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Formulation, characterization and *invitro* evaluation of floating microspheres of Verapamil Hydrochloride

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Abstract

Verapamil hydrochloride is a calcium channel blocker, widely used in management of hypertension. It has very short half life of 4h and oral bioavailability of 35.1%. The present investigation was concerned with the development of floating microspheres of verapamil hydrochloride to target the drug to its absorption site by increasing the residence time of drug in stomach and to control drug release in therapeutic range for longer period of time. Floating microspheres of verapamil hydrochloride were prepared by non-aqueous solvent evaporation technique using ethylcellulose and hydroxypropyl methyl cellulose (HPMC) in different ratios. The prepared microsheres were evaluated for particle size, surface topography, yield, percentage drug entrapment efficiency, percentage buoyancy, *in-vitro* drug release and drug release kinetics was evaluated using the linear regression method. Stability study was carried out for 3months.Results showed that particle size (45.01± 2.3 µm to 113.02±3.2 µm), yield (79.51±3.715 to 93.48±0.946), drug content (82.25±0.367 to 91.02± 1.169) and drug release of microspheres (12hour), stability was showed not much change in the optimized formulation (F4) and the best results were obtained at the ratio of drug: EC: HPMC (333.3:166.6). In most cases good *in vitro* floating behavior was observed and broad variety of drug release pattern could be achieved by variation of polymer ratio,

which was optimized to match target release profile. The developed floating microspheres of verapamil hydrochloride were used for effective management of hypertension.

Key words: Verapamil Hydrochloride, floating microspheres, ethyl cellulose, hydroxy methylcellulose

Introduction

Verapamil hydrochloride (VRP) is phenylalkylamine derivative, calcium antagonist are drugs which cause coronary and peripheral vasodilatation by reducing calcium influx through the slow channels of vascular smooth muscle and cardiac cell membranes.[1,2] Calcium antagonist are being used effectively in the treatment of several cardiovascular disorders, particularly angina pectoris, supraventricular tachycardies and hypertension[2]. It established that, as a result of oral usage, 90% of VRP is absorbed and then it reaches maximum plasma concentration within 1-2h. However, due to the extensive first pass hepatic excretion, it has such low bioavailability as 10-20% of an oral dose. VRP has nonlinear pharmacokinetic because of its saturation of presistemic metabolism leads to first pass effect which resulting in nonlinear absorption ([3-7]).

Several conventional and controlled release dosage forms of VRP with different doses have been formulated and marketed. It is from the investigations that there is no difference between the controlled release dosage from given once daily and conventional dosage from given several times daily with the same doses in respect to their bioavailability and antihypertensive effect ([1,8]) .

Gastro retentive floating dosage from remains in stomach for prolonged period and so, it releases the drug with preprogrammed rate resulting in increased gastric retension with reduced fluctuations in plasma drug concentration, maintains local concentration, improves bioavailability to drug as well as its half life, decreases the wastage of drug and also improves patient compliance by reducing repetitive doses.Gastroretetive drug delivery system can improve the controlled delivery system can improve the controlled delivery of the drug that have an absorption window by continuously releasing the drug for a prolong period of time before it reaches its absorption site thus ensuring its optimal bioavailability([9]). Floating oral microparticulate systems show several advantages in comparison with single unit dosage forms, such as more predictable gastric emptying, gastric emptying less dependent on the state of nutrition, high degree of dispersion in the digestive tract lesser risk of dose dumping and reduced local irritation.([10])

The objective of the present work is to develop floating microspheres of verpramil hydrochloride using nonaqueous solvent evaporation method. Microspheres were evaluated for surface morphology drug entrapment efficiency, yield of microspheres, particle size analysis, %Buoyancy, *in vitro* drug release study and stability studies.

Materials and Methods

Verapamil hydrochloride was obtained as a gift sample from Hetero lab, Hyderabad, India. Hydroxyl propyl methyl cellulose (HPMC), ethyl cellulose (EC), liquid paraffin was obtained from the central drug house (P) Ltd., India. All other chemicals reagents used were of analytical grade.

Fourier Transformed Infrared Spectroscopic Studies

FTIR spectral studies were carried out for pure drug verapamil hydrochloride, freshly prepared and six months old 1:1 SDs and individual substances to check the compatibility between drug and polymers using Bucker Tensor-27 (Bucker, Germany) FTIR instrument. Interaction between the components, if any, was indicated by either producing additional peaks or absence of the characteristics peaks corresponding to the drug and carrier. Interaction between the components, if any either producing additional peaks or absence of the characteristic peaks corresponding to the drug and carrier.

Preparation of Verapamil hydrochloride floating microspheres

Microspheres containing anti-hypertensive drug as a core material were prepared by a non-aqueous solvent evaporation method. Briefly, drug and different ration of polymers (EC and EC: HPMC) shown in Table I, were mixed in 30mL methanol. The slurry was slowly introduced into 30mL of liquid paraffin while being stirred at 50rpm by mechanical stirrer. The solution was stirred for 2h to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40-60°C) until free from oil. The collected microspheres were dried for 1h at room temperature and subsequently stored in a decicator over fused calcium chloride.

Table: 1 FORMULATION CHART

Batch	Amount of Drug (mg)	Total Amount of Polymer (mg)	Amount of Ethyl Cellulose (mg)		Amount of HPMC (mg)		Amount of Methanol (ml)	Amount of Liquid Paraffin (ml)
			%	Mg	%	mg		
F1	500	500	100	500	0	0	30	30
F2	500	1000	100	1000	0	0	30	30
F3	500	1500	100	1500	0	0	30	30
F4	500	500	66.66	333.3	33.33	166.6	30	30
F5	500	1000	66.66	666.6	33.33	333.3	30	30
F6	500	1500	66.66	999.9	33.33	499.9	30	30
F7	500	500	50	250	50	250	30	30
F8	500	1000	50	500	50	500	30	30
F9	500	1500	50	750	50	750	30	30

Note: Each formulation contains 5 replicate of floating microspheres equivalent to 100 mg drug in each.

Characterization

Yield of microsheres

The prepared microsheres were collected and weighed. The measured weight was divided by the total amount of all non-volatile compounds which were used for the preparation of microspheres.([11-14])

% yield= (Actual weight of product / Total weight of excipients and drug) x100

Particle size analysis

The size distributions of the microsheres were evaluated by sieve analysis, using a vibrating shaker and six standard sieves in the range 10-200μm as well as by optical microscopy. ([11,15,16])

Surface topography (SEM)

The surface morphology of the microshperes was examined by scanning microscopy electron microscopy in a Cambridge Instruments Stereo scan 360. ([17,18])

Percentage drug entrapment efficiency

Microsheres equivalent to 50mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the micropsheres and extracting with aliquots of 0.1N HCl. The extract was transferred to a 50mL volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotomectrically (UV1700, shimadzu, Japan) at 278nm ([11,12,13,17,19]). The amount of drug entrapped in microspheres was calculated by the following formula

$$\text{Percentage drug entrapment Efficiency} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

Invitro evaluation of floating ability

An *invitro* floating study was carried out using simulated gastric fluid USP containing 1% Tween 80 as a dispensing medium. Microspheres were spread over the surface of 500mL of dispensing medium at 37±0.5°C. A paddle rotating at 100rpm agitated the medium. Each fraction of microsheres floating on the surface and those settled down was collected at a predetermined time point. The collected samples were weighed after drying.

$$\text{Percentage floating Microspheres} = \frac{\text{Weight of floating microshperes}}{\text{Initial weight of floating microsheres}} \times 100$$

Invitro drug release study

Inviro drug release studies were carried out for all products and for the pure drug in USP typeII (Disso 2000, Labindia Mumbai, India) dissolution test apparatus. 100mg of pure drug was used for dissolution studies and microspheres equivalent to 100mg of the pure drug were used. Two mL of the aliquot was withdrawn at predetermined intervals and filtered. The required dilutions were made with 0.1N HCl and the solution was analyzed for the drug content spectrophotometrically (UV1700, shimadzu, Japan) at 278nm against suitable blank. Equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. Three trails were carried out for all the formulations. The simultaneous of dissolution profile of the prepared formulations were compared with that of predicted theoretical value to arrive at the optimum profile. ([20-22])

Kinetic Analysis of Drug Release

The dissolution profiles of all the solid dispersions were subjected to kinetic analysis to establish the drug – release mechanism. The release data were fitted to zero order, first order, higuchi model and similarity and dissimilarity factor equations to ascertain the kinetic modeling of drug release.

Stability studies

Stability studies were carried out on most satisfactory formulation as per ICH guidelines QIA at 30±2°C and 65±5% RH, 40±2°C and 75 ±5% RH. The most satisfactory formulation stored in a sealed in aluminum foil. These were stored at room temperature for 24 months. After 3 months % drug compartment efficiency of most satisfactory formulation was determined *invitro* release study was also carried out of best formulation.

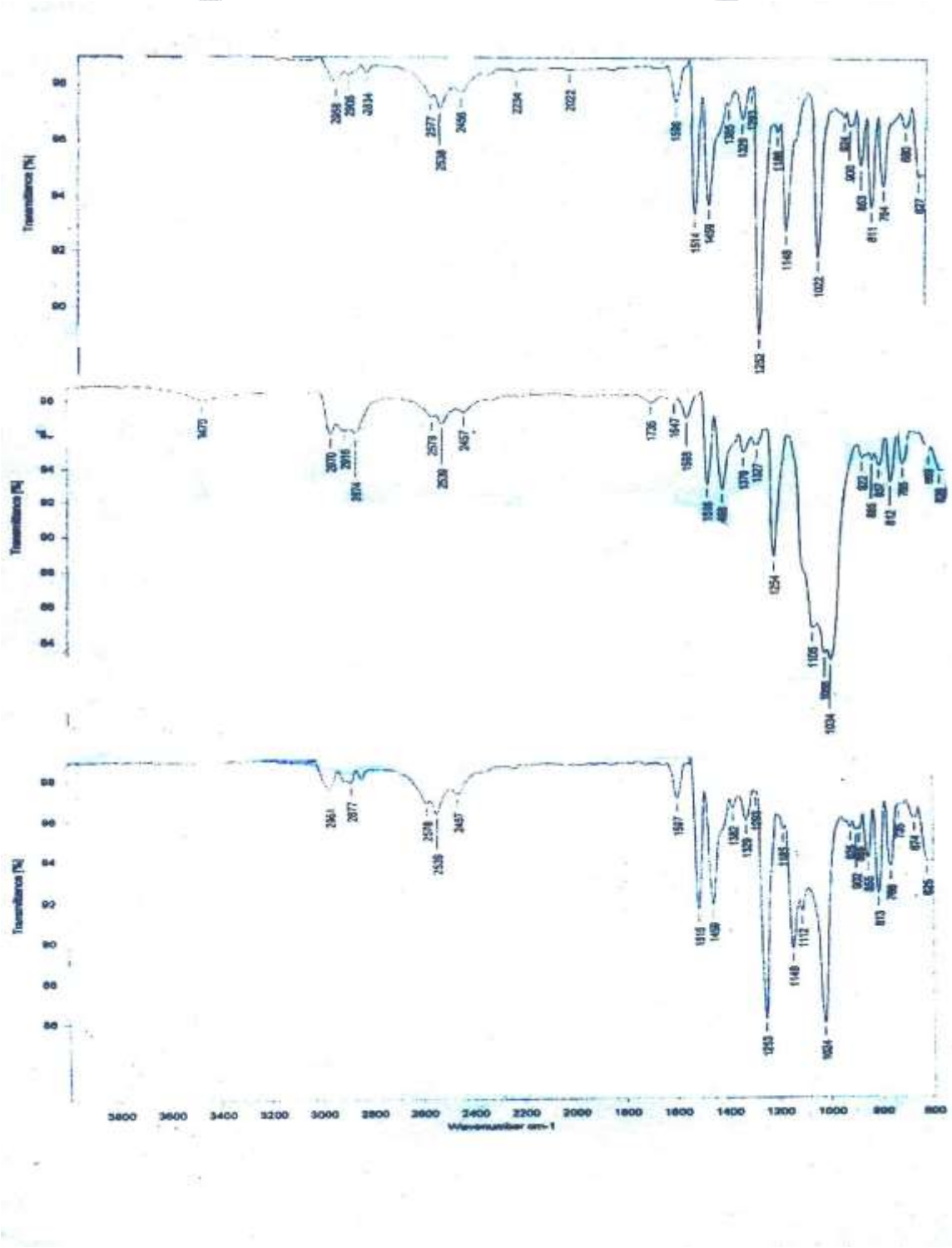
Results and Discussion

Several formulation trials were undertaken for various proportions of drug and polymer by variation of liquid paraffin and methanol volumes for qualitative and quantitative determination of microsphere characteristics. It was found that ethyl cellulose and hydroxyl methyl cellulose show desirable high drug content, flotation and accurate release characteristics and some were suitable for development of a controlled release system.

Drug- Polymer Interaction

The present study the possible interaction between the drug and excipients mixture with HPMC and EC are shown in the fig1, 2&3. Pure Verapamil HCl showed 2834, 1459, 1514, 1329, 2958 cm^{-1} wave number as major peaks. The results revealed no considerable changes in the IR peaks of Verapamil hydrochloride when mixed with excipients compared to pure Verapamil hydrochloride.

Fig:1 FTIR: a. Pure sample of Verapamil HCl. b. Verapamil HCl and Ethyl cellulose c. Verapamil HCl, Ethyl Cellulose and Methyl Cellulose.



Particle size

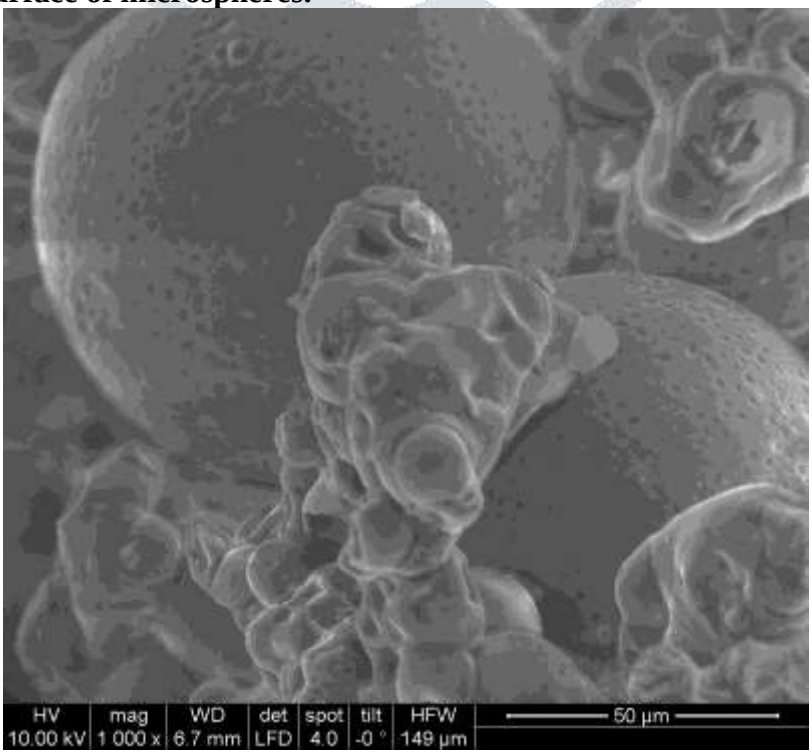
Microspheres were prepared using gradually increasing ethyl cellulose (EC) concentration in combination with increase concentration of hydroxyl propyl methylcellulose to assess the effect of polymer concentration on the size of microspheres. The mean particle of the microspheres significantly increased with increasing with different ratio of ethyl cellulose and hydroxyl methyl cellulose concentration ($P< 0.05$) and was in the range of 45.01±2.3µm to 113.02± 5.2 µm Table II. The viscosity of the medium increases at a higher polymer concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities ([23,24]). This results in the formation of larger particles.

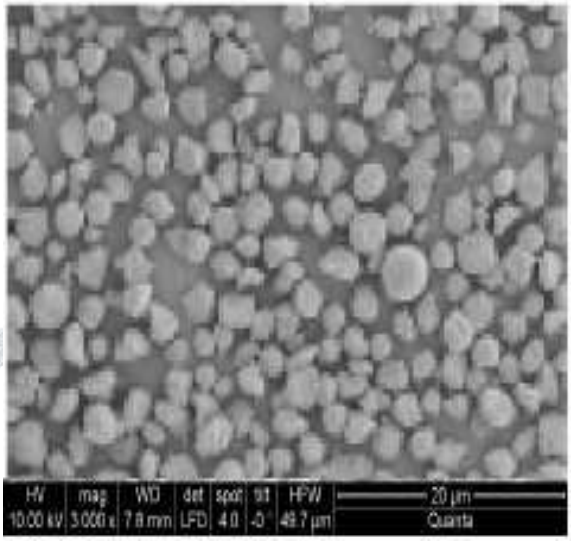
Surface Topography

Surface of all the microspheres are rough and there are several cracks on the surface. It is observed that the depth of cracks on the surface of microspheres become greater while the polymer amount of formulation was increased. This can be attributed to the increasing volume of dispensing medium i.e. liquid paraffin resulting from the extended diffusion of organic solvent to the dispensing medium. ([25]) It is thought that the surface characteristics of microspheres can be depending both on the polymer characteristics and on the amount of polymer in the formulation.

Fig: 2 Scanning electron microscope of verapamil HCl floating microsphere by the non-aqueous solvent evaporation method.

(a) General view (b) surface of microspheres.





Percentage drug entrapment efficiency

The total amount of polymer alone ethyl cellulose was increased, so percentage drug entrapment was also increased. Percentage drug entrapment of formulation F3, F5, F6 batches were found to be 91.02±1.169, 89.26± 2.806 and 90.14±2.046 respectively highly amount of drug entrapment efficiency.

Table 2: Physicochemical properties of various formulations

Batch	Percentage Yield* ± S.D.	Percentage Drug Entrapment Efficiency* ± S.D.	Percentage Buoyancy* ± S.D.	Particle size (µm)
F1	79.51 ± 3.715	83.71 ± 1.809	95.22 ± 1.550	97.13±1.123
F2	88.85 ± 0.998	88.23 ± 1.782	96.72 ± 1.559	108.13±1.012
F3	93.48 ± 0.946	91.02 ± 1.169	97.50 ± 1.533	113.02±0.946
F4	85.83 ± 6.091	86.72 ± 1.049	93.15 ± 1.859	80.13±1.133
F5	87.08 ± 2.247	89.26 ± 2.806	95.06 ± 3.216	88.20±1.001
F6	92.11 ± 1.900	90.14 ± 2.046	96.37 ± 2.712	95.73±1.020
F7	81.20 ± 1.482	82.25 ± 0.367	88.36 ± 4.858	45.01±1.029
F8	84.84 ± 1.321	85.40 ± 1.422	94.39 ± 1.427	51.88±1.033
F9	91.66 ± 1.685	88.18 ± 0.609	96.10 ± 2.444	57.95±1.044

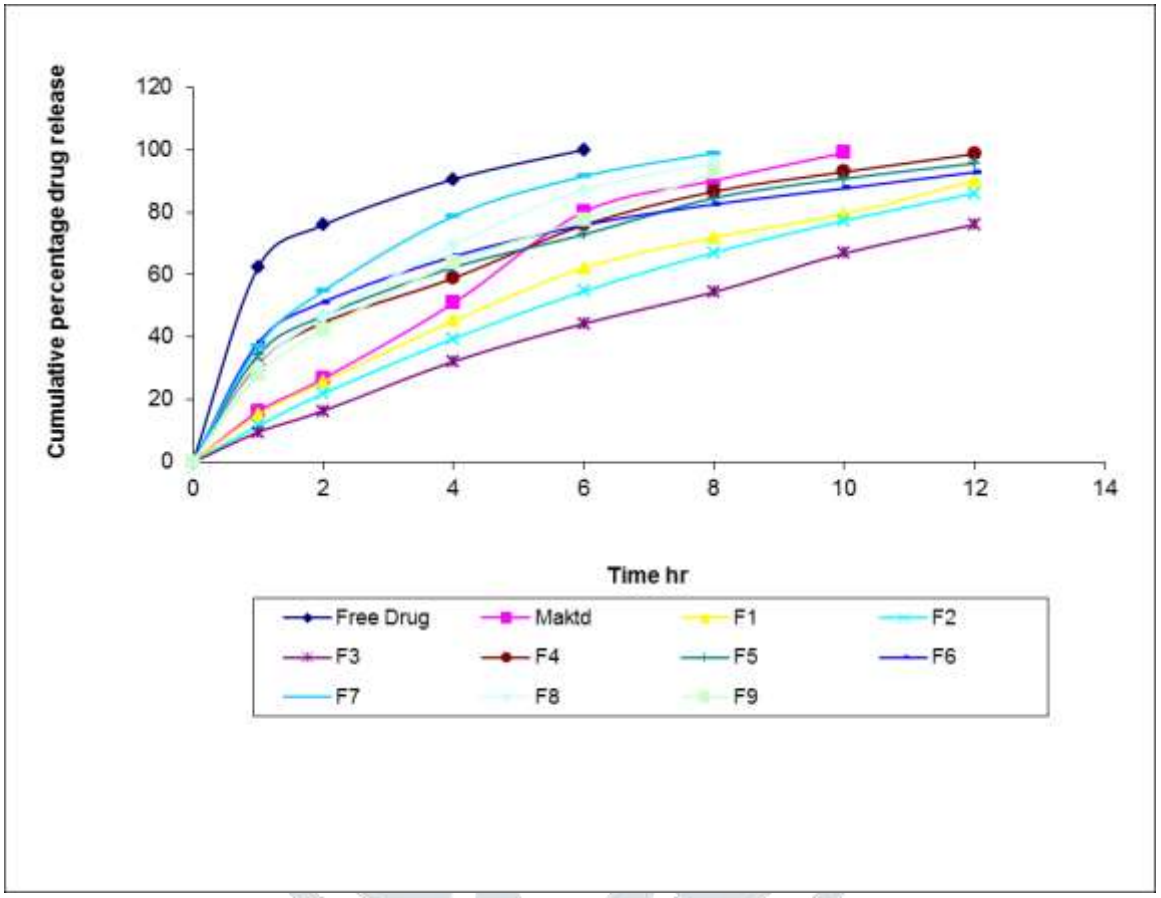
Percentage Buoyancy

Percentage buoyancy of formulation F7 batch showed lowest buoyancy (88.36±4.858) this may be due to the amount of water permeable hydroxyl propyl cellulose is decrease and F3 batch showed highest buoyancy (97.50±1.533) this may due to the amount of water impermeable ethyl cellulose is increased.

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

The formulation of F1 to F9 was showed wide range of drug release both over and below the theoretical drug release. Formulation F3 showed minimum drug release (76.08±4.47) this may be due to hydrophobic nature and increase in the concentration of ethyl cellulose compared to F1 and F2. So, it retarded the drug release at >12hour. It was also observed that increasing in ethyl cellulose concentration decreased the in vitro release of verapamil hydrochloride ($P<0.05$).Increased density of the polymer matrix at higher concentrations results in a concentrations result in an increased diffusional path length, which resulted in decrease drug release.([27]) It was observed that F4 showed maximum drug release at 12hour.this may be due to polymer nature and decreased amount of ethyl cellulose and hydroxyl propyl cellulose compared to F5 and F6. This may be due to (1:2) amount of hydroxyl methyl cellulose and ethyl cellulose. When the concentration of hydroxy propyl methylcellulose was used at higher level in the formulation F7, F8 and F9 the drug was release faster rate at 8hour.([14]) This was due to increase in hydrophilicity of the microspheres matrix fig3. Further more, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium giving rise to faster drug release ([27]).

Fig: 3 *In vitro* dissolution profile drug release studies of verapamil hydrochloride microspheres in phosphate buffer



Statistical analysis

The data obtained for *in vitro* release of microspheres was fitted into different mathematical model like zero order, first order and higuchi release model. The interpretation of data was based on the value of the resulting regression coefficients .[28,29,30] The *in vitro* drug release showed highest regression coefficient values for higuchi's model, indicating diffusion to be the predominant mechanism of drug release. Similarity (5%) and dissimilarity factors (78%) are found to be satisfactory.

Table: 3 Release kinetics of verapamil Hydrochloride from different formulations

Kinetic Profile of various formulations	For Higuchi equation		For 1st order equation		For Zero order equation	
	N	R^2	n	R^2	n	R^2
F1	24.685	0.953	-0.075	0.976	8.42	0.925
F2	22.981	0.925	-0.065	0.982	7.90	0.962
F3	19.418	0.898	-0.048	0.983	6.73	0.984
F4	29.739	0.989	-0.129	0.928	9.91	0.731
F5	29.320	0.965	-0.107	0.983	9.71	0.643
F6	29.308	0.857	-0.097	0.962	9.62	0.477
F7	36.834	0.979	-0.212	0.943	14.87	0.742
F8	34.268	0.990	-0.158	0.977	13.97	0.837
F9	31.834	0.989	-0.127	0.965	13.03	0.869

Stability studies

The best formulation F4 stored in sealed in aluminum foil. These were stored at room temperature for 3 months. Then the formulation was exposed to various temperatures for 3 months. Then the formulation was exposed to various temperature and humidity at 30±2°C and 65±5% RH, 40±2°C and 65±5% RH asses their stability as per ICH guidelines QIA. There was no significant variation in the % drug entrapment efficiency and *in vitro* release study of best formulation F4 after 3 month of stability, when subjected onway ANOVA analysis($p<0.05$).

Conclusion

In *in vitro* data obtained for floating microspheres of verapamil hydrochloride should excellent floating ability, good buoyancy and prolonged drug release. Microspheres of different size and drug content could be obtained by varying the formulation variables. The drug release was found to be diffusion method. Therefore, it may be concluded that drug loaded floating microspheres are a suitable delivery system for verapamil hydrochloride may be used management of hypertension.

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