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Preparation and Evaluation of Amoxicillin and Clarithromycin Loaded Chitosan Mucoadhesive Microspheres

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ABSTRACT
Peptic ulcer is a chronic disorder which is affecting millions of peoples all around the globe. One of the major factor which contributes to the formation of ulcer is Helicobacter pylori, which is a very highly resistant pathogen. The conventional treatment procedure has several drawback such as low permeability, low stability in acidic pH which leads to antibiotic failure. Novel delivery system can be employed as a potential solution of these drawbacks. In this present work amoxicillin and clarithromycin loaded chitosan microspheres were prepared and evaluated. The prepared microspheres were found to be capable of delivering of the drugs at the target site at a control rate.
Keywords: *Amoxicillin, Clarithromycin, Mucoadhesive, Ulcer, Microsphere*

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INTRODUCTION

Peptic ulcers are sores that form in the lining of the stomach, lower esophagus or small intestine. A mucosal rupture in the stomach or duodenum, measuring at least 3–5 mm and deep enough to be visible by endoscopy, is a characteristic and common symptom of peptic ulcer disease[1,2]. Gastric ulcer and duodenal ulcer are the two most common types of peptic ulcer. *H. pylori* and NSAID use are the two main risk factors for Peptic ulcer. *H. pylori* colonized over half of the world's population. The organism is frequently acquired in young age and remains until it is treated. Lower socioeconomic position, filthy conditions, and overcrowding are all risk factors for infection. *H. pylori* infection is more common in developing nations and among particular ethnic groups[3].Antibiotic therapy failures against the bacteria have been documented, which may be due to the antibiotics low permeability across the cell membrane, the drug's low stability in the stomach's pH, or subtherapeutic level after oral administration can be the cause[4].Clarithromycin, metronidazole, amoxicillin, and levofloxacin are some of the most commonly used medicines for H. pylori eradication. In the recent decades the resistance against these antibiotics has risen steadily. Antibiotic resistance in *H. pylori* is influenced by a variety of parameters, including age and gender, as well as host illness status and pathogen virulence factors[5].Besides antibiotics resistance other issues such as drug breakdown in the gastrointestinal environment, low oral bioavailability, and lack of vectorization to the target region, can reduce the efficacy of antibiotic therapy. As a result, the adoption of techniques to improve the efficacy of these traditional medications becomes intriguing. Drug delivery systems are now being investigated as a way to overcome drug therapy disadvantages such as antimicrobial resistance, low bioavailability, molecular breakdown in an acidic environment, and low drug concentration at the site of action [6].

Targeted drug delivery or site-specific delivery being used to improve therapy reliability, drug stability in the biological system, cost effectiveness, avoiding adverse effects, and reducing the emergence of drug resistance. The use of a bioadhesive polymer to extend the contact time of a drug with a body tissue has been shown to increase the efficiency of many drugs. These enhancements range from enhanced local pathology care to improved medication bioavailability and controlled release to increase patient enforcement. It would be beneficial to develop a novel drug delivery system that targets the antibiotic at the site of infection to achieve bactericidal concentrations[7].Gastro retentive drug delivery system (GRDDS) is one of the novel approach through which hepatic first-pass metabolism and enzymatic degradation in the GI microflora can be avoided, resulting in prompt and regulated release action[8].

Mucoadhesive drug delivery is one type of GRDDS which has been studied extensively for improving the bioavailability and retention time of drugs in the upper GIT[9].Chitosan, a D-glucosamine polymer derived from chitin, is readily available and exhibits excellent bioadhesion, biodegradability, and biocompatibility with no immunogenicity, toxicity, or side effects[10]. In the present study, chitosan mucoadhesive microspheres loaded with Amoxicillin and Clarithromycin were prepared, as the mentioned drugs were established to produced better therapeutic effect against the *H. pylori* when administered in combination. Further characterization of prepared microspheres was performed to evaluate its potential for the effective delivery of the drugs at the site of action.

MATERIAL AND METHODS

Materials

Amoxicillin and clarithromycin obtained from Yarrow chem. Product, Mumbai. All the chemicals used were of analytical grades.

Methods

Drug –excipients compatibility study

The compatibility study was performed by FT-IR and DSC

Preparation of chitosan microspheres

Microspheres were prepared by simple emulsification phase separation technique[11-14]. Weigh amount chitosan and 750 mg of drug was dissolved in 40 ml 2% acetic acid. The drug-polymer dispersion was added in a 120 ml liquid paraffin containing 1.5 ml span 80 and it was stirred with the help of magnetic stirrer, then gluteraldehyde was added and stirred continuously for 2 hours. Suspension of chitosan microspheres in paraffin oil, thus obtained, it was then allowed to stand for 20 minutes so that the microspheres settle down under gravity. Supernatant liquid was decanted then filtered. Microspheres were washed several times with solvent n-hexane to remove traces of the oil. They were finally washed with water to remove excess gluteraldehyde. The microspheres were dried at 40°C for 24 hours. The batch specification of the prepared microspheres is mentioned in table-1.

Characterization of chitosan microspheres

Percent yield: Weight of the prepared microspheres was weighed and % yield was calculated using the following equation:

Percent yield=Practical yield × 100/Theoretical yield

Practical yield=Amount of the microspheres collected after the experimental method

Theoretical yield=Total amount of the drug and the excipients used in the preparation.

Table 2 shows the % yield of the recovered microspheres.

Particle size analysis: Particle size analysis of the prepared microspheres was performed using compound microscope by calibrating eye piece micrometer. Table 2 shows the particle size of the prepared formulations with different drug: polymer ratios.

Density

a) Bulk density: Bulk density (ρb) (g/cm3) = M/Vb; Where, M = mass of powder taken, Vb = bulk volume;

b) Tapped density:

Tapped density (ρt) (g/ cm3) = M/Vt; Where, M = weight of sample powder, Vt = tapped volume

Flow properties

Where, ρt =Tapped density, ρb = bulk density

Angle of repose is determined by using funnel method. Microspheres were accurately weighed and taken in a funnel and then height of funnel is adjusted in such as way that the tip of funnel just touches the top of heap of blends. The microspheres are allowed to flow through funnel freely on to surface. The diameter of powder cone is measured and angle of repose is calculated by using following equation:

$\tan\theta = h/r$; θ - Angle of repose, h -height of pile, r - Radius of base.

Entrapment efficiency: 25 mg of drug loaded chitosan microspheres were taken in 25 ml of methanol and kept under continuous stirring for about 24 h and then the sample was centrifuged for 10 min at 2000 rpm and the supernatant layer was separated and analyzed using UV-visible spectrophotometer. Entrapment efficiency of the microspheres was calculated using the formula.

% Entrapment efficiency=Practical drug loading × 100/Theoretical drug loading

***In vitro* drug release study:** A total of 100 mg equivalent chitosan microparticle were weighed and filled in the empty capsule shells. Dissolution tests were performed in a USP Dissolution Tester Apparatus II (Paddle method) at 37 ± 0.5°C. The paddles were rotated 50 rpm speed. The dissolution medium consisted of acidic buffer pH 1.2 (900 ml). Aliquots of 5 ml were withdrawn at different time intervals,

filtered through Whatman filter paper and the content of drugs were determined spectrophotometrically by simultaneous estimation method using ultraviolet (UV) spectrophotometer.

Surface topography by scanning electron microscopy (SEM)

SEM of the microspheres shows the surface morphology of the microspheres like their shape and size.

Ex-Vivo Mucoadhesion Study

The mucoadhesive property of the prepared chitosan microspheres was evaluated on goat intestinal mucosa. 20 mg of microspheres are spread onto wet rinsed tissue specimen and immediately thereafter the slides are hung onto the arm of a USP tablet disintegrating test machine contain simulated gastric fluid (pH 1.2) with suitable support at 37˚C. The weight of microspheres leached out after 1 hour is measured [12]. The percentage mucoadhesion is calculated by the following equation

% mucoadhesion= Wa-W1/Wa x100

Where, Wa is the weight of microspheres applied W1 is the weight of microspheres leached out

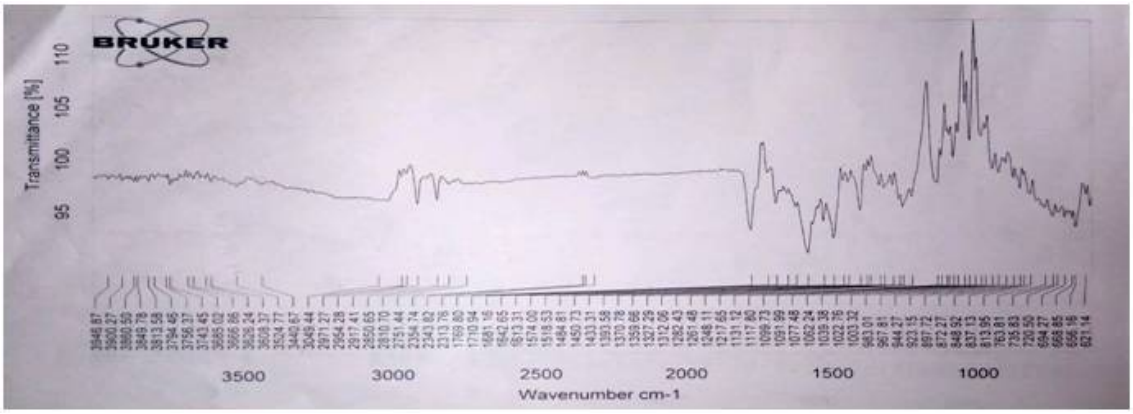

Fig 1: IR spectrum of drugs and polymer mixture

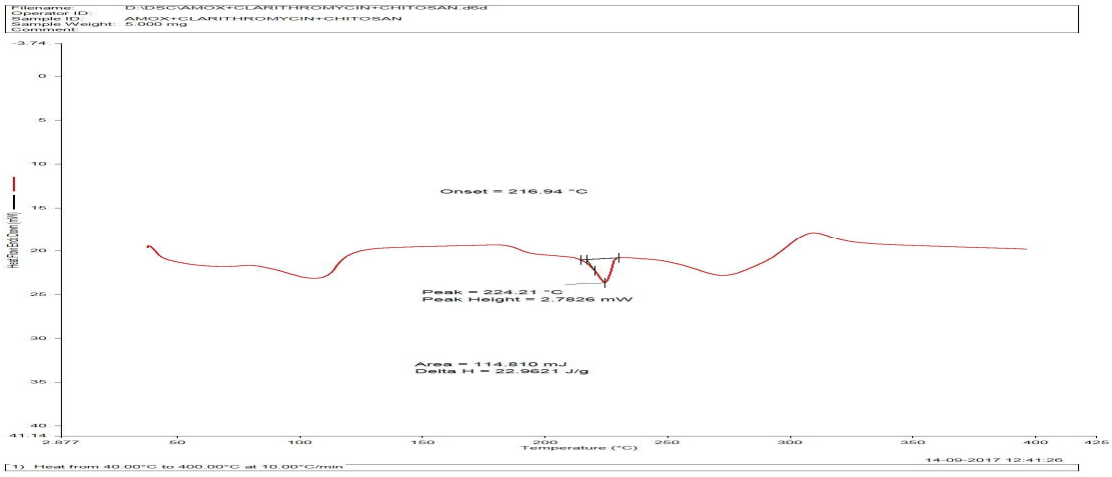

Fig 2: DSC thermogram of drugs and polymer mixture

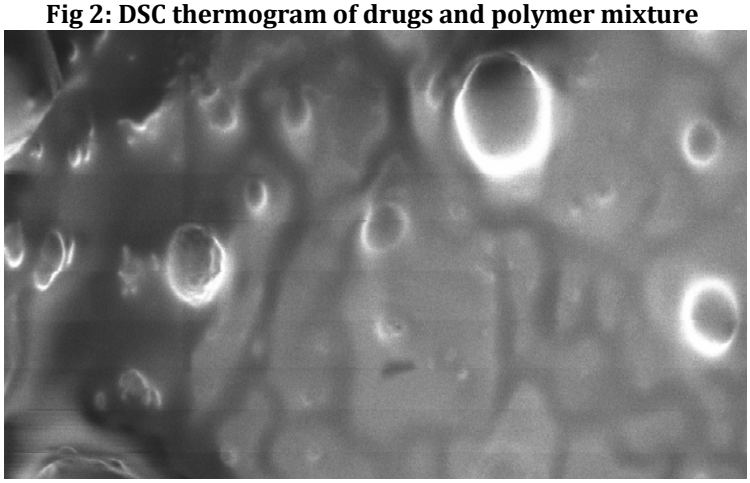

Fig 3: SEM image of prepared microsphere

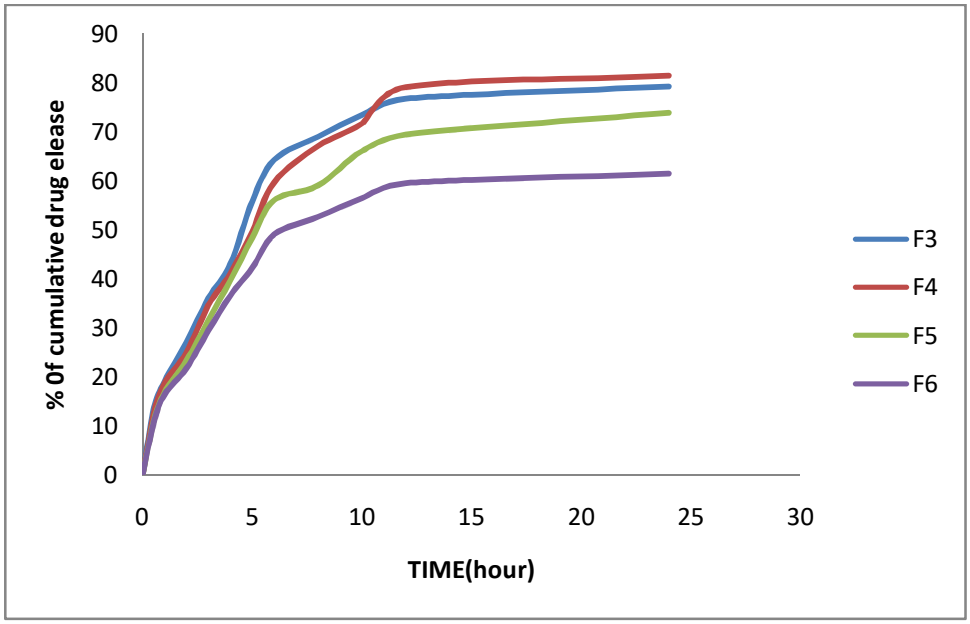

Fig 4: Drug release study

Table 1 Batch specification for chitosan microspheres

Formulation Code	Drug polymer ratio	Cross linking agent (ml)	Rotational speed(RPM)
F1	1:1	5	2000
F2	1:1	10	2000
F3	1:2	5	2000
F4	1:2	10	2000
F5	1:3	5	2000
F6	1:3	10	2000

Table 2 Results of physical evaluation of the prepared microspheres

Formulations	Avg. Particle size (µm)	% yield	% Drug entrapment efficiency	% mucoadhesion
F1	112	46.89	43.07	46
F2	101	51.22	47.4	49
F3	141	83	74.9	51
F4	134	89.2	81	49
F5	188	76.3	84.3	43
F6	179	78.8	83.2	47

Table 3 Flow properties of magnetic microspheres (as per USP30-NF25 specifications)

Formulation	F1	F2	F3	F4	F5
Bulk density	0.61±0.22	0.70±0.64	0.65±0.15	0.69±0.48	0.68±0.3
Tapped density	0.70±.55	0.76±0.32	0.71±0.44	0.76±0.62	0.75±0.23
Hausner ratio	1.14	1.08	1.09	1.10	1.10
Carr'SIndex(%)	12.23	11.34	8.45	9.21	9.33
Angle of repose	32.20	24.01	25.78	27.2	29.74

Table 4 Drug release from the microspheres

Time (hours)	% cumulative drug release			
	F3	F4	F5	F6
0.5	13.33	12.34	10.87	10.55
1	19.11	18.7	16.9	16.45
2	27.03	25	23.33	21.76
3	36.11	34.6	31.58	29.5
4	43	41.09	39.71	36.6
5	55.87	49.67	48.47	42.21
6	64.34	59.7	56.11	49.08
24	79.3	81.45	73.94	61.47

RESULT

Drug polymer compatibility study

IR spectral analysis of amoxicillin shows peaks at 1770 cm^{-1} (c=o str), 3357 cm^{-1},3443 cm^{-1}(N-H str), 3564 cm^{-1}(O-H str), 3154 cm^{-1}(N-H str, secondary amide) confirming the purity of amoxicillin . IR spectrum of clarithromycin shows peak at 1068 cm^{-1},1106 cm^{-1}(C-O-C str), 1455 cm^{-1}(C-H_2str),2875 cm^{-1}, 2937 cm^{-1}(C-H str),3465 cm^{-1}(O-H str) confirm the purity of the clarithromycin. In the IR spectrum of the drug and polymer physical mixture (figure 1) showed the peaks of pure drugs as well as the excipients with some variation in the same range indicating no interaction.

The DSC thermogram of Amoxicillin and clarithromycin showed endothermic peak at 93.71°C and 229.01°C with onset of 188.38°C and 224.17°C which is attributed to its melting point. The physical mixture of drugs and chitosan shows endothermic peak at 224.21°C and onset 216.94°C. There is no significant deviation noticed indicating negative interaction between the drugs and polymer.

Chitosan mucoadhesive microspheres were prepared by simple emulsification technique. Due to the biodegradable and mucoadhesive property chitosan it was selected for the preparation of the microspheres. The yield of the chitosan microparticle prepared between 71-89.2% (Table 2) the percentage yield increases with drug polymer ratio. The prepared cross linked microspheres were predominantly round in shape with a particle size ranges from 112 to 179 µm (Table 2). Drug and polymer ratio and volume of cross linker had positive effect on entrapment of drug. Entrapment of drug was increased with increasing in drug and polymer ratio. It was occurred due to the increased in viscosity of aqueous phase with increasing the polymer concentration that stabilize droplets and which prevent out flow of drug during the hardening phase. Drug entrapment was foundupto 84.3% (Table 2). Percentage Mucoadhesion was calculated at 1 hour, as shown in table 2 all the formulations showed satisfactory mucoadhesion on the goat intestinal mucosa. As mentioned in the table 3 all the formulation shows good flow properties. Carr's index ranges from 8.45 to 12.23 %.

SEM images (Figure 3) indicate that the microparticles are of spherical in shape with moderate rough surfaces, clumps were also present. SEM photographs revealed the absence of crystals of drug on the surface of the microshperes, indicating uniform distribution of drugs on the surface of the microspheres.

The *in vitro* drug release study from the microspheres was studied for the formulations F3, F4, F5, F6. The percentage cumulative releases are shown in the table-4. Here sustain release of drug was observed from the formulation in acidic buffer pH 1.2 for duration of 24 hours. Increasedin concentration of polymer in formulation drug release was sustained for longer period. Among all the formulations, F6 formulation showed better sustained release profile of the drugs for a period of 24 hours. (Table 4)

DISCUSSION

Helicobacter pylori infection is one of most prominent cause of stomach and duodenal ulcer which may develop to other serious complications if it is left untreated. In this study a localized drug delivery system was developed to deliver the drugs at the site of action in a sustain manner. The prepared microspheres are able to entrapup to 84 % of the drug. F6 formulation has shown very good sustain release behavior upto 24 hours. As from the findings it can be concluded that the prepared chitosan mucoadhesive microspheres has the capacity to adhere into the mucosal membrane and also can provide a sustain delivery of drugs at the affected site. Hence mucoadhesive delivery of the drugs has the potential to become one of the possible way through which the drug permeability, stability and drug targeting can be improved.

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Conflict of interests

Nil

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