry microspheres} \times 100}{\text{Weight of dry microspheres}}$$

2.2.7 Mucoadhesive studies

About 50 microspheres were taken for the study and spread over the sheep stomach mucosa of 2x2 cm. The mucosa was taken from healthy sheep and was fit to be used in the experiment. The instrument designed was, disintegration apparatus USP. The medium chosen was 0.1 N HCl, at every hour interval microspheres adhering to the mucosa were counted. The study was carried out for 8 hours [10,18].

RESULTS AND DISCUSSION

3.1. Drug content

The test for encapsulation efficiency was carried out to ascertain the amount of drug encapsulated in microspheres. Here an amount of microspheres containing 20 mg equivalent of Amoxicillin trihydrate and Metronidazole respectively is placed in 0.1N HCl solution for 24 hours. In the 0.1N HCl solution the microspheres swell and the drug is released. At the end of 24 hours, amount of Amoxicillin trihydrate and Metronidazole present in 0.1N HCl is determined spectrophotometrically at 229 & 277nm respectively. The results obtained are reported in Table 2(a), 2(b), 2.1, 2.2. From the results obtained, it can be inferred that there is proper distribution of Amoxicillin trihydrate and Metronidazole in the microspheres. The encapsulation efficiency was found to be between 55-65%. The encapsulation efficiency was studied for all formulations containing Amoxicillin trihydrate and Metronidazole individually and in combination.

Table 2. **Encapsulation efficiency of Amoxicillin trihydrate and Metronidazole loaded microspheres.**

Table 2(a): **(Metronidazole)**

SL.No	Formulation of Metronidazole	Trial 1	Trial 2	Trial 3	Average Mean (mg) ± S.D.*
1	F-9	11.1	11.5	10.9	11.16± 0.43
2	F-10	10.6	10.8	10.4	10.60± 0.41
3	F-11	11.2	11.4	11.6	11.40±0.20
4	F-12	10.8	11.2	11.2	11.06±0.23

***** *Standard deviation n=3*

Table 2(b): **(Amoxicillin trihydrate)**

SL.No	Formulation of Amoxicillin trihydrate	Trial 1	Trial 2	Trial 3	Average Mean (mg) ± S.D.*
1	F-9	11.4	11.5	10.9	11.26± 0.32
2	F-10	10.6	10.8	11.2	10.86± 0.41
3	F-11	11.7	11.4	11.6	11.56±0.25
4	F-12	10.8	11.2	11.6	11.20 ±0.23

***** *Standard deviation n=3*

Table 2.1: Encapsulation efficiency of Amoxicillin trihydrate loaded microspheres.

Sl.No.	Formulation of Amoxicillin trihydrate	Trial 1	Trial 2	Trial 3	Average Mean (mg) ± S.D.*
1	F-1	12.75	12.50	12.50	12.58±0.13
2	F-2	12.00	12.50	12.25	12.25±0.25
3	F-3	11.75	12.00	12.25	12.00±0.25
4	F-4	12.25	12.00	12.25	12.16±0.13

***** *Standard deviation n=3*

Table 2.2: Encapsulation efficiency of Metronidazole loaded microspheres

SL.No	Formulation of Metronidazole	Trial 1	Trial 2	Trial 3	Average Mean (mg) ± S.D.*
1	F-5	11.4	11.2.	11.0	11.20±0.20
2	F-6	11.5	11.8	11.2	11.50±0.25
3	F-7	11.4	11.6	11.4	11.46±0.0.23
4	F-8	11.2	11.7	11.3	11.40±0.30

***** *Standard deviation n=3*

3.2. Particle size analysis

Micromeritic property such as particle size is mainly governed by the polymer concentration. Particle size was in between range of 10 µm to 25µm. Particle size increases with increasing polymer concentration.

3.3. Morphology

The morphology of the microspheres was examined by scanning electron microscopy. The microspheres containing either the Amoxicillin trihydrate or Metronidazole had a spherical shape with a smooth surface [Fig. 1].

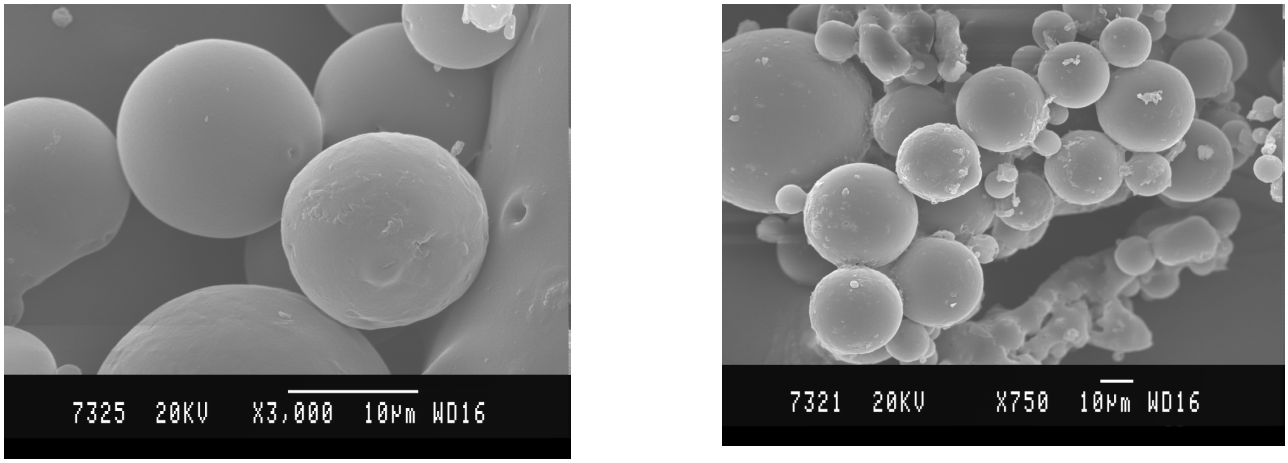

Fig. 1. Morphology of the microspheres at different magnification.

3.4. Differential scanning colorimetry

Figure No. 2 shows the DSC Thermograms and thermal transition temperatures for pure drugs and formulations. It was observed that the decomposition temperatures of pure drugs and formulations remained the same. The Amoxicillin trihydrate shows a peak at 152°C and Metronidazole shows a sharp endothermic peak at 160°C, the formulation of microspheres also show the same peaks without significant change. From the DSC studies it can be concluded that there is no significant interactions between drugs and the polymers used.

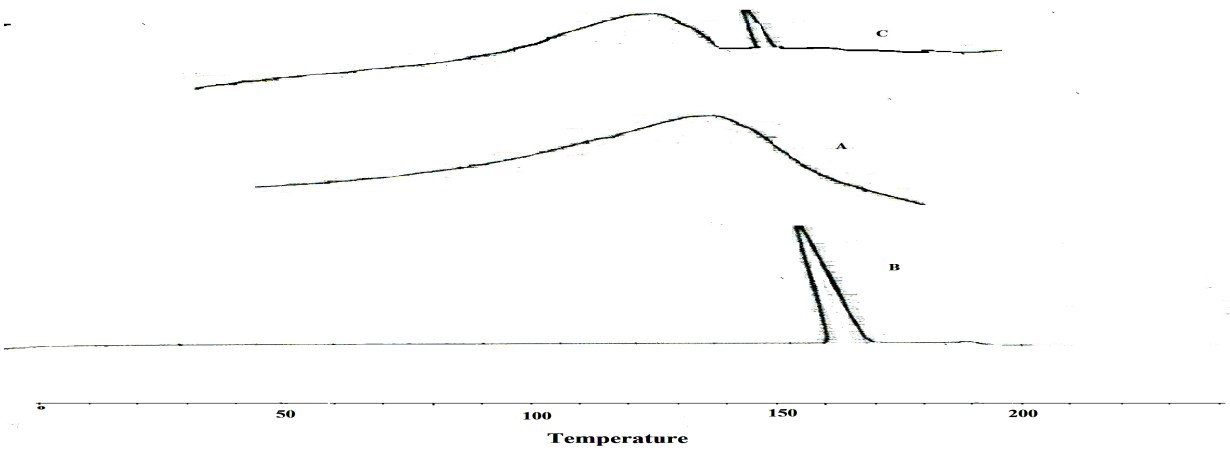

Fig. 2. DSC spectra of Amoxicillin trihydrate (A), Metronidazole (B) and formulation (C).

3.5. *In vitro* drug release of microspheres

The release profiles for both the drugs from their formulations was almost similar, hence it can be stated that release is independent of the drug used in microspheres. When concentration of STPP was increased from 2% w/v – 4% w/v and chitosan from 0.5% w/v –1% w/v the drug release was varied. It was observed that as the concentration of crosslinking agent (STPP) was increased, due to high cross linking release was slowed down hence F1 containing 4% w/v STPP showed least drug release of 79.82% as a result of high cross linking and F4 showed maximum 0f 96.50 due to loose networks. The *in vitro* drug release profiles are shown for Amoxicillin trihydrate and Metronidazole individually and in combination in [Fig.3 (a), 3(b), 3(c)].

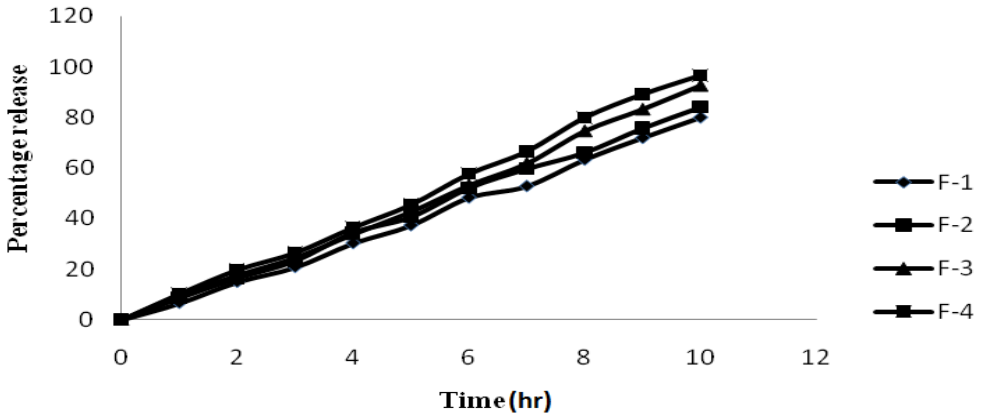

Fig.3(a) Graph showing in vitro release of (Amoxicillin trihydrate) formulations F-1 to F-4

The *in vitro* drug release profile for both the drugs from their formulations were almost similar. Hence it can be stated that release is independent of drug used. The release slowed down when concentration of cross-linking agent was kept high. The value of n determined from peppas koresmeyer equation was in the range of 0.7652-0.8293 which indicates the drug release followed non-fickian (relaxation controlled). Microspheres showed zero order release (R = 0.9985-0.9961).

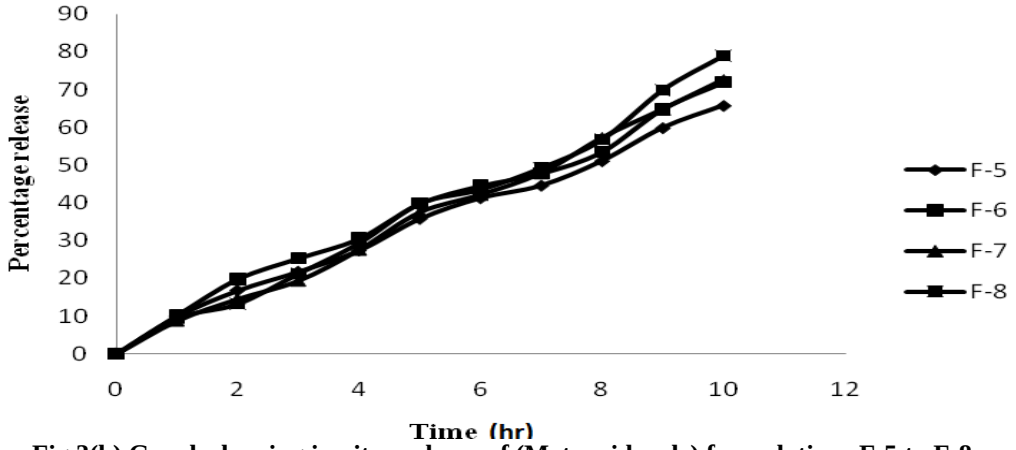

Fig.3(b) Graph showing in vitro release of (Metronidazole) formulations F-5 to F-8

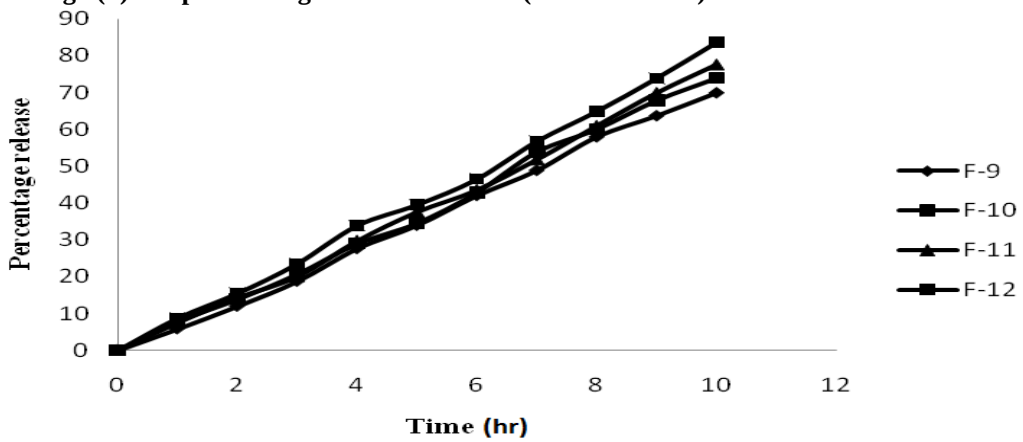

Fig.3(c) Graph showing in vitro release of (Amoxicillin trihydrate and Metronidazole) formulations F-9 to F-12.

3.6. Swelling studies

From the results of swelling studies, it was observed that in case of microspheres as the concentration of STPP was increased from 2% w/v - 4% w/v, the percentage swelling was decreased, which may be due to decrease in pore size leading to less penetration of water into the pores and hence reduced swelling which is depicted in [Fig.4].

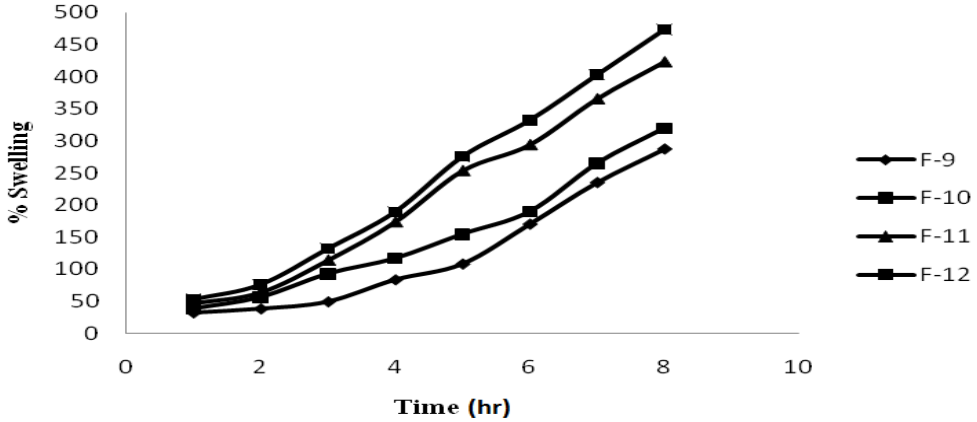

Fig.4. Graph showing percentage swelling of microspheres

3.7. In *vitro* wash- off test for mucoadhesion:

The mucoadhesive study showed that even at the end of 8 h 10% of microspheres were still adhering to the mucosa which shows that the prepared microspheres are having good mucoadhesion. The results are tabulated in [Table. 4].

Table 4: Percent of microspheres adhering to tissue

Formulations	Percent of microspheres adhering to tissue Time (in hours)				
	1hr	2hr	4hr	6hr	8hr
F-9	85	73	54	32	20
F-10	78	65	43	27	15
F-11	81	71	48	27	17
F-12	73	62	39	21	11

CONCLUSION

Mucoadhesive microspheres were prepared by a Ionic crosslinking complexation method. The prepared microspheres are spherical and have mooth surface. The drug loading was found to be satisfactory. *In vitro* drug release shows the sustained release of both drugs used and is dependent on the amount of STPP used. Swelling studies are also dependent upon the concentration of STPP used and in turn affect drug release. Microspheres exhibit good mucoadhesion. The results of this study indicate that it may be feasible to use chitosan mucoadhesive microspheres as a gastric retentive drug delivery system for treatment of Gastric ulcer. The release rate of the antimicrobial agents will be retarded due to the slower dissolution rate of the Chitosan and mucoadhesion. The combination of Amoxicillin trihydrate and Metronidazole can be used effectively in form of mucoadhsive microspheres for treatment of gastric ulcer.

Acknowledgements
The authors thank the JSS Mahavidyapeetha, Mysore and JSS University, Mysore for their valuable support to carry out this research.

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