Original Research Article

Fabrication and *in vitro* evaluation of mucoadhesive ondansetron hydrochloride beads for the management of emesis in chemotherapy

Raj Kaur Malik, Prashant Malik, Neha Gulati, Upendra Nagaich

Department of Pharmaceutics, School of Pharmacy, Bharat Institute of Technology, Meerut, Uttar Pradesh, India

Abstract

Background: Mucoadhesive beads were fabricated and evaluated for controlled release of an antiemetic drug 'Ondansetron Hydrochloride'. Ondansetron hydrochloride is a serotonin 5-HT_3 receptor antagonist mainly used for the treatment of emesis, which occurs as a side effect of chemotherapy. **Materials and Methods:** The present work was to fabricate and evaluate ondansetron-loaded microbeads by using chitosan as mucoadhesive and sustained release polymer. Sodium tripolyphosphate (Na-TPP) was used as a cross-linking agent. The microbeads were successfully prepared by ionotropic gelation technique. The particle size, entrapment efficiency, and mucoadhesive strength of drug-loaded formulations was measured by an optical microscope, direct crushing method, and *in vitro* wash-off method, respectively. **Results:** Particle size, entrapment efficiency, mucoadhesive strength, and *in vitro* drug release of optimized formulation was found to be 760.11 ± 1.02 μm, 75.09 ± 2.40%, 95.14 ± 0.27% and 87.45 ± 1.21%, respectively. The data was fitted to different kinetic models to illustrate its anomalous (non-Fickian) diffusion. **Conclusions:** The results revealed that ondansetron HCl loaded microbeads are most suitable mode of drug delivery for promising therapeutic action. Ondansetron HCl-loaded microbeads can prove to be potential pharmaceutical dosage forms for sustaining the drug release and reducing the dose frequency.

Key words: Chitosan, ionotropic gelation technique, mucoadhesion, release kinetics, sodium tripolyphosphate

INTRODUCTION

In current scenario, broad efforts are being made in pharmaceutical research laboratories for the progress of novel and targeted drug delivery system with the purpose of better therapeutic efficacy, lesser side effects, and dose for the treatment of various diseases.[1] Significant attention is focused on the development of sustained drug delivery systems which have better patient compliance than the conventional regimens that requires frequent dosing.[2] Microbeads are small, solid, and free-flowing colloidal carriers containing dispersed active agents either in solution or crystalline form which allows sustained release or multiple release profiles. The benefits of this novel dosage form are improvement in stability, enhancement of solubility, and therapeutic efficacy without major side effects.[3] Ondansetron HCl, a prototype of a new class of anti-emetic drugs controls chemotherapy-induced vomiting. Chemotherapeutic agents may cause release of 5-Hydroxytryptamine (5-HT) in small intestine which initiates vomiting reflex by activating vagal afferents via 5-HT_3 receptors. It blocks the depolarizing action of 5-HT through 5-HT_3 receptors in the gastrointestinal tract.[4,5] The shorter biological half-life (in plasma 4-5 hours) and frequent dosing of ondansetron to treat nausea and vomiting induced by chemotherapy, make it as an ideal candidate for sustained release drug-delivery system.[6] Thus, the objective of the work is to provide a pharmaceutical composition containing ondansetron in a modified release formulation to maintain the blood levels of the active ingredient for a prolonged period of time.[7] Ionotropic gelation is based on the capability of polyelectrolytes to cross-link in the presence of counter ions to form microbeads.[8] Chitosan is a polysaccharide polymer obtained by deacetylation of chitin, shows excellent mucoadhesive, and permeation enhancing effect across biological surfaces and is soluble in dilute aqueous acidic solution. In acidic medium, the amine groups of chitosan are protonated resulting in a soluble, positively charged polysaccharide that has a high-charge density.[9] It is a weak base and on reaction

Address for correspondence:
Ms. Raj Kaur Malik,
Department of Pharmaceutics, School of Pharmacy,
Bharat Institute of Technology, Meerut - 250 103,
Uttar Pradesh, India.
E-mail: rajkaurmalik18@gmail.com

Access this article online

Quick Response Code:

Website: www.jpionline.org

DOI: 10.4103/2230-973X.108962

with sodium tripolyphosphate (cross-linking agent) can convert the glucosamine units into a soluble form R-NH3$^+$. It gets precipitated in alkaline solution or with polyanions and at lower pH forms gel.[10] The microbeads were formed instantaneously due to the electrostatic attraction between NH_3^+ on chitosan and PO_4^- on sodium tripolyphosphate (Na-TPP).[11] Thus, the aim of present research work was to fabricate and evaluate mucoadhesive ondansetron hydrochloride-loaded beads for its prolonged release and reduced dosing frequency.

MATERIALS AND METHODS

Materials

Ondansetron HCl was received as a gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Chitosan (85% decetylated) and sodium tripolyphosphate (TPP) were purchased from SD Fine Chem Ltd., India and Loba Chemie Pvt. Ltd., India, respectively. All other chemicals were of analytical grade.

Methods

Preparation of ondansetron microbeads

Ondansetron HCl-loaded microbeads were prepared by dissolving chitosan in 1% v/v glacial acetic acid with agitation to have different concentration (1%, 1.5%, 2%, 2.5%, 3% w/v). Ondansetron HCl was dissolved in methanol and added to chitosan solution. Ondansetron-chitosan solution was added dropwise using 20-G hypodermic needle fitted with syringe into different concentration of sodium tripolyphosphate solution (1.5%, 2%, 2.5%, 3%, 3.5% w/v) at room temperature with continuous magnetic stirring. After 10 min of contact time, the beads were filtered and air dried.[12] The composition of the various formulations is mentioned in the Table 1.

EVALUATION OF MICROBEADS

Particle size determination

The particle size of 30 beads was measured by using optical microscope and micrometer for each formulation and mean particle size was determined as shown in Table 2.[13]

Table 1: Formulation table of ondansetron HCl-loaded chitosan beads

Formulation code	Polymer concentration (%w/v)	Cross-linking agent concentration (%w/v)	Drug (mg)
F1	1	1.5	4
F2	1.5	2	4
F3	2	2.5	4
F4	2.5	3	4
F5	3	3.5	4

Determination of drug entrapment efficiency

Ten milligrams of the crushed microbeads were dissolved in 10 ml methanol, vortexed for 5 min and filtered. The filtered samples were suitably diluted and analyzed spectrophotometrically using UV spectrophotometer (UV-VIS double beam spectrophotometer 2201, Systronics) at 248 nm against suitably constructed calibration curve.[14] The entrapped drug is calculated by using formula:

$$\text{Entrapment efficiency}\ (\%) = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Mucoadhesion testing by in vitro wash-off method

The mucoadhesive properties of various formulations of ondansetron HCl-loaded chitosan beads were evaluated by the *in vitro* wash-off method. Freshly excised pieces of goat intestinal mucosa (1 × 1 cm, collected from a slaughter house) were mounted on a glass slide (7.5 × 2.5 cm) using thread. About 50 beads were spread out on each piece of mucosa and then hang from the arm of the tablet disintegration test apparatus (PLT-280 TANCO). The tissue specimen was given a regular up and down movement in vessel containing 900 ml of phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C. The adherence of beads was regularly observed. The beads that remained adhered to the mucosa were counted at regular intervals for up to 10 h.[15]

$$\%\ \text{Mucoadhesion} = \frac{\text{Number of adhered microbeads}}{\text{Total number of applied microbeads}} \times 100$$

In-vitro drug release studies

The release of ondansetron HCl was studied in 900-ml phosphate buffer saline (PBS pH 6.8) as dissolution media using USP II (paddle type) dissolution apparatus (DS 8000, LABINDIA). The samples were withdrawn at an interval of 0, 15, 30, 45, 60, 120, 240, 360, 480 min and replaced simultaneously with fresh buffer. The collected samples were assayed with the help of UV spectrophotometer at 248 nm. Finally, the cumulative amount of drug release from the formulation was calculated with the help of calibration curve of ondansetron HCl to determine the release pattern.[16] All measurements were carried out in triplicate and average values plotted. The data for cumulative *in vitro* drug release for all formulations are given in Table 3 and Figure 1.

Table 2: Evaluation table of ondansetron HCl-loaded microbeads

Formulation code	Particle size (μm)[a]	Entrapment efficiency (%)[a]	Mucoadhesion (%)[a]	Cumulative *in vitro* drug release (%)[a]
F1	640.01±0.74	30.10±0.20	78.17±0.08	54.71±0.68
F2	690.05±1.13	49.12±1.59	83.04±0.13	72.82±0.98
F3	760.11±1.02	75.09±2.40	95.14±0.27	87.45±1.21
F4	850.09±0.97	70.09±1.37	87.23±0.17	65.29±1.34
F5	872.14±0.58	63.28±1.61	81.01±0.09	51.34±0.79

[a]Each value indicate the mean±SD (n=3)

Table 3: Data for cumulative *in vitro* drug release for all formulations

Time (min)	Formulations				
	F1[a]	F2[a]	F3[a]	F4[a]	F5[a]
0	0	0	0	0	0
15	1.06±0.12	1.37±0.05	2.61±0.07	1.47±0.08	1.29±0.04
30	1.71±0.43	2.44±0.15	4.83±0.09	1.76±0.10	1.53±0.11
45	4.62±0.96	8.35±1.98	10.97±1.83	5.39±1.76	5.19±1.23
60	19.50±1.21	27.21±2.67	32.24±3.00	22.87±1.64	18.08±2.30
120	24.23±1.28	38.19±2.34	43.81±2.31	29.16±2.47	27.04±2.13
240	29.32±2.39	54.90±1.54	61.07±2.49	41.23±1.87	38.12±1.56
360	35.26±0.98	60.10±2.32	78.98±1.87	56.74±1.94	47.19±1.62
480	54.71±1.89	72.82±1.75	87.45±2.12	65.29±1.72	51.34±1.90

[a]Each value indicate the mean±SD (n=3)

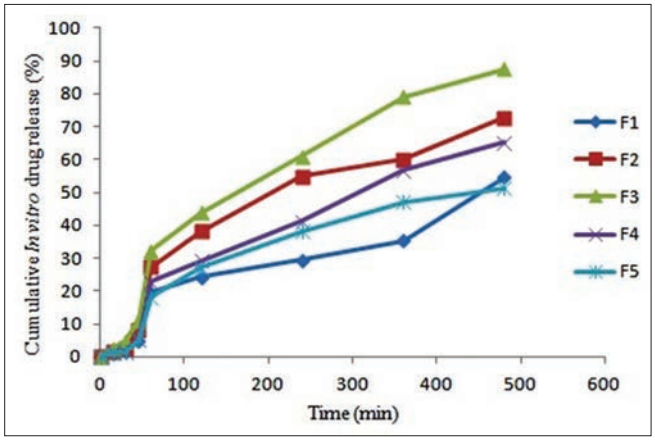

Figure 1: Cumulative *In vitro* drug release for all formulations

Release kinetic study

In order to understand the mechanism and kinetics of drug release, the drug release data of *in vitro* dissolution study was analyzed with various kinetic models like zero-order, first order, Higuchi and Korsmeyer-Peppas model. Regression (r^2) values were calculated for the linear curves obtained by regression analysis.[17] The *in vitro* release kinetics data for optimized formulation (F3) is given in Table 4. The interpretation of diffusional release mechanisms are shown in Table 5.

Stability study

The developed formulations were subjected to stability studies at different temperatures like refrigerate (2-4°C), room temperature, warm and humidity (40°C/75%RH) for 45 days. The particle size and entrapment efficiency of microbeads are determined. The results from the stability studies indicated that microbeads were physically and chemically stable for more than 45 days. The stability study data for optimized formulation (F3) is given in Table 6.

RESULTS AND DISCUSSION

The formulation of mucoadhesive ondansetron HCl-loaded beads for the management of emesis in chemotherapy was done by using chitosan as sustained release polymer and sodium tripolyphosphate as cross linking agent. An ionotropic gelation technique was used to prepare microbeads, in which chitosan converts the glucosamine units into a soluble form R-NH3$^+$, which results in the formation of gel due to interaction with sodium tripolyphosphate.[10] The particle size of microbeads was found to be 640.01 ± 0.74, 690.05 ± 1.13, 760.11 ± 1.02, 850.09 ± 0.97 and 872.14 ± 0.58 μm for F1, F2, F3, F4, and F5, respectively [Table 2]. It was observed that the particle size vary significantly either by altering the polymer ratio or variation in the exposure time to sodium tripolyphosphate.[18] A significant increase in the percentage entrapment efficiency was observed with increase in polymer concentration but decrease with

Table 4: *In vitro* release kinetics data for F3 formulation

Release kinetics	Regression value
Zero order	0.916
First order	0.621
Higuchi	0.957
Korsmeyer-Peppas	0.909

Table 5: Interpretation of diffusional release mechanisms

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45 <n=0.89	Non-Fickian transport	t^{n-1}
0.89	Case ∏ transport	Zero-order release
Higher than 0.89	Super case ∏ transport	t^{n-1}

Table 6: Stability study data for optimized formulation (F3)

Time (days)	Optimized formulation (F3)			
	Refrigeration temperature (2-4°C)		Room temperature	
	Particle size	Entrapment efficiency	Particle size	Entrapment efficiency
0	760.11±1.02	75.09±2.40	760.11±1.02	75.09±2.40
7	761.88±1.13	75.97±1.40	759.98±1.02	74.99±1.49
14	761.27±1.35	75.82±1.29	759.67±2.03	74.87±2.47
21	761.02±2.01	75.76±1.38	759.65±1.31	74.82±1.70
28	762.92±2.34	75.71±2.09	759.55±0.09	74.80±1.25
35	762.72±1.89	75.66±1.90	759.55±1.76	74.79±2.73
45	762.70±1.32	74.98±1.44	759.47±1.37	74.79±0.91

increase in cross-linking agent concentration.[11] Drug loading was found to be high for F3 as compared to other formulations. The *in vitro* wash-off test showed that as the percentage of chitosan is increased in the formulation, its mucoadhesiveness increases. But on further increasing the chitosan concentration, mucoadhesiveness decreases. This may be due to the fact that at a higher concentration of chitosan coiling of the polymer molecules occur, reducing the flexibility of the polymeric chain, thereby reducing the mucoadhesive strength.[19] The results of the wash-off test indicated that the microbeads had fairly good mucoadhesive property. *In vitro* dissolution study was carried out in USP-∏ (paddle type) dissolution apparatus in PBS (pH 6.8). The ondansetron HCl-loaded chitosan microbeads demonstrated a drug release of 87.45 ± 1.21% in 8 hours for F3 formulation. The release of ondansetron HCl from microbeads reveals more sustained nature with an increase in chitosan concentration. The results clearly indicate that the rate of drug release decreased with the increase of cross linking agent concentration, because excess sodium tripolyphosphate cause the cross-linking of drug.[20] The *in vitro* release data of ondansetron HCl was processed to understand the linear relationship which follows higuchi order of kinetics (release rate is governed by rate of diffusion through matrix) because it have highest regression value i.e. 0.9573 and then followed by zero order and first order. According to Korsmeyer-Peppas equation, the value of n is 0.815 which is more than 0.5 which indicates it follows anomalous (non-Fickian) diffusion. The stability study of optimized batch was carried out at different temperatures i.e., refrigeration, RT, and accelerated which displays that the microbeads were stable at refrigeration and RT while slight degradation was observed at accelerated conditions. This can be attributed to the fact that at accelerated conditions, chitosan gets degraded as it has tendency to get swell at high humidity conditions.

CONCLUSIONS

In conclusion, ionotropic gelation technique can be used for preparation of ondansetron HCl loaded chitosan microbeads using sodium tripolyphosphate as cross linking agent. Prepared microbeads shown higher drug entrapment and sustained release characteristics. Ondansetron HCl release from microbeads was influenced by chitosan (sustained release polymer) and sodium tripolyphosphate (cross-linking agent). From the mucoadhesion testing by *in vitro* wash-off method, it was observed that F3 formulation exhibits greater mucoadhesive strength than other formulations. Data for *in vitro* drug release indicated sustained release behaviour of all formulations. On fixing the *in vitro* release data of optimized formulation to various kinetic models, it was found that it exhibit Higuchi order of kinetics followed by zero order and first order. The formulation undergoes anomalous (non-Fickian) diffusion. Thus, ondansetron HCl-loaded microbeads can prove to be potential pharmaceutical dosage forms for sustaining the drug release and reducing the dose frequency.

REFERENCES

1. Battu S, Prasanna RI, Devi KJ, Swamy N, Chetty CM, Purushothaman M, *et al*. Preparation and characterization of cefadroxil loaded alginate microbeads. Int J Res Pharm Sci 2010;1:386-90.
2. Sherina VM, Santhi K, Sajeeth CI. Formulation and evaluation of sodium alginate microbeads as a carrier for the controlled release of nifedipine. Int J Pharm Chem Sci 2012;1:699-710.
3. Hemalatha K, Lathaeswari R, Suganeswari M, Kumar SV, Shering AM. Formulation and evaluation of metoclopramide hydrochloride microbeads by ionotropic gelation method. Int J Pharma Biol Arch 2011;2:921-5.
4. Nagaich U, Chaudhary V, Karki R, Yadav A, Sharma P. Formulation of medicated chewing gum of ondansetron hydrochloride and its pharmacokinetic evaluations. Int J Pharm Sci 2010;1:32-40.
5. Patel P, Makwana S, Jobanputra U, Ravat M, Ajmera A, Patel M. Sublingual route for the systemic delivery of ondansetron. Int J Drug Dev Res 2011;3:36-44.
6. Amish DA, Zankhana SP, Joshi J. Formulation and evaluation of transdermal ondansetron hydrochloride matrix patch: *In vitro* skin permeation and irritation. Int J Pharm Res Appl Sci 2012;1:26-34.
7. Sunitha S, Amareshwar P, Kumar SM. Preparation and characterization of ondansetron hydrochloride microspheres using various cellulose polymers. Int J Curr Pharm Res 2010;2:44-9.
8. Raja MK, Ramu, Eswarama, Anusha C. Formulation, characterization and evaluation of aceclofenac- alginate/potato starch micro beads. Indian J Novel Drug Deliv 2012;4:151-6.
9. Desai KG, Park HJ. Preparation and characterization of drug-loaded chitosan-tripolyphosphate microspheres by spray drying. Drug Develop Res 2004;64:114-28.
10. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, *et al*. Chitosan microspheres as a potential carrier for drugs. Int J Pharm 2003;274:1-33.
11. Madgulkar A, Bhalekar M, Swami M. *In vitro* and *in vivo* Studies On Chitosan Beads Of Losartan Duolite Ap143 Complex, Optimized By Using Statistical Experimental Design. Aaps Pharm Sci Tech 2009 Jun 3;10:743-51.
12. Gulati N, Nagaich U, Sharma VK, Khosa RL. Effect of polymer and cross linking agent on *in vitro* release of quercetin from microbeads. Asian J Pharm Life Sci 2011;1:401-5.
13. Bagyalakshmi J, Raj AR, Ravi TK. Fomulation, physical charecterisation and in-vitro release studies of prednisolone alginate beads for colon targeting by ionotropic gelation. Pharmacie Globale 2011;3:1-4.
14. Girhepunje KM, Krishnapiillai, Pal RS, Gevariya HB, Thirumoorthy N. Celecoxib loaded microbeads: A targeted drug delivery for colorectal cancer. Int J Chem Pharm Res 2010;2:46-55.
15. Nayak AK, Hasnain MS, Beg S, Alam MI. Mucoadhesive beads of gliclazide: Design, development, and evaluation. Sci Asia 2010;36:319-25.
16. Manjanna KM, Shivkumar B, Raichur AM. Effect of manufacturing conditions on physico-chemical characteristics of aceclofenac sodium microbeads for oral modified drug delivery. Der Pharmacia Lettre 2010;2:486-508.
17. Patil DA, Patil GB, Deshmukh PK, Belgamwar VS, Fursule RA. Chitosan coated mucoadhesive multiparticulate drug delivery system for gliclazide. Asia J Pharm Clin Res 2009;2:18-25.

18. Kulkarni PV, Keshavayya J. Chitosan sodium alginate biodegradable interpenetrating polymer network (ipn) beads for delivery of ofloxacin hydrochloride. Int J Pharm Pharm Sci 2010;2:77-82.
19. Garud A, Garud N. Preparation and evaluation of chitosan microcapsules of metronidazole using tripolyphosphate cross-linking method. Dhaka Univ J Pharm Sci 2010;9:125-30.
20. Manjanna KM, Shivakumar B, Kumar PTM. Diclofenac sodium microbeads for oral sustained drug delivery. Int J Pharm Tech Res 2009;1:317-27.

How to cite this article: Malik RK, Malik P, Gulati N, Nagaich U. Fabrication and in vitro evaluation of mucoadhesive ondansetron hydrochloride beads for the management of emesis in chemotherapy. Int J Pharma Investig 2013;3:42-6.
Source of Support: Nil. **Conflict of Interest:** None declared.