INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.6 | Issue 1 | Jan - Mar -2017
Journal Home page: www.ijpar.com

Research article *Open Access*

Design and evaluation of microspheres loaded with nizatidine

A.Shylaja Rani[1], D.V. R. N. Bhikshapathi[2*], Ganesh Kumar Gudas[3]

[1]*Research Scholar, Mewar University, Chittorgarh, Rajasthan, India*

[2]*Research Supervisor, Mewar University, Chittorgarh, Rajasthan, India*

[3]*Asst.Professor, HOD, Dept. of Pharmaceutics, Srikrupa Institute of Pharmaceutical Sciences, Siddipet. Dt: Medak,Telangana. India. 502103.*

***Corresponding Author: D.V. R. N. Bhikshapathi**

ABSTRACT

A novel ionotropic gelation technique was employed to design and develop sustained release Nizatidine microspheres for oral administration. Calcium chloride was selected as cross linking agent and sodium alginate as polymer to control the release profile of the Nizatidine from microspheres. The S1 to S14 formulations were subjected to micromeritic properties, swelling index, % yield, encapsulation efficiency and *in vitro* drug release. Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) were characterized for the prepared microspheres. The optimal formulation used for fabrication of microspheres was dispersion with 2% (w/v) sodium alginate, 10% (w/v) calcium chloride, and the drug Nizatidine content was 450mg based on solid weight in the dispersion. It was indicated that Nizatidine had no interactions with excipient by the FTIR and DSC. The optimized formulation (S6) showed the particle size, bulk density, tapped density, angle of repose, Carr's index and swelling index of 82.45±0.09 µm, 0.52g/ml, 0.59g/ml, 20˚.54, 7.95%, 97%, respectively. The % yield, %EE and Cumulative % drug release of S6 was found to be 98.3%, 96.3% and 98.54%, respectively. The *in vitro* drug release was showed the 98.07±0.46% within 12h. The optimized formulation followed the Zero order and Higuchi kinetics indicated the diffusion controlled release mechanism. SEM studies showed the optimized formulation had particles of spherical in shape. The optimized formulation S6 was found to stable. All the results proved that the ionotropic gelation is a novel and promising technique for preparing sustained-release microspheres, and suitable for industrial production due to its successive and controllable step in preparation.

Keywords: Nizatidine, Ionotropic gelation technique, Microspheres, Sustained release.

INTRODUCTION

Oral delivery is the most familiar technique for administration of drug. In recent times, a set of research works have been carried out to design and develop oral controlled release multiple unit dosage forms, particularly microspheres [1]. These are became more accepted than single unit dosage forms because of its numerous advantages, includes enhancing bioavailability, avoiding variation of gastric transit rates, emptying time and avoidance of elevated local drug level in GIT hence releasing drugs more uniformly [2]. The microspheres are prepared by several methods for instance spraying drying, phase separation and etc. However, to obtain uniform particle size, high drug entrapment efficiency may be hard due to complex post treatment procedures and removal of solvent these demerits mentioned increased the total cost of product [3]. Hence, in the present study ionotropic gelation technique was used which could successively fabricate micron sized spherical particles with high drug %EE by a simple procedure at room temperature [4].

The serious medical problem of GIT is peptic ulcer. Around 500,000 new cases are reported each year principally affecting the older population, with the peak incidence of disease occurring between at the age of 55 and 65 years [5]. 35% of patients diagnosed with gastric ulcers will suffer serious complications and in women gastric ulcers and duodenal ulcers in men are more common [6].

Polymeric drug delivery system exhibit several advantages over the conventional dosage forms and it includes enhanced efficacy, patient compliance, reduced toxicity, and also to control the encapsulated drug release. Sodium alginate is a anionic natural polysaccharide, prepared by mixture of D-mannuronic acid and L-glucuronic acid. Sodium alginate is extensively used as carrier for drug delivery due to its biocompatibility and low toxicity. Ionotropic gelation method is using in the present research for Nizatidine microspheres preparation [7]. This technique offers numerous advantages such as simple method of preparation no need to use of organic solvent, and, also easier to control [8]. Sodium alginate could form gel in the presence of multivalent cations such as Ca^{2+}, Zn^{2+}, Ba^{2+} and Al^{3+} etc... by ionic cross-linking to form microspheres, it has been widely used in sustained drug release. Hence in this study calcium chloride is selected as cross linking agent and also because of its nontoxic and biocompatibility [9].

Nizatidine is a histamine H_2-receptor antagonist that inhibits stomach acid production, and used in the treatment of peptic ulcer disease and gastroesophageal reflux disease. Nizatidine absorption and stability is found from the upper gastric mucosa, short half-life (1–2 h) and rapid clearance of it suggest is a rationale drug for gastroretentive drug delivery as microspheres [10].

The present works aims to design gastroretentive drug delivery system for Nizatidine using microspheres as the carrier system that could give site specific and controlled drug release. The prepared microspheres were evaluated for particle size, micromeritics, entrapment efficiency, % yield, drug release, and characterized by FTIR, DSC and SEM analysis. *In vitro* release properties of Nizatidine blend were studied in simulated gastrointestinal conditions. Furthermore, Stability studies were carried out as per ICH guidelines.

MATERIALS AND METHODS:

Materials

Nizatidine was obtained from Dr.Reddy's laboratories, Hyderabad., India as a gift sample. Sodium alginate was purchased from Pruthvi Chemicals, Mumbai, India, calcium chloride was obtained from SD Fine ltd, Mumbai., India. Remaining all chemicals used in this research study was of analytical grade.

Methods

Preparation of Nizatidine microspheres

The microspheres were prepared by ionotropic gelation method using the formulations as showed in Table 1. Initially, various percentages of sodium alginate (ranges from 1% to 2.2% w/v) solution was prepared by dissolving it in deionized water using gentle heat, being stirred magnetically. On complete solution, a weighed amount of Nizatidine was added to 100ml of each % solution to form homogeneous dispersions at 500rpm, maintained room temperature. The mixtures were sonicated for 30min to eliminate air bubbles that may have been

formed during the stirring process. The above dispersions was added drop wise via a 20-gauge needle fitted with a 10ml syringe into 100ml of 10% w/v and 12% w/v of calcium chloride solution, being stirred at 100rpm for 10min. Later, the solution was filtered and washed the microspheres using deionized water. The Nizatidine microspheres were thereafter dried at 60°C for 2h in a hot-air oven.

Table 1: Formulation of nizatidine microspheres

Formulation code	Nizatidine (mg)	Sodium alginate	Calcium chloride
S1	150	1%	10%
S2	150	1.2%	10%
S3	150	1.4%	10%
S4	150	1.6%	10%
S5	150	1.8%	10%
S6	150	2%	10%
S7	150	2.2%	12%
S8	150	1%	12%
S9	150	1.2%	12%
S10	150	1.4%	12%
S11	150	1.6%	12%
S12	150	1.8%	12%
S13	150	2%	12%
S14	150	2.2%	12%

Evaluation of Nizatidine microspheres [12,13]

Size analysis

Microsphere Size plays major part in analyzing the drug release from it. Particle size analysis was carried out by optical microscopy method, using calibrated eye piece and a stage micrometer, almost 100 particles were measured.

Angle of repose

Angle of repose of the microspheres, is the maximum angle possible between the surface of the pile of microspheres and the horizontal plane, was obtained by fixed funnel method using the following formula.

$\theta = \tan^{-1}(h/r)$

θ = Angle of repose, h = height of the microsphere pile and d = diameter of the microsphere pile.

Bulk density

Volume of the microspheres in the measuring cylinder was noted as bulk density.

$$\text{Bulk density} = \frac{\text{Wt of powder}}{\text{Bulk volume of powder}}$$

Tapped density: Change in the microspheres volume was observed in mechanical tapping apparatus.

$$\text{Tapped density} = \frac{\text{Wt of microspheres}}{\text{Tapped volume of microspheres}}$$

Compressibility index

Also called as Carr's index and is computed according to the following equation.

$$\text{Carr's compressibility index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio

Hausner's ratio of microspheres is determined by comparing the tapped density to the fluff density using the equation.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Swelling index studies

The swelling index of the microspheres is indication of the microspheres ability to absorbed water and swell. To determine the swelling index initially, the accurately weighed amount of microspheres suspended in simulated GI fluids after 1h microspheres were transferred onto blotting paper to remove the excess moisture then weighed the swollen microspheres using a microbalance. After that swollen microspheres were dried in oven at 60°C for 5h until showed the constant weight. The change in microspheres weight used to calculate the swelling index .

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) 100.

Drug incorporation efficiency and %yield:

10mg of drug-loaded microspheres from each batch was triturated in a mortar then transferred in 100ml conical flask containing 50ml of methanol. The microspheres were stirred to improve swelling and defragmentation of the cross-linked structure. The solution was filtered through a membrane filter. Then the Nizatidine was quantified spectrophotometrically at 224nm. The %EE and %yield were determined by using the following formulas:

% Drug entrapment = Calculated drug concentration /Theoretical drug concentration x 100

% yield = [Total weight of microspheres / Total weight of drug and polymer] x 100

In vitro drug release studies

The drug release studies were conducted at 37 ± 0.5°C in USP dissolution testing apparatus I and maintained 100rpm in 0.1N HCl about 900ml. 100mg of microspheres for each formulation was added to all drug release studies. The samples (5ml) were withdrawn at specified time intervals such as 0, 1, 2, 4, 6, 8, 10 & 12h and the same medium of fresh 0.1N HCl added immediately to maintain sink condition during the experiment. The aliquots of samples, following suitable dilution, were analyzed spectrophotometrically at 224nm in order to estimate the concentrations of Nizatidine in the test samples.

Kinetic modeling of drug release

The drug release mechanism from prepared microspheres is investigated by fitting the data into numerous kinetic models such as Zero order, First order, Higuchi's model and Korsmeyer-Peppas. Fitness of the data into several kinetic equations was determined by calculating the (r^2) correlation coefficient.

Drug excipient compatibility studies

Fourier transmission infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) used to imply the drug-excipient compatibility and Scanning electron microscopy (SEM) was used to identify the size and surface nature of microsphere.

Fourier transforms infrared spectroscopy (FTIR)

The FTIR technique can be used to identify the functional groups in the sample and drug-excipient compatibility. FTIR spectra of pure Nizatidine, and optimized formulation were recorded by using FTIR (SHIMADZU). Weighed quantity of KBr and excipients were taken in the ratio 100: 1 and mixed by mortar. The samples were made into

pellet/disk by the application of pressure. Then the FTIR spectra were recorded between 4000 and 400 cm^{-1}

SEM studies

The prepared microspheres shape and surface nature were examined with the help of Scanning Electron Microscope (HITACHI, S-3700N). The microspheres were dried completely prior to analysis and SEM was carried out at various magnifications.

Stability studies

Stability of optimized formulation was investigated for 6months at 40°C ± 2°C/75% RH ± 5% RH using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals of 0, 30, 60, 120, and 180days period according to ICH guidelines. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* drug release studies were evaluated.

RESULTS AND DISCUSSIONS

Micromeretic properties of Nizatidine microspheres

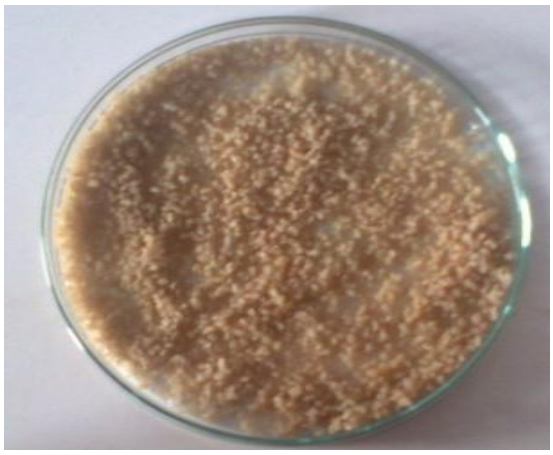

Figure 1: **Nizatidine microspheres**

Micromeretic parameters

The results of Nizatidine microspheres summarized in Table 2 and pictorial diagram was shown in Figure 1. Comparatively S6 formulation revealed the better results such as particle size, bulk density, tapped density, angle of repose, Carr's index and swelling index of 82.45±0.09µm, 0.52g/ml, 0.59g/ml, 20˚.54, 7.95% and 97% respectively.

Table 2: Micromeritic properties of Nizatidine microspheres

Formulation Code	Particle Size (µm)	Bulk density	Tapped density	Angle of repose	Carr's Index (%)	Swelling Index (%)
S1	61.12±0.08	0.66g/ml	0.69g/ml	22˚.74	9.34	44
S2	65.29±0.13	0.74g/ml	0.72g/ml	29˚.67	8.34	59
S3	67.43±0.04	0.76g/ml	0.73g/ml	30˚.54	10.12	60
S4	69.67±0.09	0.79g/ml	0.60g/ml	29˚.15	9.23	61
S5	73.45±0.04	0.89g/ml	0.75g/ml	27˚.93	14.56	69

S6	82.45±0.09	0.52g/ml	0.59g/ml	20˚.54	7.95	97
S7	95.23±0.14	0.94g/ml	0.73g/ml	22˚.91	10.32	90
S8	67.45±0.04	0.69g/ml	0.65g/ml	27˚.93	14.56	69
S9	78.45±0.09	0.77g/ml	0.72g/ml	25˚.54	13.95	70
S10	81.23±0.14	0.61g/ml	0.66g/ml	22˚.91	10.32	75
S11	85.12±0.08	0.66g/ml	0.89g/ml	23˚.74	9.34	84
S12	87.29±0.13	0.74g/ml	0.62g/ml	25˚.67	8.34	89
S13	91.43±0.04	0.76g/ml	0.73g/ml	29˚.54	10.12	92
S14	94.13±0.09	0.87g/ ml	0.78g/ ml	29˚.15	11.23	94

The results % yield and encapsulation efficiency of all formulations were evaluated and mentioned in Table 3. The S6 formulation was shown better results of % yield and entrapment efficiency, 98.30% and 96.30% respectively. As increases the sodium alginate concentration increased the percentage of entrapment efficiency but reduces inturn by increasing calcium chloride. This may be due to saturation of calcium binding sites of the polymer with drug.

Table 3: Percentage yield and entrapment efficiency Nizatidine microspheres

Formulation Code	Percentage Yield (%)	Entrapment Efficiency (%)
S1	60	63
S2	71	72
S3	73	80
S4	83.87	83.3
S5	88.3	93.2
S6	98.3	96.3
S7	91.1	92.1
S8	76	64.3
S9	61	72
S10	76	83
S11	89	85
S12	82.5	90.66
S13	75.3	94.3
S14	85.3	94.88

In vitro drug release studies

In vitro drug release studies were carried out and depicted in Table 4 and 5 and in Figure 2 and 3. Comparatively, S6 formulation showed the highest drug release was found 97.17±0.16% in 12h and the drug release from marketed product was 95.87±0.31% within 1h.

Table 4: *In vitro* Cumulative % drug release of Nizatidine microspheres S1 to S7 and marketed product

Time (h)	S1	S2	S3	S4	S5	S6	S7	Marketed product
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	15.06±0.13	14.46±0.13	13.62±0.11	12.96±0.21	11.98±0.14	11.56±0.32	11.34±0.13	95.87±0.31
2	28.13±0.14	26.97±0.34	24.62±0.14	23.61±0.17	22.83±0.52	21.62±0.16	20.37±0.16	---

4	38.62±0.15	37.42±0.23	36.42±0.12	35.26±0.18	34.56±0.28	43.79±0.18	32.18±0.17	---
6	49.31±0.16	48.87±0.11	47.42±0.17	46.71±0.27	45.63±0.32	54.96±0.42	48.60±0.23	---
8	58.41±0.17	59.20±0.16	61.32±0.17	61.98±0.42	63.29±0.15	69.46±0.16	64.20±0.11	---
10	71.32±0.13	69.90±0.16	70.31±0.16	72.17±0.17	73.34±0.71	79.45±0.12	70.20±0.15	---
12	80.42±0.51	81.51±0.23	82.53±0.12	85.53±0.14	86.32±0.26	97.17±0.16	86.13±0.17	---

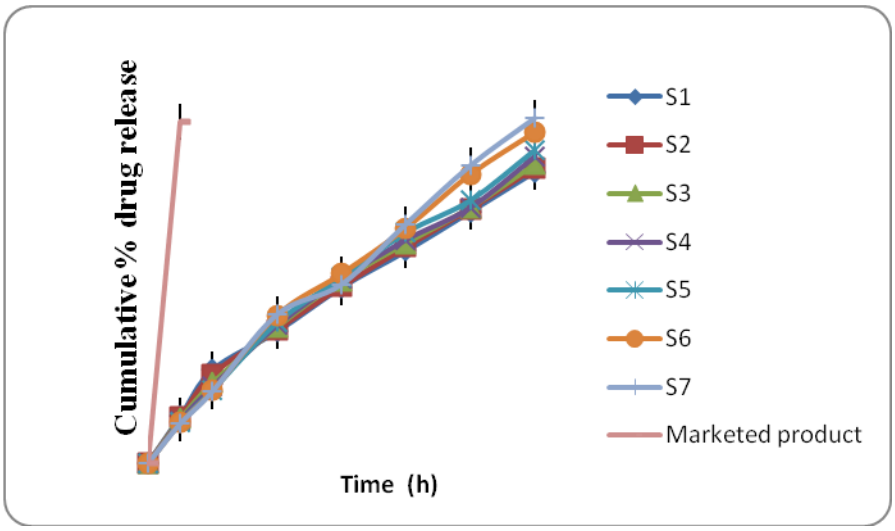

Figure 2: *In vitro* Cumulative % drug release of Nizatidine microspheres formulations S1 to S7 with marketed product

Table 5: *In vitro* Cumulative % drug release of Nizatidine microspheres formulations S8 to S14

Time (h)	S8	S9	S10	S11	S12	S13	S14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	14.32±0.15	13.15±0.12	12.56±0.23	17.36±0.12	15.56±0.24	16.56±0.11	21.39±0.52
2	25.21±0.12	26.12±0.20	24.40±0.12	23.50±0.11	23.78±0.72	21.15±0.23	32.26±0.15
4	37.30±0.42	38.10±0.12	40.27±0.23	40.43±0.15	45.27±0.16	38.14±0.24	40.35±0.32
6	51.42±0.21	52.62±0.12	53.42±0.13	53.21±0.76	53.18±0.12	52.33±0.21	53.26±0.11
8	63.46±0.27	64.57±0.14	63.26±0.67	64.82±0.71	69.20±0.13	63.53±0.32	64.17±0.16
10	68.70±0.32	70.20±0.11	72.76±0.83	78.76±0.81	82.12±0.17	73.62±0.55	75.32±0.17
12	70.20±0.24	74.18±0.15	78.54±0.72	82.65±0.92	84.26±0.65	83.51±0.51	82.70±0.21

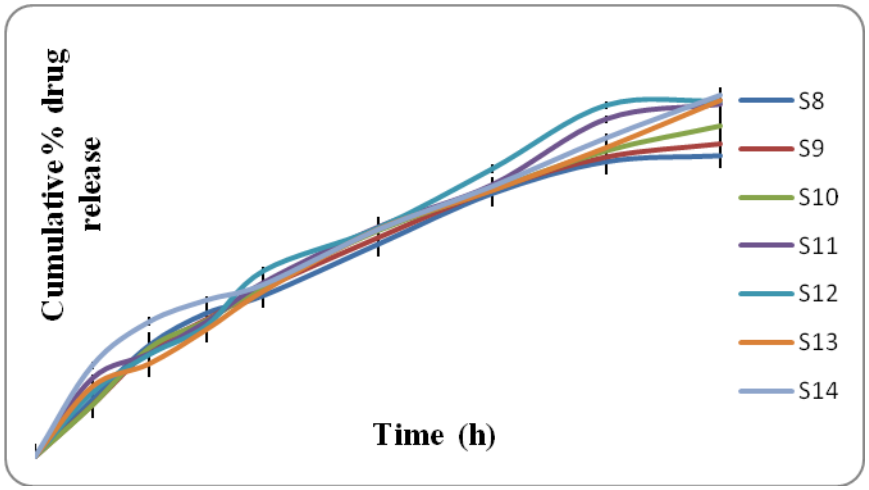

Figure 3: *In vitro* Cumulative % drug release of Nizatidine microspheres

Mathematical modeling of Nizatidine optimized microspheres (S6)

Table 6: Release order kinetics of optimized normal microspheres (S6)

Formulation code	Zero order	First order	Higuchi	Korsmeyer-Peppas	
S6	**R^2**	**R^2**	**R^2**	**R^2**	**n**
	0.983	0.897	0.974	0.970	1.884
Marketed product		0.884			

The prepared Nizatidine microspheres were fitted into various mathematical models like Zero order, First order, Higuchi and Korsmeyer-Peppas model in order to identify the mechanism of drug release and results were mentioned in Table 6. The drug release data plotted and correlation coefficients (R^2) was calculated from the linear curve slope portions. It is found that drug release from sustained release microsphere followed zero order and Higuchi model, followed the diffusion controlled.

Drug excipient compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

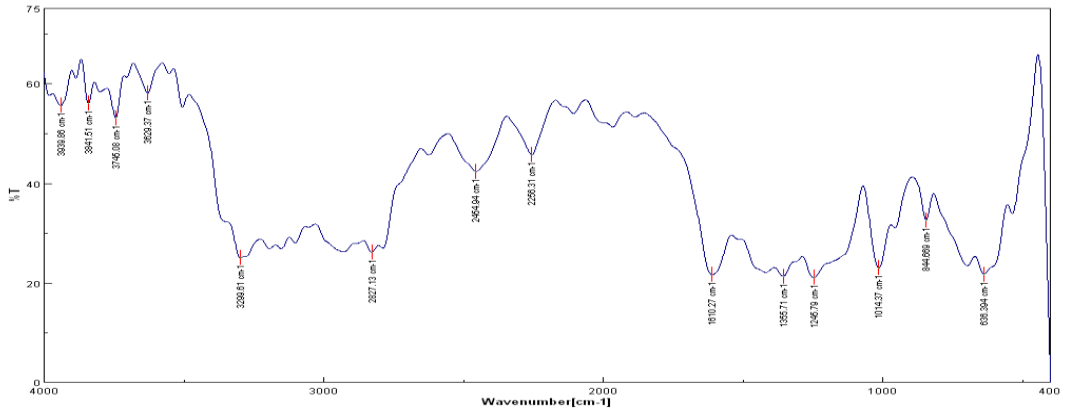

Figure 4: FTIR spectrum of pure drug Nizatidine

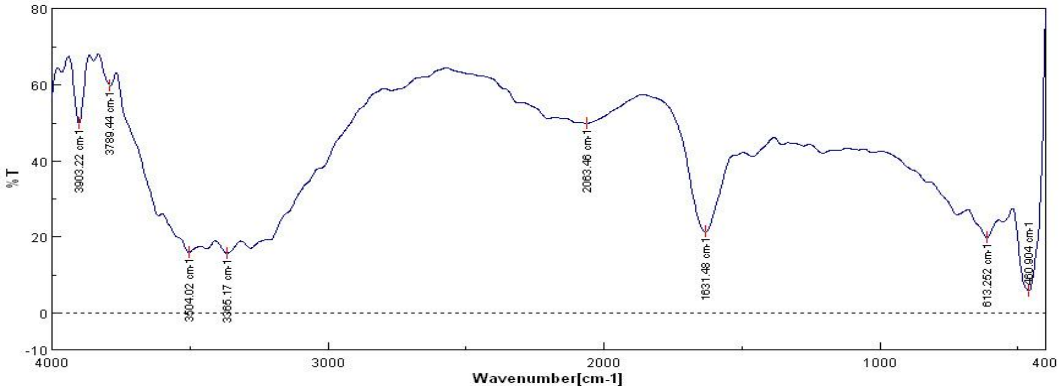

Figure 5: FTIR spectrum of Nizatidine optimized (S6) of microspheres

The FTIR spectrum of pure drug (Figure 4) showed characteristic sharp peaks of 3421 cm^{-1} (C-N stretch), 2951 cm^{-1} (C-H stretch), 1436 cm^{-1} (C=H deformation in NCH,CH), 1500 (CH & OCH groups), 1587 cm^{-1} (Conjugated with NO),1419 cm^{-1} for CH_2 bond. There were no new significant bands observed in the pure drug (Figure 4) and optimized formulation (Figure 5), which confirms that no interaction takes place between the drug and excipients.

SEM of Nizatidine microspheres

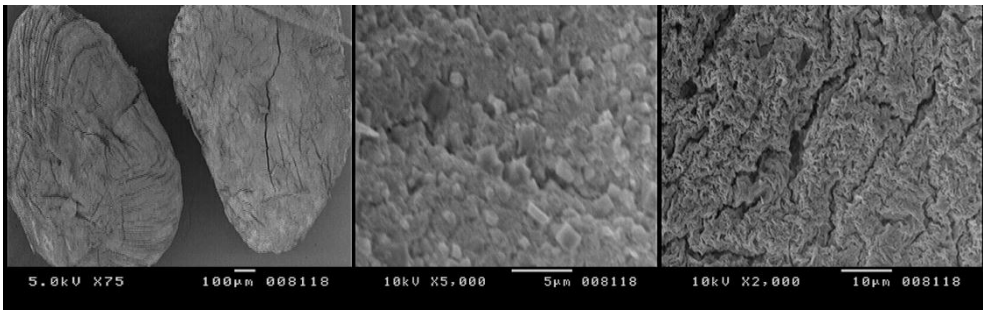

Figure 6: Scanning electron micrographs of Nizatidine microspheres

The SEM photomicrographs of the dried microspheres were shown in Figure 6. It was observed that shape of microspheres seems to be spherical with fairly rough outer surface. The surface was rough due to polymer matrix density which justifies the controlled release of the drug.

Stability studies

The results of stability studies was mentioned in Table 7 and indicated that there was no significant change in results before and after stability studies hence the optimized formulation S6 found to be stable.

Table 7: Staility studies of optimized normal microspheres

Retest time for optimized formulation (S6)	Percentage yield	Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	98.30	96.32	97.17
30 days	97.71	96.14	96.18
60 days	97.12	95.94	95.93
120 days	96.94	95.55	95.62
180 days	96.32	95.22	95.01

CONCLUSION

Nizatidine microspheres were successfully prepared by ionotropic gelation technique using sodium alginate as polymer and calcium chloride as cross linking agent and showed the higher encapsulation efficiency. Nizatidine release from the microspheres was found to be slow and controlled release over a period of 12h. The drug release from the microspheres followed the zero order, Higuchi model and Korsmeyer Peppas model which indicated the release was diffusion controlled and swelling, relaxation of polymer chain. The results of the current study indicated promising potential of microspheres in the delivery of drugs with lower half lives and less bioavailability with controlled release of Nizatidine in the management of peptic ulcer.

REFERENCES

[1]. Hailin Zhang, Weifeng Li , Xiumei Wang. Anti-ulcerogenic effect of cavidine against ethanol-induced acute gastric ulcer in mice and possible underlying mechanism. Internatinal immunopharmacology. 38, 2016, 450-459.

[2]. Jan Schulze, Stephan Hendrikx, Michaela Schulz-Siegmund. Microparticulate poly(vinyl alcohol) hydrogel formulations for embedding and controlled release of polyethylenimine (PEI)-based nanoparticles. Acta Biomaterialia. 45, 2016, 210-222.

[3]. Haogang Duan, Shaoyu Lü, Hongyan Qin. Co-delivery of zinc and 5-aminosalicylic acid from alginate/N-succinyl-chitosan blend microspheres for synergistic therapy of colitis. International Journal of Pharmaceutics 2016, 1-38.

[4]. Girod Fullana.S, Ternet.H, Freche.M. Controlled release properties and final macroporosity of a pectin microspheres–calcium phosphate composite bone cement. Acta Biomaterialia. 6, 2010, 2294–2300.

[5]. Junzi Wu, Gareth R.Williams, Christopher Branford-White. Liraglutide-loaded poly(lactic-co-glycolic acid) microspheres: Preparation and in vivo evaluation. European Journal of Pharmaceutical Sciences. 92, 2016, 28–38.

[6]. Chickering III D.E, Jacob J.S, Desai T.A. Bioadhesive microspheres: III. An in vivo transit and bioavailability study of drug-loaded alginate and poly(fumaric-co-sebacic anhydride) microspheres Journal of Controlled Release. 48, 1997, 35–46.

[7]. Sougata Jana, Arindam Das, Amit Kumar Nayak. Aceclofenac-loaded unsaturated esterified alginate/gellan gum microspheres: In vitro and in vivo assessment. International Journal of Biological Macromolecules. 57, 2013, 129– 137.
[8]. Fujioka.T, arakawa.T, shimoyama.T. Effects of rebamipide, a gastro-protective drug on the Helicobacter pylori status and inflammation in the gastric mucosa of patients with gastric ulcer: a randomized double-blind placebo-controlled multicentre trial. Aliment Pharmacol Ther. 18 (1), 2003, 146–152.
[9]. Remedios Guadalupe Gomez-Mauricio, Argia Acarregui, Francisco Miguel Sánchez-Margallo. A preliminary approach to the repair of myocardial infarction using adipose
[10]. tissue-derived stem cells encapsulated in magnetic resonance-labelled alginate microspheres in a porcine model. European Journal of Pharmaceutics and Biopharmaceutics. 84, 2013, 29–39.
[11]. Akash Jain, Vikas Pandey, Aditya Ganeshpurkar, Nazneen Dubey, and Divya Bansal. Formulation and characterization of floating microballoons of Nizatidine for effective treatment of gastric ulcers in murine model. Drug Deliv 2014, 1-6.
[12]. Sergio Benavides, Pablo Cortés, Javier Parada. Development of alginate microspheres containing thyme essential oil using ionic gelation. Food Chemistry. 204, 2016, 77–83.
[13]. Loreana Lacerda, Alexandre Luis Parize, Valfredo Fávere. Development and evaluation of pH-sensitive sodium alginate/chitosan microparticles containing the antituberculosis drug rifampicin. Materials Science and Engineering C. 39, 2014, 161–167.
[14]. Sanjay K. Motwani, Shruti Chopra, Sushma Talegaonkar. Chitosan–sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: Formulation, optimization and in vitro characterization. European Journal of Pharmaceutics and Biopharmaceutics, 68, 2008, 513–525.
[15]. Shanmuganathan.S, Shanumugasundaram.N, Adhirajan.N. Preparation and characterization of chitosan microspheres for doxycycline delivery. Carbohydrate Polymers. 73, 2008, 201–211.
[16]. Antonio J. Ribeiro Ronald J. Neufeld, Philippe Arnaud. Microencapsulation of lipophilic drugs in chitosan-coated alginate microspheres. International Journal of Pharmaceutics, 187, 1999, 115–123.
[17]. Savita Sonawane, Mangesh Bhalekar, Shamkant shimpi. Preparation and evaluation of microspheres of xyloglucan and itsthiolated xyloglucan derivative. International Journal of Biological Macromolecules 2014, 1-7.
[18]. Silvia Vicini, Maila Castellano, Marco Mauri. Gelling process for sodium alginate: New technical approach by using calcium rich micro-spheres. Carbohydrate Polymers. 134, 2015, 767–774.
[19]. Bo Li, Zhongning Liu, Jingwen Yang. Preparation of bioactive β-tricalcium phosphate microspheres as bone graft substitute materials. Material science and engineering C. 70(2), 2016, 1200-1205.
[20]. Guijin Liu, Shaomin Li, Yinxia Huang. Incorporation of 10-hydroxycamptothecin nanocrystals into zein microspheres. Chemical engineering science. 155, 2016, 405-414.
[21]. Yan Li, Haibing He, Qiao Wang, Xing Tang. Preparation, stability and pharmacokinetics evaluation of lipid microspheres loading a promising antitumor candidate, Timataxel. Asian Journal of Pharmaceutical Sciences. 11, 2016, 771–779.