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Research Article

FORMULATION AND EVALUATION OFPROPRANOLOL HYDROCHLORIDEMICROSPHERESBY IONIC GELATION TECHNIQUE

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ABSTRACT

Frequent administration and variable low bioavailability after oral administration of conventional dosage forms can be overcome by microspheres formulation. Propranolol Hydrochloride is non-selective beta blocking anti-hypertensive drugand used for angina pectoris, myocardial infraction, migraine prophylaxis, glaucoma. The aim of the present work is formulate and evaluate microspheres of Propranolol Hydrochloride to achieve sustained release. Propranolol hydrochloride microspheres loaded using combination of sodium alginate, Sodium carboxy methyl cellulose, Hydroxyl propyl methyl cellulose by ionic-gelation technique using calcium chloride as a cross linking agent. Eight formulations (F1-F8) were prepared using different polymers ratio. Depend on drug polymers ratio, the percentage yield were found to be in range between 90.17±0.16% to 98.83±0.26% and entrapment efficiency were 60.89±0.86%to 88.49±0.28% respectively.SEM study shows microspheres are spherical in nature and size distribution between 31.05±0.5 to 73.23±0.67µm. *In-vitro* drug releasestudies carried out at different pH for a period of 12 hours. All formulationsshownsustained release characteristics and particularly F8 formulated microspheres found high rate and extent of drug releaseand good fit to Higuchi model follows first order kinetics whichexhibitFickian diffusion mechanism. There is nophysical changeswere observed during stability studies and swelling indices has been done and varied between the formulations.

Keywords: Propranolol hydrochloride, microspheres, *in-vitro* evaluation, stability studies.

INTRODUCTION

For the treatment of chronic diseases it is important to take the medication in several times a day through orally and it may lead to fluctuating drug level in body. So in order to avoid frequent drug administration a Novel Drug Delivery Systems (NDDS) is popular for maintenance of therapeutic level in body for to achieve a desired concentration of the drug in the blood/tissue for a prolonged period and non-toxic by continuously releasing medication over an extended period of time after administration of single dose. Drug with short elimination half-life[1]are most suitable for prolonged release dosage design and it can be achieved by microspheres formulation which provide a larger surface area and possess an easier estimation of diffusion and mass transfer behavior also the encapsulated small molecules could diffuse out of the barrier with precise kinetics modelling and prolong release of drug to the body fluid[2]. It's also limiting fluctuate within a therapeutic range, reduction in side effects and dose frequency, hence improved patient compliance.[3-4]Hypertension is a chronic cardiac condition in which systemic arterial blood pressure is evaluated. About 90-95% of cases are categorized as 'primary hypertension' remaining was categorized as 'secondary hypertension'.Antihypertensive drugs are managing hypertension which is extensively bound to plasma proteins and cause gastrointestinal disorders neutropenia, acute hepatotoxicity, migraine and pancreatitis. It may

therefore be more desirable to deliver this drug by microspheres formulation as potential in a controlled/sustained fashion.The presently available antihypertensive conventional therapy is associated with a number of drawbacks with increase in dose, decrease in bioavailability. Propranolol Hydrochloride is non-selective beta blocking anti-hypertensive drug and conventional oral dosage form is 10-40mg in 3-4 times a day and biological half-life 3-5 hours.[5]Moreover, drug with short elimination half-life, more frequent administration is required lead risk for stroke, myocardial infraction, heart failure and chronic kidney failure.So aim and objective of the present study is to formulate a novel oral once-daily prolonged release microspheres of an antihypertensive drug likePropranololhydrochloride (Figure-1)which can provide continuous therapy and high margin of safety. These microspheres are prepared by ionic gelation technique usingwater soluble polymersand evaluated the characteristics.

MATERIALS AND METHODS

MATERIALS

Propranolol Hydrochloride was obtained as gift sample from Yarrow Chem Product, Mumbai.Sodium Carboxy Methyl Cellulose andHydroxy Propyl Methyl Cellulosefrom QualigensFine Chem Pvt Ltd, Vadodara.Sodium Alginate fromChemdyesCo-operation, Rajkot.Calcium chloride fromFlora Chemicals, Mumbai.All other chemicals and solvents wasused as an analytical grade.

METHODS

IR spectral analysis

The FT-IR spectrum of Propranolol hydrochloride and polymers was recorded using KBr mixing method on the FT-IR instrument (Schimadzu FT-IR-8400 S). The drug alone, and in a combination with polymers (mixed in the ratio of 1:1) was taken and subjected to FT-IR studies.[6]

Preparation of Propranolol hydrochloride microspheres

The microspheres formulation were prepared by an ionic cross linking technique using drug and polymers by different ratios is shown in Table 1. Initially, the polymeric solution were prepared by dissolving sodium alginate, HPMC, SCMCin distilled water. After complete dissolution of all polymers, the drug was dissolved in that polymeric solution with proper mixing. The prepared drug-polymer solution was added drop wise by a 20 gauge hypodermic needle in to a 50 ml of 5%w/v of cross linking agents such as calcium chloride and being stirred at 200rpm by mechanical stirrer for 10 minutes.The formed propranolol microspheres were further allowed to stir in the solution of cross linking agents for an additional 1 hour. The prepared microspheres was washed with 2-3 times with de-ionized water, filtered andit was dried 80^{0}C for 2 hours and were evaluated by different parameters.[7]

Characterization of Floating Microspheres

Percentage yield

The prepared microspheres were collected, dried at room temperature and then weighed and yield of microspheres preparation was calculated using the formula[8]

$$\text{Percentage yield (\%)} = \frac{\text{Amount of microspheres obtained (gm)}}{\text{Theoretical amount (gm)}} \times 100$$

Determination of particle size

The sample of prepared microspheres was randomly selected and their size was determined using an optical microscopy(Olympus, India)method.[9]

Micromeriticproperties

Bulk density, Tapped density, Carr's index, Hausner's ratio and Angle of repose of all formulationsare carried out and the results are analyzed.[10-11]

Morphological studies

Scanning electron microscopy (JEOL, JSM-6701 F, JAPAN) of formulations was carried out to study their morphological characteristics of Propranolol hydrochloride microspheres.[12]

Entrapment efficiency

The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of Phosphate buffer (pH 7.4) repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up by using buffer. The solution was filtered and the absorbance is measured by spectrophotometer against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following equation.[13]

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

Swelling index (SI)

The swelling of Microspheres were carried out in phosphate buffer (pH 7.2) for 14 hours. The excess liquid drops adhered to surface were removed by blotting and the swollen Microspheres were weighed. The Microspheres were then dried in hot air oven at 40^{0}C for 48hours until there was no change in dried mass of sample. The swelling index was calculated from following equation.[14-15]

$$SI = \frac{\text{Mass of swollen microspheres-Mass of dry microspheres}}{\text{Mass of dried microspheres}} \times 100$$

***In-vitro*drug release studies**

The *in-vitro* drug release studies were conducted in gastric pH using 0.1N Hcl for first 2 hours and in intestinal pH 7.4 phosphate buffer for remaining10 hours using USP XXIII, type-II dissolution apparatus under sink conditions. Accurately weighed 50 mg samples of the microspheres were added to dissolution medium and temperature was maintained at 37^{o}C ±1^{o}C and fluid and it was agitated at 100 rpm. Adequate volumeof mediafrom dissolution apparatus was withdrawn every frequent one hour and replaced with equal quantity of the fluid and maintains constant volume. After suitable dilution, the samples are analyzedspectrophotometrically at 360 nm. Inorder to study the exact mechanism of drug release, *in-vitro* release data were alsoanalyzed using different kinetics models and mechanism of drug release is determined.[16-17]

Stability studies

Best formulations were placed in borosilicate screw capped glass containers and stored at room temperature (27±2^{0}C and 60±5% RH)and in a stability chamber (40°C±2°C and 75± 5% RH)for a period of 3 months. At the end of specified days period, samples were withdrawn and analyzed for their drug content.[18]

RESULTS AND DISCUSSION

IR spectral analysis

FT-IR spectra of samples were taken in the wavelength region was 600-3800 cm^{-1} at ambient temperature and the resolution was 4 cm^{-1} and compared the position and relative intensity of absorption band of physical admixtures and pure drug is illustrated in Figure 2 to 5. In compatibility studies, IR spectrum of pure drug was found to be similar to the standard IR spectrum of which indicate that obtained sample was pure Propranolol hydrochloride.The IR spectra of the all pure samples and Propranolol hydrochloride physical admixtures of suitable proportion of polymers were subjected to the study. From the results, there were no considerable changes in comparison between the ratios of percent (%) transmittance and no chemical interaction and changes took place in drug and polymersalone or in combination.

Percentage yield, Particle size and Drug entrapment

From Table-2, the percentage yield of all the formulations was in the range of 90.17±0.16% to 98.83%±0.26%. From the results it was observed that the concentration of polymer increased, the percentage yield of the microspheres was slightly increased.The average particle size of all the formulations was observed in between 31.05±0.27 to 73.23±0.61µm. The mean particle sizes of microspheres significantly increase with increasing polymer concentration. This may be in increase in relative viscosity at higher concentrations of polymer and formation of large droplets during addition of the polymer solution to the cross linking agent.The percentage entrapment efficiency of microspheres was found to be in the range of 60.89±0.86% to 88.49±0.28%. It was observed that increase in polymer concentration larger microspheres were formed with greater amount of drug entrapped. This may be due to the greater availability of active calcium binding sites in polymeric chains.A maximum of 88.49% of drug entrapped in Propranolol hydrochloride microspheres (F8) which was prepared by 1:8 ratio.[19]

Micromeriticproperties

From the results of Table-3, it was observed that the bulk density and tapped density values were lies in 0.468 to 0.502 and 0.507 and 0.580 g/cm^3 i.e. less than 1.2gm/cm^3, indicates good packing. The carr's index value were lies in between 6.79 to 20.10 % indicate excellent flow characteristics of the microspheres. Angle of Repose less than 40^{0}or equal 40^0 indicates free flowing properties of the microspheres. However angle of repose greater than 40^0 indicates poor flow of material. It can be observed that, the angle of repose for various batches of the microsphere is found to be less than 40^0, it indicates good flow properties.

Morphological studies

The surface morphology of best formulation was determined by Scanning electron microscopy (SEM) for characterization of shape and size of microspheres and the scanned images are shownin Photomicrograph-1&2. The results revealed that Propranolol hydrochloride microspheres were discrete and spherical shape with rough outer surfaces due to the density of the polymer matrix which in turn justifies its desirable release.

Swelling index (SI)

From the Table-4 results,it was observed that the swelling indices of the microspheres were high and varied and F8 formulation was shown high swelling character than other microspheresand it was found in the range 0.71±0.13 to 0.92±0.11% after 14 hours. Higher swelling indices may be due to the presence of water soluble polymers. The swelling behaviour provides an indication of relative moisture absorption capacities of polymers and whether the formulations maintain their integrity after absorption of moisture.[20]

***In-vitro*drug release studies**

The *in-vitro* drug release profile of all batches of microspheres using different ratios was studied in gastric pH using 0.1N Hcl(pH 1.2) for first 2 hoursand in intestinal pH 7.4 (phosphate buffer) for remaining hours. The comparative *in-vitro*drug release curve of all batches of microspheres was shown in Figure 6&7.From the results, it was observed that the *in-vitro*drug release indicates more sustained effect with the increase in concentration of polymers and the release was achieved after initial lag time and it was directly proportional to the concentrations. The first phase must be for negligible dissociation of microspheres may be due to the swelling of polymer.The second phase exhibited a burst like release pattern, which was accomplished by the polymer disintegration due to enzymatic degradation of polymer. Both the polymers are highly branched and molecular structure of these polymer resist enzymatic breakdownin digestive tract.[21]The formulation F8 showed high rate and extent of drug release.

In order to understand the mechanism and kinetics of drug release, the results obtained were also plotted in four kinetic models of data treatment as: Cumulative percentage drug release Vs Time (Zero order rate kinetics), Cumulative % drug retained Vs Time (First order rate kinetics), Cumulative percentage drug released Vs Square root of Time (Higuchi's classical diffusion), Log cumulative percentage drug release Vs log Time (Korsmeyer-Peppa's exponential) and the kinetics data results is shown in Table-5.The kinetic model fitting of *in-vitro* drug release and coefficient of correlation value were also calculated and indicates that the drug release data was best fitted with first order kinetics. When the drug release was put in to Higuchi equation, good correlation coefficient value was obtained indicating the drug release was diffusion controlled. The release data obtained were also put in Korsmeyer-Peppas model in order to find out n values, which describe the drug release mechanism. From the results of kinetic data's, the release of drug from microspheres provide sustained manner for period of sufficient hour and the kinetic study shows that 'r' values of all formulated batches indicate compliance with Higuchi's plot which reveal that the drug release follows Fickian diffusion mechanism (Korsmeyermechanism).

Stability studies

From the *in-vitro* results, best formulation (F8) were taken for stability studiesand evaluated drug content and physical appearances. From the Table-6, it wasshownthat there is about 81.60%to 82.57% of drug is present in the formulations with no-observable physical changesduring storage. This indicates a good stability of Propranolol hydrochloride microspheres.

CONCLUSION

The objective of the work was to design and developan oral drug delivery system in the form alginate microspheres using anti-hypertensive agents to overcome the problem associated with conventional dosage form. In this study Propranolol hydrochloride microspheres was prepared by ionic gelation technique. The prepared formulations were free flowing, non sticky and evaluated for various parameters. All batches of microspheres showed prolonged release and compliance with Higuchi model

follows fickian diffusion mechanism. The best formulation (F8) showed good stability. Hence, it is concluded that the formulated Propranolol hydrochloride microspheres might be a better, practical approach to achieve a prolonged therapeutic effect by continuously releasing the medication over extended period of time in hypertensive casesand provide high margin of safety.

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Table 1: Composition of Propranolol hydrochloride microspheres

Formulations Code	Drug: Polymer ratio	Drug (mg)	Sodium alginate (mg)	HPMC & SCMC (mg)	Calcium Chloride (%w/v)
F1	1:1	200	500	500	5
F2	1:2	200	500	1000	5
F3	1:3	200	500	1500	5
F4	1:4	200	500	2000	5
F5	1:5	200	500	2500	5
F6	1:6	200	500	3000	5
F7	1:7	200	500	3500	5
F8	1:8	200	500	4000	5

Table 2: Percentage yield, Particle size and Drug entrapment of Propranolol hydrochloride microspheres

Formulations code	Percentage yield (%)	Average particle size (µm)	Drug entrapment (%)
F1	90.17±0.16	31.05±0.27	60.89±0.86
F2	91.36±0.23	38.21±0.38	67.16±0.37
F3	94.78±0.19	43.59±0.21	71.88±0.42
F4	96.17±0.13	49.46±0.46	74.46±0.64
F5	96.93±0.27	55.32±0.41	75.61±0.70
F6	97.45±0.20	62.37±0.53	82.78±0.82
F7	98.01±0.18	69.14±0.29	84.52±0.60
F8	98.83±0.26	73.23±0.61	88.49±0.28

Results are mean ± S.D of three trials (n=3)

Table 3: Micromeritic properties data of Propranolol hydrochloride microspheres

Formulations code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Angle of repose (θ)
F1	0.471±0.001	0.507±0.005	07.11±0.86	$28^0 09' \pm 0.55$
F2	0.480±0.004	0.515±0.006	06.79±0.98	$27^0 16' \pm 0.61$
F3	0.486±0.003	0.564±0.005	13.84±0.74	$26^0 19' \pm 0.70$
F4	0.461±0.001	0.577±0.005	20.10±0.60	$27^0 38' \pm 0.25$
F5	0.502±0.006	0.557±0.003	09.87±0.83	$27^0 46' \pm 0.31$
F6	0.454±0.002	0.542±0.006	16.23±0.45	$29^0 64' \pm 0.37$
F7	0.468±0.003	0.568±0.010	17.60±0.86	$28^0 26' \pm 0.45$
F8	0.469±0.003	0.580±0.010	19.13±0.58	$27^0 18' \pm 0.55$

Results are mean ± S.D of three trials (n=3)

Table 4: Swelling index of Propranolol hydrochloride microspheres

Formulations code	Average swelling index
F1	0.71±0.13
F2	0.75±0.17
F3	0.79±0.09
F4	0.81±0.12
F5	0.83±0.19
F6	0.85±0.23
F7	0.88±0.26
F8	0.92±0.11

Results are mean ± S.D of three trials (n=3)

Table 5: Kinetics analysis data of Propranolol hydrochloridemicrospheres

Formulations Code	Release model							
	Zero order		First order		Higuchi's		Korsmeyer and peppa's	
	R	S	R	S	R	S	R	S
F1	0.9651	7.701	0.9704	0.081	0.9907	33.90	0.9638	0.471
F2	0.9725	7.58	0.9801	0.076	0.9920	33.54	0.9580	0.476
F3	0.9667	7.34	0.9860	0.064	0.9857	33.05	0.9730	0.481
F4	0.9775	8.03	0.9820	0.102	0.9952	34.88	0.9672	0.485
F5	0.9590	8.12	0.9648	-0.092	0.9779	35.81	0.9750	0.489
F6	0.9625	8.27	0.9879	-0.112	0.9878	35.06	0.9628	0.492
F7	0.9457	8.31	0.9805	0.1337	0.9879	35.82	0.9635	0.495
F8	0.9468	8.30	0.9920	-0.123	0.9936	36.11	0.9710	0.498

Correlation coefficient (r), Slope(s)

Table 6: Stability studies data of Propranolol hydrochloride microspheres (F8)

At the end of period (in days)	Physical Appearance	Percentage Drug Content	
		At 27±2°C, 60± 5% RH	At 40±2°C,70± 5% RH
30	No change	82.12±0.82	81.62±0.82
60	No change	82.07±0.82	81.60±0.82
90	No change	82.42±0.82	82.57±0.82

Results are mean ± S.D of three trials (n=3)

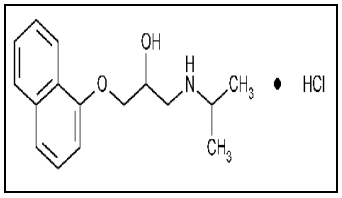


Fig. 1: Structure of Propranolol hydrochloride

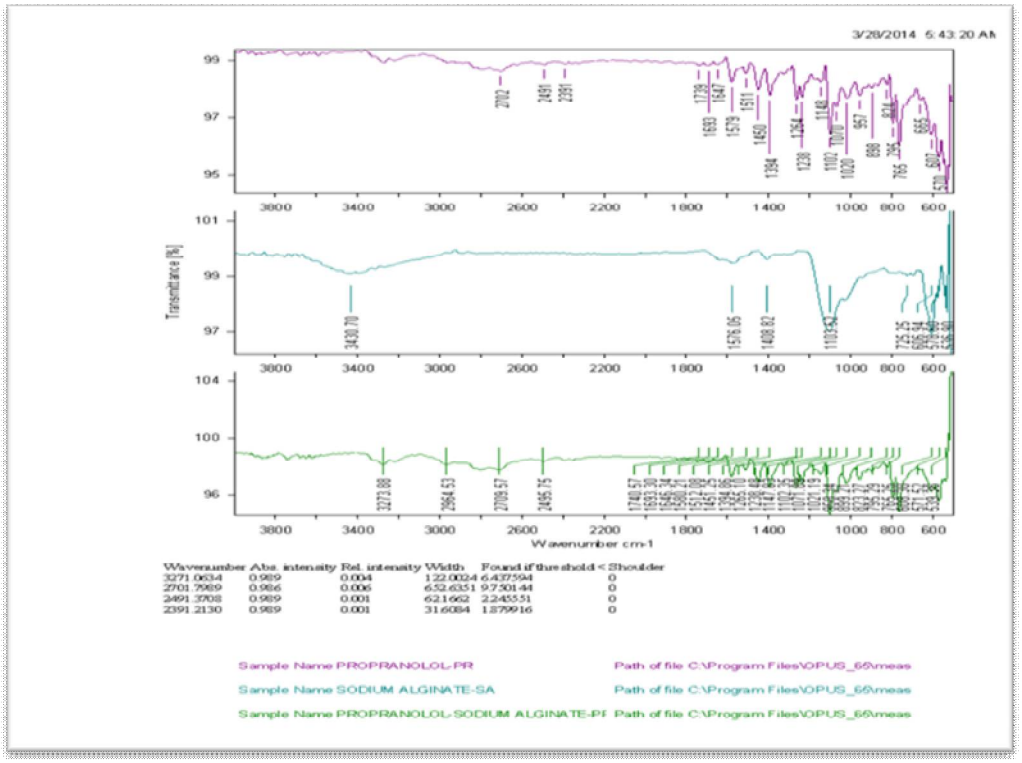


Fig. 2: IR spectra studies of pure Propranolol hydrochloride and Sodium alginate

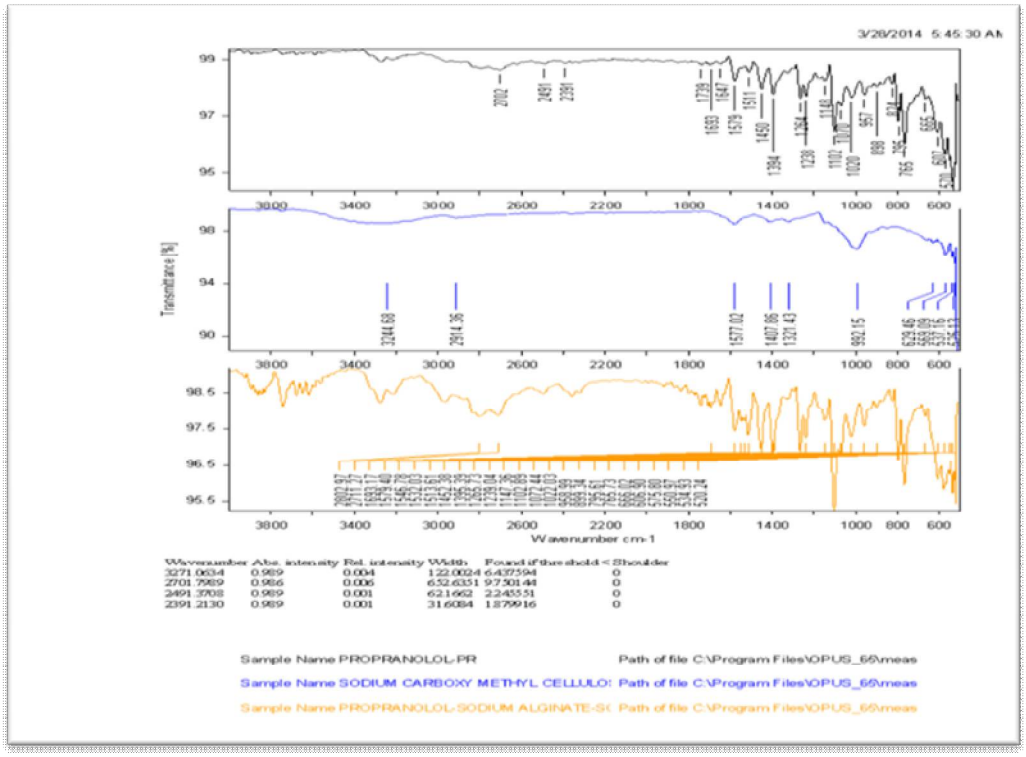


Fig. 3: IR spectra studies of pure Propranolol hydrochloride and SCMC

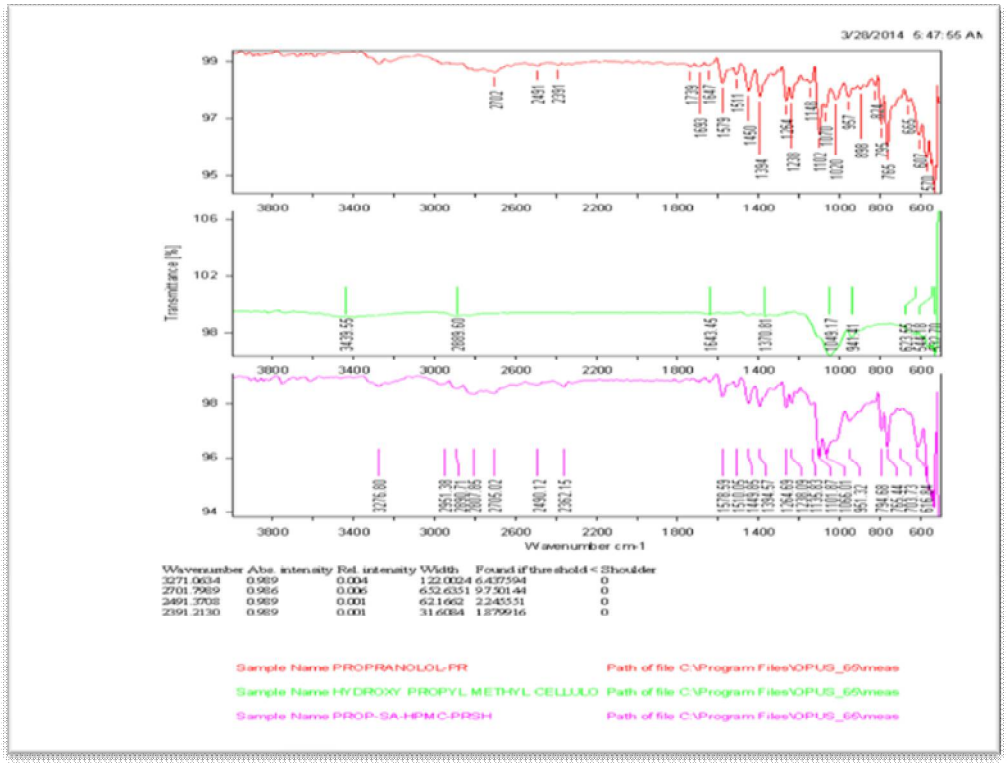


Fig. 4: IR spectra studies of pure Propranolol hydrochloride and HPMC

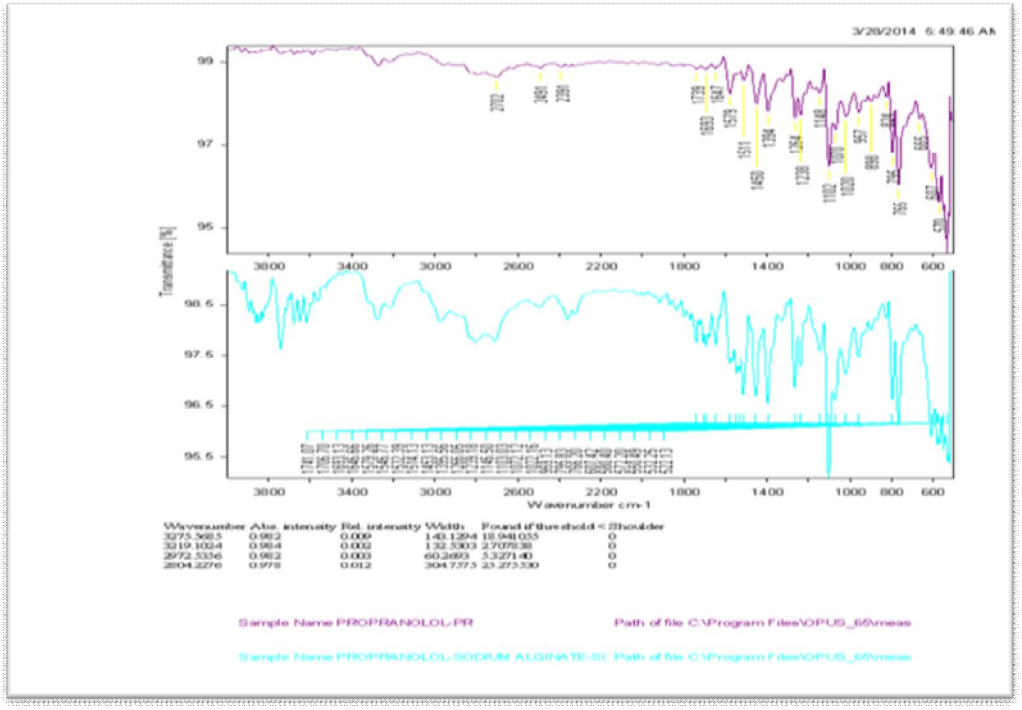


Fig. 5: IR spectra of Pure Propranolol hydrochloride and Physical admixtures of Propranolol hydrochloride,Sodium alginate, SCMC and HPMC

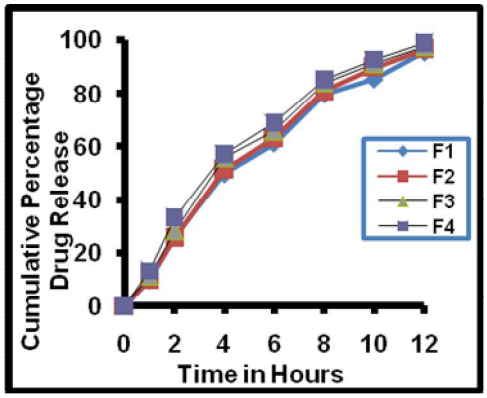


Fig. 6: Comparative *in-vitro drug* Release plot of Propranolol hydrochloridemicrospheres (F1-F4)

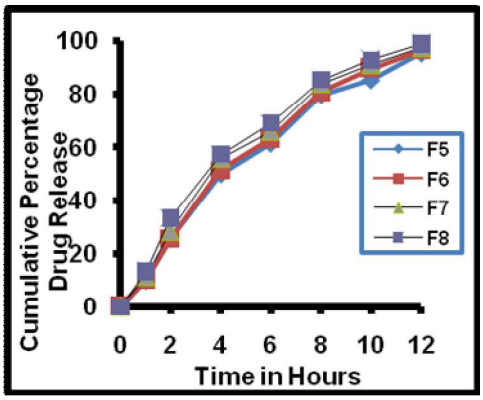
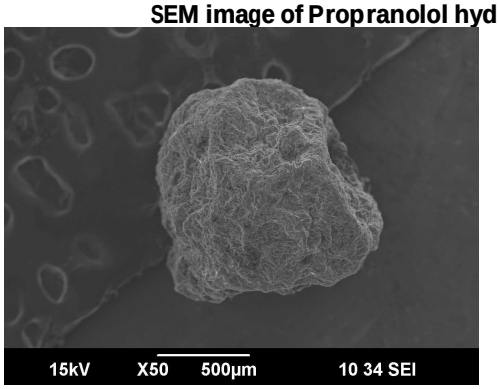
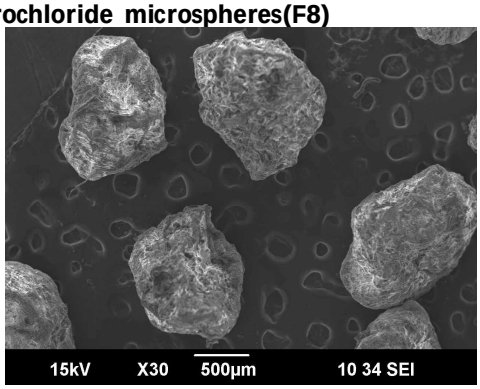


Fig. 7: Comparative *in-vitro drug* release plot of Propranolol hydrochloridemicrospheres (F5-F8)

SEM image of Propranolol hydrochloride microspheres(F8)



Photomicrograph-1 (Single)



Photomicrograph-2(Groups)

REFERENCES

1. George M, Grass IV and Robinson JR. Sustained and controlled release of drug delivery systems. Marcel Beckker, NY.1978;124-128.
2. Satoskar, RS, Bhandarkar and Ainapur. Pharma therapeutics, Popular Prakashan, Mumbai. 2008; 17th Edn: 883-913.
3. Davis. The design and evaluation of controlled release systems for the gastrointestinal tract.J control Release. 1985;2:69-77.
4. Ritschel WA. Biopharmaceutics and pharmacokinetics aspects in the design of controlled release peroral drug delivery systems. *D* Dev and Ind Pharm. 1989;15:1073-1103.
5. Gandhi TP and Gyal RK. Elements of Pharmacology. B.S Shah Prakashan, Ahmedabad. 2001;11th Edn:243-247.
6. Behera BC, Sahoo SK, Dhal S, Barik BB and Gupta BK. Characterization of glipizide-loaded polymethacrylate microspheres prepared by an emulsion solvent evaporation method. Trop J Pharm Res. 2008;7(1):879- 885.
7. Das MK. Furosamide-loaded alginate microsphere prepared by Ionic cross linking technique: Morphology and release characteristics. Indian J Pharm Sci. 2008;70:77-84.
8. Dashora K, Saraf S and Saraf S. Effect of processing variables on micro particulate system of aceclofenac. *Pak* J *Pharm* Sci. 2006;19(1):6-10.
9. Shabaraya AR and Narayanacharyulu R. Design and evaluation of chitosan microspheres of metoprolol tartrate for sustained release. Indian J Pharm Sci. 2003;65(3):250-252.
10. Aulton ME. Pharmaceutics: The Science of Dosage Form Design. Livingstone C, Elsevier Science Ltd. 2002; 2ndEdn: 312-315.
11. Lachman L, Liberman HA and Kanig JL. The Theory and Practice of Industrial Pharmacy, Varghese Publishing House, Bombay. 1991; 3rdEdn:3.
12. Chowdary KPR and Rao YS. Design and in-vitro and in-vivo evaluation of

mucoadhesive microcapsules of glipizide for oral controlled release: a technical note. AAPS Pharm Sci Tech. 2003;4:E39–E39.
13. Saxena A, Singh RK, Dwivedi A, Khan I, Singh A and Raghuvendra. Preparation and evaluation of microspheres of propranolol hydrochloride using eudragit RL as the matrix material. Am-Euras J Sci Res. 2011;6(2):58-63.
14. Patel JK, Patel RP, Amin AF and Patel MM. Formulation and evaluation of mucoadhesive glipizide microspheres. AAPS Pharm Sci Tech. 2005;6:E49- E55.
15. Soppimath KS and Aminbhavi TM. Water transport and drug release study from cross linked polyacrylamide grafted guar gum hydrogel microspheres for the controlled release application. *Eur*J.PharmBiopharm. 2002;53:87-89.
16. Dandagi PM, Manvi FV Gadad AP, Mastibolimath VS, Patil MB and Balamuralidhara V. Microencapsulation of Verapamil hydrochloride by Ionotropic gelation technique. Indian J Pharm Sci. 2004;66(5):631-635.
17. Higuchi T. Mechanism of rate of sustained-action medication. J Pharm Sci. 1963;52(11):1145-1149.
18. Nair AB, Gupta R, Kumria R, Jacob S and Attimarad M. Formulation and evaluation of enteric coated tablets of proton pump inhibitor. J Basic Clin Pharma. 2010;1(4):215-221.
19. El-Kamal AH, Al-Gohary OM and Hosny EA. Alginate-diltiazem hydrochloride beads: optimization of formulation factors, in-*vitro* and *in-vivo* availability. J Microencapsul. 2003;20:211–25.
20. Panomsuk SP, Hatanaka T, Aiba T, Katayama K and Koizumi T. A study of the hydrophilic cellulose matrix: effect of drugs on the swelling properties. Chem Pharm Bull. 1995;126:147-153.
21. Ankit Vajpayee, Suresh Fartyal and Alok Pratap Singh Sajal Kumar. Formulation and evaluation of colon targeted curcumin microsphere using natural gum. J Pharm Res Opi. 2011;14:108-112.