



Development and evaluation of stable microsphere of Diltiazem hydrochloride, an antihypertensive drug

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Abstract

Drug delivery system that can precisely control the release rates or target drugs to specific the body sites had an enormous impact on the health care systems carrier Technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microsphere nanoparticle liposome etc that modulates the and absorption characteristic of the drug microspheres constitute an important part of the particulate drug delivery systems by virtue of their small size and efficient carrier characteristic. The present paper deals with the development and evaluation of stable microsphere of Diltiazem HCl. various parameters have been reported in present communications.

Key-Words: Microsphere, Diltiazem hydrochloride, Antihypertensive drug

Introduction

Diltiazem hydrochloride are easily absorbed from gastrointestinal tract (GIT) and have a short half-life are eliminated quickly from blood circulation. This drug undergoes substantaintly hepatic first pass effect it shows to oral bioavailability 40%. So they require frequently dosing to avoid these drawback, the oral sustained control release formulation have been developed in an attempt to release the drug surely in to the GIT and maintained an effective drug concentration in the serum for longer period of time Diltiazem hydrochloride an effective drug in treatment of hypertension and angina pectoris is a benzothiazepine derivative calcium and turbonist are drug which cases coronary and peripheral vasodilation by reducing calcium influx through the slow channel of vascular smooth muscle and cardiac cell membranes. Administration of conventional tablet of Diltiazem Hydrochloride has been reported to exhibit fluctuations in plasma drug level resulting either in side effect of reduction in drug concentration at receptor side also the maintenance of constant plasma concentration of cardiac vascular drug is important in ensuring the designed therapeutic response, again since the half life of Diltiazem HCl is 3-4 hrs multiple dose of drug need to maintained constant plasma concentration for good therapeutic response and improve patients compliance¹⁻².

Hence the objective of study was made to develop control release microsphere system of Diltiazem HCl using polymer like Ethyl Cellulose which will controlled the released of drug increase the bioavailability of drug and dose decreasing the dosing frequency of drug.

In the patients study microsphere formulation was preferred over conventional tablet or capsule formulation has it as several advantage like it control the release pattern thus decreasing the dosing frequency.

- To carry out the preformulations studies.
- To formulate the microsphere of Diltiazem hydrochloride.
- To carry out physicochemical characterization of the prepared microsphere.
- To carry out in vitro drug release studies.
- To carry out stability studies of the optimized formulation.

Material and Methods³⁻⁹

Preparation of standard curve

The ultraviolet spectrophotometer method was selected in the present study for estimation of Diltiazem hydrochloride. The drug solution (10µg/ml in methanol) was scanned in between the wavelength 400-200 nm in the UV visible spectrophotometer (Shimadzu1800).The maxima were found to be 211nm. and selected for quantitative analysis.

Preparation of phosphate Buffer, pH 7.4

2.38 gram of Disodium hydrogen phosphate, 0.19 gram of potassium dihydrogen phosphate and 8 gram of

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sodium chloride was dissolve in little amount of distilled water and volume was made up to 1000 ml with distilled water. Stock solution of Diltiazem Hydrochloride of concentration 100µg/ml was Prepared. Serial dilutions of ranging from 2-20 µg/ml were then prepared using the stock solution. The absorbance was measured at 211nm against a blank solution using UV-Visible spectrophotometer (Jasco V-530). The experiment was repeated in triplicate and the average of three readings was taken to plot the standard curve.

Method of preparation of diltiazem microsphere

The microsphere will be prepared using emulsion solvent evaporation technique. The polymeric solution will be prepared by dissolving Ethyl Cellulose 5% in water. The drug will be dissolving in the polymeric solution forming the internal phase. The prepared drug polymer solution will be added drop wise by a syringe with a needle gauge 21 to liquid paraffin (internal phase) containing 1.5% span 80 and will be emulsified by stirring at 1000 rpm. The stirring will be continue at room temperature until the polymer solvent will be evaporated to produce microsphere were decanted and washed three time with 20 nm and dryovernite in a hood.

Drug identification

Colour

A small quantity of Diltiazem Hydrochloride powder was taken in butter paper and viewed in well-illuminated

Table 1: Composition of microsphere for formulation with varying stirring speed and varying ethyl cellulose

Ingredient	F-1	F-2	F-3	F-4	F-5
Liquid paraffin	25 ml	25 ml	25 ml	25 ml	25 ml
Span 80	0.37 ml	0.37 ml	0.37 ml	0.37 ml	0.37 ml
Acetone	25 ml	25 ml	25 ml	25 ml	25 ml
Ethyl Cellulose	1.8gm	1gm	1.3gm	1gm	2.0 gm
Drug	20 mg	20 mg	20 mg	20 mg	20 mg
Stirring Speed	700 rpm	1000 rpm	1000 rpm	1000 rpm	500 rpm

Taste and odor

Very less quantity of Diltiazem Hydrochloride was used to get taste with the help of tongue as well as smelled to get the odor.

Melting point

A small quantity of Diltiazem HCl will be placed into a Capillary tube. That tube will be placed in the melting

point determining apparatus. The temperature of the apparatus will be gradually increase automatically and read the temperature at which powder stated to melt and the temperature when all the powder gets melted.

Solubility Study

A semi quantitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute or vice versa. After each addition, the system was vigorously shaken and examined visually for any undissolved solute particles. The solubility is expressed in terms of ratio of solute and solvent.

Physical compatibility study

In the microspheres the drug is in intimate contact with one or more excipients, the latter could affect the stability of the drug. Knowledge of drug excipients interaction is therefore very useful to the formulator in selecting appropriate excipient. This information may already be inexistence for known drugs. For new drugs of new excipient the excipients the formulation scientist must generate the needed information. Diltiazem hydrochloride was well mixed with excipient according to the formula selected for microspheres and kept small portion of this mixed powder in cleaned and dried vial in hot air oven at 40° C + 2% C and at room temperature physical observation has been carried out visually for 7 days.

Particle size analysis

The microspheres were first suspended in 100ml of distilled water (containing 1% span 80) and subjected to sonication for 30 seconds and vortex mixing for 10 second before analysis. For the formulation f-1,f-2,f-3,f-4,and f-5,partical size was determined by optical microscopy To optimize the stirring speed particle size of microspheres was affected by stirring rate the formulation were prepared at five different stirring rates i.e. 700rpm,500rpm, 1000rpm.outOf which 1000rpm was selected as optimum speed.

Production yield

The production yield of microspheres of various batches was calculated using the weight of final product after drying with respect to initial total weight of the drug and polymer used for preparation of microspheres and percent production yields are calculated as per the formula mentioned below.

$$\text{Percent production Yield} = \frac{\text{Observed mass of microsphere}}{\text{Calculated mass of microsphere}} \times 100$$

After drying of microspheres in the round bottom flask, the microspheres were collected and weighted accurately.

Encapsulation efficiency

Encapsulation efficiency was determined by direct method wherein the 20mg microspheres were crushed

and immersed in the water for 24hour with constant shaking which resulted in the extraction of drug from microspheres in the water, which was then cumulative estimated though UV spectroscopy by taking its absorbance at 211nm and the value thus obtained was used to determine the encapsulation efficiency of the microspheres using the formula mentioned and the encapsulation efficiency values are reported.

Percentage encapsulation efficiency
 = $\frac{\text{Observed Drug Content}}{\text{Calculated Drug content}} \times 100$

In Vitro release study

For in vitro release, weighed microspheres containing equivalent amount of 20 mg diltiazem hydrochloride was suspended in 2 ml of phosphate buffer ph 7.4 was placed in diffusion cell. The tube containing dispersion of microspheres was then introduced into a 200ml beaker containing 100 ml release media (phosphate buffer ph 7.4), which was stirred at 400±20rpm using magnetic stirrer. Drug release was assessed by intermittently sampling the receptor media (2ml) at predetermined time interval and each time 2 ml of fresh phosphate buffer pH 7.4 was replaced. The amount of diltiazem hydrochloride released in the buffer solution was quantified by a uv spectrophotometer at 211nm. The in vitro release of diltiazem hydrochloride from microsphere of different formulation in shown in f-1, f-2, f-3, f-4 and f-5.

Drug release kinetic

The mechanism of drug release will be determined by fitting the release data to the various kinetic equations such as zero order and first order Higuchi and Korsmeyer-Peppas finding the R² values of release profile corresponding to each model. Korsmeyer-Peppas model will be widely used, when the release mechanism is not well known or when more than one type of release phenomenon can be involved the n value can be used characterized different release mechanism. The release of active agent from the matrix involved initial swelling will be followed by diffusion of the drug.

Stability Study

Stability studies of optimized formulation will be carried out as per condition only at different time period. Microspheres will be kept at different time period of room temperature for few days. Stability samples were analysed for the physical compatibility. All regulatory bodies accept only real time stability data of any formulation for the purpose of assessing shelf life. Accelerated stability studies may only serve as tool for formulation screening and issues related during shipping or storage. On storage as per ICH guidelines as the accelerated condition (40±0 °C).

Results and Conclusion

The aim of this study was to develop microspheres of diltiazem hydrochloride by solvent evaporation method, using ethyl cellulose as a polymer, ethyl cellulose microspheres are used to provide controlled release of diltiazem hydrochloride and to enhance the uptake of hydrophilic substance across epithelial layer. Liquid paraffin light was used as oil phase and span 80 was used as emulsifier or stabilizing agent in the formulation of primary emulsion. During the preparation of microspheres ethyl cellulose in acetone containing Diltiazem Hydrochloride.

Fig. 1. Standard curve of drug

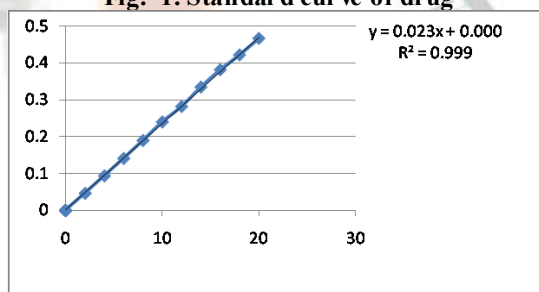


Table 2: Drug Identification

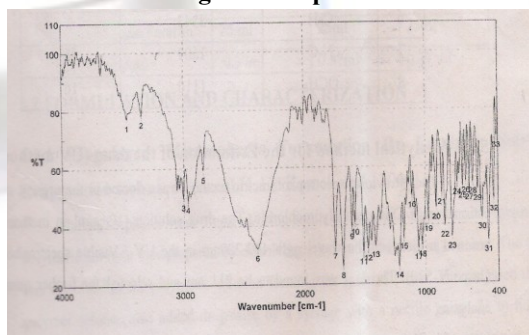
Test	Specification	Observation
Colour	White	White almost
Taste	Bitter	Bitter
Odour	Almost odorless	Odorless
State	Solid	Crystalline Solid

Table 3: Melting Point

Test	Specification	Observation
Diltiazem HCL	214-218° C	215° C

Solubility Study

Fig. 2. IR Spectra



Quantity of Diltiazem hydrochloride	Solvent(ml)	Solubility
100mg	5 ml of water	Freely soluble
100mg	5 ml of acetone	Freely soluble
100mg	5 ml of methanol	Freely soluble
100mg	5 ml of chloroform	Sparingly soluble
100mg	5 ml of Formic acid	Freely soluble
100mg	5 ml of Alcohol	Freely soluble
100mg	5 ml of pH 7.4 phosphate buffer	Freely soluble

Table 4: Solubility profile

Test	Observation	Inference
Physical compatibility	No change of color	These materials are compatible for formulation

Table 5: Physical Compatibility Study

Table 6: Production yield of Microsphere of Diltiazem Hydrochloride

S. No	Formulation code	% Production Yield
1.	F-1	62
2.	F-2	73
3.	F-3	68.45
4.	F-4	75
5.	F-5	73

Table 7: Effect of different process variable on encapsulation efficiency

S. No	Formulation Code	Encapsulation efficiency %
1.	F-1	70
2.	F-2	68
3.	F-3	66
4.	F-4	64
5.	F-5	72

Scanning electron microscopy study revealed that diltiazem hydrochloride microsphere was discrete and spherical in shape with a porous outer surface the roughness on the surface of the microsphere was attributed to the removal of crystal result on the surface during washing slightly aggregation was observed it may be due to higher concentration of polymer which was not washed completed during washing with hexane.



Fig. 3. Formulated Microsphere

Differential Scanning Calorimetry

Differential Scanning Calorimetry was performed by using DSC- Jade. The sample were placed in aluminium pans and were crimped, followed by heating under nitrogen flow (30ml/min) at the scanning rate 5°C/min from 25°C- 250°C. Aluminium pan containing same quantity of indium was used as reference. The heat flow as a function of temperature was measured for both the drug- excipient mixture.

Sample	Ingredient
A	Diltiazem Hydrochloride
B	Ethyl cellulose
C	DiltiazemHCl + Ethyl Cellulose

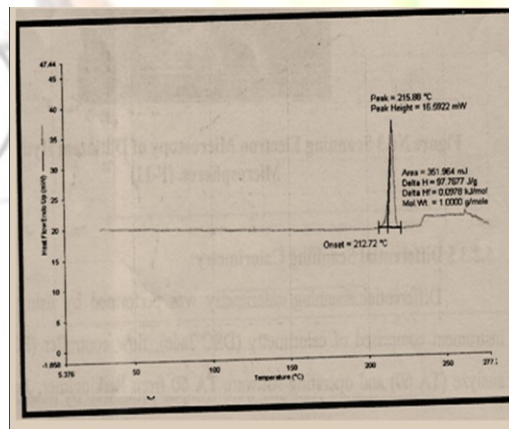


Fig. 4. DSC of Pure Diltiazem HCL

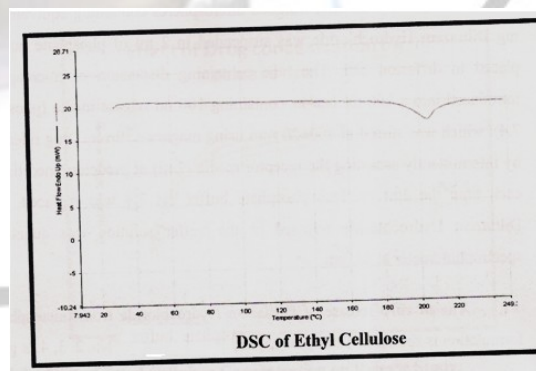


Fig. 5. DSC of Pure ethyl cellulose

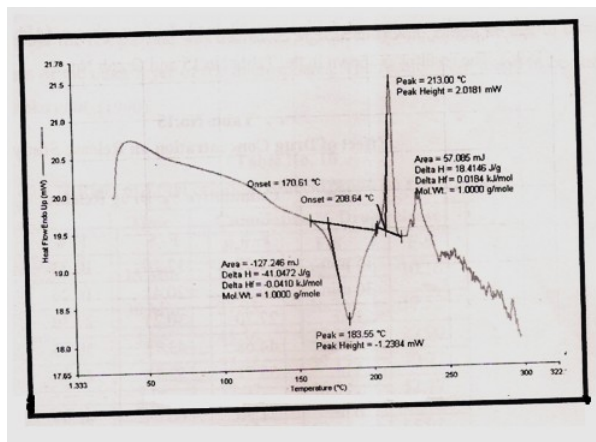


Fig. 6. DSC of Microsphere

Table 8: Release Study Pattern

Time	F-1	F-2	F-3	F-4	F-5
15 min	16.91	10.23	10.16	8.24	9.34
30 min	27.93	16.23	17.52	17.28	19.47
1 hrs	43.56	27.30	29.90	26.20	27.42
2 hrs	52.48	39.15	40.27	38.20	40.27
3 hrs	57.07	46.50	52.77	53.70	52.28
4 hrs	65.07	53.21	57.54	57.80	56.35
6 hrs	71.19	62.04	65.08	68.80	67.87
8 hrs	75.19	65.90	68.23	71.70	70.35
12 hrs	80.51	70.67	73.23	74.72	76.34
24 hrs	88.48	81.42	80.37	79.81	78.87

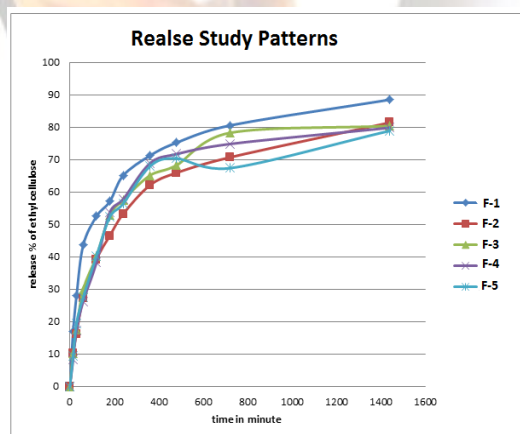


Table 9: Release Exponent (n) Values

Release Component (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion (Case I transport)	$t^{-0.5}$
$0.5 < n < 1.0$	Anomalous transport (Non Fickian diffusion)	t^{-n-1}
1.0	Case-II transport	Zero order release
> 1.0	Super Case- II transport	T^{-n-1}

Table 10: Calculated values of n and R²

Formulation Code	"n" Slope Value	R ² Regression Coefficient
F-1	0.478	0.93
F-2	0.432	0.94
F-3	0.461	0.93
F-4	0.412	0.93
F-5	0.503	0.93

Table 11: Kinetics of in vitro Diltiazem Hydrochloride Release from Microsphere

FC	Zero order		First order		Higuchi		KorsmeyerPeppas	
	K ⁰	R ²	K ¹	R ²	K ^h	R ²	n	R ²
F-1	2.99	0.68	0.029	0.83	16.61	0.89	0.48	0.96
F-2	2.97	0.66	0.027	0.85	16.60	0.91	0.47	0.95
F-3	2.96	0.68	0.028	0.82	16.59	0.89	0.46	0.94
F-4	2.73	0.68	0.029	0.86	16.77	0.89	0.51	0.93
F-5	2.65	0.73	0.027	0.84	16.00	0.92	0.38	0.97

Fig. 7. Cumulative % Drug release Vs Time (Zero order Kinetics)

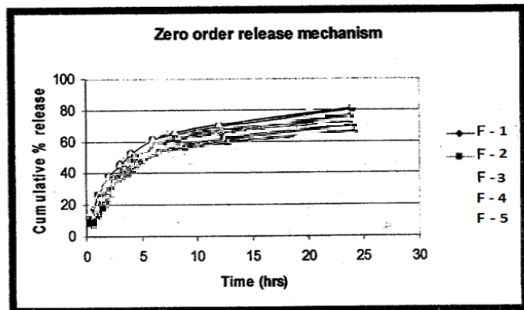


Fig. 8. Log Cumulative % Drug unreleased Vs Time (First order Kinetics)

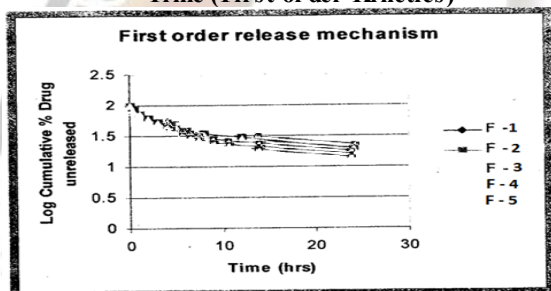


Fig. 9. Cumulative % Drug released Vs Square root of time (Higuchi Diffusion mechanism)

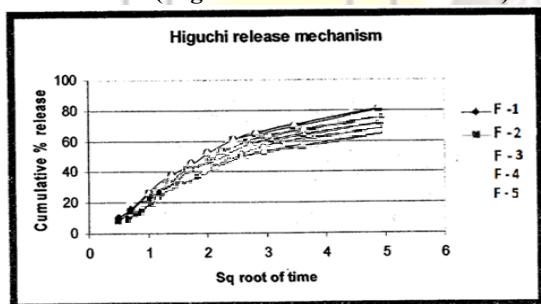
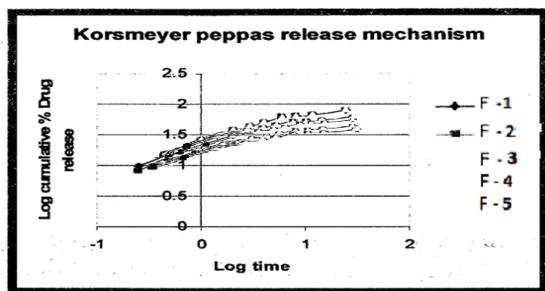


Fig. 10. Log Cumulative % Drug released Vs Log time (KorsmeyerPeppas Exponential mechanism)



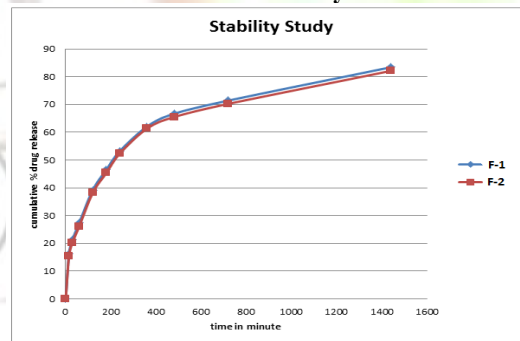
In the drug release kinetic study the different graph plot shows that R^2 value is regression coefficient values is approx near to one so it is following a zero order release and by the n value indicates that the

formulation is following diffusion and fickian case 1st transport.

Table 12: Cumulative % of after 15 days and after 30 days Stability study

Time	Cumulative % of a drug release	
	15 Days	30 Days
15 min	16.23	15.54
30 min	21.26	20.31
1 hrs	27.3	26.11
2 hrs	39.15	38.21
3 hrs	46.5	45.37
4 hrs	53.21	52.4
6 hrs	62.04	61.31
8 hrs	66.67	65.48
12 hrs	71.42	70.23
24 hrs	83.42	82.11

Fig. 11. Stability study release mechanism after day 15 and after day 30



The objective of this study was to develop and evaluate stable microsphere of Diltiazem Hydrochloride, an antihypertensive drug using ethyl cellulose as polymer which deliver the drug and at a control rate for prolonged period of time. Following conclusion have been drawn from present study: The analytical method used in present study was found to be suitable for the estimation of Diltiazem HCl in different media, which was indicated by the high regression values obtained in the standard plot. It was found that Diltiazem HCl is practically fairly soluble in chloroform, acetone, methanol, water, formic acid and in phosphate buffer solution pH 7.4 and sparingly in ethanol. The DSC studies revealed that there was no interaction between Diltiazem HCl and ethylcellulose used in the formulation of microsphere. The size of microsphere mainly effected by stirring speed, as stirring speed increased the size of microsphere was decreased. The encapsulation efficiency was increased by increase in polymer conc. decreased with increase in drug polymer ratio. SEM studies of the formulation were carried out

for the confirmation of shape and surface morphology of microsphere. SEM revealed that microsphere was discrete and spherical in shape with porous outer surface. The optimum formulation was found to be stable at storage condition for a period of 15 days and 30 days with respect to physical characteristics, drug content and release pattern to conclude, the present study demonstrated the successful preparation of diltiazem HCl microsphere using ethyl cellulose as polymer.

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