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Research Article

FORMULATION AND EVALUATION OF CHITOSAN PRAZOSIN BEADS BY IONOTROPIC GELATION METHOD

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ABSTRACT

The hydrogels or polyelectrolyte systems are based on ionotropic gelation and ionic cross linking. They have been employed to prepare a controlled release dosage form. These systems are based upon the fact that their structure can entrap the drug within them. They normally employ a hydrophilic matrix system. Prazosin loaded chitosan polyelectrolyte complex (PEC) hydrogel beads were prepared via ionotropic gelation and ionotropic crosslinking with sodium tripolyphosphate (TPP). A combination of Eudragit polymer was studied with chitosan having Prazosin dispersed within them. Thus, prazosin dispersed in 2% glacial acetic acid and having chitosan and polymer dispersed within. It was cross linked with 2% sodium tripolyphosphate solution adjusted to a pH of 4.5-6. The beads prepared were examined for the optimal stirring conditions and curing time in order to obtain spherical beads. Beads were prepared by three different drug: polymer ratios (1:1, 1:1.5, 1:2). Spherical to oval beads with varying particle size, weight, drug entrapment efficiency (DEE), and sustained release profile were obtained depending on the drug and polymer combination used. These beads were able to sustain the release of Prazosin from the beads. The *in vitro* dissolution rate profile showed a sustained release of the drug from the beads over a 7 hour study period. Prazosin release decreased with an increasing concentration of chitosan.

Keywords: Beads, chitosan, Prazosin, Eudragit RL 100, Ionotropic gelation.

INTRODUCTION

Gastroretentive drug delivery systems are defined as systems that increase the retention of a per-oral dosage form in the stomach offering numerous advantages for drugs exhibiting an absorption window in the gastro intestinal (GI tract), drugs that are poorly soluble in the alkaline medium, and drugs that are intended for local action on the gastro-duodenal wall. The retention of oral dosage forms in the upper gastro intestinal tract (GIT) causes prolonged contact time of drug with the GI mucosa, leading to higher bioavailability, and hence better therapeutic efficacy, reduced time intervals for drug administration, potentially reduced dose size and thus improved patient compliance. Therefore, extended release drug delivery systems (DDS) possessing gastric retention properties may be potentially useful (Eldeen et al., 2006).

Over the last three decades, various approaches have been pursued to design gastroretentive delivery systems including floating systems, modified shape systems, swelling and expanding systems, bioadhesive systems, and high density systems. Based on these approaches, floating drug delivery systems seems to be the promising delivery systems for controlled release of drugs (Rani et al., 2010). Gastro-retentive multiparticulate drug delivery systems include Floating beads, Floating Microspheres and Floating Granules (Dasharath et al., 2011). The Floating Beads are prepared using polymers having the property of ionotropic gelation like sodium alginate and pectin. Floating microspheres are free flowing powders consisting of proteins or synthetic polymers. Floating granules can be

prepared using a drug with suitable lipophilic polymer having low density (Rani et al., 2010). Gastro-retentive multiparticulate drug delivery system offers several applications for drugs having poor bioavailability because of the narrow absorption window in the upper part of the GIT. It retains the dosage form at the site of absorption and thus enhances the bioavailability. These systems are particularly advantageous for drugs that are specifically absorbed from stomach or the proximal part of the small intestine, e.g., Riboflavin, Furosemide. Bilayer-floating capsule was developed for local delivery of Misoprostol, which is a synthetic analog of prostaglandin E1 used as a protectant of gastric ulcers caused by administration of Non steroidal Antiinflammatory Drugs (NSAIDs), thus exhibiting site specific drug delivery. Hollow microspheres of non-steroidal anti-inflammatory drugs are very effective for controlled release as well as it reduces the major side effect of gastric irritation; for example floating microspheres of Indomethacin are quiet beneficial for Rheumatic patients (Dasharath et al., 2011).

Beads are multiple unit drug delivery systems and better suited because they reduce the inter-subject variability in absorption, lower the probability of dose dumping and overcome all- or non emptying process prominent in case of single unit floating drug delivery systems. The beads have inbuilt advantages as floating drug delivery with good floating efficiency and zero floating lag time. The processing does not require sophisticated instrumentation and techniques as in case with microspheres. In addition, processing and finished product handling is quite easy and practical in case of beads (Khobragade et al., 2009).

Prazosin is a Antihypertensive drug classified as a Adrenergic Receptor antagonist, alpha blocker (Li et al., 1991). In the present study Prazosin beads were prepared by ionotropic gelation method by using two different polymers Chitosan and Eudragit RL 100 and tripolyphosphate (TPP). Chitosan has been proposed as a useful excipient for obtaining sustained release formulation and for enhancing the bioavailability of poorly water-soluble drug (Prazosin) (Josias, 2010, Pillai et al., 2009, Pradip et al., 2004, Motohiko et al., 1999). The purpose of this study was to prepare and evaluate the chitosan gel beads as a new controlled drug release system for Prazosin. Another purpose is to investigate the conditions in which the polymer bead is formed, and hence the dependence of the drug releases on bead formation. This study reports the effects of matrixing polymers on *in vitro* dissolution profile of the controlled release beads of Prazosin.

MATERIALS AND METHODS

Materials

Prazosin was supplied as a gift sample from Sun Pharmaceuticals, Gujarat. Chitosan was procured from India Sea Foods, Cochin and Eudragit RL-100 was procured from Yarrow Pharmaceuticals, Mumbai, India. Sodium Tripolyphosphate was purchased from Rajesh Chemicals, Mumbai. All other chemicals and reagents used were of laboratory or analytical grade.

Methods

Preparation of Prazosin Chitosan beads by Ionotropic gelation method

Drug-loaded hydrogel beads were produced by ionotropic gelation method using TPP as the gelling counterion. The complexation mechanism is an ionotropic crosslinking or interpolymer complex. Chitosan with eudragit RL was dissolved in acetic acid (2% v/v) and stirred for 6-7 hours at 1000 rpm (Remi Equipments, Mumbai). Weighed amount of Prazosin (Table 1) was added to the polymeric solution and stirred on a magnetic stirrer for 2 hours, and allowed to stand till the removal of the entrapped air bubbles. The pH of the drug-chitosan solution was adjusted to pH 4.5-6 with dilute alkali solution (0.1 M, NaOH). Chitosan solution containing drug was added dropwise using a syringe fitted with a flat-end needle (23G, 0.7 mm id) into sodium tripolyphosphate solution (2% w/v, pH 5, 60°). Beads were left for 20-30 min, unless otherwise specified, and after curing, were collected by filtration, washed twice with distilled water and dried at 50° for 4 hours and then at room temperature (25°) for 12 hours (Payam et al., 2008, Singh et al., 2011).

Initially only the drug and chitosan beads were prepared. But beads were having problem in drying and were not strong enough to hold integrity. So beads were formulated using drug, chitosan and the second polymer eudragit. The detailed compositions of each formulation are seen in the (Table 1).

Evaluation of Prazosin Beads

Appearance

The general appearance and elegance of the beads were identified visually, which include analyzing the beads size, shape, color, presence or absence of an odor, taste, surface texture etc.

Bead Size, Weight, Yield and Swelling

The average diameter for both wet and dry beads (10 beads) was measured using a slide caliper scale. The weight of 10 beads from each batch was taken and the average was calculated. The total yield (%) and percent swelling were calculated as follows:

$$\text{Yield (\%)} = \frac{\text{(weight of the dry beads)}}{\text{Total weight of raw material}} \times 100$$

$$\text{Swelling (\%)} = \frac{\text{(weight of wet beads – weight of dry beads)}}{\text{Weight of weight beads}} \times 100$$

The swelling kinetics for the hydrogels can be classified as diffusion controlled and relaxation controlled.

Drug Entrapment Efficiency

The filtrate obtained after bead collection on the filter medium and diluting with phosphate buffer 7.4 was analyzed using a UV-Visible spectrophotometer and the absorbance value of the solution was noted from the UV spectrophotometer. This value was then compared using the calibration curve. The calibration curve was first obtained by scanning the wavelength at maximum absorbance. This occurred at about 254 nm. The concentration of the drug was known at this wavelength (Takka and Gurrel, 2010).

$$\text{DEE \%} = \frac{\text{(Total drug – Drug in solution)}}{\text{Total drug}} \times 100$$

DEE = Drug entrapment efficiency

Scanning electron microscopy (SEM)

The shape and surface morphology of the beads were studied using scanning electron microscope (National Institute of technology, Suratkal, Mangalore, India). Beads were mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film (thickness 200 nm) under reduced pressure (0.001 mm of Hg). The beads were viewed at an accelerating voltage of 10KV (Shishu et al., 2006).

Drug release

The dried beads were suspended in 900 mL of Phosphate Buffer (pH 7.4) in a glass flask and maintained at 37°C, and at 50 rpm. A 5 ml sample of the solution was removed from the flask after 15, 30, 45 and 60 minutes and then after 2, 3, 4, 5, 6 and 7 hours. The volume of each sample was replaced with the same volume of phosphate buffer (pH 7.4) to maintain the sink conditions (22). The amount of Prazosin released from the beads was analyzed using a UV spectrophotometer at 254 nm. The *in vitro* release studies were performed in triplicate for each of the samples (Arica et al., 2005).

Characterization of Prazosin Beads

FTIR spectroscopy

The drug - excipients interaction were studied using Fourier transform infrared spectrophotometer (FTIR 1615, Perkin Elmer, USA). An IR spectrum for the drug was recorded in a FTIR with Potassium Bromide (KBr) pellets. The spectra were scanned over the 3600 to 400 cm-1 range.

Statistical analysis of data

Data were expressed as mean±S.D. Statistical evaluation was performed by one-way analysis of variance (ANOVA) at a significance level of $p<0.05$ by Dunnett's multiple comparison test using GraphPad Prism software version 4.03.

RESULTS AND DISCUSSION

Percentage yield

The percentage yields of formulations F1 to F3 were calculated and the yield of F_1 was found to be 70%. The other formulations show comparatively less percentage yield. Percentage yield was found to be 64.7% and 56.79% for formulations F_2 and F_3 respectively as shown in table 2, 3 and 4. The loss of material during preparation of beads may be due to process parameters as well as during filtration of beads.

Measurement of the Bead Size, Weight and Swelling

The mean particle size of the beads containing Chitosan and Eudragit RL 100 was found to be 1.45 ± 0.05mm, 1.50 ± 0.09mm, and 2.50 ± 0.20mm. With increase in polymer concentration in beads, the particle size of beads increased. This may be because of viscosity of polymer solution which increases as the polymer concentration increases. The stirrer speed was maintained at 1000 rpm. The pKa value for chitosan was 6.3. Thus, at pH 6.0, a higher amount of precipitation of the complex took place. Chitosan acted as a polymeric drug carrier because of its biocompatibility, biodegradability, low toxicity and natural source of origin.

The swelling rate was found to be around (12 % to 17 %) which describes the amount of hydrophilicity, the beads can sustain on interaction with water. The yield was found to be less due to the small batch size and thus

can be improved by making bigger batches and improving the crosslinking methods.

Drug Entrapment Efficiency

The drug entrapment efficiency from the hydrogel beads was obtained using UV spectrophotometry. The filtrate obtained after bead collection on the filter medium was diluted with phosphate buffer 7.4 and was analyzed using a UV-Visible spectrophotometer and the absorbance value of the solution was noted at 254nm. This value was then compared with the calibration curve.

The percentage entrapment efficiency (%EE) for the Chitosan beads prepared using various compositions were calculated using the formula.

$$\text{DEE \%} = \frac{(\text{Total drug} - \text{Drug in solution}) \text{ X } 100}{\text{Total drug}}$$

DEE = Drug entrapment efficiency

The %EE was found to be 73.42%, 71% and 59% for F_1, F_2 and F_3 respectively. It was observed that increasing polymer concentration decreased the drug entrapment efficiency.

***In vitro* drug release studies**

The *in vitro* drug release from the beads in phosphate buffer pH 7.4 was performed using the USP II Dissolution test apparatus. The *in-vitro* drug release study was done for the chitosan –polymer-TPP drug loaded beads for a 6-7 hour study. The *in-vitro* dissolution results showed that beads were sustaining the drug release. The dissolution characteristics are used to compare the release rates of drugs prepared as chitosan beads.

It can be seen that the beads released the drug within 1-2 hours to its maximum over the entire 6-7 hour study period. The beads were able to achieve the maximum solubility of the encapsulated drug. This may be due to the crosslinking. The reason for the sustained release might be due to the entanglement of polymer chains in the beads because of strong ionic interactions. Thus, the penetration of the dissolution medium into the hydrogel beads was made difficult.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy of formulation F_1, F_2 and F_3 were carried out. It was observed that beads were almost spherical in shape and not having a smooth surface. When the concentration of polymer increased from F_1 to F_3, the beads were not having complete spherical shape and smooth surface.

Release Kinetics

To investigate the drug release mechanism, the *in vitro* release data was fitted into various kinetic models and the release mechanism of Prazosin from various formulations was determined by comparing their respective correlation coefficient. The best fit with the highest correlation coefficient was observed in zero order plots than Higuchi and first order, indicating that the predominant drug release mechanism is controlled release (Table 6) (Figure 4). From the kinetic studies, formulations F1 having higher correlation coefficient, was selected as the best formulation.

FTIR spectral studies

In order to further study whether Prazosin undergoes a polymorphic change during preparation of beads and to test for possible intermolecular interactions between Prazosin and excipients, FTIR was used. The FTIR spectra of pure Prazosin, Chitosan, Eudragit RL-100 and Prazosin with Chitosan and Prazosin with Eudragit RL-100 are shown in (Figure 5 and 6). The main spectral assignments of the drug are explained in (Table 7). From the graphs and datas it clearly indicates that there is no interaction between Prazosin and excipients.

CONCLUSION

Chitosan / TPP beads were successfully prepared by ionotropic gelation and the ionic cross–linking of cationic chitosan and polyanionic TPP. The polyanion interacts with the cationic chitosan to form a polyelectrolyte complex hydrogel in which drug molecules are trapped. This type of system shows a controlled drug release behavior due to the diffusion and swelling process.

The influence of combination of chitosan and eudragit RL showed improvement in the drug entrapment efficiency (DEE). The replacement of half of the chitosan with eudragit improved drug entrapment efficiency due to the hydrogel forming nature of the polymer.

The particle size analysis revealed that the size of beads was found to increase with increase in the concentration of polymers. The formulations gave particle size in the range of 1.45 ± 0.05mm to 2.50 ± 0.20mm. Swelling percentage was found to be in the range of 12 to 17.89%. SEM showed that the beads were almost spherical and had rough surface.

FTIR studies showed the presence of functional groups. Characteristic IR absorption

peaks were found to be superimposed in the beads. No significant chemical interaction was observed. The *in vitro* drug release study showed a slow release profile for chitosan / eudragit / TPP beads. From the *in vitro* studies it was observed that the drug release decreased with increase in the polymer concentration. The prepared beads may provide a possible sustained drug release of a poorly water soluble molecule Prazosin. This should be confirmed using an *in-vivo* study which was not part of this research project.

Finally this study demonstrated the observations of spherical to oval shaped particles with high drug entrapment efficiency and a possible sustained drug release profile from the Chitosan / eudragit /TPP polyelectrolyte complexes or beads. Thus, the prepared drug delivery system could be utilized for a possible sustained release product of Prazosin.

Table 1: Formulation of prazosin beads

Formulation code	Drug Quantity (mg)	Chitosan Quantity (mg)	Eudragit RL Quantity (mg)	Volume of polymer solution	Volume of 1% TPP	Curing time (min)
F_1 (1:1)	200	200	200	10mL	50mL	30min
F_2 (1:1.5)	200	300	300	10mL	50mL	30min
F_3 (1:2)	200	400	400	10mL	50mL	30min

Table 2: Bead characteristics of formulation F_1

Bead Characteristics	Prazosin and Chitosan(F_1)
Yield (%)	70
Wet diameter (mm)	2.5 ± 0.5
Dry diameter (mm)	1.45 ± 0.05
Weight(10 wet beads) gms	0.380 ± 0.25
Weight(10 dry beads) gms	0.120 ± 0.5
Wt % swelling	17.89
%Drug Entrapment Efficiency	73.42%

Table 3: Bead characteristics of formulation F_2

Bead Characteristics	Prazosin and Chitosan(F_2)
Yield (%)	64.7
Wet diameter (mm)	2.8 ± 0.35
Dry diameter (mm)	1.50 ± 0.09
Weight(10 wet beads) gms	0.417 ± 0.041
Weight(10 dry beads) gms	0.285 ± 0.034
Wt % swelling	13.22
%Drug Entrapment Efficiency	71%

Table 4: Bead characteristics of formulation F_3

Bead Characteristics	Prazosin and Chitosan(F_3)
Yield (%)	56.79
Wet diameter (mm)	3.1 ± 0.25
Dry diameter (mm)	2.50 ± 0.20
Weight(10 wet beads) gms	0.452 ± 0.031
Weight(10 dry beads) gms	0.328 ± 0.020
Wt % swelling	12.00
%Drug Entrapment Efficiency	59%

Table 5: *In vitro* drug release studies of formulations F1, F2 and F3

Time(hrs)	Cumulative drug release*		
	F_1	F_2	F_3
0.25	1.69	1.77	1.03
0.5	2.88	6.74	2.88
1	21.15	29.68	12.12
2	34.61	41.83	18.48
3	38.46	52.63	21.94
4	50.96	54.63	26.56
5	65.38	56.68	29.45
6	72.11	60.72	32.91
7	84.61	66.12	37.53

Table 6: Correlation coefficients of different mathematical models for Prazosin beads

S. No.	Formulations	Zero order	First order	Higuchi
		R^2	R^2	R^2
1	F_1	0.970	0.9469	0.9341
2	F_2	0.9027	0.9722	0.9152
3	F_3	0.6953	0.7477	0.892

Table 7: FTIR spectral assignment's of Pure Prazosin

Frequency (cm-1)	Description
3128	C-H streching
1725	C=O ketone streching
1600	1º amine bending, C=C alkene streching
1283	O-H streching
1110.8	C-O esters streching
717	C-Cl streching

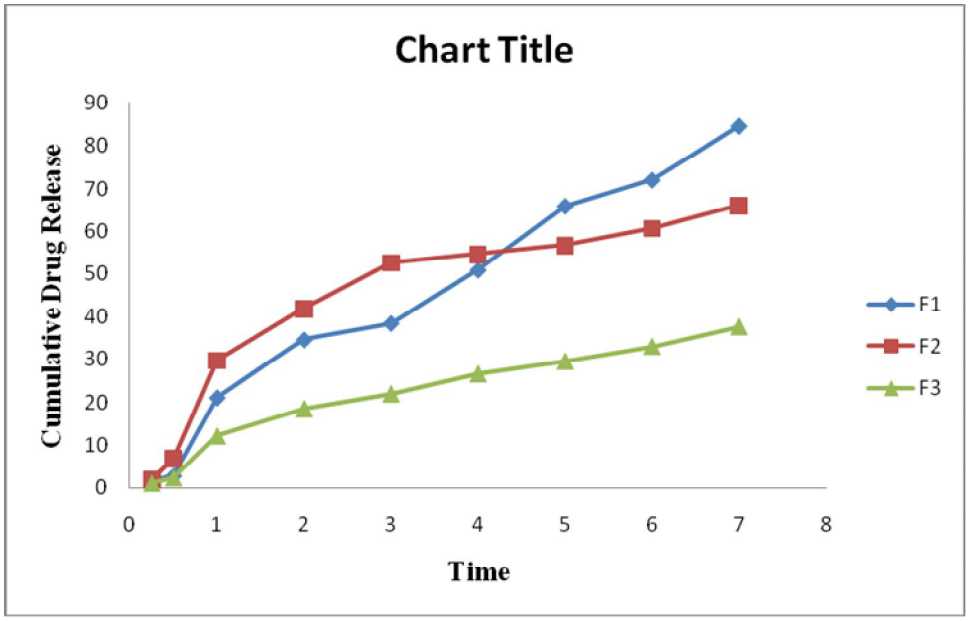


Fig 1: *In vitro* drug release studies of formulations F1, F2 and F3

Fig 2: Scanning electron microscopy of formulation F_1

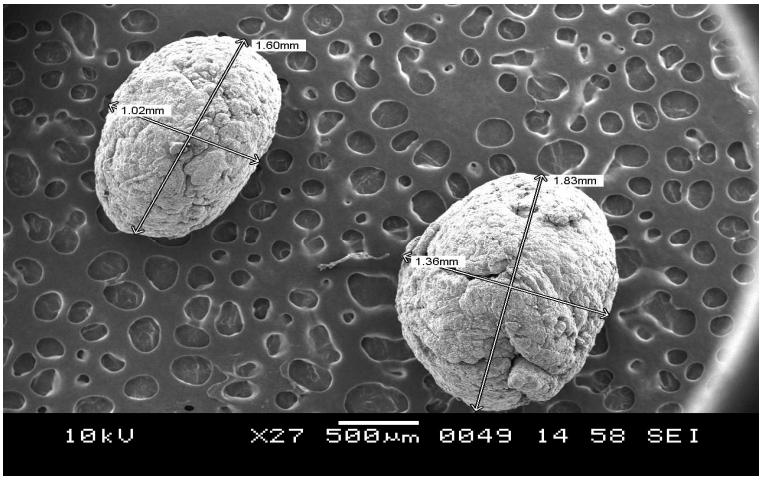
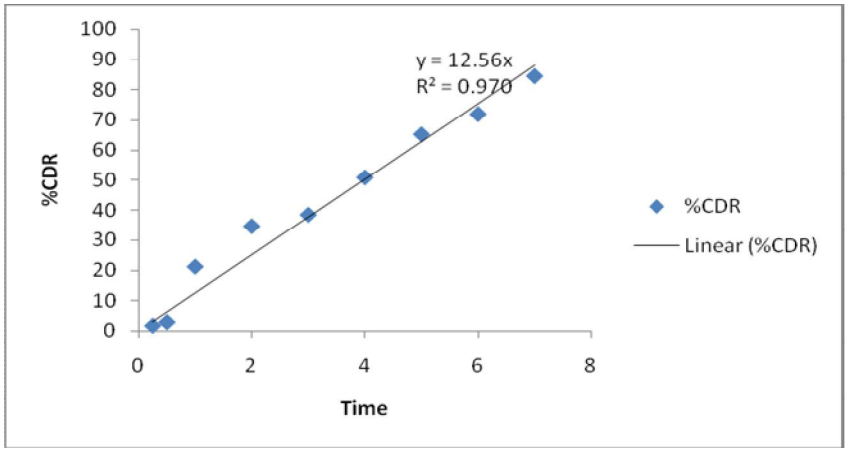


Fig 3: Scanning electron microscopy of formulation F_3

a)



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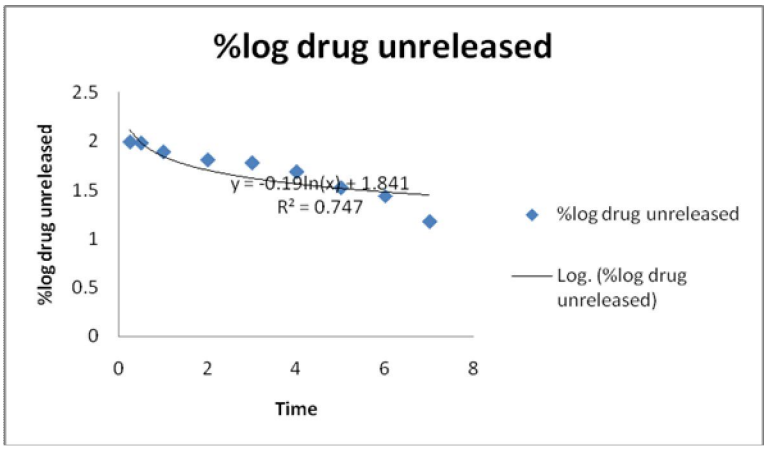


c)

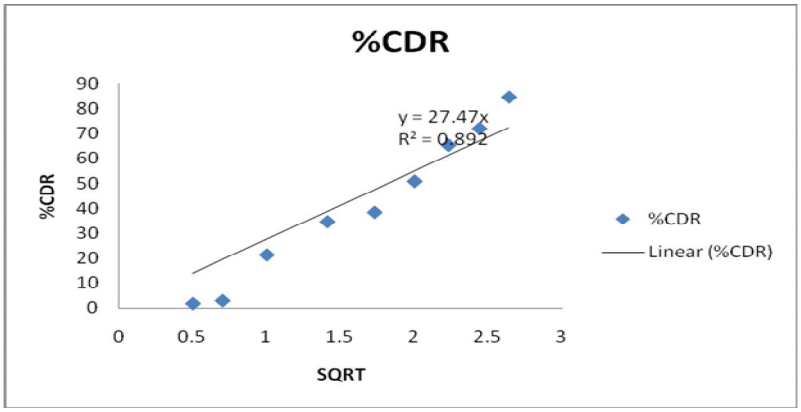
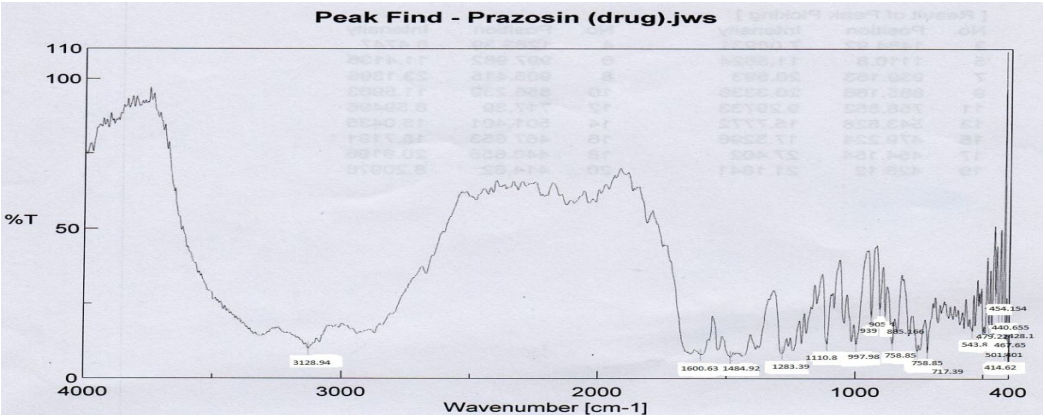


Fig 4: Model plot of Prazosin release from formulation F_1. a) Zero Order b) First order c) Higuchi Model

A



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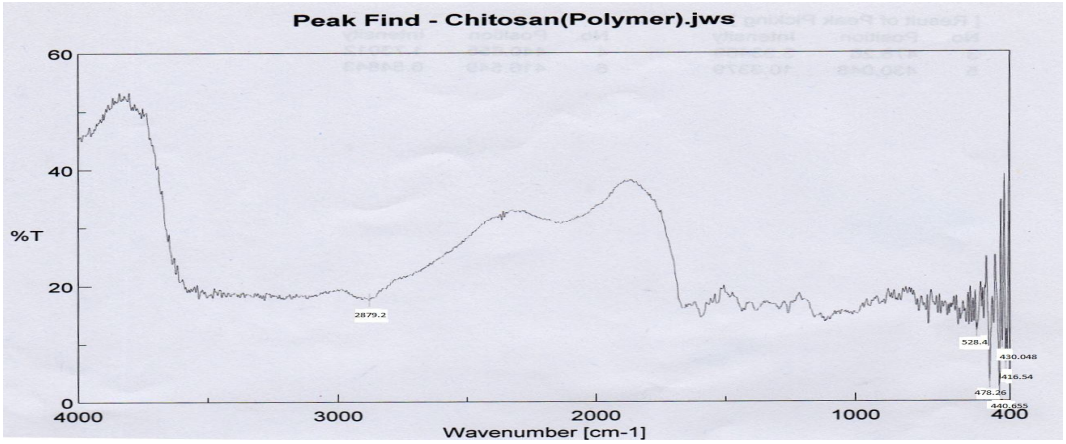

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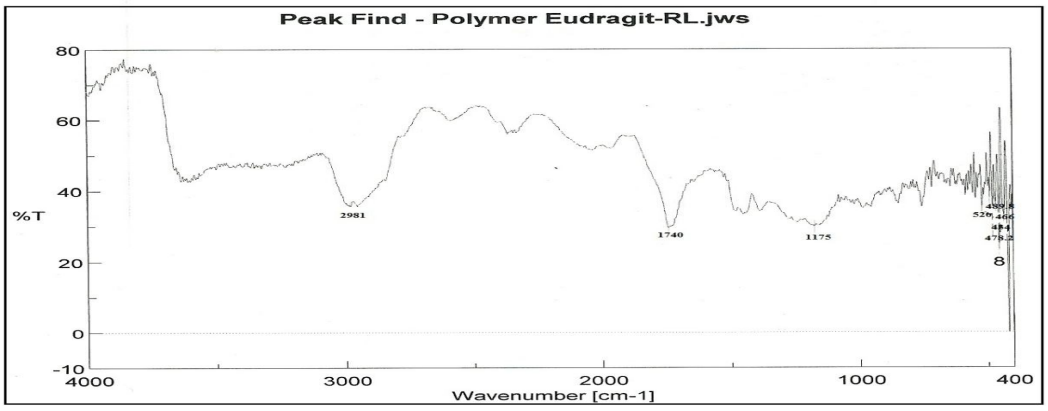
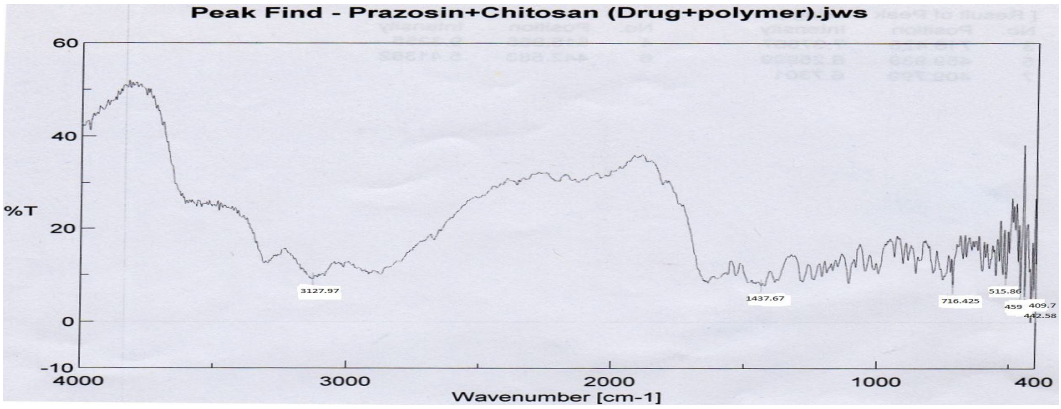


Fig. 5: A) IR Spectra of Prazosin, B) Chitosan C) Eudragit – RL100

A



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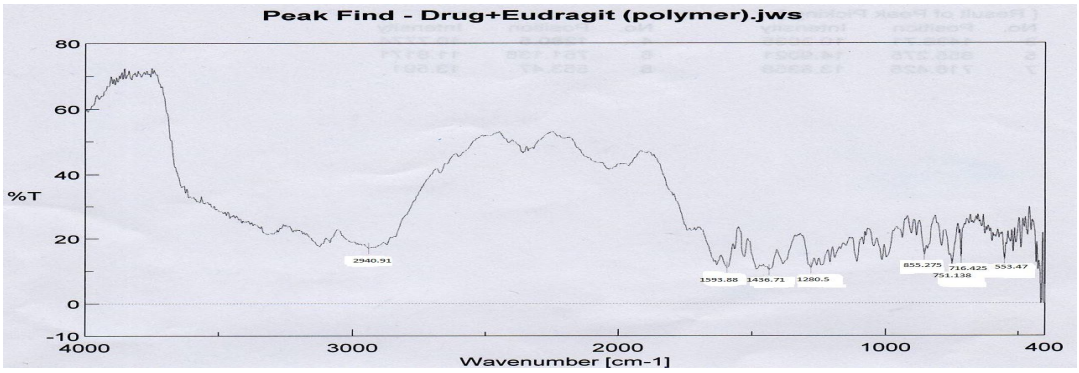

Fig. 6: A) IR spectra of Prazosin with Chitosan B) Prazosin with Eudragit RL-100

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REFERENCES

1. Alaa Eldeen BY, Ibrahim AA and Abdulah MA. Chitosan beads as a new gastroretentive system of verapamil. Sci pharm 2006; 74:175-188.
2. Arica B, Calis S, Atilla P, Durlu NT, Cakar N, Kas HS, Hincal AA. J Microencapsul 2005; 22(2):153-165.
3. Dasharath MP, Mehul JP, Chhagan NP. Multiparticulate System: A Novel Approach in Gastro-Retentive Drug Delivery. Int J Pharm sci 2011; 02(04):96-106.
4. Josias HH. Chitosan Based Polyelectrolyte Complexes as Potential Carrier Materials in Drug Delivery Systems. Mar Drugs 2010; 8(4): 1305–1322.
5. Khobragade DS, Parshuramkar PR, Ujjainkar AP, Mahendra AM, Phapal SM, Patil AT. Conception and Evaluation of Sustained Release Polymeric Matrix Beads for Enhanced Gastric Retention. Curr Drug Deliv 2009; 06(03):249-254.
6. Li XL, Adams NW, Bennett DB, Feijen J, Kim SW. Synthesis of poly(hydroxypropylglutamine- prazosin carbamate) and release studies. Pharm Res 1991; 08(04):527-530.
7. Motohiko S, Osamu H, Shose T, Toshinobu S, Takeo K, Kenji S, Kazuhiko J, Yasunori M. Relationship between Solubility of Chitosan in Alcoholic Solution and Its Gelation. Chem Pharm Bull 1999; 47(7):1044-1046.
8. Payam K, Abbas P, Fereshteh H. Formulation of Ibuprofen Beads by Ionotropic Gelation. IJPR 2008;7(3):163-170.
9. Pillai CKS, Paul W, Chandra PS. Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Prog Polym Sci 2009; 642-669.
10. Pradip KD, Joydeep D, Tripathi VS. Chitin and chitosan: chemistry, property and applications. JSIR 2004; 63:20-31.
11. Samyuktha Rani B, Vedha Hari BN, Brahma RA, Punitha S, Parimala Devi. The recent developments on gastric floating drug delivery system: An overview. Int J Pharm Sci 2010; 1(2):524-534.
12. Singh A, Jha KK, Singh PS, Shah G. Formulation and Evaluation of Cefixime beads. IJRPC 2011; 1(4):922-924.
13. Shishu, Gupta N, Aggarwal N. Stomach Specific drug delivery of 5-Fluorouracil using floating alginate beads. AAPS PharmSci Tech 2007; 8(2):E1-E7.
14. Takka S and Gurel A. Evaluation of Chitosan/Alginate beads using experimental design: Formulation and Invivo characterization. AAPS PharmSci Tech 2010; 11(1):460-466.