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Preparation, Characterization and Evaluation of Ranitidine Hydrochloride-Loaded Mucoadhesive Microspheres

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of the article

Abstract

Background. Mucoadhesion enables localization of drugs to a defined region of the gastrointestinal tract through attractive interactions between polymers composing the drug delivery devices and the mucin layer of the intestinal epithelium. Thus, this approach can be used for enhancement of the oral bioavailability of the drug.
Objectives. The current communication deals with the development of ranitidine hydrochloride-loaded chitosan-based mucoadhesive microspheres.
Material and Methods. Microspheres were prepared by water-in-oil emulsion technique, using glutaraldehyde as a cross-linking agent. The effect of independent variables like stirring speed and polymer-to-drug ratio on dependent variables, i.e. percentage mucoadhesion, percentage drug loading, particle size and swelling index, was examined using a 3^2 factorial design.
Results. The microspheres were discrete, spherical, free-flowing and also showed high percentage drug entrapment efficiency (43–70%). An *in vitro* mucoadhesion test showed that the microspheres adhered strongly to the mucous layer for an extended period of time. The RC 4 batch exhibited a high percentage of drug encapsulation (70%) and mucoadhesion (75%). The drug release was sustained for more than 12 h. The drug release kinetics were found to follow Peppas' kinetics for all the formulations and the drug release was diffusion controlled.
Conclusions. The preliminary results of this study suggest that the developed microspheres containing ranitidine hydrochloride could enhance drug entrapment efficiency, reduce the initial burst release and modulate the drug release (**Polim. Med. 2014, 44, 2, 75–81**).

Key words: chitosan microspheres, gastric ulcers, factorial design, mucoadhesive microspheres, ranitidine hydrochloride.

The oral route of drug administration is the most accepted and popular route for drug administration [1]. The concentration of the drug in the blood vacillates over consecutive medications using the conventional delivery system and leads to a rise in the blood drug concentration to a high value followed by a subsequent fall to a very low level as a result of elimination. The drawbacks associated with the conventional delivery systems can be overcome by developing a system with predetermined drug release profiles [2]. There has been considerable interest in developing a controlled or sustained drug delivery system using microspheres or microcapsules [3]. Within the past decade, gastric retention has received much attention with the purpose of maximizing the residence of the dosage vehicle in the stomach, thus solving a specific absorption window issue or for localized drug delivery [4]. Mucoadhesion enables localization of the drugs to gastrointestinal mucosa through attractive interactions between polymers composing the drug delivery devices and the mucin layer of the intestinal epithelium [5–8].

Ranitidine HCl is an H_2 blocker which decreases the amount of acid produced in the stomach; used to treat ulcer, gastroesophageal reflux disease (a condition in which a backward flow of stomach acid causes heartburn and injury of the esophagus), and conditions where the stomach produces too much acid, such as Zollinger--Ellison syndrome. Over-the-counter ranitidine is used to prevent and treat symptoms of heartburn associated with acid indigestion and sour stomach [9].

Material and Methods

Material

The ranitidine HCl was a generous gift sample received from Win-Medicare Pvt. Ltd., Meerut, India. The chitosan was received from Colorcon Asia Pvt. Ltd., Goa, India. The glutaraldehyde was purchased from Central Drug House (P) Ltd., New Delhi, India. All other ingredients were of analytical grade and used as such without any further purification.

Preparation of Mucoadhesive Microspheres

The mucoadhesive microspheres containing ranitidine HCl were prepared using the water-in-oil emulsion method. A 3^2 full factorial design was used for the optimization of process parameters (Table 1). Chitosan was dissolved in a 1% acetic acid solution. The drug (200 mg) was dispersed in the polymer solution as a water phase. The chitosan solution was added into the oil phase of liquid paraffin (100 mL) containing 1% Span 80 as an emulsifying agent with constant stirring using a stirrer (LT400A, Yamato, Japan). The emulsion was stirred for 40 min with a drop wise addition of 1.5 mL glutaraldehyde solution at 10, 20 and 30 min, respectively. The microspheres obtained were separated by centrifugation and washed with isopropyl alcohol to remove liquid paraffin. The microspheres were dried at 40°C and stored in vacuum desiccators.

Table 1. Various formulations with the levels of independent variables used in 3^2 full factorial design layout

Formulation Code*	Polymer Concentration	Stirring Speed
RC_1	–1 (1.0 g)	–1 (500 rpm)
RC_2	–1 (1.0 g)	0 (1000 rpm)
RC_3	–1 (1.0 g)	+1 (1500 rpm)
RC_4	0 (1.5 g)	–1 (500 rpm)
RC_5	0 (1.5 g)	0 (1000 rpm)
RC_6	0 (1.5 g)	+1 (1500 rpm)
RC_7	+1 (2.0 g)	–1 (500 rpm)
RC_8	+1 (2.0 g)	0 (1000 rpm)
RC_9	+1 (2.0 g)	+1 (1500 rpm)

* Drug concentration (200 mg) was kept constant in all the formulations

Characterization of Developed Microspheres

Determination of Particle Size and Morphology

The mean particle size of the developed microspheres was determined using an optical microscope (SZ-6045, Olympus, Tokyo, Japan). The calibrated eyepiece was fitted with a micrometer which was used to determine the particle size. The surface morphology of the drug-loaded microspheres of optimized formulation (RC_4) was studied by using a scanning electron microscope (SEM) (EVO-50, ZEISS, Birmingham, UK). Before observation, the silver coating of the sample was done under vacuum using a Polaron SEM coater; Polaron, Birmingham, UK.

Drug-Excipients Compatibility Study

The compatibility study between the drug and excipients, to find out any interaction at the molecular level, was done by observation of physical changes and by FTIR spectroscopy.

Determination of Swelling Index

The swelling behavior of the microspheres was observed by soaking the microspheres in 5 mL 0.1 N hydrochloric acid (pH 1.2). The volume of the microsphere bed was determined after 12 h. The swelling ratio was calculated as follows [10]:

$$\text{swelling index} = \frac{\text{volume after 12 h}}{\text{original volume}} .$$

Determination of Yield and Percentage Drug Encapsulation

Precisely weighed (50 mg) microspheres were crushed using a glass mortar and pestle and dispersed into 50 ml phosphate buffer (pH 6.8) to extract the drug, assuring that there was no material loss during the processing. The mixture was agitated for 24 h at 37 ± 0.5°C. After 24 h agitation, the sample was filtered and analyzed for the content of ranitidine HCl by a UV-spectrophotometer (UV-1700 Shimadzu, Japan) at 314 nm after suitable dilution. The yield and percent encapsulation efficiency were calculated as follows [11]:

$$\text{yield (\%)} = \frac{\text{amount of microspheres obtained}}{\text{total weight of all non volatile components}} \times 100,$$

$$\text{encapsulation efficency (\%)} = \frac{\text{actual amount of drug present in the microspheres}}{\text{theoretical amount of drug added in the microspheres}} \times 100,$$

In vitro Mucoadhesion Study

The *in vitro* mucoadhesive properties of the microspheres was determined by the wash-off test method using freshly excised goat stomach mucosa (2 × 7 cm) obtained from a local slaughter house within an hour of slaughter and transported to the laboratory in Tyrode's solution. About 50 microspheres were spread onto the rinsed tissue specimen tied onto a glass slide. The slide containing the sample was hung to the tablet disintegration test apparatus. The disintegration test apparatus was operated for regular up and down movements of the tissue specimen in the beaker of the disintegration apparatus, containing simulated gastric fluid. The microspheres still adhering to the tissue were counted every 30 min in the first hour and then every hour till the 12th hour. Adhesion was calculated by the following formula [12]:

$$\text{adhesion number} = \frac{\text{no. of particle attached to mucosa after washing}}{\text{initial no. of microspheres in the mucosa}} \times 100.$$

In Vitro Drug Release Study

The drug release from different formulations was examined using USP basket apparatus (TDT-068, Electrolab, India) at 37 ± 0.5°C and 50 rpm. To examine the effects of the release medium on drug release, the release studies were carried out in 900 mL of simulated gastric fluid (pH 1.2) and phosphate buffer (pH 6.8). Microspheres equivalent to 150 mg of ranitidine HCl were placed in the basket. The samples were taken at appropriate time intervals and analyzed by a UV-spectrophotometer (UV-1700 Shimadzu, Japan) at 314 nm. All the dissolution runs were performed in triplicate for both the dissolution media.

The release data obtained in both of the dissolution media was analyzed for similarity factor (f_2) and dissimilarity factor (f_1) to confirm the effect of various process parameters on *in vitro* drug release [13, 14]. The similarity factor is a logarithmic reciprocal square-root transformation of the sum of squared error and is a measurement of the similarity in the percentage of dissolution between the 2 curves. Similarity (f_2) and dissimilarity (f_1) factors were determined using the following formula:

$$f_2 = 50 \times \log\{[1 + (1/n) \sum_{j=1}^{n} |R_j - T_j|^2]^{-0.5} \times 100\},$$

$$f_1 = \left\{ \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} R_j} \right\} \times 100$$

where, n is the number of dissolution time points, Rj and Tj are percent dissolved of the reference product and test product at each time point j, respectively.

Results and Disscussion

The percentage yield of microspheres was found in the range of 57% to 83.3%. Formulation RC_1 and RC_3 showed yields of 57.00% and 63.00%, indicating the effect of stirring speed and polymer concentration on the yield of microspheres. The results of a particle size analysis of the developed microspheres showed that the size of microspheres was in the range of 43.74 to 292.82 μm (Table 2). A significant increase in particle size was ob-

Table 2. Observations of various formulations for dependent variables

Formulation code	Shape	Yield (%)	Mean particle size (μm)	Swelling index	Drug encapsulation (%)	Mucoadhesion after 1 h (%)
RC_1	spherical	57.00	72.43	2.38	52.85	50
RC_2	spherical	60.00	58.76	2.24	43.63	48
RC_3	spherical	63.00	43.74	1.80	40.58	43
RC_4	spherical	62.50	90.89	3.60	70.72	75
RC_5	spherical	65.00	80.63	2.60	68.48	68
RC_6	spherical	67.50	52.85	5.00	64.49	60
RC_7	spherical	72.50	292.82	4.40	68.22	71
RC_8	spherical	75.00	193.29	4.60	63.48	66
RC_9	spherical	83.30	107.76	4.40	62.19	60

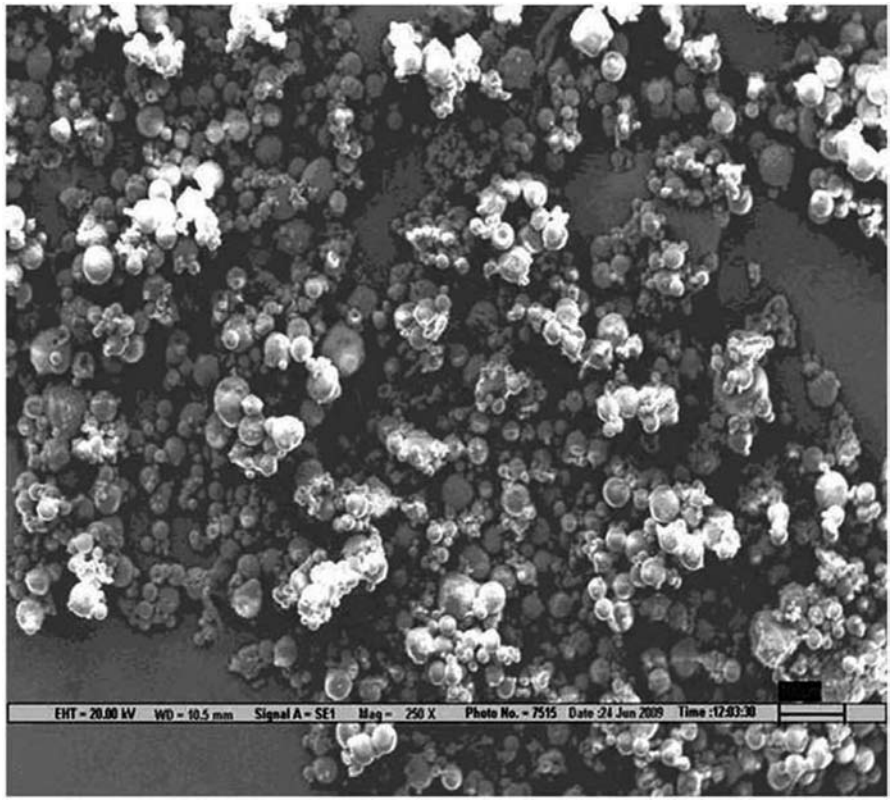

Fig. 1. Scanning electron micrograph of the drug loaded chitosan based microspheres (formulation RC 4)

served with an increase in polymer concentration. This might be due to the fact that the increasing concentration of polymer increases the cross linking and viscosity, hence the matrix density of the microspheres increased, which may result in an increase in the particle size of the microspheres. The above results indicate that the polymer concentration had a positive effect on particle size, whereas stirring speed had a negative effect on particle size. Therefore, a lower level of polymer is suggested for producing microspheres of smaller particle size. It was evident from the SEM photograph of optimized formulation (Fig. 1) that the microspheres were spherical in shape.

In order to fully characterize the starting materials, the FTIR spectrum of pure ranitidine hydrochloride and chitosan were recorded. FTIR spectroscopy study of the formulation was carried out to ascertain that the processing condition has not led to any interaction between the drug and the polymer in the formulation. These results suggested that there was no interaction between the drug and excipients. The FTIR spectrum of drug, polymers and formulation are shown in Fig. 2. The bands around 3260, 3190 and 3100 cm^{-1} present in the FTIR spectrum of ranitidine hydrochloride are assigned to stretching vibrations of OH groups and symmetric stretching peaks of NH. In addition, the bands around 3066 and 3017 cm^{-1} are assigned to the CH furan in the chemical structure of the compound. The peaks at 2970, 2950 and 2908 are due to the CH aliphatic group. The other characteristic peaks in the spectrum of ranitidine hydrochloride were 2653, 2560 and 1620 (NH), 1590 (NO_2), 1570 (NH), 1472 and 1428 (CC), 1380 (NO_2), 1226 and 1137 (CN), 990, 806 and 760 (CH). The peaks in the range of 1680–1480 cm^{-1} were due to the vibrations of C=O of the amide group. The peak near 1150 cm^{-1} in the FTIR spectrum of chitosan is due to the asymmetric vibrations of CO. The peaks near 1080–1025 cm^{-1} are assigned to the CO of the ring COH, COC and CH_2OH. The peak near 890 cm^{-1} matches to a wagging of the saccharide structure of chitosan. Some of the peaks were shifted with a very slight change in the wave number. The characteristic peaks of the drug were also present in the FTIR spectrum of formulation.

The swelling behavior of drug-loaded microspheres after rehydration in 0.1 N HCl (pH 1.2) revealed that the extent of swelling increased with an increase in polymer concentration. The swelling index varied from 2.38 to 5.00 (Table 2).

The percentage of drug encapsulation was increased with an increase in polymer concentration. The percentage of drug encapsulation varied from 40.00% to 70.00% (Table 2). Among all the formulations RC_4 shows the highest drug encapsulation (70%) when compared to other formulations. The effect of the polymer concentration is more significant than stirring speed. Moreover, stirring speed had a negative effect on drug encapsulation (i.e., as the stirring speed increased, the particle size decreased and thus drug encapsulation also decreased). The high drug encapsulation might be due to the increased viscosity of the dispersion and higher degree of cross-linking, which restricts leaching of the drug from

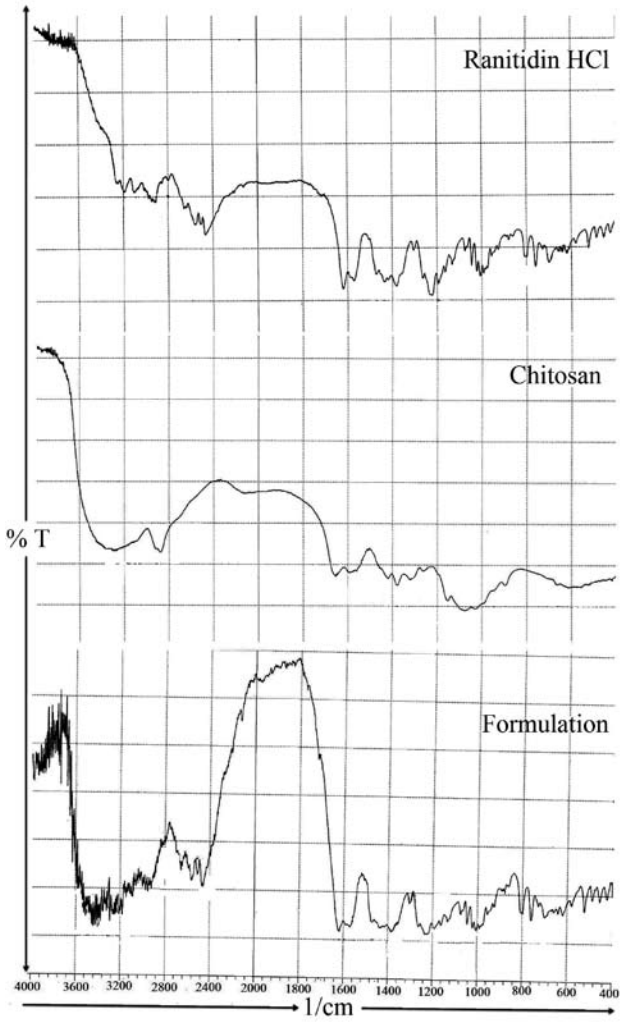

Fig. 2. FTIR spectrum of Ranitidin HCl, chitosan and optimized formulation (RC4)

microspheres. The encapsulation of ranitidine was found to be high in all the formulations. Drug loss in the continuous phase occurs while the dispersed phase stays in a transitional, semisolid state. If the solubility of the drug in the continuous phase is higher than in the dispersed phase, then it will easily spread out into the continuous phase during this transitional stage. But in the present study the drug is poorly soluble in the continuous phase. Diffusion of the drug into the continuous phase occurs during the first 10–15 min of particle formation. Thus, as the duration of the polymer phase staying in a semisolid state is extended, encapsulation efficiency gets reduced. In the present study, solidification/cross-linking of the polymers occurs immediately when it comes in contact with a glutaraldehyde solution. Hence, the drug will not get diffused into the surrounding aqueous medium. Both these factors might be responsible for the increased drug encapsulation efficiency [15].

In the mucoadhesion test, it was observed that the percentage mucoadhesion after 1 h varied from 43% to 75% for a period of 1 h. The effect of various dependent variables on the prepared microspheres is given in Table 2. The higher level of polymer produced a viscous gel and increased the mucoadhesion property. This also might be due to the increased number of amino groups available for binding with the sialic acid residues in mucus membrane.

Gastric fluid pH is usually steady and approximates 1.2 in a fasting state. Food and buffers neutralize gastric acid, thus increasing the pH up to about 6.0. Dosage forms are usually administered in a fed state, as during the fed state, the onset of MMC is delayed resulting in a slowdown of the gastric emptying rate. The drug release studies were performed in a phosphate buffer (pH 6.8) and simulated gastric fluid (pH 1.2) for 24 h. A slight increase in the dissolution of the drug in a phosphate buffer (pH 6.8) was observed when compared to the SGF (pH 1.2). The drug release profile was mainly influenced by the mechanical properties of the gel barrier that formed during the swelling of the polymer. The degree of cross-linking also influences drug release. A slightly more sustaining effect on the drug release was observed in the microspheres prepared at a higher polymer level, which might be due to the higher cross-linking. However, this difference in the drug release was

Table 3. Peppas' kinetic equation parameter of the formulation in phosphate buffer (pH 6.8) and SGF (pH 1.2)

Formulation code	Phosphate buffer (pH 6.8)		SGF (pH 1.2)	
	r^2 values	n values	r^2 values	n values
RC_1	0.9912	0.210	0.9906	0.210
RC_2	0.9906	0.790	0.9869	0.960
RC_3	0.9875	0.854	0.9909	0.950
RC_4	0.9932	0.781	0.9917	0.830
RC_5	0.9915	0.807	0.9760	0.904
RC_6	0.9907	0.856	0.9762	0.903
RC_7	0.9953	0.777	0.9666	0.867
RC_8	0.9983	0.826	0.9712	0.843
RC_9	0.9908	0.774	0.9525	0.896

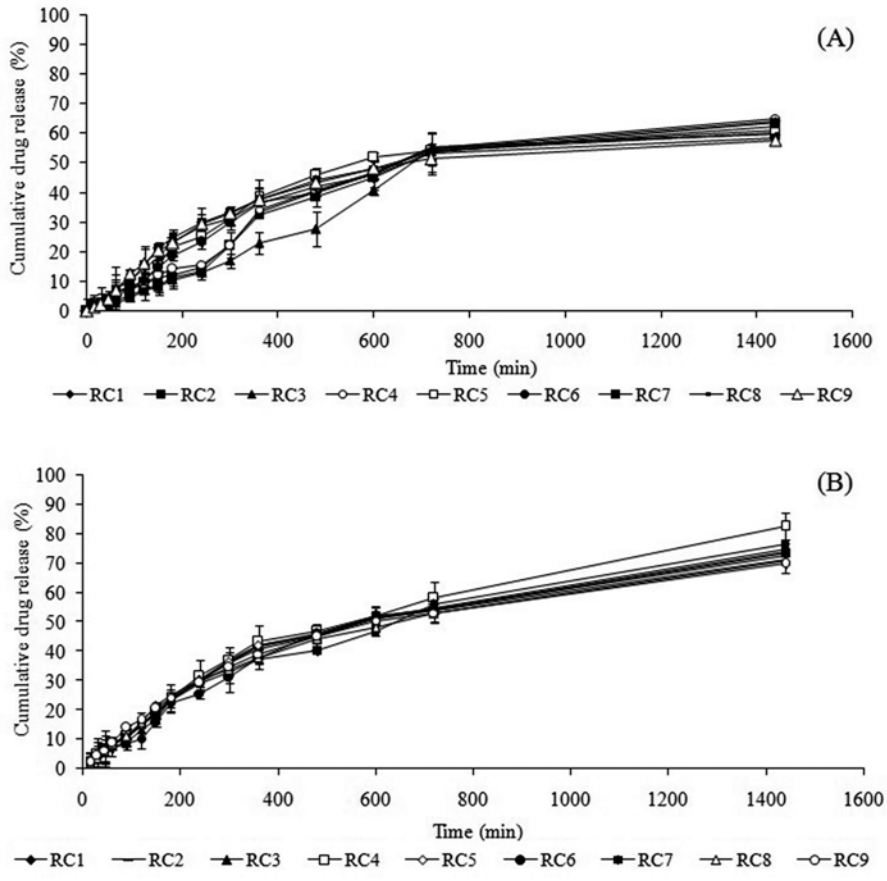

Fig. 3. *In vitro* drug release profiles of different batches of microspheres containing ranitidine HCl in SGF, pH 1.2 (A) and phosphate buffer, pH 6.8 (B) at 37°C (mean ± SD, n = 3)

not significant, which is also supported by the similarity and dissimilarity results (Table 4). The higher dissolution rate at a lower polymer level might also be due to the formation of smaller particles at lower polymer concentration, leading to a larger surface area exposed to the dissolution medium. Fig. 3 shows release profile of ranitidine HCl from formulations RC_1 to RC_9 in a phosphate buffer (pH 6.8) and SGF (pH 1.2) at 37°C.

In the present study, the Peppas' plots (log percent release against log time) were straight lines for all the formulations, with correlation coefficients ranging between 0.9666 and 0.9983. All the formulations, except formulation RC_1, had "n" values greater than 0.5 and less than 1. The regression coefficient (r^2) and 'n' values from *in vitro* release profiles of ranitidine HCl in a phosphate buffer pH 6.8 and SGF pH 1.2 were calculated and are reported in Table 3.

From the kinetic data, it was evident that the drug release kinetics were found to follow Peppas' kinetics for all the formulations and the drug release was diffusion controlled. The calculated slope values of Peppas' equation for all the formulations except batch RC_1 gave a value close to 1 but less than 1 in both of the dissolution media, which confirmed that the release mechanism of ranitidine HCl from the microspheres was Fickian diffusion with swelling, in both the media.

Similarity (f_2) and dissimilarity (f_1) factors were employed to find out the degree of closeness between the release profiles of different batches. US-FDA suggests that if the value of the similarity factor (f_2) lies within 50–100, the two formulations have similar release profiles. If the similarity factor (f_2) is 100 and dissimilarity factor (f_1) is 0, then the two formulations are considered as identical regarding their release profile. In the present study, the results suggested that the release profiles were almost similar in all the formulations, except formulation RC_3, as all the formulations had similarity values greater than 50 (Table 4).

Table 4. Values of similarity (f_2) and dissimilarity factor (f_1) for dissolution profiles of different formulations in phosphate buffer (pH 6.8) and SGF (pH 1.2)

Formulation	Similarity Factor (f_2)	Dissimilarity factor (f_1)
	pH 1.2 and pH 6.8	pH 1.2 and pH 6.8
RC_1 and RC_1	56	27
RC_2 and RC_2	52	36
RC_3 and RC_3	48	46
RC_4 and RC_4	51	32
RC_5 and RC_5	61	16
RC_6 and RC_6	61	15
RC_7 and RC_7	66	09
RC_8 and RC_8	69	09
RC_9 and RC_9	66	11

Discrete and spherical microspheres of ranitidine HCl with mucoadhesive polymer chitosan were prepared by a water-in-oil emulsification method. The major advantage of the preparation technique includes a short processing time, the lack of exposure of the drug to high temperature, due to which drug stability increases during the processing. The process parameters supported the formation of microspheres with a high level of drug encapsulation and good mucoadhesion. The developed microspheres prolonged the drug release. This prolonged drug release and local residence of delivery system may lead to effective management of gastric ulcer.

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