Review Article
SGVU Journal of Pharmaceutical Research & Education ISSN: 2456-4508

JPRE

Journal homepage: http://www.gyanvihar.org/researchjournals/

COMPARATIVE EVALUATION OF IVABRADINE HYDROCHLORIDE LOADED NATURAL AND SYNTHETIC MICROSPHERES

Prashant Singh*[1], T. Tamizh Mani[2], Shambaditya Goswami1, Pradeep Singh[3]
[1]Department of Pharmaceutics, NIMS University, Jaipur, Rajasthan, India.
[2]Dept. of Pharmacognosy, Bharathi College of Pharmacy, Mandya, Karnataka, India.
[3]Department of Pharmaceutics, Kashi Institute of Pharmacy, Varanasi (U.P.), India

ABSTRACT

The present work is a comparative evaluation of Ivabradine HCl (IBH) microspheres formulated using natural (egg albumin) and semi-synthetic (ethyl cellulose) polymers with the aim to get the best possible drug- polymer ratio giving the sustained drug release. IBH loaded egg albumin and ethyl cellulose microspheres were prepared by heat denaturation technique and solvent evaporation technique, respectively. Various evaluation parameters were assessed, with a view to obtain sustained release of drug. The prepared IBH microspheres were then subjected to FTIR, SEM, particle size and size distribution, % yield, % drug loading, entrapment efficiency, *in vitro* dissolution studies, release kinetics and DSC. Different concentrations of natural and semi-synthetic polymers were used individually to maintain a suitable lag period. The FTIR Spectras revealed that, there was no interaction between the polymer and drug. IBH microspheres were spherical in nature, which was confirmed by SEM. Microspheres with normal frequency distribution were obtained. A maximum of 86.12% and 86.20% of drug entrapment efficiency was obtained in the drug loaded natural and semi-synthetic microspheres, respectively. The *in-vitro* dissolution data maximum of 73.26% and 90.40 % cum. drug release was obtained in the IBH loaded natural and semi-synthetic microspheres, respectively. The *in-vitro* performance of IBH microspheres showed that sustained release was dependent upon the polymer concentration. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. The DSC pattern shows that there was decrease in the crystallinity of the IBH. The present study conclusively demonstrates the feasibility of effectively encapsulating Ivabradine HCl into natural (egg albumin) and semi-synthetic (ethyl cellulose) microspheres to form potential sustained release drug delivery system. On comparing the dissolution data of all the formulation, the best release was obtained from AF2 formulation (natural polymer) and CF1 formulation (semi-synthetic polymer). Therefore, on comparative evaluation it can be concluded that among all four drug: polymer ratios of egg albumin and ethyl cellulose, CF1 is the best suitable formulation of IBH microspheres as a sustained drug delivery system.

Keywords: Ivabradine HCl; sustained drug delivery; egg albumin microspheres; ethyl

cellulose microspheres; natural polymers; heat denaturation method; solvent evaporation method.

INTRODUCTION

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[1-3]. They have varied applications and are prepared using assorted polymers[4]. However, the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[5-8]. Ivabradine is a specific heart rate lowering agent, acting by reducing the rate of pacemaker activity in the sinoatrial node. Within the sinoatrial node, IBH is a selective inhibitor of I_f, an important current involved in generating the early phase of spontaneous diastolic depolarisation in pacemaker cells, thereby reducing the frequency of action potential initiation and lowering heart rate. The cardiac effects are specific to the sinus node with no effect on intra-atrial, atrioventricular or intra-ventricular conduction times, nor on myocardial contractility or ventricular repolarisation or coronary vasomotricity.[9] Hence, there is a need to develop an oral drug delivery system that is convenient for patients. The objective of the present investigation was to compare the developed, an extended and controlled release composition and formulation of IBH microspheres using egg albumin (published earlier)[10] and ethyl cellulose (published earlier)[11] polymer to reduce dose/ dosing frequency in the angina pectoris.

MATERIALS AND METHODS

Materials

IBH was received as a gift sample from Ind. Swift, Jammu, India. Egg albumin, ethyl cellulose, and paraffin liquid light was obtained from S D fine-chem limited, Mumbai. Tween 80, gluteraldehyde solution was obtained from Central drug house (p) Ltd, Mumbai. All other solvents and chemicals used were of analytical grade. FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan).

Preparation of Microspheres

Preparation of Egg Albumin Microspheres[12]

Microspheres were prepared by heat denaturation method. In this method a solution of albumin in 25ml of distilled water was prepared and the drug was added to the albumin solution. The formulation was carried out with 1:1, 1:2, 1:3, 1:4 drug: polymer ratios. The contents were slowly added to a beaker containing 100 ml of preheated (60^0C) liquid paraffin containing 0.5ml of span 80 as emulsifying agent and stirred for 1h. The temperature was reduced to 40^0C for hardening process and was maintained for 25min. The resulting

microspheres were stabilized using gluteraldehyde solution (25%v/v) for a period of 15min. The microspheres were collected by decantation and washed with n-hexane and dried at room temperature.

Preparation of Ethyl Cellulose Microspheres[13]

Ethyl Cellulose microspheres were prepared by solvent evaporation method. In this method 10ml of dichloromethane and methanol in 1:1 ratio was taken and various drug: polymer ratios (1:1, 1:2, 1:3, and 1:4) were added simultaneously. This above solution was dispersed drop wise in a separate 200ml beaker containing 100ml of liquid paraffin and 0.5ml of span 80. The stirring speed was 1000rpm and stirring was carried out for 30 minutes. Then later obtained microspheres were washed with petroleum ether and dried.

EVALUATION OF MICROSPHERES

Drug polymer interaction (FTIR) study

The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000-600 cm-1. FTIR study was carried on IBH, physical mixture of IBH and polymer, IBH loaded egg albumin microspheres (Fig.1 to Fig.4) and ethyl cellulose microspheres (Fig.5 to Fig.8).

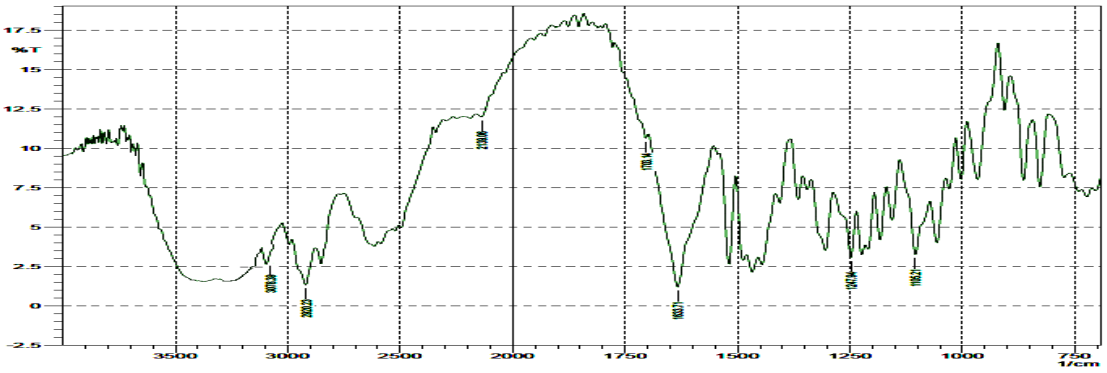


Fig.1: IR Spectrum of Ivabradine Hydrochloride (pure drug)

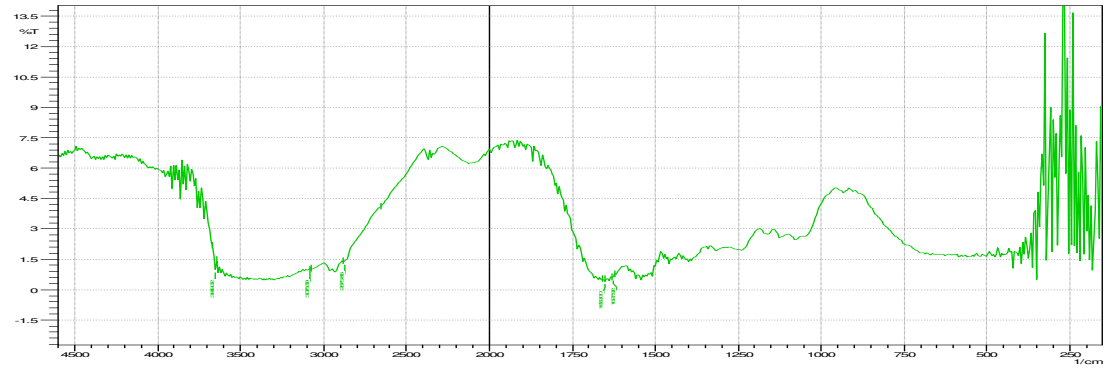


Fig.2: IR Spectrum of egg albumin (polymer)

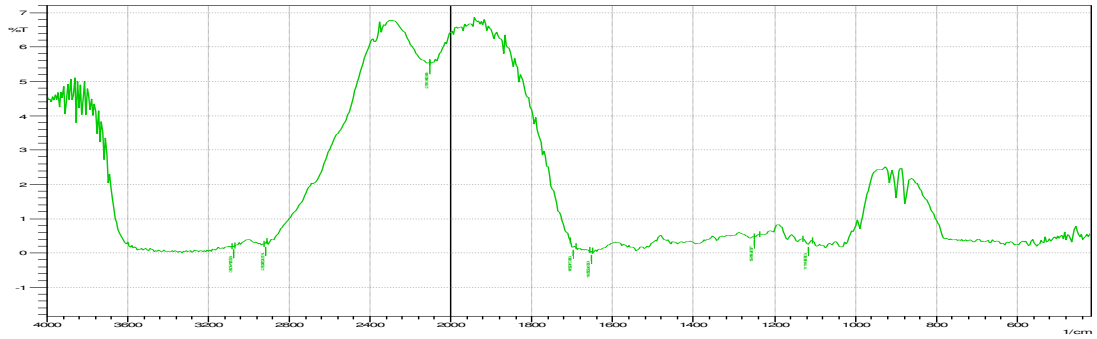


Fig.3: IR Spectrum of physical mixture of IBH and EA

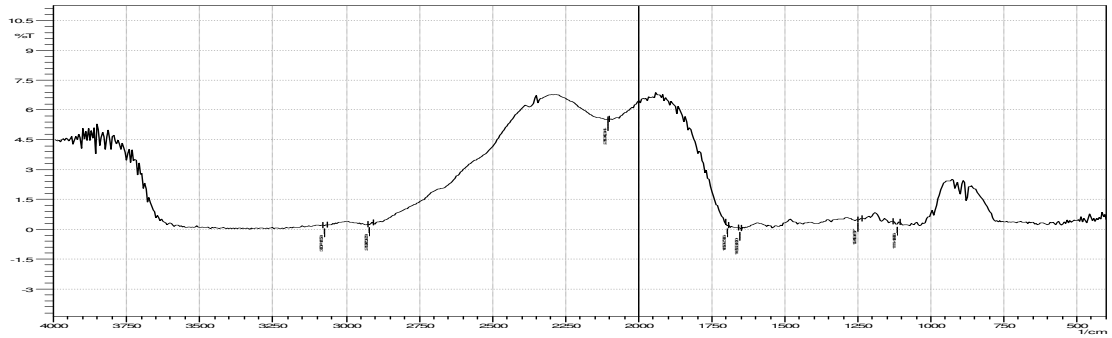


Fig.4: IR Spectrum of IBH loaded EA microspheres

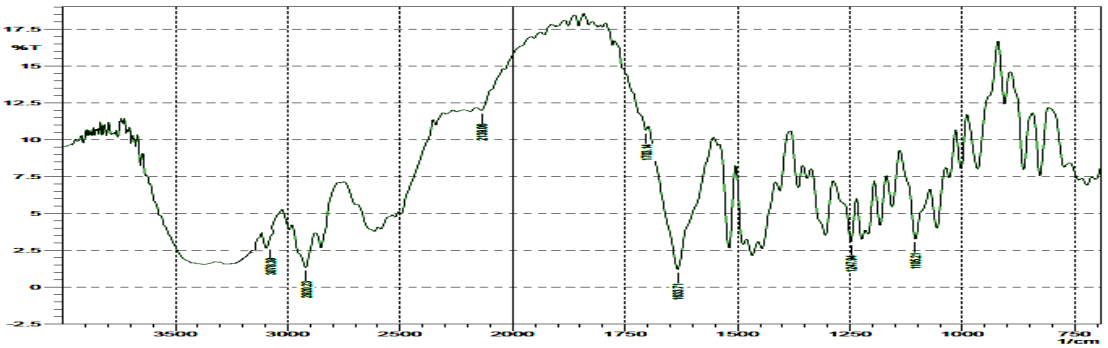


Fig.5: IR Spectrum of Ivabradine Hydrochloride (pure drug)

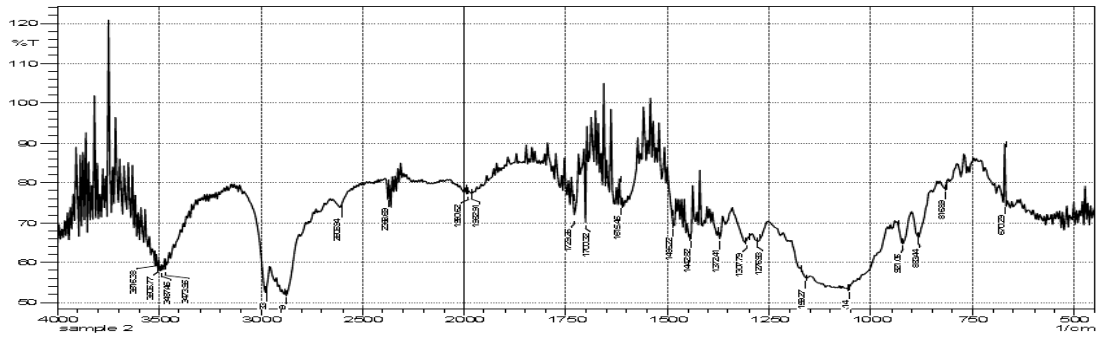


Fig.6: IR Spectrum of ethyl cellulose (polymer)

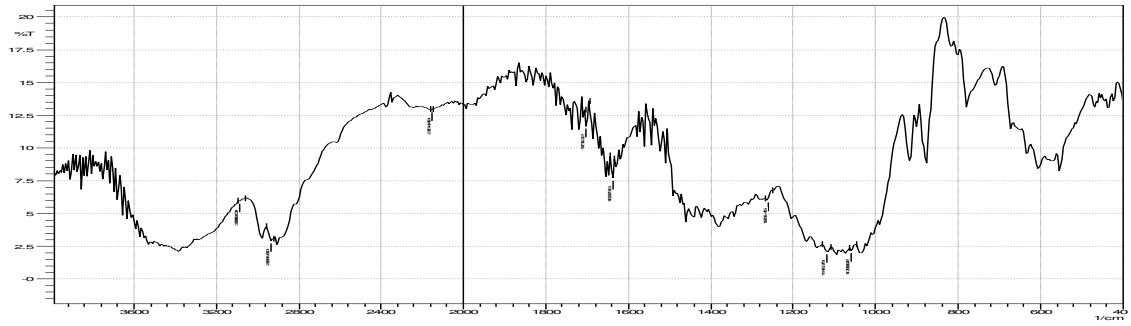


Fig.7: IR Spectrum of physical mixture of IBH and Ethyl cellulose

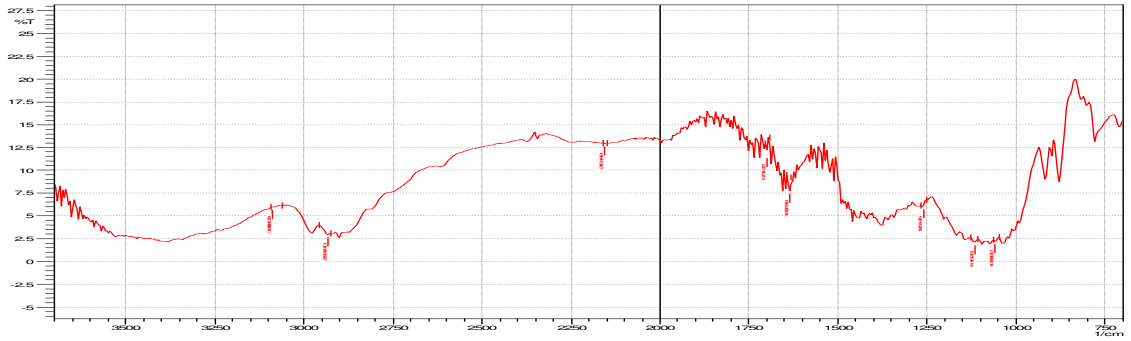
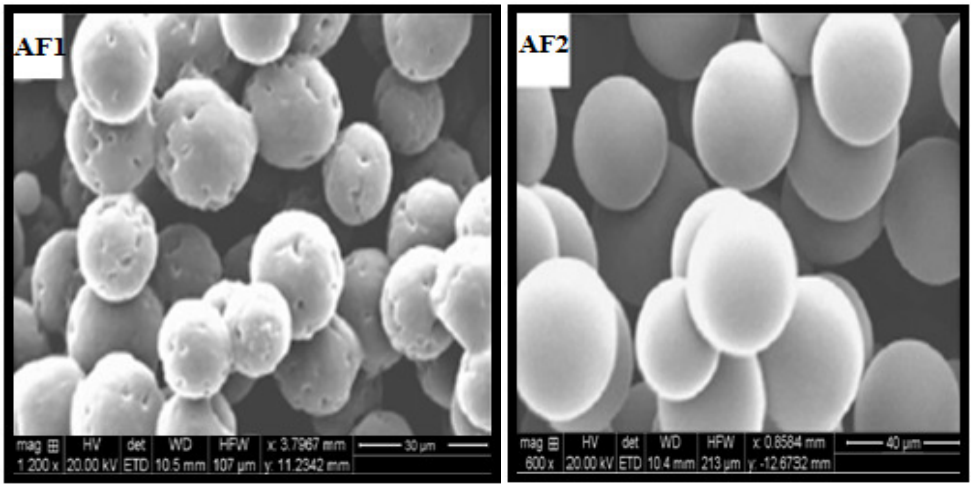


Fig.8: IR Spectrum of IBH loaded ethyl cellulose microspheres

Scanning electron microscopy (SEM) [14]

Scanning electron microscopy has been used to determine particle size distribution, texture and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry IBH microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of IBH loaded egg albumin microspheres (Fig.9) and ethyl cellulose microspheres were taken by random scanning of the stub. (Fig.10)



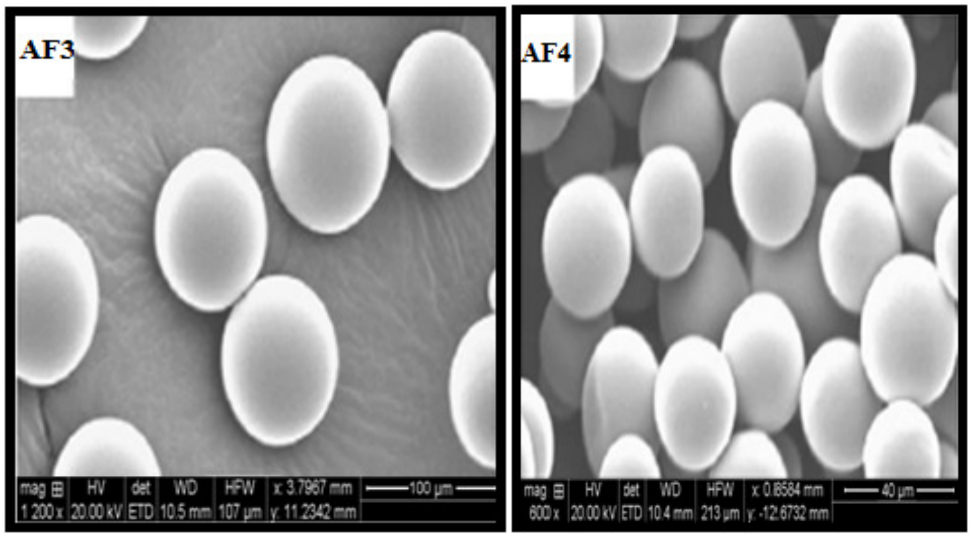


Fig. 9: SEM photographs of IBH loaded EA microspheres: AF1 (1:1 ratio); AF2 (1:2 ratio); AF3 (1:3 ratio); AF4 (1:4 ratio)

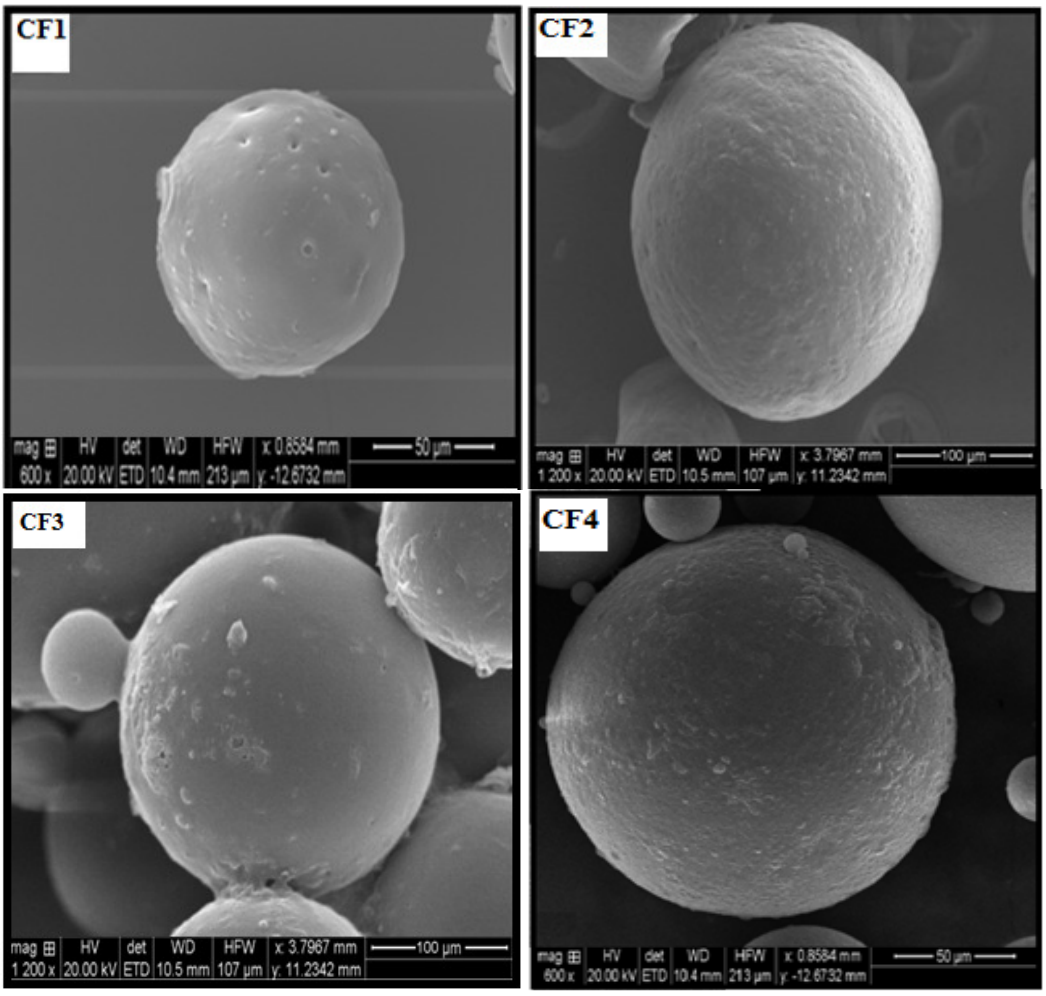


Fig. 10: SEM photographs of IBH loaded Ethyl cellulose microspheres: CF1(1:1 ratio); CF2 (1:2 ratio); CF3 (1:3 ratio); CF4 (1:4 ratio)

Percentage yield

Determining whether the preparation procedure chosen for incorporating a drug into the polymers is efficient and is of prime importance. The raw materials, amount of active compound, polymer(s) and other process parameters are deciding factors for the yield of the product during the preparation of microspheres. The yield was determined by weighing the microspheres and then finding out the percentage yield with respect to the weight of the input materials, i.e., weight of drug and polymers used. (Table.1)

The formula for calculation of % yield is as follows;

The percentage yield of prepared Ivabradine Hydrochloride microspheres was determined by using the formula:

$$\% \text{ yield} = \frac{\text{wt. of microparticles}}{\text{wt. of drug + wt. of polymers}} \text{ X } 100$$

Table 1: Percentage yield, drug content, encapsulation efficiency and average particle of Ivabradine Hydrochloride microspheres and Diffusion exponent (n) of Peppas model and Regression coefficient (r^2) of Ivabradine Hydrochloride release data from microspheres according to different kinetic models.

Parameters		Ethyl Cellulose Microspheres				Egg Albumin Microspheres			
		CF1	CF2	CF3	CF4	AF1	AF2	AF3	AF4
% Yield		81.74	84.21	88.37	90.28	67.33	69.42	72.57	74.86
Drug Content %		15.32	15.86	16.80	17.24	15.60	15.69	16.76	17.20
Drug Encapsulation Efficiency (%)		30.61	47.62	67.26	86.20	31.20	47.89	67.08	86.12
Avg. Particle Size (µm)		72.20	77.28	86.10	128.33	69.48	71.11	74.92	85.83
Zero order		0.9805	0.9814	0.9714	0.9769	0.9797	0.9736	0.9640	0.9774
First order		0.0455	0.0788	0.1410	0.1751	0.9337	0.9018	0.9060	0.9414
Higuchi		0.8511	0.8412	0.8183	0.8307	0.8339	0.8210	0.8069	0.8305
Peppas model	r^2	0.9608	0.9651	0.9731	0.9819	0.9819	0.9796	0.9884	0.9909
	n	1.7402	1.7087	1.6392	1.6121	1.6121	1.6465	1.5631	1.5635

Percentage drug entrapment efficiency (PDE) [15, 16]

Drug loading is important with regard to release characteristics. Generally, increased drug loading leads to an acceleration of the drug release. Drug entrapment efficiency represents the proportion of the initial amount of drug, which has been incorporated into the

microparticles.(Table. 1; Fig.11 and Fig.12)

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

$$\text{PDE} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

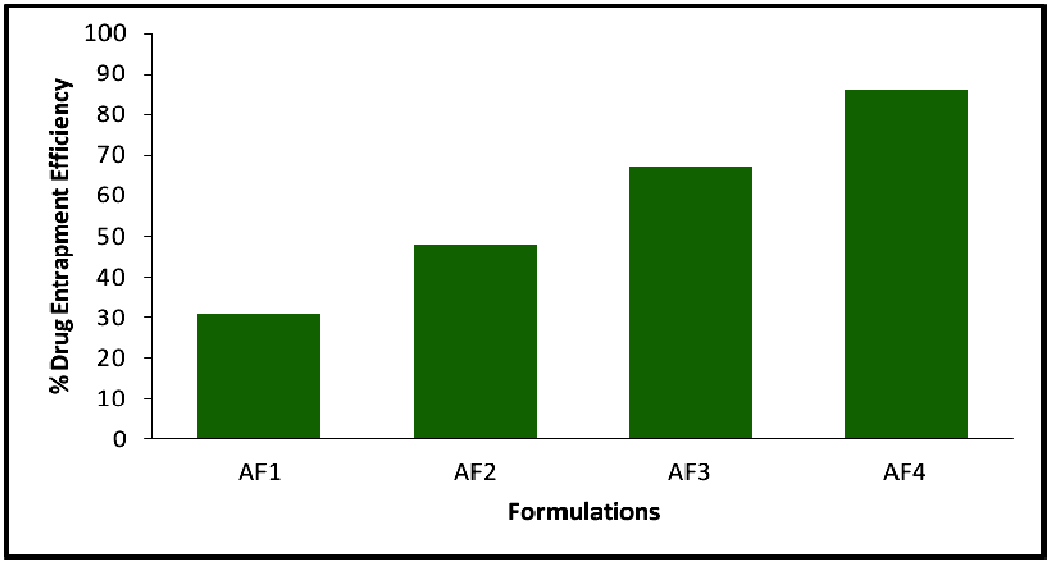


Fig. 11: Percentage drug entrapment efficiency of Egg Albumin Microspheres

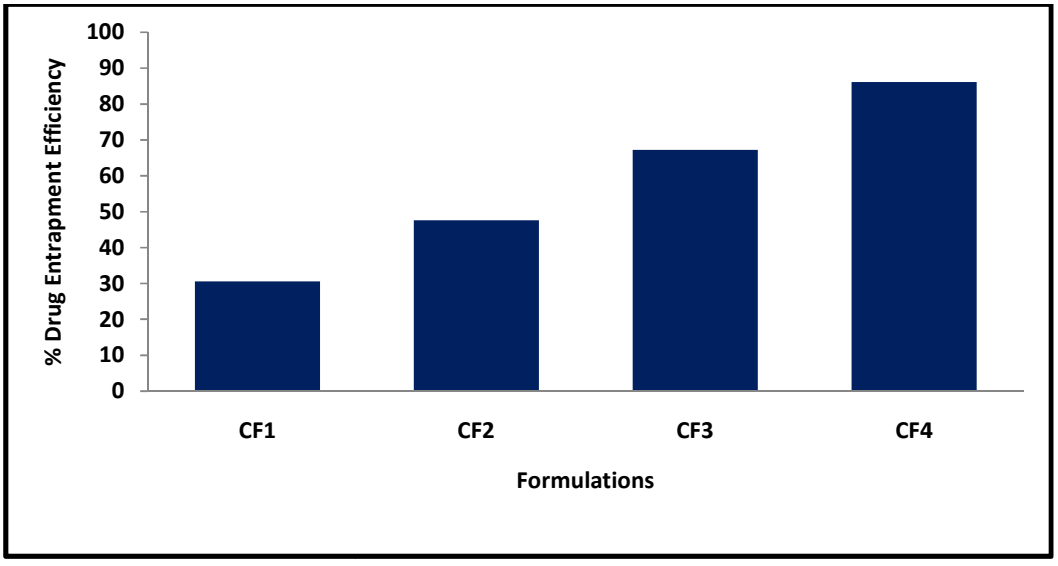


Fig. 12: Percentage drug entrapment efficiency

In vitro dissolution studies

The *in vitro* release of drug from the microparticles was carried out in basket type dissolution tester USP XXIII, TDT-08L, with auto sampler containing 500 ml of pH 1.2 buffer for the first 2 hrs and in 7.4 pH phosphate buffer for the next 10 hrs. The volume of the dissolution

media was maintained at 500 ml with constant stirring (50 rpm) and temperature of bath was maintained at 37 ± 0.5°C. Aliquots (10 ml) of dissolution media were sampled at specified time intervals and replaced with fresh media immediately after sampling. Samples were analyzed for drug content by UV visible spectroscopy (Shimadzu UV 1601).The release data obtained were fitted into various mathematical models. Dissolution studies were carried out for all the batches of the prepared formulations.(Table.1; Fig.13 and Fig.14)

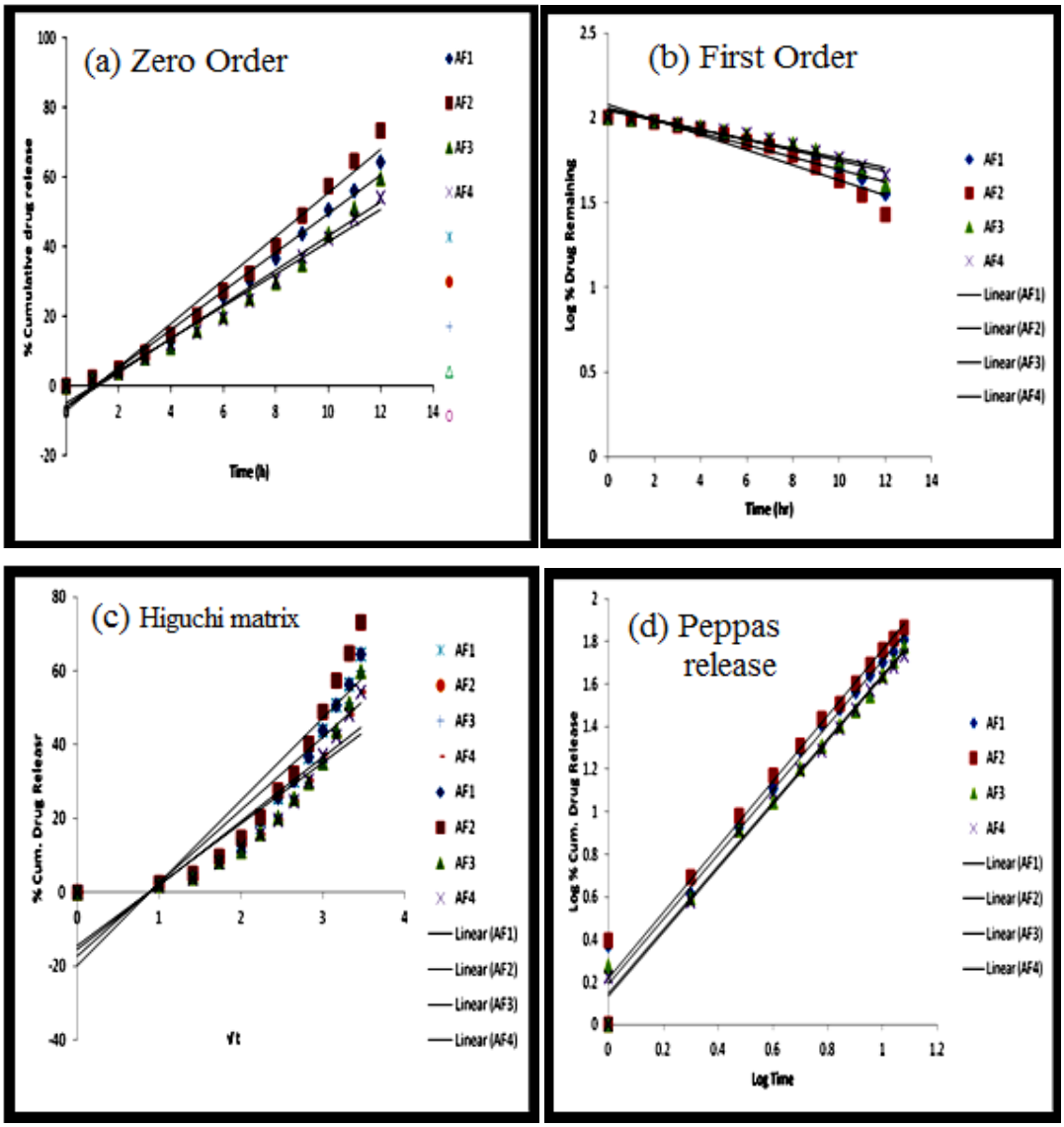


Fig. 13: *In vitro* release kinetics profile of IBH loaded egg albumin microspheres. (a) In vitro drug release was tested for Zero order; (b) first order; (c) Higuchi; (d) Peppas release in pH 1.2 for 2 hrs and changes to pH 7.4 from 2 to 12 hrs

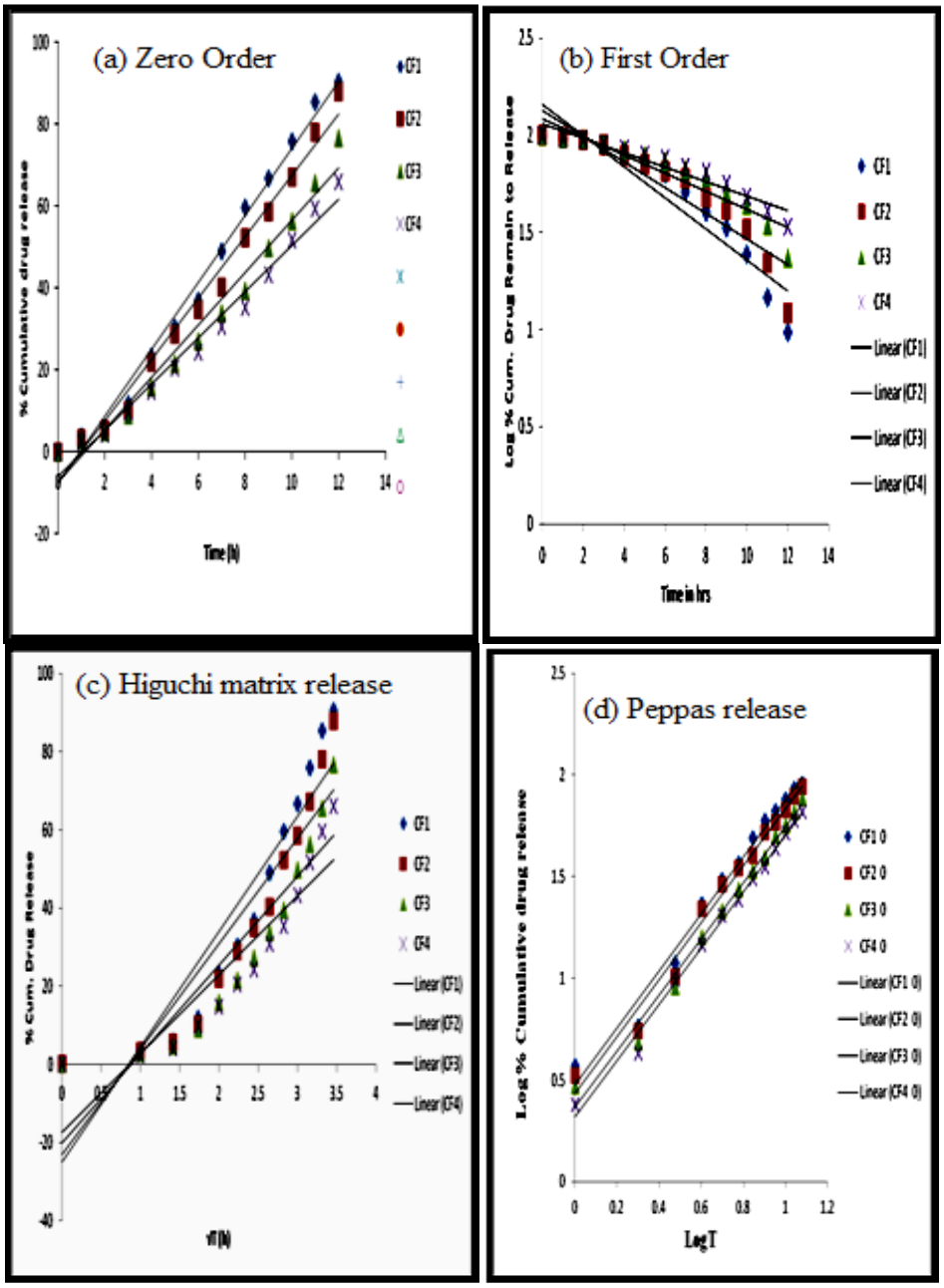


Fig. 14: *In vitro* release kinetics profile of IBH loaded ethyl cellulose microspheres. (a) In vitro drug release was tested for Zero order; (b) first order; (c) Higuchi; (d) Peppas release in pH 1.2 for 2 hrs and changes to pH 7.4 from 2 to 12 hrs

Differential Scanning Calorimetry (DSC) [17]

The physical state of KP in the microspheres was analyzed by Differential Scanning Calorimeter (Mettler-Toledo star 822^{e} system, Switzerland). The thermograms of the IBH, physical mixture of IBH and polymer, IBH microspheres and blank microspheres were obtained at a scanning rate of 10°C/min conducted over a temperature range of 25–300°C, respectively.(Fig.15 and Fig. 16)

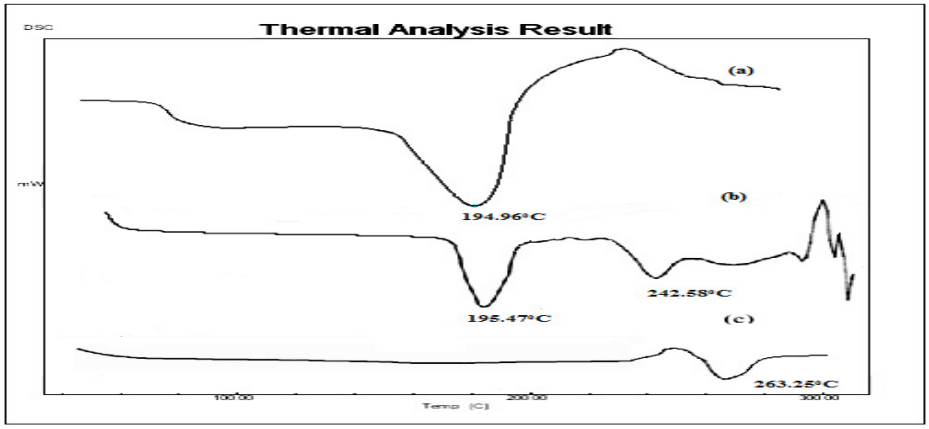


Fig.15: DSC thermogram of (a) IBH, (b) IBH loaded Egg Albumin microspheres, (c) blank Egg Albumin microspheres

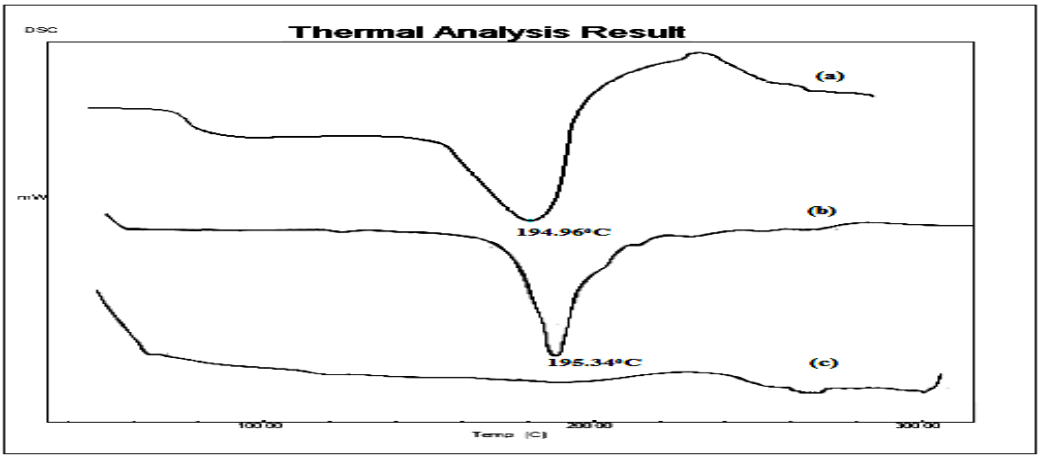


Fig.16: DSC thermogram of (a) IBH, (b) IBH loaded Ethyl cellulose microspheres, (c) blank Ethyl cellulose microspheres

RESULT AND DISCUSSION

In the present work controlled release microspheres of Ivabradine hydrochloride were formulated using egg albumin and ethyl cellulose polymer by heat denaturation technique and solvent evaporation technique, respectively. Four batches each were prepared with different polymer ratios were evaluated for physical properties like FTIR, SEM, particle size, Percentage yield, percentage drug content, encapsulation efficiency, *in vitro* dissolution, release kinetics and DSC of Ivabradine hydrochloride microspheres. The % cum. Drug release data shows that among all the four formulations of ethyl cellulose microspheres CF1 shows the maximum release of 90.40 ± 0.53 whereas, maximum % Cum. drug release formulation AF2 shows 73.26± 0.60 among the egg albumin microspheres. On comparing the results of natural and semi-synthetic polymers it can be concluded that among these two polymers, IBH shows better results with semi-synthetic (ethyl cellulose) polymer.

ACKNOWLEDGEMENT

Authors are thankful to Dr. TT Mani, Director, Bharathi College of Pharmacy, Mandya,

Karnataka, India for his support & encouragement in carrying out this work.

REFERENCES

1. Remington. The science and practice of pharmacy 21st ed, vol. 1, Philadelphia, PA: Lippincott Williams and Wilkins; 2005. p.939.
2. Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics-A treatise. 1st Ed. Delhi: Vallabh Prakashan; 2000. p. 335-75.
3. Kreuter J, Nefzger M, Liehl E, CzokR, Voges R. Microspheres – A Novel Approach in Drug Delivery System. J Pharm sci.1983;72: 1146.
4. Margel S, Wiesel E. Surface Functionalization of Polymer Latex Particles. J Polym Sci.1984;22:145.
5. http://www.doyouknow.in/Articles/Pharmaceutical/Controlled-Release-Drug-Delivery-System-Cdds.aspx, Nov 2010.
6. Hoffman A. Pharmacodynamic aspects of sustained release preparations. Adv Drug Del Rev 1998;33:185-99.
7. Vyas S, Khar R. Targeted and Contolled drug delivery: Novel carrier systems. First edition, CBS Publishers; New Delhi; 2006. 417-57.
8. Watts PJ, Davies MC, Melia CD. Microencapsulation using emulsification/solvent evaporation: an overview of techniques and applications. Crit. Rev Ther Drug Carrier Syst 1990;7:235-59.
9. http://circep.ahajournals.org/content/2/5/588.full.
10. Prashant Singh, S.K.Senthil Kumar, S. Parthiban, Peeyush, T.Tamizh Mani. Formulation and Evaluation of Ivabradine Hydrochloride Loaded Egg Albumin Microspheres. Journal of Pharmaceutical Research and Opinion 2012; 2(2): 25–28.
11. Prashant Singh, Anuj Kumar Srivastava, Devprakash, Kashinath B.P. Formulation and Evaluation of Ivabradine Hydrochloride Loaded Ethyl Cellulose Microspheres. IJPRD, 2012; 4(10):57 – 63.
12. Rajamanickam D, Rajappan M, Varadharajan M, Srinivasan B. Formulation and evaluation of albumin microspheres containing aceclofenac; IJPSRR 2010;4(1): 112-117.
13. K.P.R. Chowdary, N. Koteswara Rao, K. Malathi. Ethyl Cellulose microspheres of glipizide: characterization, *in-vitro, in-vivo* evaluation; Indian J. Pharma. Sci., 2004;66(4):412-416.
14. Magharla DD, Nandhakumar S, Vankayalu DS, Suresh C. Preparation of poly (epsilon-caprolactone) microspheres containing etoposide by solvent evaporation method. Asian J of Pharm Sci 2010;5(3):114-22
15. Dandagi PM, Manvi FV, Gadad AP, Mastiholimath VS, Patil MB, Balamuralidhara V. Microencapsulation of Verapamil hydrochloride by ionotropic gelation technique. Ind J Pharm Sci 2004;66(5):631-5.
16. Deore BV, Mahajan HS, Deore UV. Development and characterization of sustained release microspheres by quassi emulsion solvent diffusion method. Int J Chem Tech Res 2009;1(3):634-42
17. Jeevana JB, Sunitha G. Development and evaluation of gelatin microspheres of Tramadol Hydrochloride. J Young Pharma 2009;1:24-7.