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## Development and Evaluation of Biodegradable Chitosan Microspheres Loaded with Ranitidine and Cross Linked with Glutaraldehyde

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### ABSTRACT

The present study aimed at the formulation of biodegradable chitosan microspheres loaded with ranitidine to overcome the poor bioavailability and frequent dose administration. Chitosan microsphere was prepared by simple emulsification technique by glutaraldehyde crosslinking. Various process variable and formulation variable such as speed of emulsification, cross linking time, drug/polymer ratio, volume of cross linking agent and volume of surfactant were optimized. Formulated microspheres were characterized for its entrapment efficiency, drug loading, *in vitro* drug release, Kinetics of drug release, surface morphology, particle size analysis, Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) thermal analysis. The characterized ranitidine microsphere formulations were investigated for *in vivo* gastric and duodenal antiulcer activity. The characterization of the fabricated microspheres showed smooth surface with narrow particle size distribution and entrapment efficiency upto 84%. The prepared microspheres exhibited a controlled drug release of 74% over a period of 24 h with initial burst release of 35% in the first 2 h. The FTIR and DSC reports showed that there was no potential drug interaction between the drug and polymer. *In vivo* studies shows that gastric volume, pH, total acidity and ulcer index of formulated ranitidine microspheres were significantly reduced as 2.67 mL, 5.59, 110 mEq L<sup>-1</sup> and 1.74, respectively and also there is no evidence of extra tissue damage as seen in the biopsy report. From the data obtained it can be concluded that the chitosan microspheres could be considered as a potential biodegradable carrier for controlled drug delivery of ranitidine.

**Key words:** Chitosan, controlled release, ranitidine, Glutaraldehyde Saturated Toluene (GST), *in vivo* gastric activity, duodenal antiulcer activity

### INTRODUCTION

Chitin is chemically (1→4)-2-acetamido-2-deoxy-β-D-glucan, i.e., abundant in nature and chitosan is its deacetylated derivative (Fig. 1), has many industrial applications such as lubricant, disintegrant, thickening, stabilising and suspending agent in textile and paper industry (Upadrashta *et al.*, 1992), as a chelating agent for removal of harmful metals in industrial nuclear wastes as a support for ion exchange, chelation affinity chromatography (Qurashi *et al.*, 1992). The industrial source of chitin is shells of shrimp, lobster and crab. Chitin and Chitosan are distinguished by their solubility profile.

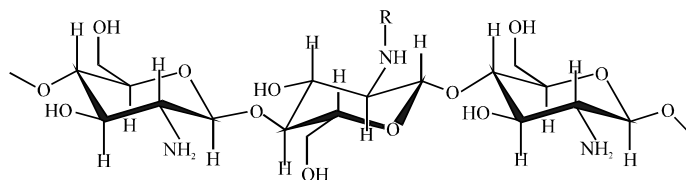


Fig. 1: Structure of chitosan

The characteristic properties of chitosan render them suitable for pharmaceutical and biomedical application. Chitosan has antacid, antiulcer, hypocholesterolemic, wound healing, haemostatic, spermicidal properties (Hillyard *et al.*, 1964). Chitosan has favourable biological properties like biodegradability, biocompatibility and nontoxicity. Chitosan was found to improve the fluidity of powder mixtures (Kumar, 2001). Chitosan has good mucoadhesive property and show good application potential (Wittaya-Areekul *et al.*, 2006). Sustained release formulations have been successfully prepared by using chitosan (Agnihotri and Aminabhavi, 2004; Kofuji *et al.*, 2005). Chitosan controlled drug delivery systems for hormones (Berthold *et al.*, 1996; Cheng *et al.*, 2005), vitamins (Shi and Tan, 2002; Murata *et al.*, 2002), proteins (Remuman-Lopez *et al.*, 1998; Grenha *et al.*, 2005) and enzymes (Chen and Chen, 1998; Jiang *et al.*, 1998) have been reported. The chitosan has antitumour activity, thus chitosan microspheres bearing antineoplastic agents are promising carriers for cancer treatment (Ouchi *et al.*, 1989). Chitosan also holds immense promise for ophthalmic delivery (Paolicelli *et al.*, 2009).

The pH dependant solubility of chitosan is a function of amino groups present and is a drawback for oral delivery. Chitosan microspheres formed by electrostatic interaction between a polyion and counterions become unstable in gastric fluid. This problem can be countered by irreversible chemical cross linking (Berthold *et al.*, 1996). It is also eminent that the drug diffusion from a chitosan microsphere could be effectively controlled by cross linking with a dialdehyde such as glutaraldehyde (Thanoo *et al.*, 1992).

The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting mucosal barrier and act on the central nervous system (Manonmani *et al.*, 1995). Even though wide range of drugs available for the treatment of ulcer, many do not fulfill the requirements and have many side effects such as arrhythmias, impotence and hemopoietic changes are noted (Austin and Jegadeesan, 2003).  $H_2$  antagonists unlike anticholinergics they do not cause side effects like dry mouth, urinary retention etc. They do not delay gastric emptying time which may reflexly stimulate gastric secretion because of food remaining in the stomach for long time. Also, it does not cause abdominal colic and diarrhea caused by proton pump inhibitors (Goel and Shah, 2008). Out of the available category of drugs for the treatment of ulcer,  $H_2$  antagonists class of drugs like famotidine, ranitidine are considered to be the safest drugs. Hence this drug has promising future if controlled release formulations are developed.

Ranitidine is a  $H_2$  receptor antagonist. It is widely prescribed in gastric ulcers, duodenal ulcers Zollinger-Ellison syndrome, systemic mastocytosis (Aboofazeli and Shafaati, 2002) and gastroesophageal reflux disease (Bruntan *et al.*, 2006).  $H_2$  receptor antagonists not only inhibit gastric secretion, induced by histamine, gastrin and cholinergic stimulation. They also promote healing of duodenal ulcers (Sharma, 2007). The effects of factors such as food intake, formulation, age and hepatic diseases, on blood concentrations of ranitidine have been described by several researchers (Alkaysi *et al.*, 1989; Smith *et al.*, 1984).

The H<sub>2</sub> anti histamines like ranitidine is a very successful drug due to its prominent clinical parameters. They block more than 90% of nocturnal acid and 60-70% of day time secretion. The relative potency of ranitidine is also higher when compared to other H<sub>2</sub>-antihistamines. The recommended dose of ranitidine for duodenal, gastric ulcers, reflux esophagitis, NSAID ulcers and Zollinger-Ellison syndrome is 150-300 mg BD (Reynolds, 1996).

Two types of polymorphic crystalline forms of ranitidine hydrochloride, Form 1 and Form 2 have been described. The basic form or Form 1 can be obtained from the ethanolic solution of ranitidine base by salt formation with hydrochloric acid. The filtration and drying characteristics of Form 1 are known to be unfavourable, moreover, it exhibits considerable hygroscopicity. Form 2 is obtained upon the isopropanolic recrystallization of Form 1. The preparation of Form 2 is described in U.S. Pat. No. 4,672,133. The two above crystalline forms of ranitidine hydrochloride are well distinguishable by X-ray powder diffraction patterns. From a technological standpoint Form 2 is more advantageous, consists of larger crystals, is easy to filter, to dry and less sensitive to moisture, Upon storage Form 1 slowly gets converted into Form 2. The existence and spontaneous transformations of polymorphic forms of drug substances are of disadvantage, because they cause difficulties to fulfill exacting pharmaceutical requirements and specifications. The physicochemical properties of products with such polymorphics change according to the actual ratios of polymorphic forms. The sharp endothermic heat low peak characteristic for the melting of Form 2 ranitidine hydrochloride is seen at 143-145°C and this is the same on the DSC curve of the mechanical mixture (Wilfried and Karin, 1997).

It has been reported that the oral treatment of gastric disorders with H<sub>2</sub> antagonist like ranitidine or famotidine used in combination with antacids promotes local delivery of these drugs to the receptor of parietal cell wall. Local delivery also increases the stomach wall receptor site bioavailability and increases efficacy of drugs to reduce acid secretion. Hence this principle may be applied for improving systemic as well as local delivery of ranitidine, which would efficiently reduce the gastric acid secretion (Coffin and Parr, 1995).

From the above facts, a need was felt to develop a preparation that deliver ranitidine in the stomach and would increase the efficiency of the drug, providing sustained action. Thus an attempt was made to prepare ranitidine loaded chitosan biodegradable microspheres.

## **MATERIALS AND METHODS**

**Materials:** Chitosan (medium mol wt. ca 40 kDa) was obtained from Central Institute of Fisheries and Technology, Cochin, India. Ranitidine was obtained as a gift sample from Novartis Bombay. Sorbitan sesquioleate, glutaraldehyde (25% aqueous), liquid paraffin hght with viscosity of 18 CPS, petroleum ether were obtained from Loba Chemie Pvt. Ltd., Bombay. The entire research project was carried out in Research Lab., GIET School of Pharmacy, Rajahmundry, India during 15 June 2007 to 14 Sep., 2010.

### **Methods**

**Preparation of Ranitidine loaded chitosan microspheres:** Famotidine containing chitosan microspheres were prepared by simple emulsion technique. In the prehiminary preparations various ratios of drug polymer were tried. Four percent solution of chitosan in 5% aqueous acetic acid containing 2% NaCl was prepared and the drug was loaded by mixing the required amount of drug with 6 g of chitosan paste and it was dispersed in a mixture of 35 mL liquid paraffin and 25 mL of petroleum ether containing 0.85 g of sorbiton sesquioleate in a 100 mL round bottomed flask at

room temperature (Jameela and Jayakrishnan, 1995). The dispersion was stirred using stainless steel half moon shaped paddle stirrer at 2000 rpm for 5 min and 10 mL of Glutaraldehyde Saturated Toluene (GST) prepared according to the Patel method (Patel and Patel, 2007), was introduced into the flask while stirring. At the end of 30 min, glutaraldehyde (25% v/v aqueous solution) was added and stirring was continued. The volume of cross linking agent and cross linking time was varied in preliminary trial batches from 0.5-15 mL and 1-3 h, respectively. The stirrer speed was also varied from 1500-3000 rpm. The stirring was continued for a total duration of 90 min, at the end the hardened microspheres were filtered, washed several times with petroleum ether followed by acetone, a 5% solution of sodium metabisulphate and finally with water. The microspheres thus obtained were dried overnight in an air oven at 60°C. The microspheres were stored in a dessicator.

**Determination of loading efficiency:** Ranitidine content in the preparation was determined by extracting the drug containing microspheres using pH 6.8 phosphate buffer. Fifty milligram of microspheres were taken and triturated and dissolved in 50 mL of pH 6.8 phosphate buffer. The solution was filtered through Millipore filters and the amount of drug was measured after suitable dilution at 226 nm by spectrophotometry. The amount of drug loaded in microspheres was calculated by the following formula (Gladiziwa and Klotz, 1993).

$$\text{Loading efficiency, } L = (Q_m/W_m) \times 100$$

Where:

$W_m$  = Weight of microspheres in grams

$Q_m$  = Quantity of drug present in  $W_m$  g of microspheres

**Entrapment efficiency:** Fifty milligrams of accurately weighed microspheres were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10 mL of pH 6.8 phosphate buffer solution. After 24 h the solution was filtered and the filtrate was analysed for drug content. The drug entrapment was calculated using the formula (Patel and Patel, 2007).

$$\text{Percentage drug entrapment} = W/T \times 100$$

Where:

$W$  = Weight of drug present in microspheres (practical drug content)

$T$  = Theoretical weight of drug

**Particle size analysis:** The size distribution in terms of average diameter ( $d_{avg}$ ) of microspheres was determined using the optical microscopic method. Scanning Electron Microscopy (SEM) was performed to characterize the surface morphology of formed microspheres (Thanoo *et al.*, 1993) by using Hitachi S-520 SEM.

**Fourier transform infrared spectroscopy:** The FT-IR spectrum of chitosan, ranitidine and ranitidine loaded chitosan microspheres were recorded on a PerkinElmer (model No-Spectrum Rx, Serial No. 83806) instrument using KBr discs in the range of 4000-400  $\text{cm}^{-1}$ . FT-IR spectrum could be a useful tool in determination of structural change undergone by the drug or the polymer in the microsphere formulation.

**Differential scanning calorimetry:** Differential Scanning Calorimetry (DSC) of ranitidine, blank microspheres and drug loaded microspheres were performed with DSC 821e Mettler Toledo. The DSC tracings were performed from 20 to 240°C at a rate of 10°C min<sup>-1</sup>.

**In vitro release study:** *In vitro* release study was carried out in pH 6.8 phosphate buffer solution at 37±1°C. Drug loaded microspheres (50 mg) were added to 50 mL of dissolution medium in a stoppered bottle. The bottle was fixed in the orbital shaker (Remi) and shaker speed was adjusted to 50 rpm. The samples were collected at predetermined time intervals (30 min, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36 and 48 h) for analysis. The medium was replenished with an equal volume of phosphate buffer solution after withdrawal of each sample. Values reported are the average of three determinations using the same technique (Jaimini *et al.*, 2007).

**Kinetics of drug release:** In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of microspheres were fitted with various kinetic equations, such as zero-order (percentage release versus time), first-order (log percentage of cumulative drug remaining versus time), Higuchi's model (percentage drug release versus square root of time) (Tamizharasi *et al.*, 2008).

#### **In vivo gastric and duodenal antiulcer activity**

**Pyloric ligation:** Wistar albino rats of both sex were grouped into eight each containing 6 animals. They were kept in the animal house at room temperature 25±2°C, with relative humidity of 45-55% maintained under 12 h light and dark cycle and were fed with standard rat feed and were acclimatized for a week before the study (Bhave *et al.*, 2006; Kath and Gupta, 2006). Group I served as normal control in which distilled water was administered orally in which no pyloric ligation was done, group II served as disease control, group III received Ranitidine 50 mg kg<sup>-1</sup> orally and it was considered as standard, group IV served as Ranitidine Formulation group and the dose equivalent to ranitidine 50 mg kg<sup>-1</sup> was administered.

Rats were fasted for 36 h prior to the surgical procedure and kept in raised mesh-bottomed cages to avoid coprophagy. Under ether anesthesia the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was identified, slightly lifted, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. Animals were deprived of both food and water during the post operative period and were sacrificed at the end of 19-20 h after the operation. The stomach was dissected out as a whole by passing a ligature at the esophageal end.

The stomach was separated from the surrounding tissues and organs and thus brought out as a whole along with its contents. The contents were subjected to centrifugation (3000 rpm for 10 min) and then analyzed for mean volume of gastric secretion, mean pH and mean total acid. The pH was estimated by using indikrom pH strips (Glaxo India Limited, India) with pH ranges of 2-4.5 and 5-8.5 with a difference range of 0.5. Free acidity and total acidity were estimated by titrating 1 mL of centrifuged sample with 0.01 N NaOH, using Topfers reagent as indicator and phenolphthalein indicator respectively. Acidity was expressed in clinical units that are the amount of 0.01 N NaOH base required to titrate 100 mL of gastric secretion (Kulkarni, 1985).

Acidity was expressed as:

$$\text{Total acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1} \text{ mEq L}^{-1}$$

**Aspirin induced ulcer:** In Aspirin induced ulcer models four groups of albino rats of either sex weighing 150-175 g, with each group consisting of six animals were used. The first group served as a normal control the second group served as disease control and the third group served as standard group that received ranitidine 50 mg kg<sup>-1</sup> and group four received Ranitidine formulation equivalent to ranitidine 50 mg kg<sup>-1</sup>. All the animals received above treatment once daily for eight days orally. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of aqueous suspension of aspirin (200 mg kg<sup>-1</sup> orally) on the day of sacrifice. The animals were sacrificed 4 h later and stomach was opened to calculate the ulcer index by kunchandy method (Kunchandy *et al.*, 1985).

The antiulcer activity was carried out after the ethical approval from CPCSEA and it was done as per the recommended guidelines of CPCSEA reg. No. 1069/AC/07/CPCSEA.

**Statistical analysis:** Statistical analysis was performed using graph pad InStat 3 software. All the tests were run in triplicate (n=3). Experimental results were expressed as Mean±SD and one way ANOVA for significance at p<0.05 was conducted for the release profiles.

## RESULTS

Ranitidine loaded chitosan microspheres were prepared by simple emulsification phase separation technique. Chitosan was selected as a polymer for the preparation of microspheres owing to its biodegradable, antiulcer, mucoadhesive properties and it may give better synergistic effect for the treatment of ulcer. Different concentrations of acetic acid from 1 to 6% w/v were used for preparing polymer solution, but 5% w/v acetic acid was finally used owing to its good solubility of chitosan and maximum sphericity was observed. Therefore from the preliminary trials it was decided that 4% chitosan solution and 5% acetic acid was found to be optimum concentration for the polymer solution. Liquid paraffin was used as the dispersion medium. 0.85 mg of sorbiton sesquioleate was added to dispersion medium and it was found to minimize aggregation of microspheres (Patel and Patel, 2007).

Preliminary trial batches were prepared to study the effect of the volume of cross-linking agent (glutaraldehyde), time of cross linking and stirring speed, drug entrapment efficiency and characteristics of the microspheres. The volume of glutaraldehyde saturated in toluene was varied from 0.25 to 15 mL. Discrete spherical spheres were obtained using 15 mL of glutaraldehyde saturated in toluene. Batches prepared using 0.25-5 mL of GST yielded irregular microspheres. The higher amount of glutaraldehyde appears to favour the cross linking reaction and spherical free flowing microspheres were obtained using 10 mL of GST. The entrapment efficiency was also good and it was found to be 84%. Thus, we can conclude that 10 mL GST was the optimum volume for the preparation. Increase in cross linking time (1-3 h) in all preliminary trial batches affected the release rate. The cross linking polymer and time probably becomes more rigid and thus decrease the release of drug. The cross linking time did not have significant effect on percentage drug entrapment efficiency.

The chitosan ranitidine microspheres made with 1:2 drug polymer ratio at 2000 rpm stirring speed with 10 mL GST as cross linking agent had higher loading efficiency of ranitidine and further increase in drug polymer ratio does not give increase in loading (Table 1).

Analysis of particle size showed that 35% were below 75 um, while 40% had particles sizes from 75-150 um and 25% were in the range from 150-300 um. Extensive analysis on particle size gave us the information that lower stirring speed of 2000 rpm has produced higher percentage of larger

Table 1: Preparation and characteristics of chitosan microspheres (n = 3)

Batches	Drug/polymer ratio	Mean diameter ( $\mu\text{m}$ )	Drug loading	Entrapment efficiency
			------(wt. %)------	
R <sub>1</sub>	1:1	125 $\pm$ 18	16 $\pm$ 3	53 $\pm$ 2
R <sub>2</sub>	1:2	189 $\pm$ 13	25 $\pm$ 3	84 $\pm$ 3
R <sub>3</sub>	1:3	219 $\pm$ 12	24 $\pm$ 1	82 $\pm$ 4
R <sub>4</sub>	1:4	225 $\pm$ 11	23 $\pm$ 2	81 $\pm$ 2
R <sub>5</sub>	1:5	236 $\pm$ 14	22 $\pm$ 4	79 $\pm$ 4

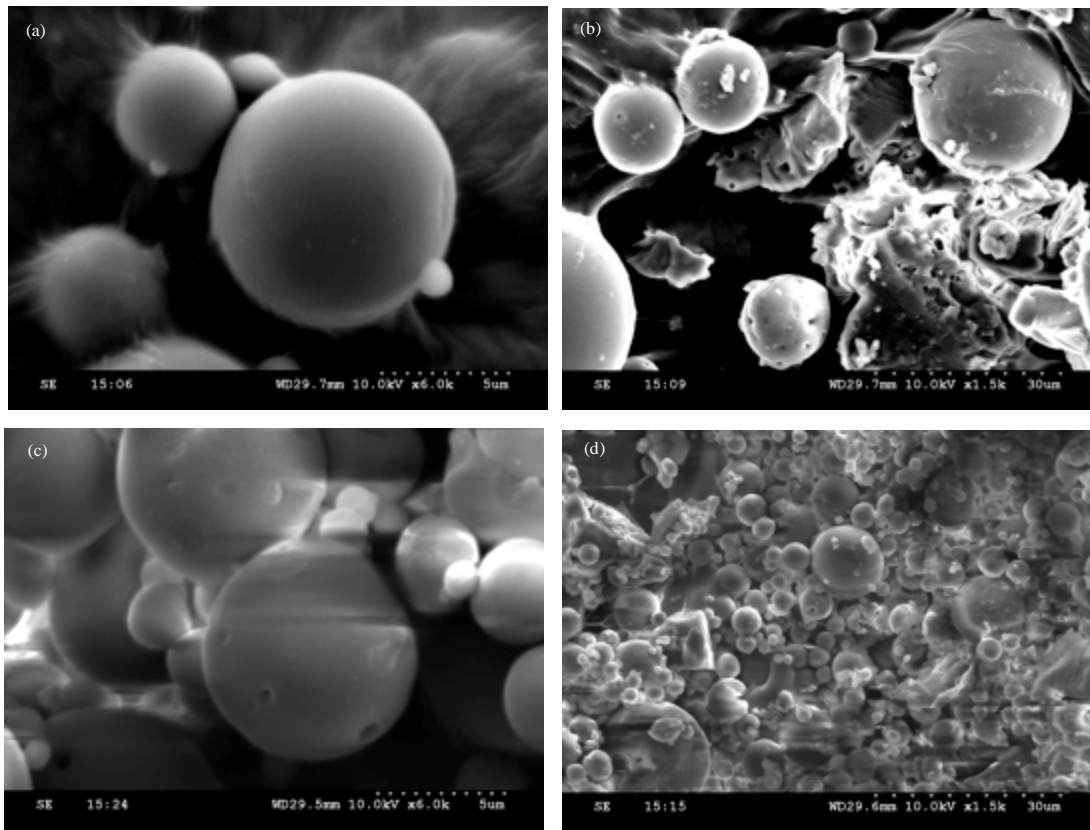


Fig. 2: Scanning electron micrographs of (a, b) Batch R2 Chitosan ranitidine microspheres and (c, d) batch R3 Chitosan ranitidine microspheres

spheres ranging between 150-300  $\mu\text{m}$ . The release rate was extended upto 24 h for the batch having larger spheres. Drug release was found to be more faster from the spheres of smaller size due to the increased surface area in contact with the dissolution medium (Jameela and Jayakrishna, 1995).

The microspheres obtained were fairly spherical in shape, as evidenced by scanning electron microscope analysis (Fig. 2). The morphology of the drug loaded microspheres appeared to be little rough.

UV spectrophotometric method was employed to determine the encapsulation efficiency of ranitidine in the microsphere prepared. The entrapment efficiency was determined by measuring the absorbance at 226 nm using pH 6.8 phosphate buffer solution. The maximum percentage of



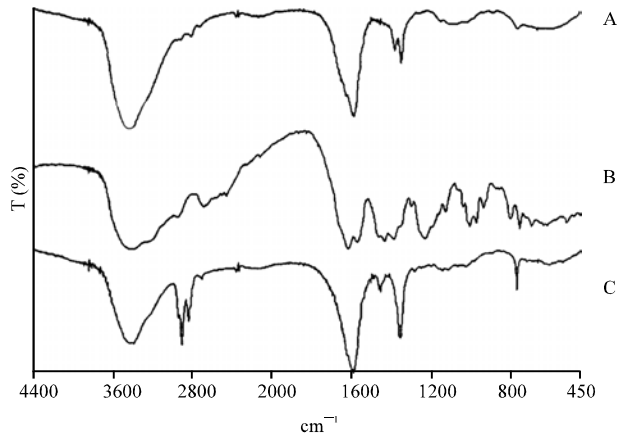


Fig. 3: FTIR spectras of (A) Chitosan microspheres, (B) Pure ranitidine, (C) Ranitidine loaded chitosan microspheres

encapsulation was found to be 84% for the batch R2 cross linked with 10 mL of GST at slow stirring. Many factors affect the entrapment efficiency of drugs in chitosan microspheres, e.g., nature of drug, chitosan concentration, drug polymer ratio, stirring speed etc. Generally lower concentrations of chitosan shows low entrapment efficiency (Orienti *et al.*, 1996), however at higher concentrations chitosan forms highly viscous solution which are highly difficult to process.

The FTIR spectra of chitosan depict characteristic absorption band at  $3437\text{ cm}^{-1}$  which represents the presence of hydrogen bonded OH group. The amino group has a characteristic absorption band in the region of  $3400\text{-}3500\text{ cm}^{-1}$  which must have been masked by the absorption band due to O-H group. Chitosan showed the characteristic bands of the amide at  $1654$ ,  $1608$  and  $1323\text{ cm}^{-1}$ . The ether linkage has a characteristic band at  $1091\text{ cm}^{-1}$ . The FTIR spectra of ranitidine showed characteristic absorption band at  $3435\text{ cm}^{-1}$  which represents the presence of N-H str of secondary amines. The weak absorption band at  $3050\text{ cm}^{-1}$  represents the presence of furan ring. Ranitidine showed the characteristic Nitro band at  $1357\text{ cm}^{-1}$ , The absorption band at  $650\text{ cm}^{-1}$  represents L-S Str.

FTIR spectra of ranitidine loaded microspheres showed absorption bands at  $283$ ,  $1458$  and  $1367\text{ cm}^{-1}$  of glutaraldehyde crosslinked chitosan microspheres are rather intense as a consequence of enhanced aliphatic C-H str absorption. The shift of the sharp peak from  $1600$  to  $1620\text{ cm}^{-1}$  stands for the stretching vibrations of C=N in Schiff's base formed by the reaction of glutaraldehyde and chitosan. The presence of N-H band at  $3402\text{ cm}^{-1}$ , C-H band at  $650\text{ cm}^{-1}$  proves that there is no change of functional groups present in ranitidine. The FTIR spectra of chitosan, ranitidine and ranitidine loaded chitosan microspheres are shown in Fig. 3.

As DSC is a useful tool to monitor the effects of additives on the thermal behavior of materials, this technique was used to deliver qualitative information about the physicochemical status of drug in the preparations (Dhanikula and Panchagnula, 2004). Thermogram of chitosan showed a broad peak at  $58^\circ\text{C}$  over a large temperature range is attributed to water loss due to evaporation of absorbed water and this represents the energy required to vapourise water present in the samples. DSC thermograms of chitosan, ranitidine and ranitidine loaded chitosan microspheres are shown in Fig. 4. Under the experimental conditions no degradation DSC peak was observed for chitosan polymer that normally occurs at  $280^\circ\text{C}$  (Cervera *et al.*, 2004; Liao and Hung, 2004). DSC

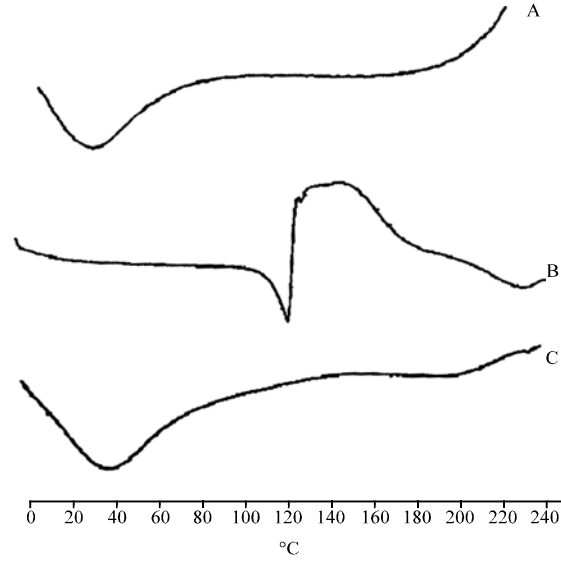


Fig. 4: A: DSC of chitosan, B: DSC of pure ranitidine and C: DSC of ranitidine loaded chitosan microsphere

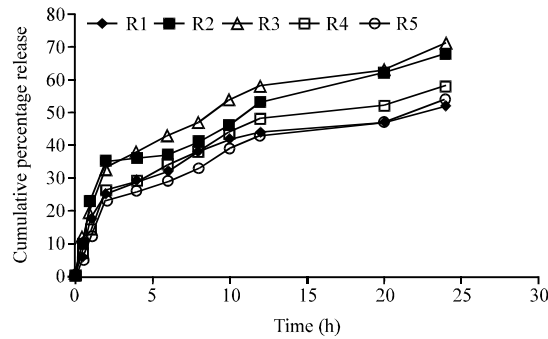


Fig. 5: Release profiles of ranitidine microspheres batches-R1, R2, R3, R4 and R5

thermogram of ranitidine form II (Fig. 4) shows sharp endothermic peak at 144°C which corresponds to their melting point temperature range of 143-145°C. The peak disappeared for the drug loaded chitosan microspheres, which indicated that the drug was molecularly dispersed inside of the matrix of chitosan as a solid solution.

The ranitidine release profiles from chitosan ranitidine microspheres at 37°C pH 6.8 were shown in Fig. 5. The drug release from the microsphere formulations were found to be 52, 74, 71, 58 and 54% at the end of 24 h for the batches R1, R2, R3, R4 and R5, respectively. From the release profile, it is clear that the release of ranitidine from the microsphere was characterized by initial rapid release (burst release) phase of 35% which is related to the drug entrapped near the surface of the microsphere, followed by a slow release phase. The drug release was found to be rapid in batch R2 due to its smaller particle size and it also exhibited initial rapid burst release. The release was found to be highest at 24 h and 74% release was found. This was exhibited by 1:2 drug polymer ratio batch. 1:3 batch did not show much variation. The remaining batches exhibited less drug release. As the cross linking agent and time increases the release rate is extended, this may be due to

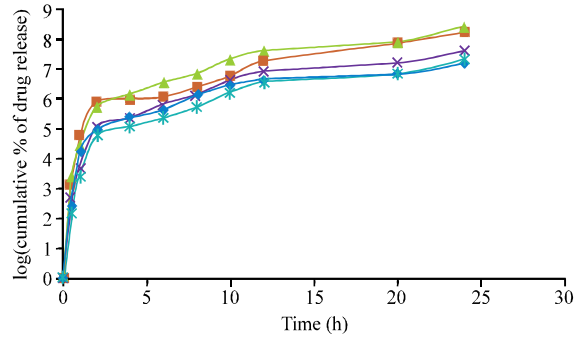


Fig. 6: First order release kinetics

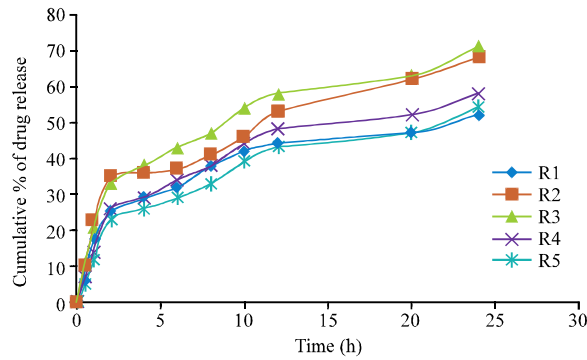


Fig. 7: Zero order drug release

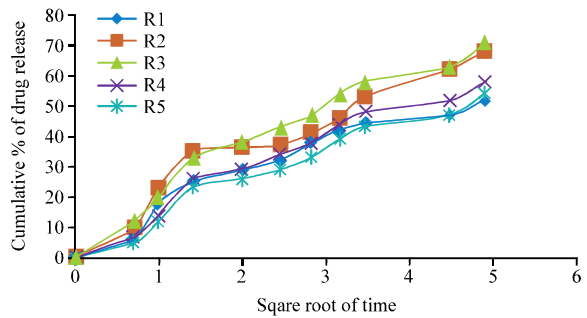


Fig. 8: Higuchi release kinetics

hardening of the microspheres as the time increases. Ten milliliter cross linking agent (GST) and 3 h cross linking period exhibited formation of good microspheres with 74% release within 24 h.

The *in vitro* release data were applied to various kinetics models to predict the drug release mechanism and kinetics (Fig. 6-8). The drug release mechanism from the microspheres thus can be described as diffusion controlled. The drug release was proportional to square root of time. When percentage of cumulative drug released versus time was plotted in accordance with first-order and zero-order equations, the  $r^2$  values obtained were found to be better for first-order plot compared to zero-order drug release, indicating that the drug release was described better with first-order release kinetics.

Table 2: Antiulcer effect of ranitidine microsphere formulation on pyloric ligation induced gastric ulcer in rats

Groups	Parameters			
	Mean volume of gastric secretion	Mean pH	Mean total acid	Ulcer index
Control	3.44±0.18	4.45±0.15	96.2±1.32	2.34±0.43
Disease control	5.79±0.25	2.41±0.21	160.4±1.76	5.61±0.53
Standard Ranitidine	2.48±0.25**	5.92±0.74***	107.0±1.02**	1.32±0.14***
Ranitidine microsphere formulation	2.67±0.22**	5.59±0.62**	110.4±0.89**	1.74±0.34**

Values are expressed as mean ±SEM, n=6 in each group. \*\*p<0.01, \*\*\*p<0.001

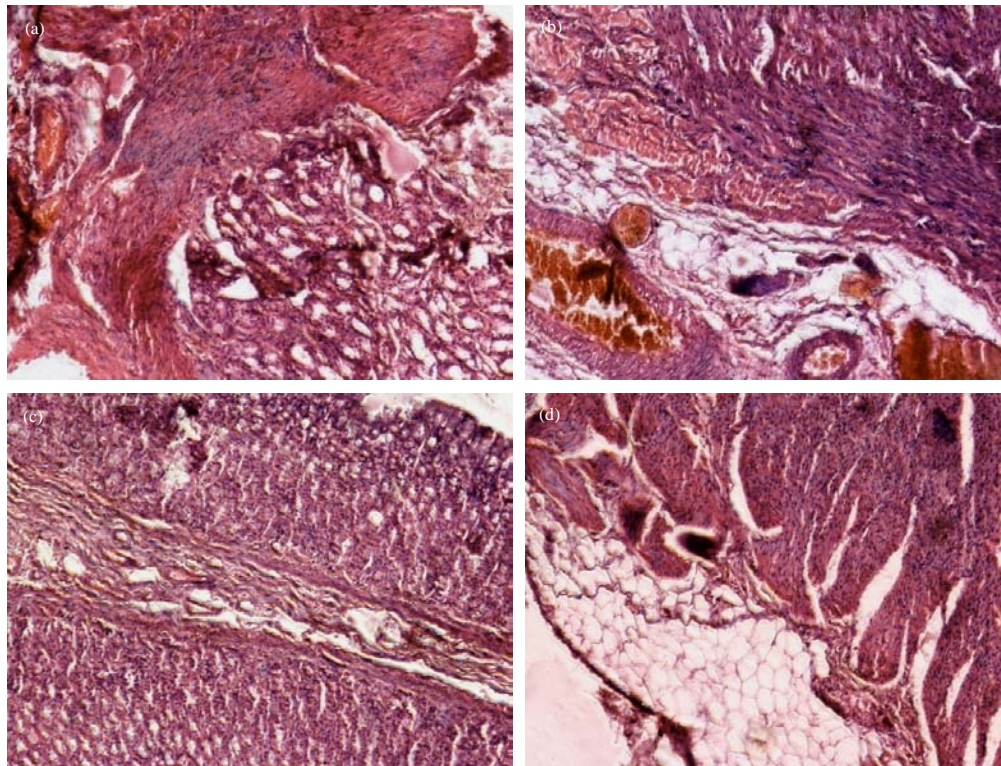


Fig. 9: Biopsy of Rat Stomach induced with ulcer. (a) Section of stomach from normal control rat shows normal architecture, (b) Section of stomach from disease control rat shows severely damaged cells, (c) Section of stomach from Ranitidine treated rat shows mild damaged cells and (d) Section of stomach from Ranitidine microsphere formulation treated rat shows mild damaged cells

In aspirin and pylorus ligation induced gastric ulcer models the microsphere formulation reduced the gastric volume, total acidity and ulcer index (Table 2) thus showing the anti secretory mechanism involved in the antiulcerogenic activity through H<sub>2</sub> receptors. Ulcer index parameter (Table 3) was used for the evaluation of antiulcer activity since ulcer formation is directly related to the factors such as gastric volume and total acidity (Goel and Bhattacharya, 1991). From the results it is clear that gastric volume, pH, total acidity and ulcer index of formulated ranitidine microspheres were significantly reduced as 2.67 mL, 5.59, 110 mEq L<sup>-1</sup> and 1.74, respectively. The biopsy reports of all the groups of rats were analyzed and shown in Fig. 9a-d.

Table 3: Antiulcer effect of Ranitidine microsphere formulation on aspirin induced gastric ulcer in rats

Groups	Parameters	
	Dose	Ulcer score
Control	Normal saline 2 mL kg <sup>-1</sup>	2.47±0.87
Disease control	Normal saline 2 mL kg <sup>-1</sup>	4.79±0.13
Standard ranitidine	Ranitidine 50 mg kg <sup>-1</sup>	1.39±0.26**
Ranitidine microsphere formulation	Formulation equivalent to Ranitidine 50 mg kg <sup>-1</sup>	1.51±0.63**

Values are expressed as Mean±SEM, n = 6 in each group. \*\*p<0.01, \*\*\*p<0.001

## DISCUSSION

Chitosan was selected as a polymer for the preparation of microspheres owing to its biodegradable, antiulcer, mucoadhesive properties and it may give better synergistic effect for the treatment of ulcer.

The production of ranitidine microspheres is based on the solubility behavior of chitosan, which is poorly soluble in water. Addition of an acid improves the solubility as a result of the protonation of the amino groups. The solubility is also dependent on other anions present in the solution. The presence of acetate, lactate or glutamate, chitosan shows good solubility. Where as phosphate, polyphosphate, sulphate and glutaraldehyde decreases the solubility. For this reason acetic acid was selected to dissolve the chitosan and glutaraldehyde was used for microsphere formation. Glutaraldehyde leads to a poorly soluble chitosan derivative where by microsphere formulation become possible.

The microspheres obtained were fairly spherical in shape, using 15 mL of glutaraldehyde saturated in toluene and similar results were obtained by Guerrero *et al.* (2010). When the volume of glutaraldehyde used in the microsphere preparation was 15 mL, the cumulative percentage of drug released was lowered. This is because increase in glutaraldehyde concentration caused highly crosslinked spheres and become dense.

Jameela and Jayakrishnan (1995) described, during the cross-linking and hardening process, water is exuded from the microspheres along with the dissolved drug and this appears to be responsible for the rather low incorporation efficiency. Increase in stirring speed beyond 3000 rpm has decreased the particle size upto 10 µm. In this case the drug release was found faster due to its increased surface area in contact with the dissolution medium. Increase in the cross linking time favoured the controlled release of drug from the spheres. This is also due to the hardening of the spheres with longer cross linking time. The high entrapment efficiency was similar to that reported for the encapsulation of drugs that were soluble in the same solvent as polymers using the spray drying technique (Blanco *et al.*, 2003). The drug content was increased upto the drug polymer ratio 1:3 and the entrapment efficiency was increased upto the drug polymer ratio 1:4. But at higher drug polymer concentration both the drug content and entrapment efficiency was decreased. At higher polymer concentrations viscosity of chitosan became too high, as a consequence a homogeneous distribution of the added glutaraldehyde was not possible, which leads to the formation of larger particles with reduced drug content and entrapment efficiency. Exactly the insufficient amount of crosslinking agent for higher polymer concentration and inadequate homogeneous dispersion cause slower crosslinking and insufficient entrapment ability that leads to decrease in drug content and entrapment efficiency.

Chitosan microparticles prepared by Huang *et al.* (2003) using a spray-drier showed a particle size of 2.12 µm and their external surface appeared smooth. In our study the average particle size

was increased with increase in drug polymer ratio. When the polymer amount was increased more viscous internal phase occurred during the emulsification process, the internal phase was hardly dispersed in the outer phase and larger microspheres were produced, which lead to increase in average particle size.

The *in vitro* release profile of R1, R2 and R3 mainly depends on entrapment efficiency. Even though the drug polymer ratio was increased upto 1:3 in formulation R3, it showed higher cumulative percentage of drug release due to its higher entrapment efficiency. Thereby the increase in drug release was in the order of R1 < R2 < R3. Incase of formulation R4 even though having higher entrapment efficiency than R3, it showed lesser drug release due to its higher polymer concentration. Batch R5 had both lower entrapment efficiency and higher polymer concentration which lead to a marked reduction in the drug release.

From the *in vitro* release profiles of various ranitidine loaded microspheres, the formulation R3 which contains 1:3 drug polymer ratios could be produced successfully with rapid burst release of 26.93% which is related to the drug entrapped near the surface of the microsphere, followed by subsequent slow sustained release of 85.61% upto 24 h. This burst phase has been also described for the release of different drugs from chitosan microspheres (Blanco *et al.*, 2000; Corrigan *et al.*, 2006; Huang *et al.*, 2003). The burst effect observed in drug release from micro particulate system is not itself an advantage or disadvantage of the formulation; it depends on the type of drug entrapped and also on the type of application of the microspheres (Trapani *et al.*, 2003). Further Higuchi release kinetics was also achieved. So, the formulation R3 will be considered as best formulation to overcome the frequent administration of ranitidine tablets and to improve the patient compliance. It was also confirmed that there was no potential drug interaction produced between polymer, drug and other ingredients as evidenced from the FTIR and DSC reports.

The biopsy reports of all the groups of rats were analyzed and shown in Fig. 9a-d and it was found that the section of stomach from normal control rat showed normal architecture, section of stomach from disease control rat showed severely damaged stomach cells with chronic inflammation, section of stomach from Ranitidine treated rat showed mild damaged cells and the section of microsphere formulation treated also showed mild damaged cells confirming the antiulcer effect of formulated Ranitidine microspheres and also there is no evidence of extra tissue damage as seen in the biopsy report.

## CONCLUSIONS

Ranitidine loaded chitosan microspheres using glutaraldehyde as crosslinking agent by simple emulsion technique could be produced successfully with rapid burst release of 35% and subsequent slow sustained release of 74% upto 24 h. Chitosan being natural biodegradable polymer gives no toxicity when incorporated in formulations. The present ranitidine loaded microspheres are suggested to be useful for the improvement of ranitidine efficacy against peptic ulcers.

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