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Research Article

Design, Characterization and Anti-ulcerogenic Effect of Amoxicillin trihydrate Thiolated Chitosan Mucoadhesive Microspheres for Effective Treatment of *H. pylori*

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ABSTRACT

Amoxicillin (α -amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in the standard eradication treatment of gastric and duodenal ulcers, which are associated with *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent. The aim of this study was to develop controlled release mucoadhesive microspheres of amoxicillin trihydrate for the treatment of gastric and duodenal ulcers caused by *Helicobacter pylori* (*H. pylori*) and evaluated anti-ulcerogenic effect of microsphere. Amoxicillin loaded thiolated chitosan microspheres were prepared by emulsifying method using liquid paraffin light and heavy in ratio of 50:50 as a dispersing medium and glutaraldehyde used as a cross-linking agent. The prepared microspheres were evaluated for mean particle size and particle size distribution, shape and surface morphology, drug content, mucoadhesion measurement and in-vitro drug release. FT-IR spectroscopic analysis was performed to ascertain drug polymer interaction. Antiulcer activity of amoxicillin microspheres was investigated on Indomethacin induced gastric ulcer model in Albino rats. The antiulcer capability of amoxicillin microspheres was compared to 100mg/kg cimetidine. Gastric ulcers were induced in Wistar albino rats by oral administration of indomethacin (5 mg/kg), antiulcer activity of amoxicillin microspheres (100, 200mg/kg, p.o) was observed on ulcer index and gastric pH. Statistical analysis was done by One-way ANOVA followed by Tukey's post hoc test. The release profiles showed Korsmeyer-Peppas release behavior up to 48 hours where the highest drug release was 76.7 \pm 3.52% of the amoxicillin loaded in the thiolated chitosan microspheres, indicating a strong crosslinking between chitosan and glutaraldehyde. Amoxicillin microspheres produced significant ($P < 0.05$) decrease in ulcer index and gastric pH as compared to control. The anti-ulcer effects of amoxicillin microspheres were dose dependent manner. From the results of the present investigation it may be concluded that drug loaded chitosan microspheres can be prepared by a simple technique which avoids the use of complex apparatus and special precautions and microsphere produced significant anti-ulcer effects in experimental models.

Keywords: Amoxicillin, *Helicobacter pylori*, Microspheres, Antiulcer activity, Indomethacin induced gastric ulcer model, Cimetidine,

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INTRODUCTION

Microsphere carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems¹⁻³. They have varied applications and are prepared using assorted polymers⁴. However, the success of these microspheres is limited, owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate

contact of the drug delivery system with the absorbing membranes⁵⁻⁸. This can be achieved by coupling mucoadhesion characteristics to microspheres and developing mucoadhesive microspheres. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site⁹⁻¹². One of the polymers included in the multifunctional polymer is chitosan. Chitosan has mucoadhesive properties, permeation-enhancers, and enzyme-inhibitor¹³. Chitosan obtained from chitin deacetylation resulting the free amino group that can make it be policationic¹⁴. Chitosan has been shown to have mucoadhesive properties due to electrostatic interactions

between positively charged chitosan and negatively charged mucosal surface. Chitosan has one primary amino group and two free hydroxyl groups for each monomer. Free amino group in chitosan is positively charged subsequently react with the surface/mucus are negatively charged¹⁵. Various modifications have been made to the existing mucoadhesive polymer resulting in better mucoadhesive properties. One modification is done is with the immobilization of thiol groups to mucoadhesive polymer so as to form disulfide bonds with cysteine-rich subdomains of mucus glycoproteins. Unlike the first generation mucoadhesive polymers attached to the mucus gel layer through noncovalent bonding, the new generation of mucoadhesive polymers capable of forming covalent bonds to the layer of mucus¹⁵. Modification of the thiol group attachment has also been made to the chitosan. This modification is based on the immobilization of thiol bearing movement on chitosan backbone, thus known as thiolated chitosan. This modification was developed to improve the solubility of chitosan, mucoadhesive property, and/or property of permeation¹⁶. Improved properties of mucoadhesive thiolated chitosan expected to increase the contact time of the drug in the gastrointestinal tract that it can increase the bioavailability of the drug. *Helicobacter pylori* (*H. pylori*), a Gram-negative human gastric bacterium, infects approximately 30-50% of adults in the developed world and over 90% of inhabitants in the developing world¹⁷. *H. pylori* normally cause a lifelong chronic gastritis and peptic ulcer disease. The infection plays an important role in peptic ulcer disease and gastric B-cell MALT (mucosa-associated lymphoid tissue) lymphoma and is associated with gastric adenocarcinoma¹⁸⁻²⁰ and it predicted that by 2020 to enter the top ten of leading causes of death worldwide²¹. The International Agency for Research and Cancer (IARC, USA) classified *H. pylori* as a group I carcinogen, a definite cause of human gastric cancers²². Amoxicillin (α -amino-hydroxybenzylpenicillin) is a semisynthetic antibiotic, belonging to the β -Lactam family, which is effective for bacterial infection treatment, especially for *H. pylori* infection²³. However, therapies using conventional oral amoxicillin capsules cannot completely eradicate *H. pylori* infections, allowing recolonization^{24,25}. The incomplete eradication of *H. pylori* is mainly due to the short residence time of antimicrobial agents in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists^{26,27}. The minimum inhibitory concentration of less than or equal to 0.01-0.1 mg/L determined *in vitro* implies that if successful local delivery were achieved, lower doses of antibiotic may be effective²⁸. It has therefore been proposed that local delivery could increase drug levels in the gastric mucus and mucosa to effective bactericidal levels and extend the contact time of drugs with the organism²⁹. Mucoadhesive drug carriers may prolong the residence time in the GI tract because they can adhere to the mucus surface, resulting in an effective localized drug concentration³⁰. The aim of this work was to prepare amoxicillin trihydrate loaded mucoadhesive microspheres using the mucoadhesive polymers for *H. pylori* eradication therapy and evaluated prepared microspheres for antiulcer activity.

MATERIALS AND METHODS

Materials

Amoxicillin trihydrate was obtained as gift sample from Sandoz Pharma Ltd. Mumbai, India. Thiolated Chitosan,

Cimetidine and Indomethacin were acquired from Sigma-Aldrich (St. Louis, MO, USA). Tween-80 and span-80 from Qualigens, Mumbai. Glacial acetic acids were purchased from Merck Specialties Pvt. Ltd., Mumbai, All other chemicals and reagent used was of analytical grade. Ultrapure water was used throughout the study.

Determination of absorption maxima

A solution of containing the concentration 30 μ g/ml was prepared in 0.1N HCl and methanol separately. UV spectrum was taken using double beam UV/VIS spectrophotometer (Labindia-3000+). The solution was scanned in the range of 200-400nm.

Preparation calibration curve

Accurately weighed 10 mg of drug was dissolved in 10 ml of 0.1N HCl and methanol solution in 10 ml of volumetric flask separately. The resulted solution 1000 μ g/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 0.1N HCl and methanol solution separately. Prepare suitable dilution to make it to a concentration range of 10-50 μ g/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). Linearity of standard curve was assessed from the square of correlation coefficient (r^2) which determined by least-square linear regression analysis.

Preparation of thiolated chitosan microsphere

Thiolated Chitosan was selected for preparing microsphere. Microspheres were prepared by emulsifying method using liquid paraffin light and heavy in ratio of 50:50 as a dispersing medium and glutaraldehyde used as a cross-linking agent. Thiolated chitosan dispersion (1.5 %w/v) was prepared by mixing of thiolated chitosan in glacial acetic acid (4%w/v) with Tween 80 (0.5% w/w). Drug was dissolved in chitosan solution. The prepared, 10 ml of thiolated chitosan solution with drug was added dropwise in a beaker containing 100 ml of liquid paraffin light and heavy in ratio of 50:50 containing Span 80 (1.0% w/v). The system was kept under stirring at 3000-4000 rpm using two blade mechanical stirrers. 1.5 ml of glutaraldehyde saturated toluene was added to above solution after 30 min of stirring. Stirring was continued for 4hr at 40°C at 4000 rpm. The microspheres were separated from dispersion medium by centrifugation and washed two times with petroleum ether to remove liquid paraffin and then washed three times with acetone. Dispersion was poured in petridish to remove acetone. After complete evaporation of acetone, dried drug loaded microsphere were collected and stored in tight container for further evaluation. The compositions of formulation were given in Table 1.

Optimization of process Variable

The effect of formulation process variables such as stirring time, stirring speed on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations. Different amoxicillin incorporated microspheres were prepared using effect of Thiolated chitosan, Tween-80, Span-80 quantity and stirring speed. The prepared microspheres were further evaluated for entrapment efficiency, particle size, zeta potential and *In Vitro* drug release study.

Table 1 Formulation optimization of microsphere

F. Code	Thiolated Chitosan (%w/v)	Tween-80 (%)	Span-80 (%)	Stirring Speed	Drug Conc. (% w/w)
TCM-1	0.5	0.5	0.5	2000	-
TCM-2	1.5	0.5	0.5	2000	-
TCM-3	2.0	0.5	0.5	2000	-
TCM-4	2.5	0.5	0.5	2000	-
TCM-5	1.5	1.0	0.5	2000	-
TCM-6	1.5	1.5	0.5	2000	-
TCM-7	1.5	2.0	0.5	2000	-
TCMS-8	1.5	1.0	1.0	2000	-
TCMS-9	1.5	1.0	1.5	2000	-
TCMS-10	1.5	1.0	2.0	2000	-
TCMS-11	1.5	1.0	2.5	2000	-
TCMS-12	1.5	1.0	1.5	2000	-
TCMS-13	1.5	1.0	1.5	3000	-
TCMS-14	1.5	1.0	1.5	4000	-
TCMD-15	1.5	1.0	1.5	4000	10
TCMSD-16	1.5	1.0	1.5	4000	20
TCMSD-17	1.5	1.0	1.5	4000	30
TCMSD-18	1.5	1.0	1.5	4000	40

Evaluation of microspheres

Measurement of mean particle size and polydispersity index

Average particles size of prepared microsphere was determined using particle size analyser (Malvern particle size analyser). The microsphere formulation was diluted with deionized water (1:9 v/v) and analysed for average size.

Shape and Surface characterization of microspheres by scanning electron microscopy (SEM)

From the formulated batches of microspheres, formulations (TCMSD-17) which showed a suitable balance among the percentage releases were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was practical in a high vacuum evaporator. The acceleration voltage was set at 10KV during scanning. Microphotographs were taken on dissimilar magnification and higher magnification (200X) was used for surface morphology.

Determination of drug content

The amount of drug entrapped in the microspheres was determined using a UV spectrophotometer. The weighed amount of the microspheres was incubated with 0.1 N HCl, pH 1.2, for 48 h. It was centrifuged at 10,000 g for 30 min and the supernatant was diluted 10 times before analysis into the UV spectrophotometer system at λ_{max} 238nm.

Mucoadhesion measurement study

Mucoadhesiveness of prepared microsphere was determined by taking a 5-6 cm length of piece obtained from freshly cut pig intestine which was procured from a local abattoir within 1 h after sacrificed of animal. It was washed with isotonic saline solution. The pig intestine piece was attached to a polyethylene plate and placed 10 mg of microspheres on the mucosal surface. Plate was positioned at 40° angle relative to the horizontal plane. The time required for shedding all the microspheres from mucosal surface was noted.

In Vitro drug release from microspheres

The drug release was performed in 0.1 N HCl (1.2 pH) for prepared microsphere using dialysis bag technique. In this study suspension of microsphere equivalent to 20 mg of drug was taken in dialysis tubing (MWCO, 15KDa, himedia) and placed in a beaker containing 50ml of 0.1 N HCl (1.2 pH). The dialysis bag retains microsphere and allows passing of free drug into the dissolution media. Temperature was maintained at 37±1°C throughout the study. The samples were withdrawn after specified time intervals that are 0.5, 1, 2, 3, 4, 8, 10 and 12hrs and replaced with the same volume of fresh 0.1 N HCl and analyzed for drug concentration by using UV spectrophotometer a λ_{max} 238nm³¹⁻³³.

Drug release kinetic data analysis

A number of kinetic models have been planned to explain the release characteristics of a drug from matrix. The next three equations are usually used, because of their simplicity and applicability. Equation 1, the zero-order model equation (Plotted as cumulative percentage of drug released vs time); Equation 2, Higuchi's square-root equation (Plotted as cumulative percentage of drug released vs square root of time); and Equation 3, the Korsmeyer-Peppas equation (Plotted as Log cumulative percentage of drug released vs Log time). To study the release kinetics of stavudine from the mucoadhesive microspheres the release data was fitted to these three equations.

Zero order equation

When a graph of the cumulative percentage of the drug released from the matrix against time is plotted, zero order release is linear in such a plot, indicating that the release rate is independent of concentration.

$$Q_t = k_0.t \dots\dots\dots (1)$$

Where Q_t is the percentage of drug released at time t and k_0 is the releaserate constant;

First order equation

$$\ln (100-Q_t) = \ln 100 - k_1.t \dots\dots\dots (2)$$

Where k_1 is the release rate constant;

Higuchi's equation

$$Q_t = k_H.t^{1/2} \dots\dots\dots (3)$$

Where k_H is the Higuchi release rate constant

Korsmeyer-Peppas

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsmeyer's equation.

$$Q_t/Q_\infty = k_{KP} \cdot t^n$$

Where Q_t/Q_∞ is the fraction of drug released at time t , k_{KP} constant comprising the structural and geometric characteristics of the device and n is the release exponent. The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When $n = 1$, the release rate is independent of time (typical zero order release / case II transport); $n = 0.5$ for Fickian release (diffusion/ case I transport); and when $0.5 < n < 1$, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when $n > 1.0$ super case II transport is apparent. 'n' is the slope value of $\log M_t/M_\infty$ versus \log time curve³⁴.

Anti-Ulcer Activity

Animals

30 Wistar rats of either sex weighing between 150-250 g were used for the study. Prior to the experiments, the rats were kept in the central animal house in rat cages and were given standard rat feed with water ad libitum. Cages were fitted with wire mesh floor to avoid coprophagy.

Experimental procedures

Indomethacin induced gastric ulcer

Rats were divided into 4 groups of 6 each, for indomethacin induced gastric ulcers.

Group -1: Control

Group -2: Cimetidine (Standard)

Group -3: Amoxicillin microspheres (100mg/kg, p.o.)

Group -4: Amoxicillin microspheres (200mg/kg, p.o.)

The animals were fasted for 24 h prior to the experiment. Under anaesthesia, ulcers were induced by applying indomethacin (5 mg/kg, p.o.) over the anterior serosal surface of the stomach for 60 seconds. The animals were treated with cimetidine (100 mg/kg, p.o.), low dose of amoxicillin microspheres (100 mg/kg p.o.) or high dose of amoxicillin microspheres (200 mg/kg p.o.) [Once daily, for 5 days after the induction of ulcer, while the control group received only the vehicle]. The rats were sacrificed on the 5th day, the stomachs removed and cut open along the greater curvature³⁵. The ulcer index was determined using the formula:

$$\text{Ulcer index} = 10/X$$

Where X = Total mucosal area/Total ulcerated area.

Based on their intensity, the ulcers were given scores as follows:

0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

Statistical Analysis

The data is expressed as mean \pm Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Tukey's post hoc test. Differences were considered as statistically significant at $P < 0.05$, when compared with control.

RESULTS AND DISCUSSION

The λ_{\max} of Amoxicillin was found to be 234 and 238 nm by using U.V. spectrophotometer (Labindia-3000+) in linearity range 10-50 μ g/ml in methanol and 0.1N HCl respectively Figure 1-4. The Particle size of different formulations was in range of 5.37 \pm 2.16-58.15 \pm 4.25 μ m. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of amoxicillin microspheres Table 2. The amount of drug entrapped in the microspheres was determined using a UV spectrophotometer. The weighed amount of the microspheres was incubated with 0.1 N HCl, pH 1.2, for 48 h. It was centrifuged at 10,000 g for 30 min and the supernatant was diluted 10 times before analysis into the UV spectrophotometer system at λ_{\max} 238 nm Table 2. The results of mucoadhesiveness of prepared microsphere were given in Table 2. Shape and surface characteristic of amoxicillin microspheres examine by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnifications 55X which illustrate the smooth surface of microspheres Figure 5. The drug release rate from mucoadhesive microspheres was passed out using the USP type II (Electro Lab.) dissolution paddle instrument. A weighed amount of mucoadhesive microspheres equivalent to 20 mg drug were dispersed in 900 ml of 0.1 N HCl (pH=1.2) maintained at 37 \pm 0.5 $^\circ$ C and stirred at 55rpm. The release study of optimized formulation TCMSD-17 was given in Table 3 and Figure 6. The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order, Higuchi and Korsmeyer-Peppas kinetic models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of Korsmeyer-Peppas was maximum i.e 0.926 hence indicating drug release from formulations was found to follow Korsmeyer-Peppas release kinetics Table 4, 5 and Figure 7-10. Amoxicillin microspheres (200 mg/kg, p.o.) produced significant ($P < 0.01$) increase in the gastric pH as compared to the control in a models of gastric ulcer. A significant ($P < 0.05$) reduction in ulcer index was also observed in indomethacin induced ulcers. However, these effects were less or equal than as compared to cimetidine. In the stomach, prostaglandins and nitric oxide play a crucial protective role of stimulating the secretion of bicarbonate and mucus³⁶, maintaining mucosal blood flow and regulating mucosal cells turnover and repairs³⁷. Therefore, suppression of prostaglandin synthesis by non-steroidal anti-inflammatory drugs like indomethacin results in increased susceptibility to gastric mucosal lesions and mucosal injury^{38,39} which were observed in indomethacin control. Amoxicillin microspheres at concentration of 200mg/kg significantly protected the mucosa from being damaged by indomethacin suggesting that amoxicillin mimicking prostaglandin and nitric oxide Table 6 and Figure 11, 12.

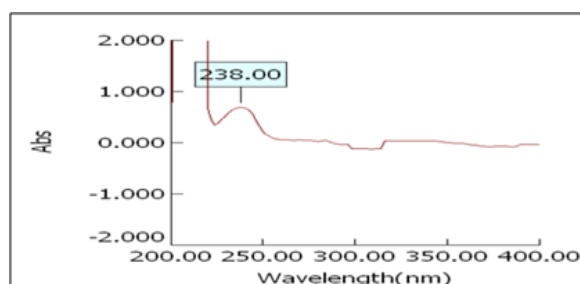


Figure 1 Wavelength maxima of amoxicillin trihydrate in 0.1 N HCl

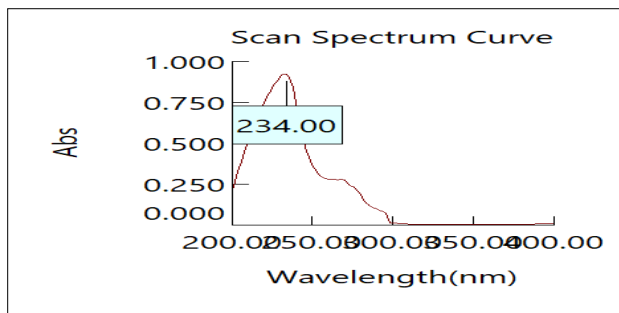


Figure 2 Wavelength maxima of amoxicillin trihydrate in methanol

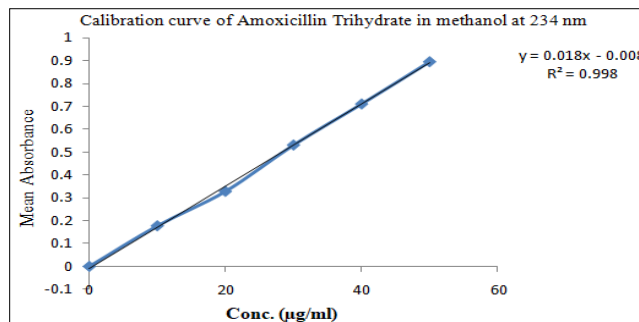


Figure 4 Calibration curve of amoxicillin trihydrate in methanol at 234 nm

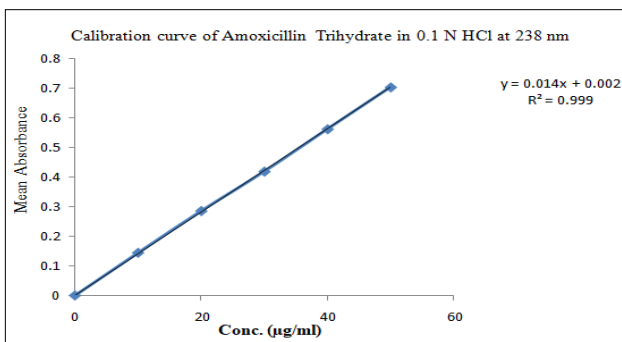


Figure 3 Calibration curve of amoxicillin trihydrate in 0.1 N HCl at 238 nm

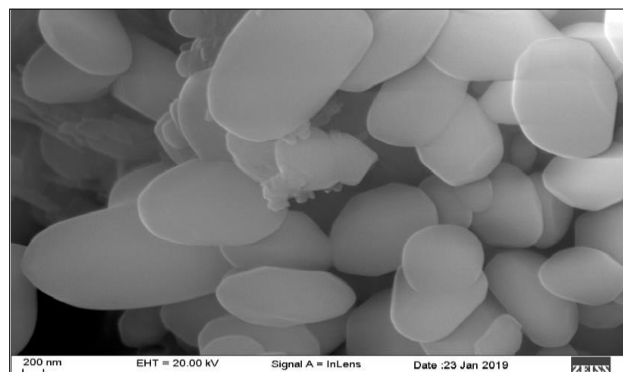


Figure 5 SEM Photomicrographs of optimized formulation TCMSD-17

Table 2 Evaluation of amoxicillin trihydrate mucoadhesive microspheres

F. Code	PDI	Particle size (µm)	% Drug Entrapment	Mucoadhesion time (hr.)
TCM1	0.31±0.01	26.70±2.23	-	
TCM2	0.46±0.02	34.34±3.45	-	
TCM3	0.55±0.02	41.62±1.46	-	
TCM4	0.43±0.03	58.15±3.41	-	
TCM5	0.26±0.01	32.45±2.12	-	
TCM6	0.34±0.02	28.56±4.56	-	
TCM7	0.41±0.03	17.36±2.32	-	
TCM8	0.35±0.02	18.62±3.12	-	
TCM9	0.13±0.01	14.62±2.14	-	
TCM10	0.32±0.03	13.54±1.74	-	
TCM11	0.37±0.01	12.32±2.32	-	
TCM12	0.23±0.04	11.63±2.14	-	
TCM13	0.25±0.05	10.54±3.65	-	
TCM14	0.12±0.02	6.37±2.12	-	
TCMSD15	0.25±0.03	8.63±3.21	58.63±3.52	7.2±0.05
TCMSD16	0.26±0.04	6.54±2.14	64.54±2.12	7.1±0.02
TCMSD17	0.29±0.02	5.37±3.78	67.37±2.16	6.9±0.05
TCMSD18	0.18±0.03	6.37±2.41	67.62±3.43	6.5±0.04

Table 3 In vitro drug release of optimized formulation TCMSD17

S. No.	Time interval (h)	Plain drug	Amoxicillin Trihydrate Loaded Microsphere
1	0.5	26.6±1.63	5.4±0.15
2	1	39.4±2.38	8.5±0.83
3	2	62.8±2.35	12.6±0.52
4	3	86.4±3.26	18.7±1.05
5	4	99.5±3.37	26.4±1.25
6	5	-	34.2±1.23
7	6	-	42.4±2.09
8	8	-	51.4±2.34
9	12	-	60.5±3.52
10	24	-	68.3±2.25
11	48	-	76.7±3.52

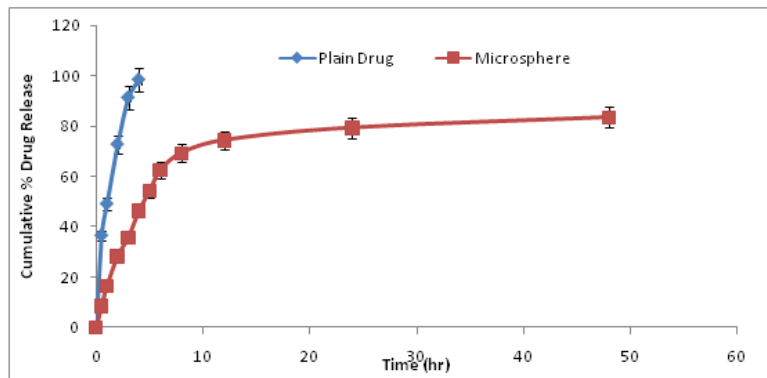


Figure 6 cumulative % amoxicillin trihydrate releases

Table 4 *In-vitro* drug release data for optimized formulation TCMSD-17

Time (h)	Square Root of Time(h) ^{1/2}	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	5.4	0.732	94.6	1.976
1	1.000	0.000	8.5	0.929	91.5	1.961
2	1.414	0.301	12.6	1.100	87.4	1.942
3	1.732	0.477	18.7	1.272	81.3	1.910
4	2.000	0.602	26.4	1.422	73.6	1.867
5	2.236	0.699	34.2	1.534	65.8	1.818
6	2.449	0.778	42.4	1.627	57.6	1.760
8	2.828	0.903	51.4	1.711	48.6	1.687
12	3.464	1.079	60.5	1.782	39.5	1.597
24	4.899	1.380	68.3	1.834	31.7	1.501
48	6.928	1.681	76.7	1.885	23.3	1.367

Table 5 Regression analysis data

Batch	Zero Order	First Order	Higuchi	Korsmeyer-Peppas
	r ²	r ²	r ²	r ²
TCMSD-17	0.675	0.821	0.865	0.926

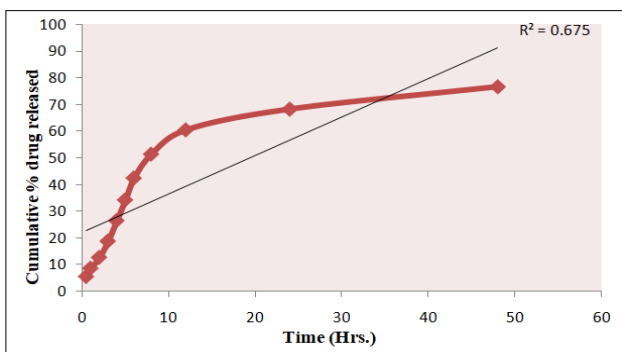


Figure 7 Zero order release kinetics

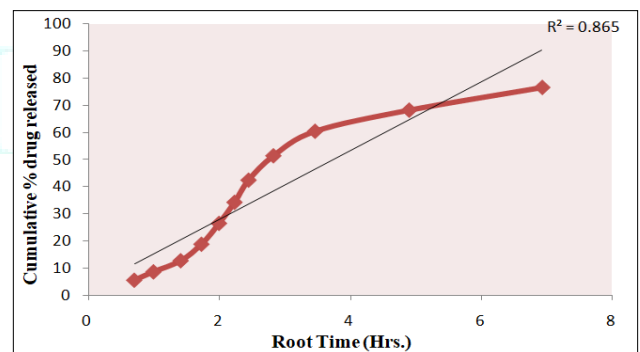


Figure 9 Higuchi release kinetics

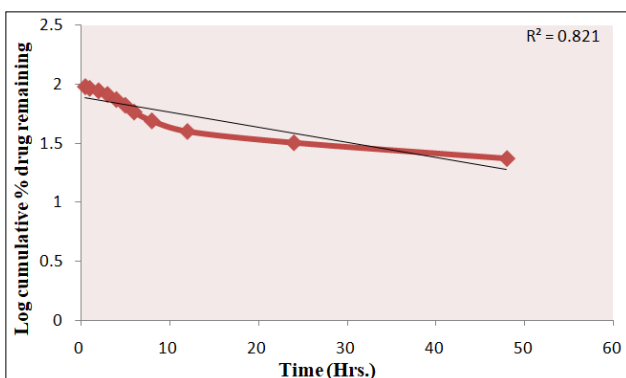


Figure 8 First order release kinetics

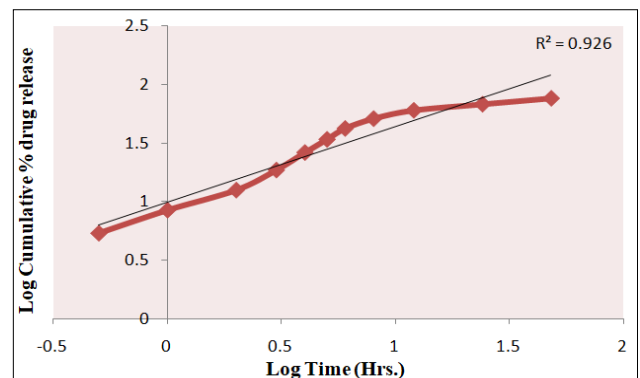
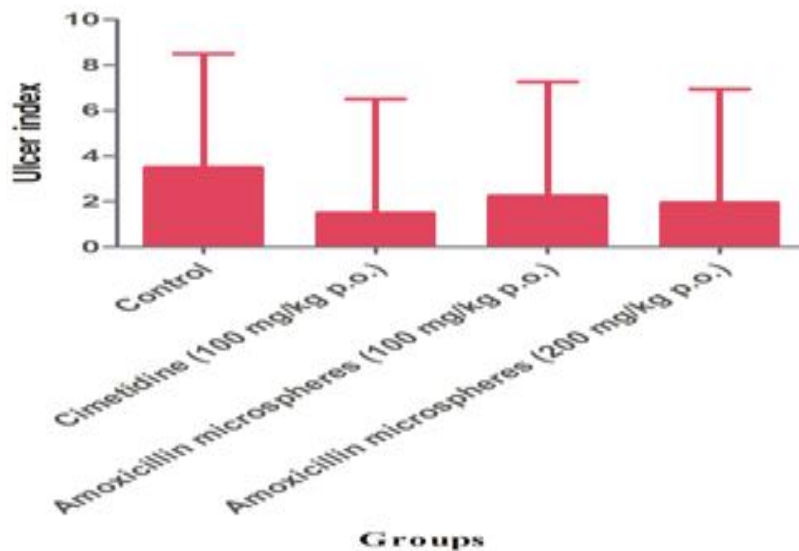
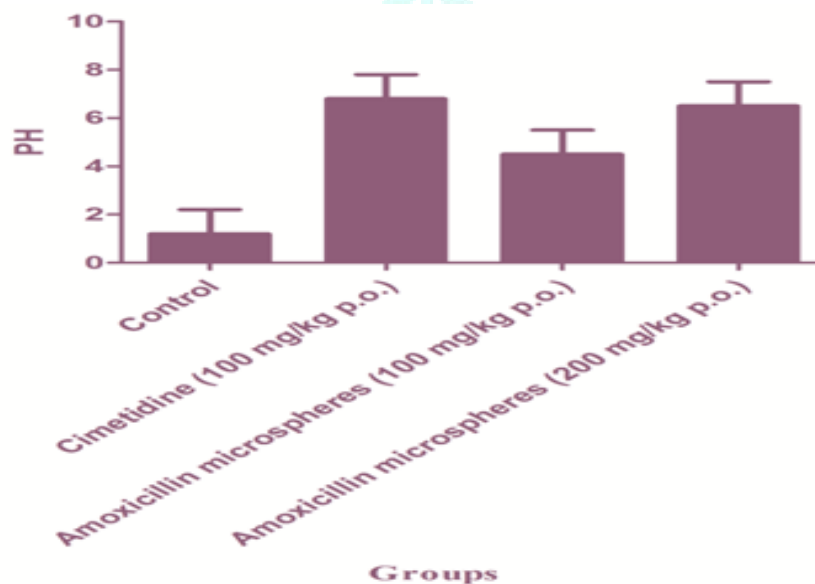


Figure 10 Korsmeyer-Peppas release kinetics

Table 6 Anti-ulcerogenic effect of amoxicillin microspheres against ulcerogenic agents in rats

Treatment and dose	Ulcer index	pH
Control	3.50 ± 5.0	1.20 ± 1.0
Cimetidine (100 mg/kg, p.o.)	1.50 ± 5.0***	6.80 ± 1.0***
Amoxicillin microspheres (100 mg/kg, p.o.)	2.25 ± 5.0**	4.50 ± 1.0*
Amoxicillin microspheres (200 mg/kg, p.o.)	1.95 ± 5.0***	6.50 ± 1.0***

Values are expressed as mean±S.E.M. (n = 6).Percent inhibition calculated as compared to control group.*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (One-way ANOVA followed by Tukey's post hoc test).

**Figure 11** Anti-ulcerogenic effect of amoxicillin microspheres against ulcerogenic agents in rats (Ulcer index)**Figure 12** anti-ulcerogenic effects of amoxicillin microspheres against ulcerogenic agents in rats (pH)

CONCLUSION

From the above experimental results, it can be concluded that oral controlled release of amoxicillin from microspheres can be achieved by emulsifying method using thiolated chitosan as polymer and glutaraldehyde used as a cross-linking agent. The IR spectra's revealed that, there was no interaction between polymer and drug. The entire polymer used was compatible with the drug. Prepared microspheres

exhibited Korsmeyer-Peppas release kinetics. The present study demonstrates the ulceroprotective effects of amoxicillin microspheres in Indomethacin-induced gastric ulcer model in rats by dose dependent manner. From the study, it is evident that a promising controlled release micro-particulate drug delivery of amoxicillin can be developed. Further, *in-vivo* investigation is required to establish efficacy of these formulations.

REFERENCES

1. Gohel MC, Amin AF. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. *J Contr Rel* 1998; 51:115-22.
2. Woo BH, Jiang G, Jo YW, DeLuca PP. Preparation and characterization of a composite PLGA and poly (acryloyl hydroxymethyl starch) microsphere system for protein delivery. *Pharm Res* 2001; 18:1600-6.
3. Capan Y, Jiang G, Giovagnoli S, DeLuca PP. Preparation and characterization of poly (D,L-lactide-co-glycolide) microsphere for controlled release of human growth hormone. *AAPS PharmSciTech*. 2003; 4:28.
4. Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. *Int J Pharm* 2003; 255:13-32.
5. Ikeda K, Murata K, Kobayashi M, Noda K. Enhancement of bioavailability of dopamine via nasal route in beagle dogs. *Chem Pharm Bull* 1992; 40:2155-8.
6. Schaefer MJ, Singh J. Effect of isopropyl myristic acid ester on the physical characteristics and in-vitro release of etoposide from PLGA microspheres. *AAPS PharmSciTech*. 2000; 1: 32.
7. Govender S, Pillay V, Chetty DJ, Essack SY, Dangor CM, Govender T. Optimisation and characterisation of bioadhesive controlled release tetracycline microspheres. *Int J Pharm* 2005; 306:24-40.
8. Patel JK, Patel RP, Amin AF, Patel MM. Formulation and evaluation of mucoadhesive glipizide microspheres. *AAPS PharmSciTech*. 2005; 06:E49-55.
9. Lehr CM, Bouwstra JA, Schacht EH, Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int J Pharm* 1992; 78:43-8.
10. Henriksen L, Green KL, Smart JD, Smistad G, Karlsen J. Bioadhesion of hydrated chitosans: An in vitro and in vivo study. *Int J Pharm* 1996; 145:231-40.
11. Rao SB, Sharma CP. Use of chitosan as biomaterial: Studies on its safety and hemostatic potential. *J Biomed Mater Res* 1997; 34:21-8.
12. Chowdary KPR, Rao YS. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. *AAPS Pharm Sci Tech*. 2003; 4: 39.
13. Vigil C. Multifunctional Polymeric Excipients in Oral Macromolecular Drug Delivery in Oral Delivery of Macromolecular Drugs, Andreas Bernkop-Schnurch (editor), Springer Dordrecht Heidelberg London New York, 2009; p.137-152.
14. Khan, T.A., Peh, K.K., dan Ch'ng, H.S., 2002, Reporting degree of deacetylation values of chitosan: the influence of analytical methods, *J Pharm Pharmaceut Sci* 5(3):205-212.
15. Bernkop-Schnurch A, Guggi D, Dan Pinter Y. Thiolated chitosans: development and in vitro evaluation of a mucoadhesive, permeation enhancing oral drug delivery system. *J Control Rel* 2004; 9: 177-186.
16. Bernkop-Schnurch A. Thiomers: A new generation of mucoadhesive polymers, *Advanced Drug Delivery Reviews* 2005; 57: 1569-1582.
17. Kleantous H, lee CK, Monath TP. Vaccine development against infection with *Helicobacter pylori*. *Br Med Bull* 1998; 54:229-41.
18. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; 1:1311-5.
19. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991; 302:1302-5.
20. Wotherspoon AC, Ortiz-hidalgo C, Falzon MR, Isaacso PG. *Helicobacter pylori* associated gastritis a primary B-cell gastric lymphoma. *Lancet* 1991; 338:1175-6.
21. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997; 349:1498-504.
22. Schistosomes, liver flukes and *Helicobacter pylori*. IARC working group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994; 61:1-241.
23. Risbud MV, Hardilar AA, Bhat SV, Bhonde KR. pH sensitive freeze-dried chitosan-polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. *J Control Rel* 2000; 68:23-30.
24. Kawabami E, Ogata SK, Portorreal AC, Magni AM, Pardo ML, Patrício FR. Triple therapy with clarithromycin, amoxicillin and omeprazole for *Helicobacter pylori* eradication in children and adolescents. *Arq Gastroenterol* 2001; 38:203-6.
25. Lin CK, Hsu PI, Lai KH, Lo GH, Tseng HH, Lo CC, et al. One-week quadruple therapy is an effective salvage regimen for *Helicobacter pylori* infection in patients after failure of standard triple therapy. *J Clin Gastroenterol* 2002; 34:547-51.
26. Cooreman MP, Krausgrill P, Hengels KJ. Local gastric and serum amoxicillin concentrations after different oral application forms. *Antimicrob Agents Chemother* 1993; 37:1506-9.
27. Atherton JC, Cockayne A, Balsitis M, Kirk GE, Hawley CJ, Spiller RC. Detection of the intragastric sites at which *Helicobacter pylori* evades treatment with amoxicillin and cimetidine. *Gut* 1995; 36:670-4.
28. Hirschl AM, Rotter ML. Amoxicillin for the treatment of *Helicobacter pylori* infection. *J Gastroenterol* 1996; 31 (9):44-7.
29. Conway BR. Drug delivery strategies for the treatment of *Helicobacter pylori* Infections. *Curr Pharm Des* 2005; 11:775-90.
30. Nagahara N, Akiyama Y, Nakao M, Tada M, Kitano M, Ogawa Y. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. *Antimicrob Agents Chemother* 1998; 42:2492-4.
31. Kalyankar TM, Nalanda T. Formulation and Evaluation of Mucoadhesive Pioglitazone Hcl Microspheres. *Int J Pharma World Res* 2010; 1(3):1-14.
32. Gavini V, Ganesh NS, Joshi H, Jayanthi C. Formulation and evaluation of mucoadhesive microspheres of macromolecular polymers using flurbiprofen as model drug. *Der pharmacia Lettre* 2012; 4(5): 1560-1566.
33. Nagahara N, Akiyama Y, Nakao M, Tada M, Kitano M, Ogawa Y. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. *Antimicrob Agents Chemother* 1998; 42: 2492-2494.
34. Brahmankar D M and Jaiswal S B. *Biopharmaceutics and Pharmacokinetics: A Tretise*, Vallabh Prakashan, New Delhi, 1st edition, 2006, 335-357.
35. Vogel GH, Vogel WH, Scholkens BA, Sandow J, Muller G, Vogel WF. J.3.7. Antiulcer activity. Drug discovery and evaluation. *Pharmacological assays*. 2nd ed. Berlin: Springer-Verlag; p.867-71.
36. Archana J, Sakat SS, Preeti T. Gastroprotective Effect of *Oxalis corniculata* (Whole Plant) on Experimentally Induced Gastric Ulceration in Wistar Rats. *Indian J Pharma Sci* 2012; 74(1):48-53.
37. Hayllar J, Bjarnason I. NSAIDs, COX-2 inhibitors, and the gut. *Lancet* 1995; 346:521-522.
38. Malfertheiner P, Chan FKL, McColl KEL. Peptic ulcer disease. *Lancet* 2009; 374:1149-1161.
39. Kushima H, Nishijima CM, Rodrigues CM, Rinaldo D, Sassa MF, Bauab TM et al. Gastroprotective, anti-inflammatory immunomodulatory and anti-*Helicobacter pylori* action. *J Ethnopharmacol* 2009; 123:430-438.