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Research Article

FORMULATION AND EVALUATION OF RANITIDINE LOADED CHITOSAN MICROSPHERES

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ABSTRACT

The present study aimed at the formulation of Ranitidine hydrochloride loaded chitosan microspheres to prolong the release rate so as to decrease the necessity of multiple dosage and to increase bioavailability. Ranitidine loaded chitosan microspheres were prepared using coacervation-phase separation technique and characterized for the effective delivery of ranitidine. The biodegradable property and good mucoadhesive nature of chitosan on ranitidine drug release was studied. The formulation RCP3 showed rapid drug release after 4 h due to the result of polymer erosion in the surface of microspheres. The prepared microspheres were evaluated for percentage drug loading, entrapment efficiency, and *In vitro* release characteristics to identify the effect of addition of these polymers. The formulation variables influenced the drug release profile. The results of in vitro release study fitted with kinetic equations indicated that ranitidine drug release followed Higuchi's matrix model.

Key words: Ranitidine hydrochloride, bioavailability, chitosan.

INTRODUCTION

Microencapsulation is a process of coating the small drug particles with a suitable coating material to give small capsules. The material inside the microcapsules is referred to as core, and the wall material is called as shell. Most microcapsules have diameters between a few micrometers and few millimeters. Depending on the methods of preparation the product may form either microcapsules or microspheres. Microencapsulation and resulting microcapsules/microspheres possess good advantages in terms of sustained, oral and parenteral controlled release and drug targeting[1-2]. Micro particulate drug delivery systems offer improve bioavailability and stability of the drugs having problem with low bioavailability and less stability in the GI environment. It can also minimize the side effect, dosing frequency and to improve better therapeutic performance.

Ranitidine, a H_2 receptor blocker is widely used for the treatment of ulcers, hyper acidity and gastro-esophageal reflux diseases (GERD).[3] Chitosan is a natural polymer commonly used for the development of sustained release dosage form. The bio-adhesive property of chitosan made it more popular for developing the mucoadhesive drug delivery system[4] In the present study ranitidine loaded chitosan microspheres were prepared using coacervation phase separation technique characterized and evaluated for the effective delivery of ranitidine. The developing chitosan microspheres can improve the therapeutic efficacy of the loaded drug and also reduce the dosing frequency of the ranitidine.

MATERIALS AND METHODS

Ranitidine was obtained as gift sample from Burgeon Pharmaceuticals, Chennai, Chitosan and Poly vinyl alcohol were obtained from Sigma, Mumbai. Dichloromethane and Acetonitrile (HPLC grade) were obtained from Qualigens, Mumbai and Potassium dihydrogen orthophosphate & Sodium hydroxide from SD Fine Chemicals, Mumbai, India. Distilled- deionized water was prepared with Milli-Q plus System (Elix 10, Millipore corp. India). All other reagents were of analytical grade.

Preparation of ranitidine microspheres by coacervation- phase separation method

The ranitidine loaded chitosan microspheres were prepared by coacervation-phase separation technique by using various ratios of drug and polymer (Table 1). The polymeric solution was prepared by dissolving the chitosan in DCM. Then ranitidine was dissolved in the polymeric solution and sonicated for 5 min. Then liquid paraffin was added to the polymeric solution at the rate of 1ml/min under continuous stirring using mechanical stirrer with 600 rpm. The slow addition of liquid paraffin coacervates the polymer in the mixture. The coacervate phase is then added to the hexane (non-solvent) under gentle stirring to harden the coating layer. The formed microspheres were then centrifuged for 10 min at 10,000 rpm. The precipitate was then collected and washed a least three times with distilled water. The prepared microspheres were lyophilized and stored in container for further studies. Six batches of microspheres were prepared using various ratios of drug: polymer. [5-7]

CHARACTERIZATION OF MICROSPHERES

Entrapment efficiency and drug content

The entrapment efficiency of ranitidine in the prepared microspheres was estimated by UV-spectrophotometry. A standard curve of ranitidine was prepared by serial dilution of standard stock solution. The standard stock solution was prepared by dissolving the weighed quantity of ranitidine in hydrochloric acid. For measuring the entrapment efficiency the weighed quantity of ranitidine was extracted from microspheres with alcoholic hydrochloric acid after dissolving the microspheres in acetonitrile. The absorbance of the resulted solution was measured in a UV- spectrophotometer (Shimadzu, Japan) at 313 nm after suitable dilution. The amount of ranitidine was estimated from standard curve. The entrapment

efficiency (EE) and drug content (DC) were calculated using the following formula[8]

Entrapment efficiency (%/w/w) = Estimated drug content/ % drug content (theortical) x 100

Drug content (%/w/w) = Weight of drug in microspheres/ weight of polymers and drug added (theoretical) x 100

Particle size analysis and surface morphology

The particle size of ranitidine microspheres were analyzed using Malvern particle size analyzer (Malvern Instruments Ltd, UK) . About 10 mg of samples were suspended in 5 ml of Milli-Q water and analyzed with an obscuration index of about 5% (Obscuration index is a measure of amount of light lost due to introduction of sample against light path). The measurements were carried out at a fixed angle of 90°. The analysis was carried out at a temperature of 25°C. The mean particle diameter and size distribution of the suspension were assessed. Analysis was carried out in triplicate for each batch of sample under identical conditions and mean values were reported.[9]
Surface morphological study of prepared microspheres was carried out using scanning electron microscope (SEM) (JEOL JSM-5610LV, Japan). Samples were prepared on 10 x 10 mm brass stub and coated with gold using sputter coater (Jeol auto fine coater) at accelerating voltage of 20 KV at high vacuum mode.

Poured Density, Tapped Density and Carr's Index[10]

Poured density, tapped density Carr's Index (%) and Hausner's Ratio of prepared microspheres were calculated[28] by the following formula:

Carr's Index (%) = {(Tapped density – Poured density) / Tapped density} × 100

Hausner's Ratio = Tapped density / Poured density

The angle of repose was also determined by funnel method and was calculated using the following formula:

$$\theta = \tan^{-1}(h/r)$$
Where, θ = angle of repose, h = height of heap, and r = radius of base of the heap.

***In-vitro* evaluation**

Drug release study

In-vitro release study of ranitidine from the microspheres was carried out in 900 ml of simulated gastric fluid (pH 1.2) maintained at $37^0C \pm 0.5\ ^0C$, with stirring speed 50 rpm, using USP Dissolution test apparatus (USP TDT 06PL, Electrolab, Mumbai) type II (Paddle type) for 24 h. Microspheres, equivalent to 5 mg of ranitidine, were used for the study and placed in dissolution test apparatus. At predetermined time intervals 10 ml of the sample solution was withdrawn and filtered through 0.45 µm of membrane filter. The samples were analyzed using UV spectrophotometer at 313 nm after suitable dilution. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of each test sample. It was diluted suitably and absorbance was measured in triplicate.[11]

Yield and entrapment efficiency

The product yield was in the range of 50.3 to 80.7% for coacervation-phase separation method (Table 2). The entrapment efficiency was in the range of 56.74 ± 1.12 to 82.97 ± 1.07 % for coacervation-phase separation method (Table 2).

Analysis of dissolution data

The kinetics and mechanisms of drug release from the prepared ranitidine loaded chitosan microspheres was studied. The *in-vitro* drug release data from the microspheres were fitted into zero order and first order kinetic models and to find out the mechanisms of drug release, the same data were fitted into Higuchi, Hixson-Crowell and Korsemeyer-Peppas equations. [13-18]

Stability study

The stability study was carried out by keeping the ranitidine microspheres in glass vials and placed in three different storage conditions [ICH Q1A (R2)] viz. long term study (25 ± 2 °C / 60% RH ± 5% RH) for one year, intermediate study (30 ± 2 °C / 65% RH ± 5% RH) for six months and accelerated study (40 ± 2 °C / 75% RH ± 5% RH) for six months.

The microspheres were evaluated at specific time intervals of 0, 3, 6 and 12 months for long term study and 0, 3 and 6 months for intermediate study and accelerated study. During stability testing samples were evaluated for physical appearance, particle size and drug content.

RESULTS AND DISCUSSION

Preparation of microparticles

The ranitidine loaded chitosan microspheres were prepared by three different methods viz. solvent evaporation, double emulsion (w/o/w) and coacervation-phase separation. The various drug polymer ratios (Table 1) were used for preparing the microspheres. The biodegradable nature and good mucoadhesive property of chitosan made it more promising carrier for sustained release drug delivery system. In the present study chitosan was selected keeping the view to slow release of ranitidine in the stomach.

Chitosan possesses positive surface charge which helps the microspheres for effective adhesion to the negatively charged mucous membrane in the GI tract

Particle size analysis

The particle size analysis of ranitidine microspheres was carried out by Malvern particle size. All the parameters were optimized for getting the particle sizes ranges from 2 to 8 µm. The size distribution data showed that microspheres were distinct, freely flowable, and mono-dispersed with spherical shape.

The increase in particle size of microspheres was due to the increase of polymer content in the formulations. The increased in the viscosity of the coating solution phase results the increase of particle size. The drug-polymer ratio played an important role for the formation of microspheres. The sizes of the microspheres were increased in formulations with drug: polymer ratios beyond 1:4. The larger particle size of the microspheres was observed at highest drug to polymer ratio 1:10. The increased concentration of chitosan polymer in the coating phase increases the viscosity which results the agglomeration of the particles leads to the bigger particle size

The encapsulation efficiency of ranitidine in the prepared microspheres was increased with the increasing proportion of polymer in the formulation. But the low yield was observed in the in some cases. It is because of losses occurring during the various processing steps involved in microspheres preparation. The major reason for getting low yield was adherence of polymeric solution to glass container and the washing of microspheres [19]

The formulation RCP3 prepared by coacervation-phase separation method with drug: polymer ratio 1:4 selected for further *in-vitro* and *in-vivo* evaluation.

Poured Density, Tapped Density and Carr's Index

The poured density and tapped density were increased with increase of polymer ratio in the formulations which is justified with relatively higher bulk density of the polymer. The angle of repose, poured density and tapped density; Carr's Compressibility Index and Hausner's Ratio were given in the table (Table 3). The value of angle of repose (between 23º and 29º) of the microparticles indicates the good flowability of the microspheres.The Carr's index between 5 and 15 is the indication of excellent flowability. But, the Hausner's Ratio 20 less than 1.2, indicates good flow properties and results corroborates with values of angle of repose of the microspheres.

Validation of microsphere formulation RCP3

The reproducibility of the prepared microspheres was assured by preparing the formulation RCP3 in triplicate by coacervation-phase separation technique. The drug to polymer ratio (1:4), organic phase to aqueous phase ratio (1:5) and stabilizer concentration (1% w/v) was kept constant for three batches.

The particle size, yield and entrapment efficiency of three batches of microspheres were analyzed and given in (Table 4). The results showed no prominent dissimilarity among the batches and produce the reproducibility of the formulation.

Particle size and surface morphology of ranitidine microspheres (RCP3)

The particle size of the ranitidine loaded microspheres was measured by Malvern instrument. The size distribution plots of formulation RCP3 showed sharp and steep peak specifies narrow size distribution (Figure 1)

The SEM image showed the particles are of spherical in shape with relative smooth surface (Figure 2) and smaller than the particle size measured by Malvern particle size analyzer. It is because of the hydrodynamic layer surrounding the particles.

***In vitro* drug release study**

The drug release from the microspheres was carried out in acidic pH 1.2 and the release data was depicted in table 5.4 The drug release study from the ranitidine loaded chitosan microspheres was carried out in simulated gastric fluid (pH 1.2). The release data is shown in (Table 5).

Drug release kinetics: To estimate drug release pattern the data are plotted in different kinetic models, viz. zero order, first order, Higuchi and Korsmeyer-Peppas equation respectively. Derived data and corresponding plots with respect to various models are furnished in tables and figures as follows. The drug release data was fitted into the models like zero and first order which indicates that the ranitidine release from the microspheres followed zero order and first order kinetics during 1 to 24 h. Drug release from the microspheres also obeyed Higuchi as well as Peppas models, indicating that the drug release was by diffusion mechanism. The release exponent 'n' value (0.542) was calculated from the slope of the Peppas model and indicates that the mechanism of drug release follows non- fickian (anomalous) diffusion diffusion (Table 6). The model fitting data suggests that the drug release from the microspheres obey first order kinetics, followed by diffusion and erosion mechanism.

Stability study

Drug decomposition was also less than 5 % at 6 months of storage. The particle size was not changed during storage at 25 °C/60% RH and 30 °C/65% RH over 6-12 months, but in accelerated storage condition the appreciable change was observed due to the aggregation of particles. The drug release study of the microspheres showed noticeable changes when stored in accelerated condition but no changes were observed when the microspheres were stored in 25 °C/60% RH and 30 °C/65% RH (Figure 6).

Table 1: Formulation of Ranitidine Chitosan microspheres

Sl. No	Formulation	Drug: polymer	Wt. of Ranitidine (mg)	Wt. of polymer (mg)	Vol. of OP (ml)	Vol. of AP (1% PVA) (ml)	% Yield
Coacervation-phase separation method							
1	RCP1	1:1	50	50	6	30	50.3
2	RCP2	1:2	50	100	6	30	67.1
3	RCP3	1:4	50	200	6	30	80.7
4	RCP4	1:6	50	300	6	30	64.4
5	RCP5	1:8	50	400	6	30	58.8
6	RCP6	1:10	50	500	6	30	75.3

Note: RCS, RCD and RCP indicate ranitidine loaded chitosan microspheres prepared by solvent evaporation, double emulsion and coacervation-phase separation method respectively; OP: organic phase; AP: aqueous phase

Table 2: Particle size, entrapment efficiency, drug content and % yield of ranitidine loaded chitosan microspheres prepared by three different methods.

S. No	Formulation	Drug: polymer	Particle size (µm)*	Entrapment efficiency (%w/w)*	Drug content (%)*	% Yield
Coacervation-phase separation method						
1	RCP1	1:1	2.54 ± 1.04	50.23 ± 1.12	36.42 ± 1.96	49.8
2	RCP2	1:2	2.81 ± 2.06	64.18 ± 1.39	32.24 ± 0.88	67.1
3	RCP3	1:4	2.93 ± 1.18	74.28 ± 1.09	24.43 ± 1.08	80.7
4	RCP4	1:6	22.38 ± 2.36	76.1 ± 1.48	19.08 ± 1.06	64.4
5	RCP5	1:8	30.22 ± 1.51	82.63 ± 0.99	11.59 ± 0.94	58.8
6	RCP6	1:10	43.26 ± 2.93	82.97 ± 1.07	8.18 ± 1.47	75.3

* The values are expressed as mean ± SD for n=3

Table: 3. Physical Characteristics of ranitidine loaded chitosan microspheres

S. No	Form. Code	Poured Density* (g/cm^3)	Tapped Density* (g/cm^3)	Carr's * Index (%)	Hausner's Ratio *	Angle of Repose * (degrees)
1	RCS2	0.181 ± 0.004	0.189 ± 0.002	4.232 ± 0.137	1.044 ± 0.005	29.74 ±0.83
2	RCS3	0.204 ± 0.001	0.221 ± 0.004	7.692 ± 0.09	1.083 ± 0.002	28.14 ±1.08
3	RCD2	0.238 ± 0.003	0.247 ± 0.004	3.643 ± 0.076	1.037 ± 0.001	23.76 ± 1.02
4	RCD3	0.262 ± 0.002	0.273 ± 0.002	4.029 ± 0.108	1.041 ± 0.004	25.92 ± 0.82
5	RCP2	0.244 ± 0.002	0.256 ± 0.003	4.687 ± 0.25	1.049 ± 0.003	25.19 ± 0.71
6	RCP3	0.292 ± 0.001	0.292 ± 0.002	3.767 ± 0.174	1.039 ± 0.003	27.07 ± 1.09

* The values are expressed as Mean ± SD; n= 3.

Table 4. Yield, particle size and % Entrapment Efficience of reproducible batches of RCP3

Formulation	Yield (%)	Particle size (µm)	Entrapment efficiency (%w/w)
RCP3a	81.26	2.84 ± 1.16	76.97 ± 0.84
RCP3b	82.02	2.76 ± 1.28	75.79 ± 1.02
RCP3c	81.46	2.91 ± 0.97	75.83 ± 1.09

Table 5. Régression coefficient (r^2) values obtained from the drug release kinetic data of ranitidine - Chitosan microspheres (RCP3).

Formulation	Zero order	1st order	Higuchi	Korsmeyer-Peppas	Peppas 'n'
RCP3	0.971	0.998	0.914	0.944	0.542

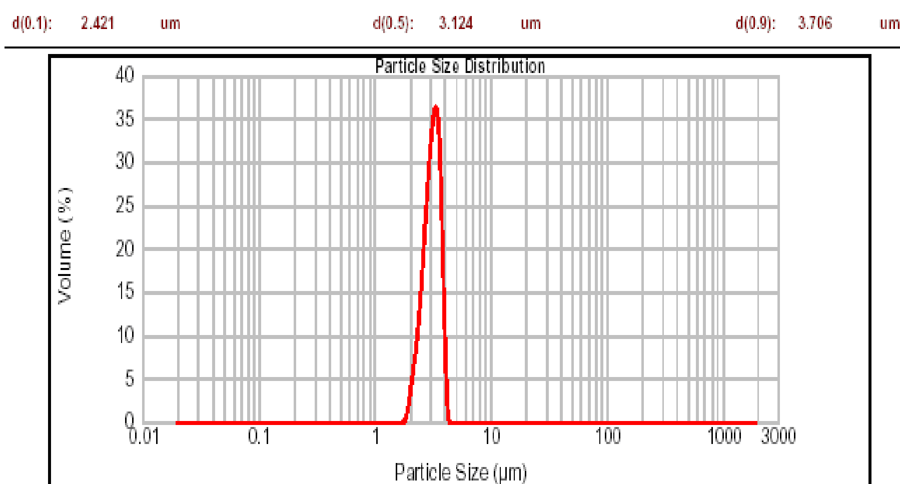

Figure 1: Particle size distribution of ranitidine-chitosan microspheres (RCP3) measured in Malvern particle sizer

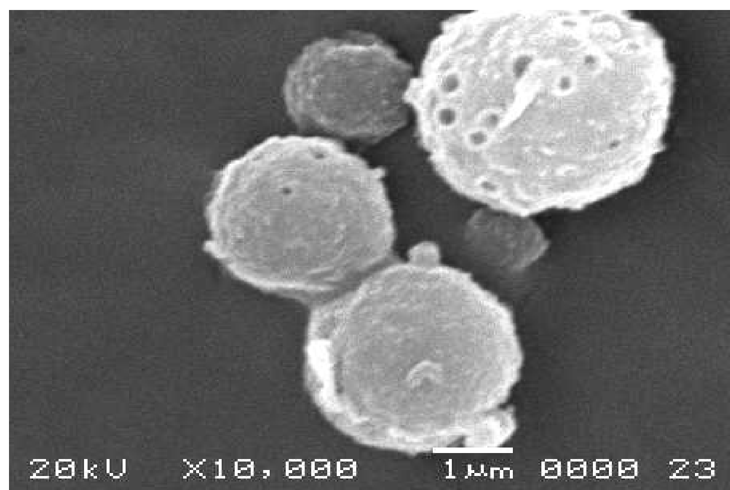

Figure 2: SEM image of ranitidine-chitosan microspheres (RCP3)

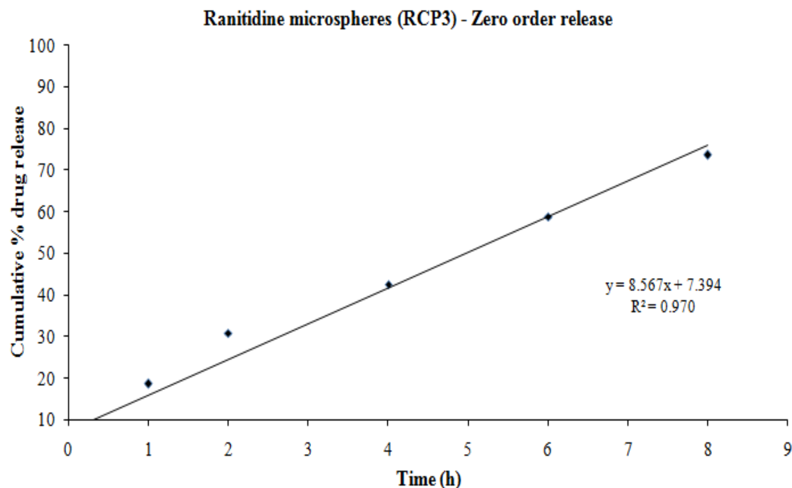

Figure 3. Ranitidine loaded Chitosan microspheres (RCP3) - Zero order release in simulated gastric fluid (pH 1.2)

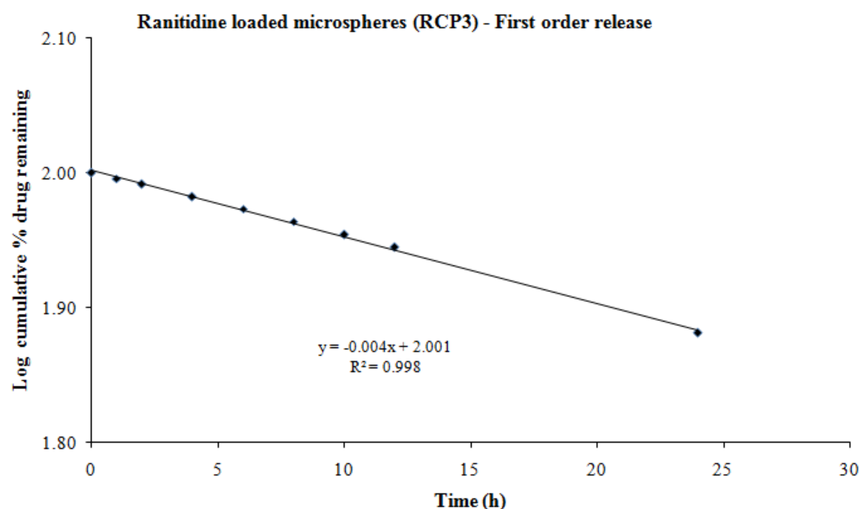

Figure 4. Ranitidine loaded Chitosan microspheres (RCP3) – first order release in simulated gastric fluid (pH 1.2).

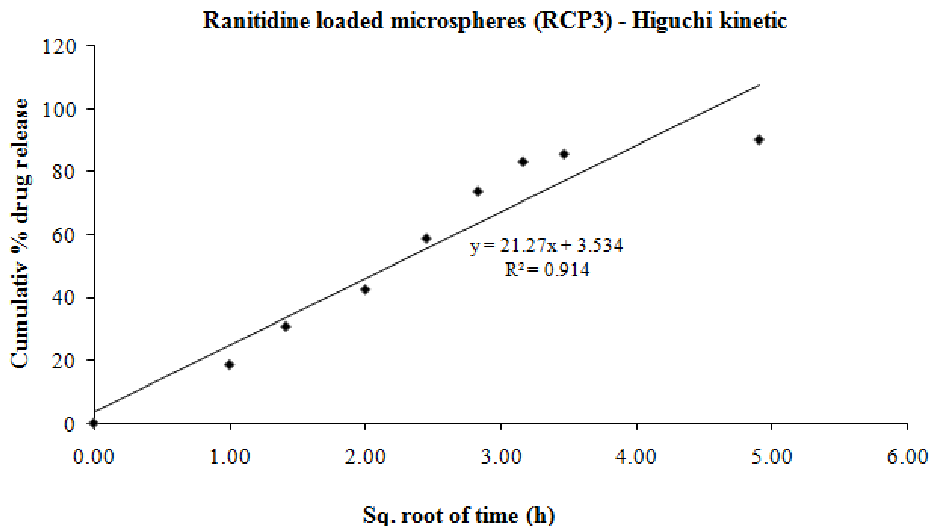

Figure 5. Drug release from ranitidine loaded Chitosan microspheres (RCP3) in simulated gastric fluid pH 1.2 – Higuchi kinetic.

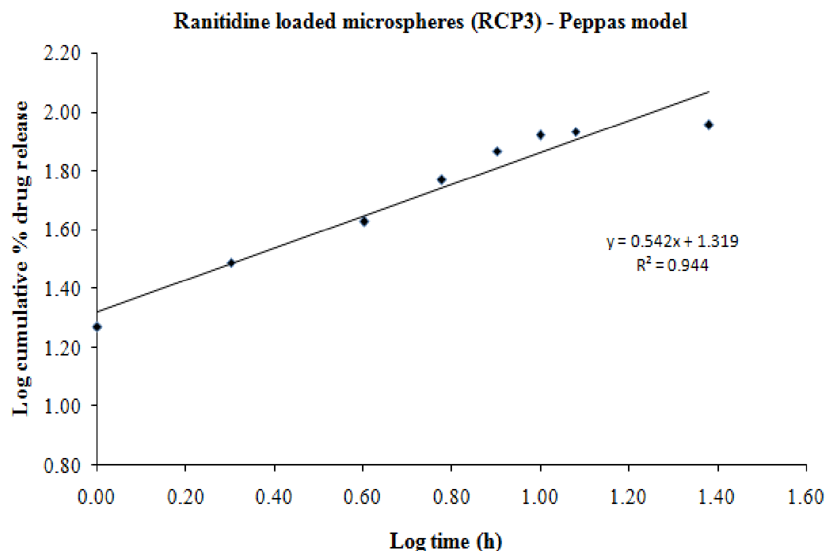

Figure 6 Drug release from ranitidine loaded Chitosan microspheres (RCP3) in simulated gastric fluid pH 1.2 – Peppas model.

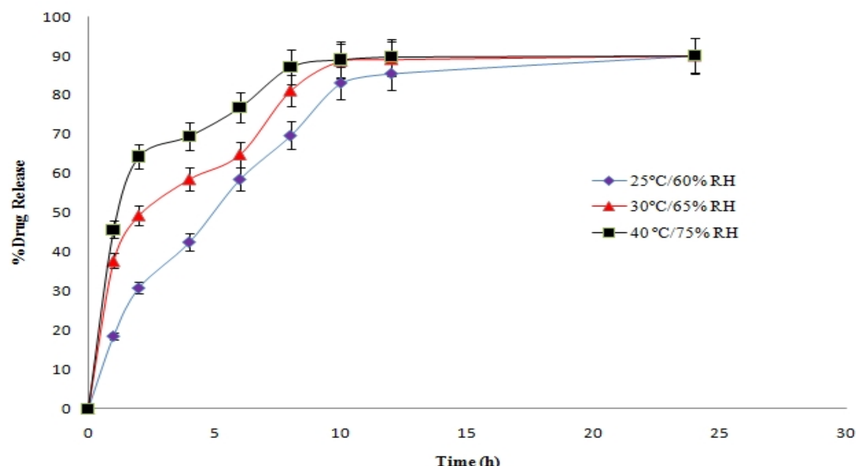

Figure 7. Drug release from Ranitidine loaded Chitosan microspheres (RCP3) in simulated gastric fluid pH 1.2 placed in three different storage conditions, (25ºC ± 2ºC/60% RH ± 5% , 30ºC ± 2ºC/65% RH ± 5%RH, 40 ºC ± 2ºC/75% RH ± 5% RH).

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