FORMULATION AND CHARACTERIZATION OF NATURAL POLYMERIC MICROSPHERES LOADED IVABRADINE HYDROCHLORIDE

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ABSTRACT

The aim of the study was formulation and characterization of polymeric Ivabradine hydrochloride loaded microspheres (IH-MS) in a sustained manner, to inhibit the *I*f pacemaker current. The drug selectively targets the heart rate and to beta-blockers, that does not reduce myocardial contractility. The drug with different concentration ratio of natural polymers Gelatin, Ethyl cellulose and Methocel K4M (1:1, 1:2, 1:3, 1:4 and 1:5) were used to develop microspheres by emulsion cross-linking and emulsion solvent evaporation method. These were evaluated for % production yield, %Entrapment efficiency (EE) and Arithmetic mean particle size. On the basis of the highest values of these parameters, formulations G-F4, EC-F9 and MC-F14 were in each kind of polymer. The *In-vitro* drug release kinetics study and morphology were performed on selected batches. It was results that % production yield was in the range of 61.5±0.84 to 78.3±0.48, %EE was 59.7±0.51 to 87.2±0.13 and mean particle size was observed 71.4±0.95 to 93.2±0.47 µm in all formulations. In comparison, the formulation EC-F9 has maximum % production yield 78.3±0.48; maximum %EE 87.2±0.13 and suitable arithmetic mean size of 82.3±0.53 µm. The % cumulative drug release rate was found maximum in EC-F9 formulation in 12 h, which was 1.52 times greater than drug alone. SEM study showed spherical

shape of microspheres. The sustained effect was shown and data were most fitted into the Korsemeyer-pappas kinetic model. The calculated n value showed anomalous transport release profile of drug from polymers. Stability studies at 30±2ºC/60%RH±5% condition as per ICH guideline suggested that prepared microspheres were most stable at refrigeration conditions. Thus, developed polymeric microspheres encapsulated IH could be chosen for different drug delivery systems in future.

Key words: Polymeric Microspheres, Ivabradine hydrochloride, Gelatin, Ethyl cellulose, Methocel K4M, Microspheres

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INTRODUCTION

Microencapsulation is a polymeric developed concept under sustained or targeted drug delivery system that has been used widely to enhance the bioavailability, stability, drug release rate. This approach was successful in masking the taste and odor and helps to reduce the dosing frequency of drug[1]. Polymeric microspheres encapsulation was found to be the effective way to control some chronic diseases like cancers, genetic disorders, cardiovascular disorders etc. Ivabradine hydrochloride (IH), is the cardiovascular drug used to treat chronic stable angina pectoris. Results of a post marketing surveillance study on patients, where therapy with Ivabradine tablets were associated with a significant reduction in the frequency of angina attacks and consumption of short-acting nitrates of 87%. This includes an increase in myocardial diastolic perfusion time[2], enhancement in coronary flow reserve[3], improvement in endothelial function[4], and enhancement in coronary collateral flow in patients with chronic stable coronary artery disease[5]. This makes IH an important anti-anginal and anti-ischemic therapeutic strategy. The most common adverse effects were luminous phenomena (14.5%), bradycardia (2%), and headaches (2.6-4.8%) were reported[6]. These problems might be overcome to some extent by using the concept of encapsulation of IH within a microspheres core having lipophilic and biodegradable outer matrix in sustained delivery. Microspheres are homogeneous monolithic particles (0.1-1000μm) that provide protection of drugs from gastric irritation and incompatibilities. IH is hydrophilic, low bioavailability (40%), low biological half-life (about 2 h) and has a

melting point less than 200^0C. Thus, the drug was found to be a suitable candidate for encapsulation within a polymeric cavity. For development of IH-MS, three natural polymers gelatin, ethyl cellulose (water insoluble) and HPMC (water soluble) have been chosen in different proportions. These are inert, colorless, tasteless, non-toxic, biocompatible and widely accepted in various pharmaceutical formulations[7]. Glutaraldehyde is used as a cross-linking agent. Sesame oil, a heart healthy fat that reduces the blood pressure was used as oil phase to prepare the emulsion. Sustained action of the drug is achieved by passing through cell/tissue membrane to systemic circulation due to its small size range. This work summarizes the formulation and characterization of IH-MS using natural polymers. This might become a helpful tool in development of various dosage forms in oral, topical, parenteral and other routes by enhancing the stability, bioavailability and dissolution rate of drugs.

MATERIALS AND METHODS

Materials

The IH drug was generously gifted by Ind swift Ltd., Chandigarh, India. Ethyl cellulose, Methocel K4M, Petroleum ether, Dichloromethane, Liquid paraffin light and Glutaraldehyde from CDH fine chemicals, New Delhi, Span-80 from Croda industrial chemicals, Methanol, Toluene, Isopropyl alcohol from Spectrochem Pvt. Ltd., Gelatin from Hi media Laboratories Ltd., Sesame oil from Recon oil industries, Mumbai were purchased. All chemicals and solvents used in this study were of analytical grade. Freshly prepared distilled water was used throughout the studies.

Methods

Identification of drug

IR spectra and Differential scanning calorimetry (DSC) methods were used for identification of drugs. Potassium bromide pellet technique was used in the Shimadzu instrument at frequency range of 500-4000 cm^{-1} with scanning speed of 4.0 cm^{-1} and the graph was recorded. For DSC, a weighted sample was placed in a crucible, placed in the measurement cell of STAR*SW 12.10 instrument and scanned at a rate of 10°C per min and at 40-150°C. The Thermogram was recorded.

Quantitative Estimation of Drug

The Spectrophotometric method (UV) was used for estimation of the drug by UV-2400 PC series, Shimadzu, Japan, in the present study. The drug concentration in the range

of 10-55µg/ml in phosphate buffer pH 6.8 solutions (PB-6.8) was estimated in range of 200-400nm. [8]

Solubility Profile

The drug concentration of 5.0mg was mixed with different solvents (Distilled water, methanol, dimethyl sulfoxide, dimethyl formamide, PB-6.8, 95% ethanol, chloroform, dioxane, dichloromethane, 0.1 N HCl and acetone) separately to attain the equilibrium in amber color volumetric flask. Solutions were filtered and analyzed spectrophotometrically at 286.0nm and calculated by the following equation.

Solubility (mg/ml) = Initial - Final concentration of drug

Drug–excipients compatibility study

Compatibility of drug and polymers was evaluated through physical mixture preparation by kneading method. These mixtures of drug and different polymers (Gelatin, Ethyl cellulose and Methocel K4M) were prepared in 1:1 molar ratio, stored and analyzed.

Preparation of Gelatin IH-MS

These microspheres were formulated by modified emulsion cross-linking method[9]. The gelatin solution of different concentrations (1%, 2%, 3%, 4% and 5%) was prepared in distilled water separately in beakers at room temperature. Then, 100 mg of drug was added in each beaker slowly and homogenized using a magnetic stirrer. Sesame oil 50ml each was added gradually at 1000rpm with continuous agitation. Then 25ml glutaraldehyde saturated toluene was added and stirred continuously for 4 h for cross linking. Thus formed microspheres were collected, filtered and washed with cold isopropyl alcohol and were dried at room temperature for 24h. These were kept in vacuum desiccators for removing of any traces of solvent, labeled (G-F1 to G-F5) and stored in airtight container for further work.

Preparation of Ethyl cellulose IH-MS and Methocel K4M IH-MS

These microspheres were formulated by modified emulsion solvent evaporation method[10]. Different concentration ratios 1:1, 1:2, 1:3, 1:4 and 1:5 of IH: Ethyl cellulose and IH: Methocel K4M was placed separately in beakers of suitable size at room temperature, respectively. Then, 10 ml of mixture of Methanol: Dichloromethane (1:1v/v) was added in each beaker slowly through stirring to prepare an organic dispersed phase. The aqueous phase was prepared by dissolving 100ml of Liquid

paraffin and 0.5ml of Span-80 in another beaker separately. The organic dispersed phase of each beaker was added slowly drop wise to each beaker of aqueous phase respectively, using a magnetic stirrer at 1000rpm for half an hour. Thus formed microspheres were collected and washed through Petroleum ether and were dried in an oven at 40ºC for 24h, separately. These were kept in vacuum desiccators for removing of any traces of solvent, labeled (EC-F6 to EC-F10 and MC-F11 to MC-F15) and stored in airtight container for further study (Table-1).

Table 1 Formulation composition of Polymeric IH-MS

Formulation code	Polymer concentration (mg)			
	Gelatin	Ethyl cellulose	Methocel K4M	Drug*: Polymer ratio
G-F1	100	-	-	1:1
G-F2	200	-	-	1:2
G-F3	300	-	-	1:3
G-F4	400	-	-	1:4
G-F5	500	-	-	1:5
EC-F6	-	100	-	1:1
EC-F7	-	200	-	1:2
EC-F8	-	300	-	1:3
EC-F9	-	400	-	1:4
EC-F10	-	500	-	1:5
MC-F11	-	-	100	1:1
MC-F12	-	-	200	1:2
MC-F13	-	-	300	1:3
MC-F14	-	-	400	1:4
MC-F15	-	-	500	1:5

* Drug concentration was 100mg in each formulation.

Characterization of IH-MS

Determination of % Production yield

The Initial and final weight produced of each IH-MS of different concentration ratio was measured. The production yield was calculated in triplicate by following formula and reported.

$$\text{Production yield (\%)} = \frac{\text{Practical weight}}{\text{Theoretical weight}} \times 100$$

Determination of %Entrapment efficiency (EE)

Accurately measured 5.0ml of each preparation was centrifuged through Remi Equipments, Mumbai, India, at 4ºC, 22000 rpm for 30 min. The pellets of microsphere

and the supernatant containing free drug were obtained from each of the centrifugal tubes and were washed again with distilled water to remove any non entrapped drug by centrifugation at the same speed. The combined supernatant which was obtained before and after washing was analyzed at 286.0nm for the drug content by formula. Where, Q_1=Initial amount of drug; Q_2=Amount of diffused drug; (Q_1–Q_2)= Amount of entrapped drug.

$$\%EE = \frac{Q1-Q2}{Q1}100$$

Determination of Mean Particle size

Mean vesicle size of prepared formulations was observed by a calibrated electron optical microscope (Olympus, India)[11]. This is suitable for a size range of 0.2-100µm in small sample size. A thin film of formulation was spread on slide; a cover slip was placed over it and observed under the optical microscope. The arithmetic mean vesicle size was determined by following equations[12]. Where, n is the total number of particles counted and d is projected diameter. $Arithmetic\ mean = \frac{\sum nd}{\sum n}$

***In-vitro* drug release kinetics study**

A drug release study assured that a drug carried by a vehicle is able to reach the systemic circulation of the receiver compartment at an adequate rate and in sufficient amounts. Formulations G-F4, EC-F9 and MC-F14 were selected on the basis of maximum % production yield, %EE and suitable arithmetic mean size diameter in each kind of polymer. The treated cellophane membrane was used as a dialysis membrane. The diffusion conditions were maintained and 50ml of PB-6.8 was used as receptor fluid at 37ºC. The assembly was kept at 100 rpm on a magnetic stirrer. 10ml each sample was placed on a cellophane membrane separately and these assemblies were touched onto a dissolution medium surface. 2ml samples were withdrawn at regular intervals of 2h and replaced with the same amount of phosphate buffer. Same procedure was adopted for 1%w/v of 10ml of plain drug solution in PB-6.8. The withdrawn samples were suitably diluted and analyzed at 286.0 nm by UV method. The readings were taken in triplicate and released data were fitted in various mathematical models (Zero order $Q_t=Q_0+K_0t$; First order $Q=In\ Q_0-K_1t/2.303$; Higuchi $Q=K_Ht^{1/2}$ and Korsemayer-pappas $M_t/M_\infty=K\ t^n$ model). Where, Q is the quantity of drug released in time t at initial concentration Q_0, rate constant of zero-

order, first-order and Higuchi rate equations is K_0, K_1 and K_H, respectively. In pappas model, the quantity of drug released at time t and ∞ are M_t and M_∞, n is diffusion exponent[13-14].

Scanning electron microscopy (SEM)

Morphological examination helps to study the texture and size of the particles suitable for drug delivery systems. The evaluated IH-MS were examined through SEM (JSM-T-330A microscope, JEOL, Japan) at 20KV. The sample was placed on a brass stub with double adhesive tape and coated under vacuum using a metallizer to image the particles.

Stability study

To examine the aggregation and leakage from prepared vesicles throughout storage, physical stability studies were conducted. A protocol of stability study was carried out using a humidity chamber (Kesar control system) as per ICH guideline under intermediate testing conditions (30±2ºC/60%RH±5%)[15]. The selected formulations G-F4, EC-F9 and MC-F14 were stored in sealed amber colored glass ampoules separately at refrigeration temperature (4±2)ºC, room temperature (25±2)ºC and body temperature (37±2)ºC for a period of at least 6 months. After every month time interval physical appearance, mean vesicle size, and residual drug content was determined and reported.

RESULT AND DISCUSSION

Identification tests of IH indicated that the Infrared spectra of the drug exhibited peaks of functional groups present in its structure. It included the different peaks of N-H stretching amine (2850.0nm), C=C=O stretching (2339.65nm), C=C stretching conjugated alkene (1629.85nm), O-H bending (1487.12nm), C-H bending methylene group (1465.9nm), C-N stretching aromatic amine (1303.88), C-O stretching (1247.94nm), C-N stretching amine (1058.92-1224.8nm) and C=C bending alkenes tri-substituted (825.53nm). DSC Thermogram showed the thermal behavior of a drug that exhibited an endothermic peak at 198.63 ºC. The UV absorption maximum of the drug after scanning in PB-6.8 was observed at 286 nm. Standard curves of the drug were prepared in PB-6.8. The absorbance data obtained was subjected to linear regression in the range of 10-55 µg/ml. All data were analyzed statistically with mean±SD. Solubility profile of drug in different

solvents was determined and results showed that the drug is freely soluble in water, methanol, PB-6.8, 95% ethanol; soluble in chloroform and dioxane; sparingly soluble in dichloromethane, 0.1 N HCl and slightly soluble in acetone, 0.1 N NaOH etc. The drug–excipients compatibility study of physical mixture of IH and Polymers were found not to interfere in the estimation of the drug. Microspheres were formulated using three natural polymers, which are biocompatible and pharmaceutically acceptable[16]. Gelatin microspheres were formulated by a modified emulsion cross-linking method. Ethyl cellulose and Methocel K4M microspheres were formulated by modified emulsion solvent evaporation methods in different concentration ratios. In this method surfactant plays a key role in microencapsulation[17-18]. Prepared vesicles were evaluated for % production yield, %EE, Mean particle size, *In-vitro* drug release kinetics study and morphology. Physical observation of all formulations showed that all were turbid and colloidal. The % production yield was found in the range of 61.5±0.84 to 78.3±0.48; %EE was 59.7±0.51 to 87.2±0.13. Study showed that a higher polymer ratio leads to higher %EE, because it protects the drug molecules from leaching out toward the external phase during the microencapsulation process[19]. Mean particle size was observed 71.4±0.95 to 93.2±0.47 µm, depending on the concentration of drug: polymer ratio in formulation G-F1 to MC-F15 (Table 2). For stabilization of emulsion droplets, due to the surfactant effect smaller size of microspheres developed that helps against coalescence[20-22].

Table 2 Evaluation parameters of prepared IH-MS*

Formulation code	Production yield (%±SD)	EE (%±SD)	Arithmetic mean size diameter (µm±SD)
G-F1	66.2±0.14	63.9±0.25	91.7±0.15
G-F2	69.6±0.26	71.4±0.28	86.4±0.32
G-F3	71.5±0.97	80.5±0.39	88.6±0.53
G-F4	77.8±0.34	85.2±0.69	79.4±0.81
G-F5	72.9±0.13	82.6±0.87	68.9±0.24
EC-F6	64.4±0.72	59.7±0.51	83.2±0.17
EC-F7	67.5±0.29	64.3±0.92	90.5±0.38
EC-F8	70.9±0.11	70.4±0.74	89.1±0.27

EC-F9	78.3±0.48	87.2±0.13	82.3±0.53
EC-F10	73.2±0.17	75.3±0.57	71.4±0.95
MC-F11	61.5±0.84	60.4±0.68	88.9±0.14
MC-F12	64.6±0.91	65.9±0.15	93.2±0.47
MC-F13	68.7±0.83	69.2±0.47	84.5±0.82
MC-F14	76.5±0.27	83.1±0.24	73.4±0.68
MC-F15	71.9±0.67	78.6±0.93	82.1±0.12

*****All values expressed as mean± SD, (n=3)

On the basis of maximum % production yield, %EE and suitable vesicle size, microsphere formulations G-F4, EC-F9 and MC-F14 were selected from each kind of polymer. The comparative study showed that the formulation EC-F9 has maximum % production yield 78.3±0.48; maximum %EE 87.2±0.13 and suitable arithmetic mean size of 82.3±0.53 µm. These selected formulations were studied for *In-vitro* drug release profile and were compared with plain drug solution. Obtained data were represented graphically in Figure 1. It was observed that maximum drug release (94.7 %) was found in EC-F9 in 12 h as compared with drug alone (62.4%). The release kinetic data were fitted into various mathematical models. Table 3 showed Rate constant (K) and correlation constant (R^2) of selected IH-MS. The best suited model was found in the Korsemeyer-Pappas model for microspheres. The n- value for different drug: polymer ratio was ranged from 0.574 to 0.744, indicating that drug release mechanism results combined controlled effects of diffusion and dissolution/erosion[23-24].

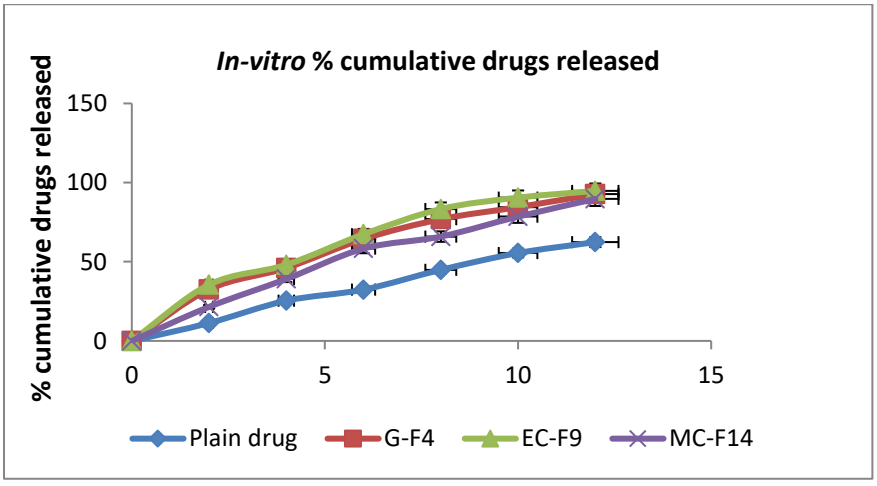

Fig 1 Comparative study of *In vitro* % cumulative drug release profile from Selected formulations*

***Expressed as mean± SD, n=3 trials, where G-F4= Gelatin microsphere, EC-F9= Ethyl cellulose microsphere,

MC-F14= Methocel K4M microsphere

Table 3 Various Mathematical models Rate constant (K) and correlation constant (R^2) of selected IH-MS

Formulation code	Rate constant (K) and correlation constant (R^2)							
	Zero order		First order		Higuchi		Korsemeyer-Pappas	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
Plain drug	5.454	0.9926	0.076	0.9873	15.853	0.9065	0.903	0.9957
G-F4	8.814	0.8920	0.179	0.9916	26.232	0.9884	0.595	0.9957
EC-F9	9.274	0.8714	0.201	0.9854	27.651	0.9857	0.574	0.9889
MC-F14	8.071	0.9625	0.144	0.9835	23.733	0.9536	0.744	0.9948

The SEM study showed spherical shape. It was due to the binding of the polymer heads of the molecules, encapsulated in the presence of a hydrating medium shown in figure 2.

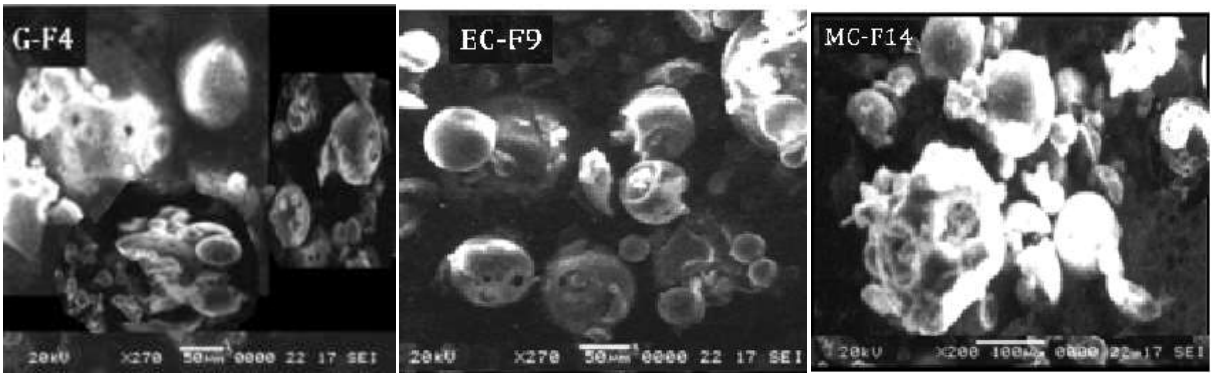

Fig. 2: SEM study of selected formulations (Mean ± SEM)

These formulations were subjected for physical stability studies for a period of at least 6 months as per ICH protocol and represented graphically in figure 3. After 6 months, at refrigeration condition insignificant changes (p>0.05) in color, residual drug content and vesicular size of stored microspheres were detected in all

formulations. As the temperature increased, physical in-stability at these conditions was found. It might be swelling or aggregation of vesicles. The decrease in residual drug content was detected representing leakage drug from formulations due to raised temperature[25-26].

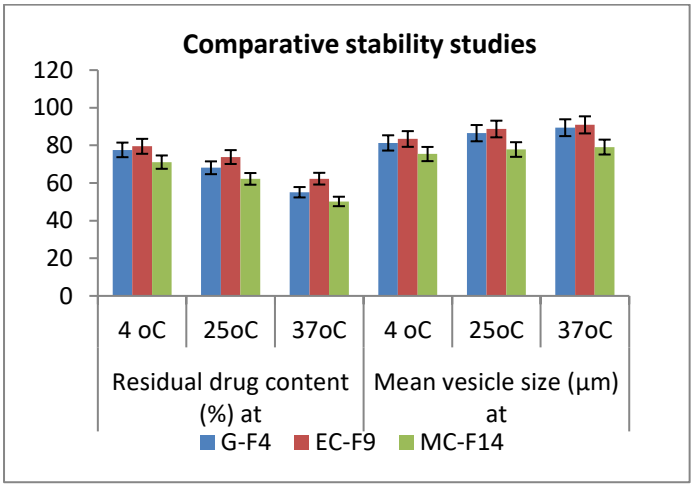

Fig 3 Comparative stability studies of selected formulations at different temperature after 6 months

(Expressed as mean± SD, n=3 trials, where G-F4= Gelatin microsphere, EC-F9= Ethyl cellulose microsphere, MC-F14=Methocel K4M microsphere)

CONCLUSION

The present work was aimed to develop and evaluate IH microspheres using different concentrations of natural polymer: drug ratio to produce sustained effect of drug. Each kind of polymeric formulation G-F4, EC-F9 and MC-F14 were selected, on the basis of highest % production yield, %EE and suitable vesicle size. These were of turbid, colloidal and spherical shape. The *in vitro* drug release study indicated that EC-F9 has maximum % cumulative drug release in 12 h, which was 1.52 times greater than drug alone. These formulations showed sustained effect and data were fitted in various mathematical models of zero, first order, Higuchi and Korsemeyer-pappas. The calculated n value showed non-fickian/ anomalous transport release profile suitable for sustained drug delivery. As per ICH guideline stability studies suggested that prepared microspheres were most stable at refrigeration conditions and could be chosen for the development of the different drug delivery systems in future.

FUNDING

Nil

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Kumar D. and Kalakuntala RR. Evaluation of zidovudine encapsulated ethyl cellulose microspheres prepared by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique. Acta Poloniae Pharm-Drug Res. 2006;63(2):141-8.
2. Dillinger JG., Maher V., Vitale C., Henry P., Logeart D., Manzo S.S., Allee G. and Levy BI., Impact of ivabradine on central aortic blood pressure and myocardial perfusion in patients with stable coronary artery disease. Hypertension, 2015, 66, 1138–44.
3. Tagliamonte E., Cirillo T., Rigo F., Astarita C., Coppola A., Romano C. and Capuano N. Ivabradine and bisoprolol on Doppler-derived coronary flow velocity reserve in patients with stable coronary artery disease: beyond the heart rate. Adv. Ther., 2015, 32, 757–67.
4. Mangiacapra F., Colaiori E., Ricottini E., Balducci F., Creta A., Demartini G. and Di Sciascio G. Heart rate reduction by ivabradine for improvement of endothelial function in patients with coronary artery disease: the randomized open-label RIVENDEL study. Eur. Heart J., 2015, 36 (suppl 1), 1105.
5. Gloekler S., Traupe T., Stoller M., Schild D., Steck H., Khattab A., Vogel R. and Seiler C. The effect of heart rate reduction by ivabradine on collateral function in patients with chronic stable coronary artery disease. Heart, 2014, 100, 160–6.
6. Tardif JC., Ford I., Tendera M., Bourassa M.G. and Fox K. Efficacy of ivabradine, a new selective I(f) inhibitor, compared with atenolol in patients with chronic stable angina. Eur. Heart J., 2005, 26 (23), 2529–36.
7. Yamuda T., Onishi H. and Machida Y. Sustained release ketoprofen microparticles with ethyl cellulose and carboxymethyl ethyl cellulose. J Controlled Release 2001;75:271–82.
8. Chaurasia G. and Lariya N. Formulation and evaluation of Ivabradine hydrochloride loaded transfersomal gel for transdermal delivery. Int. J. App. Pharm., 2020, 12(5), 295-301.
9. Jeevana JB and Sunitha G. Development and Evaluation of Gelatin Microspheres of Tramadol Hydrochloride. J Young Pharm. 2009;1:24-7.
10. Prashant Singh, T. Tamizh M., Shambaditya G. and Pradeep S. Comparative evaluation of Ivabradine hydrochloride loaded natural and synthetic microspheres. SGVU Journal of

Pharmaceutical Research & Education, 2019, 4(1), 368-379.

11. Martin A., Swarbrick J. and Cammarata A., Physical Pharmacy, Verghese publishing house, Bombay, third edition, 1991, 492-506.
12. Hadkar UB. A Handbook of Practical Physical Pharmacy and Practical Pharmaceutics, Nirali prakashan, Pune, Edition 7, 2012: 82-7.
13. Jelvehgari M., Hassanzadeh D., Kiafar F., Delf Loveym B. and Amiri S. Preparation and determination of drug-polymer interaction and *In-vitro* release of Mefenamic acid microspheres made of cellulose acetate phthalate and/or ethyl cellulose polymers. Iran J Pharm Res., 2011 Summer;10(3):457-67.
14. Mohapatra PK., and Sahoo SK. A study on the effects of surfactant concentration and particle size on ethylcellulose microspheres containing indinavir sulphate. Indian J Pharm Sci 2022;84(6):1556-65.
15. González-González O, Ramirez IO., Ramirez BI., O'Connell P., Ballesteros MP., Torrado JJ. and Serrano DR. Drug Stability: ICH versus accelerated predictive stability studies. Pharmaceutics. 2022;14(11):2324-30.
16. Panwar A, MM., Lee BH. andTan LP. Synthesis and fabrication of gelatin-based elastomeric hydrogels through cosolvent-induced polymer restructuring: RSC. Adv. 2022;12(13):7922-34.
17. Lalduhsanga P., Sushanta S. and Bhaskar M. The study of the effects of surfactants on ethyl cellulose microspheres containing salbutamol sulphate. Der Pharm Lett., 2009;1:65-74.
18. Yadav VK., Rai AK. and Ghosh AK. A study on the effects of different surfactants on morphology and drug release of repaglinide microspheres. Int J Res Dev Pharm Life Sci., 2017;6(5):2786-92.
19. Fu X., Ping Q. and Gao Y. Effects of formulation factors on encapsulation efficiency and release behavior *in vitro* of huperzine A-PLGA microspheres. J Microencapsul. 2005;22(7):705–14.
20. Akbari J., Enayatifard R., Saeedi M. and Saghaf M. Influence of hydroxyl-propyl methyl cellulose molecular weight grade on water uptake, erosion and drug release properties of diclofenac sodium matrix tablets. Trop J Pharm Res., 2011;10(5):535–41.
21. Heiskanen H., Peter D., Markku H., Paivi P and Marita O. Effect of physical properties and emulsification conditions on the microsphere size prepared using a solvent extraction process. J Dispers Sci Technol. 2012;33(2):234–44.
22. Banerjee S., Siddiqui L., Bhattacharya SS., Kaity S., Ghosh A., Chattopadhyay P, et al. Interpenetrating polymer network (IPN) hydrogel microspheres for oral controlled release application. Int J Biol Macromol. 2012;50(1):198-206.

23. Jose S., Fangueiro JF., Smitha J., Cinu TA., Chacko AJ., Premaletha K., et al. Predictive modeling of insulin release profile from cross-linked chitosan microspheres. Eur J Med Chem., 2013;60: 249-53.
24. Haya Y., Bashar AT., and Sheikh MS. Preparation and characterization of ethylcellulose microspheres for sustained-release of pregabalin. Research in Pharmaceutical Sciences, February 2021; 16(1): 1-15.
25. Puthli S. and Vavia PR. Stability studies of microparticulate system with piroxicam as model drug. AAPS Pharm. Sci. Tech. 2009;10(3):872-80.
26. Prashanth AK, Formulation and development of hydrochlorothiazide floating microspheres. Int. J. Pharm. Sci. Rev. Res., 2014; 25(1): 274-80.