FORMULATION AND EVALUATION OF CONTROLLED RELEASE MICROSPHERES OF DILTIAZEM HYDROCHLORIDE BY SOLVENT EVAPORATION TECHNIQUE

Malay paul*, Keerthy H.S , Dr. Shivanand K Mutta, F R Sheeba, Dr. Ashvini H M, Mukesh Sharma

Mallige College of Pharmacy
#71 Silvepura, Chikkabanavara post,
Bangalore-560090

Corresponding Author: Malay paul
Department of Pharmaceutics,
Mallige college of pharmacy,
Rajiv Gandhi university of Health Sciences,
Bangalore, Karnataka – 560090, India.
E-mail address: *paulmalay550@gmail.com*
Mob: 9609990176

ABSTRACT

The aim of the present work was to prepare controlled released microspheres of Diltiazem Hydrochloride to sustain the release of the drug, Diltiazem Hydrochloride. Diltizam Hydrochloride loaded controlled microspheres were prepared by solvent evaporation technique with combination of polymers (Ethyl cellulose, HPMC K4M and Gelatin). Nine formulations were prepared with different ratios of combination of EC, HPMC K4M & Gelatin. The prepared Microspheres were characterized for physical characteristics such as particle size, particle shape and surface morphology by scanning electron microscopy, percentage yield, drug entrapment efficiency, and *In-vitro* drug release studies. The obtained microspheres were found to be spherical, free flowing and have a particle size ranging between 127.8-158µm suitable for oral delivery. Percentage drug entrapment efficiency was found to be 75.37 – 89.24%. Scanning electron microscopy and particle size analysis revealed differences between the formulation as to their appearance and size distribution. Diltiazem Hydrochloride loaded Microspheres that effectively sustain the drug release more than 12 h. Finally the study confirmed that various Diltiazem Hydrochloride loaded Ethyl cellulose, HPMC K4M & Gelatin microspheres formulations could be developed that effectively sustain the drug release for a desired period by varing the ratio of EC , HPMC K4M & Gelatin polymers.

KEYWORDS: Diltiazem hydrochloride, microsphere, Ethyl cellulose, HPMC, Gelatin, *in-vitro* evaluation

INTRODUCTION:

Oral route is by far the most preferable route of drug administration. However, the therapeutic potential of many drugs are limited due to their short biological half-life and their restricted absorption. Such a pharmacokinetic limitation demands for frequent dosing of medication to achieve the best therapeutic effect. A rational approach to enhance bioavailability and to improve pharmacokinetic and pharmacodynamic profile is done by making the drug release in a controlled and site specific manner. Microspheres are small spherical particles, with diameters 1 µm to 1000 µm. They are spherical free flowing particles consisting of proteins or synthetic polymers, which are biodegradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall

and micromatrices in which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microsphere play an important role to improve bioavailability of conventional drugs and minimizing side effects.

MATERIALS AND METHODS:

Materials: Diltiazem hydrochloride was a purchased from Balaji chemicals, Gujrat. Ethyl cellulose, HPMC K4M & Gelatin are from Research-Lab Fine chem and all the other ingredients used in the study were analytical grade.

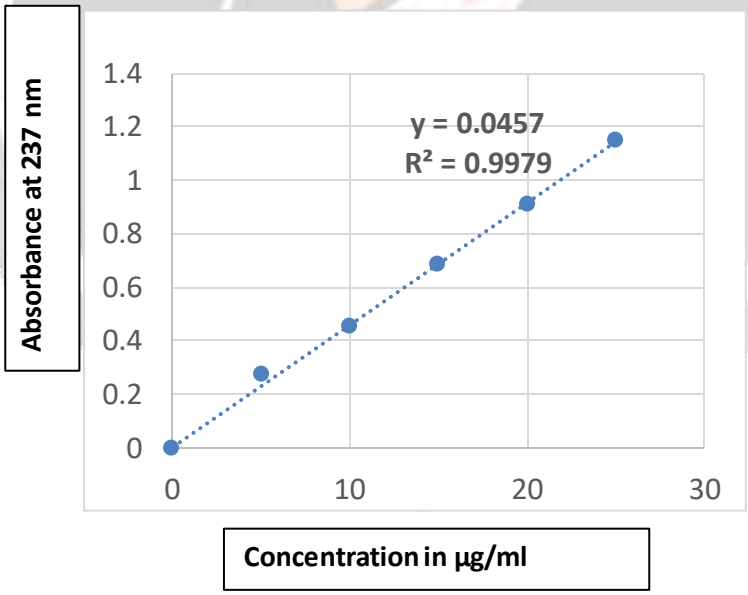
CALIBRATION CURVE FOR DILTIAZEM HYDROCHLORIDE:

An accurately weighed quantity of diltiazem hydrochloride (100 mg) was dissolved in small quantity of phosphate buffer pH 1.2. The volume was made up to 100 ml with phosphate buffer pH 1.2. Working standard solution having concentration 2 to 25 ug/ml were prepared by appropriately diluting the stock solution. The absorbance of the working standard solution was recorded and a graph of concentration of the solution was plotted against absorbance.

Table no. 1 : Standard Calibration curve data of Diltiazem HCl

CONCENTRATION	ABSORBANCE			
	TRIAL-1	TRIAL-2	TRIAL-3	AVERAGE
5	0.278	0.279	0.280	0.279
10	0.454	0.461	0.458	0.457
15	0.684	0.685	0.689	0.685
20	0.905	0.920	0.918	0.914
25	1.144	1.150	1.152	1.148

Fig no. 1 : Standard Calibration curve of Diltiazem HCl



Formulation of diltiazem hydrochloride controlled microspheres:-

The microspheres containing the antihypertensive drug Diltiazem Hydrochloride, as the core material were prepared by a non-aqueous solvent evaporation method. Here, the combinations of Polymers at various ratios were added to 50ml of organic solvent (acetone) kept under magnetic stirrer for about 15mins, to form a uniform polymer solution. To this polymer solution, weighed amount of Diltiazem Hydrochloride was added and continued stirring for 15mins to form a uniform dispersion of drug in polymer solution. This solution was slowly poured into the dispersion medium containing 100ml of light liquid paraffin and 0.5ml of span80 (oil soluble surfactant). The whole system was stirred at constant speed with a mechanical stirrer equipped with a three-blade propeller at room temperature.

Stirring was continued over 2-3 h to ensure complete evaporation of solvent. As the solvent evaporates, it leaves an empty space or hollowness within the micro particles and simultaneously, this hollowness is filled with air and makes microspheres less dense and resulting in the formation of microspheres. After completion of stirring, the formed microspheres are separated by filtration through Whatman filter paper, and washed thrice with n-hexane and air dried for 24 h. the dried microspheres were stored in airtight container.

Table no 2: Formulation of controlled release diltiazem hydrochloride microspheres

SL NO	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Diltiazem HCl(mg) Equivalent to Diltiazem 100mg	108.8	108.8	108.8	108.8	108.8	108.8	108.8	108.8	108.8
2	Ethyl cellulose (mg)	100	200	300						
3	HPMC K4M (mg)				100	200	300			
4	Gelatin (mg)							100	200	300
5	Acetone(ml)	50	50	50	50	50	50	50	50	50
6	Span 80 (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
7	Liquid paraffin(ml)	100	100	100	100	100	100	100	100	100

EVALUATION OF FORMED MICROSPHERES

Percentage yield: Microspheres dried at room temperature were weighed and the Percentage yield of microspheres was calculated using formula.

$$\%\ yield = \frac{\text{practically obtained microspheres}}{\text{theoritical weight of drug and polymer}}$$

Flow property: Angle of repose method was employed to assess the flow ability. Microspheres were allowed to fall freely through the funnel, which was fixed at 1cm above the horizontal flat surface until the apex of pile just touches the tip of the funnel. The angle of repose (ø) was determined by formula.

$$\theta = \tan - 1\ (h/r)$$

Where, h= height of pile formed by microspheres,

r= radius of circular base formed by the microspheres on the ground.

Particle Size determination of microspheres: Microspheres were separated into different size fractions by sieving for 5 minutes using standard sieves having nominal mesh apertures of 1.0 mm, 0.71 mm and 0. 5 mm (sieve no.16, 22 and 30respectively). The particle size distributions of the beads were determined and mean particle sizes of beads were calculated using following formula.

$$D_{mean} = \sum nd / \sum n$$

Where; n-number of microspheres observed
d-mean size range

Shape and surface morphology :- The shape and surface characteristics of the prepared microspheres were evaluated by means of scanning electron microscopy.The samples for scanning electron microscopy were prepared by gently sprinkling the microspheres on a double adhesive tape, which is sticked to an aluminum stub. The stubs were then coated with gold using a sputter coater under high vacuum and high voltage to achieve a film thickness of 30nm. The samples were than imaged using a 20KV electron beam.

Drug Entrapment Efficiency: About 50 mg of accurately weighed drug-loaded microspheres were added to 50ml of phosphate buffer pH 7.4. The resulting mixture was kept shaking on mechanical shaker for 24 hrs. Then the solution was filtered and the drug content was estimated at 236.2 nm spectrophotometrically after appropriate dilution with phosphate buffer solution pH 7.4 using the standard calibration curve. All experiments were carried out in triplicate (Mishra, 2010). The drug entrapment efficiency was determined using the following relationship

$$\text{Drug entrapment efficiency} = \frac{\text{Experimental drug content X 100}}{\text{theoretical drug content}}$$

Swelling index: This technique was used for characterization of microspheres. The microspheres (100mg) were placed in a petri dish with distilled water and swelling was allowed at room temperature and changes in weight variation between the initial weight of microspheres and weight due to swelling was measured by taking weight periodically. Swelling ratio was found by using the formula,

$$\text{Swelling index} = \frac{(We - Wo)}{Wo} \text{ X } 100$$

Where, Wo = Initial weight of the dry microspheres,

We = Final weight of the swollen microspheres at equilibrium swelling in the media.

FTIR study: The IR spectra of pure drug (DTZ HCl), blank polymer and drug loaded polymeric beads crosslinked was obtained separately at room temperature in KBr pellets using a Simadzu Prestige spectrophotometer between the range of 400 to 4000 cm-1 and resolution was 2 cm-1.

In-vitro drug dissolution studies

Dissolution studies were carried out for all the formulation. A weighed amount of microspheres equivalent to 100 mg of drug were filled into a capsule and placed in the basket. Dissolution media used was 900ml of 0.1N HCl maintained at 37±0.5˚C and stirred at 100rpm for 2 hours and followed by 7.4 phosphate buffer. At predetermined time intervals, 5 ml of sample was withdrawn and replaced with equal amount of respected buffer. The collected samples were filtered and suitably diluted with buffer solution and analyzed spectrophotometrically at 237nm to determine the amount of drug released in the dissolution medium.

RESULT AND DISCUSSION:

The prepared microspheres were a free flowing off White crystalline powder. Soluble in methanol, chloroform and water . The melting point of Diltizam Hydrochloride pure drug was found to be 187-188^{0} C .

PERCENTAGE YIELD

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to microspheres lost during the washing process. A 100% yield could not be achieved principally due to of microspheres to the stirring rod of the Mechanical stirrer. The percentage yield was found to be in the range of 71.00% to 82.70%. The percentage yield of the prepared Microspheres is recorded in **Table no 3** .

FLOW PROPERTIES

Angle of Repose for all the formulations (F1 toF9) was found to be within the range 25.13±0.07 to 29.14±0.019, showing good flow characteristics.

PARTICLE SIZE ANALYSIS

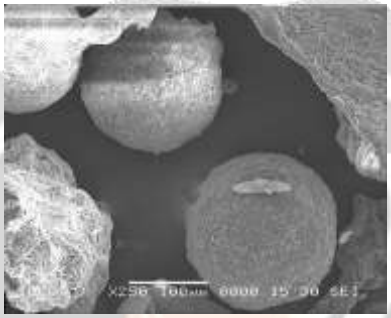
The prepared hollow microspheres were in a size range suitable for oral delivery. The mean size increased with increasing polymer concentration. Diltiazem Hydrochloride microsphere had a size range of 127.80 to158.00 µm. The particle size data is presented in **table no.3.**

SHAPE AND SURFACE MORPHOLOGY

Morphology of the Microspheres was investigated by Scanning electron microscopy (SEM). The photographs of the optimized formulations taken by scanning electron microscope are shown in the **Fig 2.**

The results of SEM (Fig: 2) revealed that the Microspheres were discrete and spherical in shape with a rough outer surface morphology which might be due to surface associated drug. Surface topography of optimized formulation was carried out. SEM study showed that pores were found on the surface of microspheres which indicates that drug is released by diffusion mechanism.

Fig 2: SEM picture of Optimized Formulation-02



DRUG ENTRAPMENT EFFICIENCY

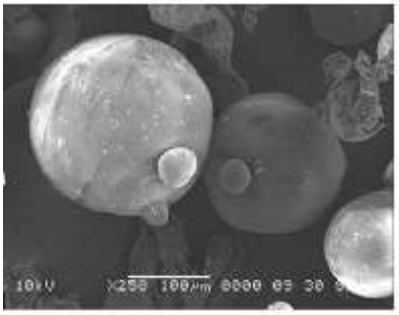
The % of Drug Entrapment Efficiency of all the formulations was in the range of 75.00 % 90.50%. The % drug entrapment efficiency of the prepared Microspheres is displayed in **table no.3.** The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers.

Table no :-3: Evaluation datas of different formulations:

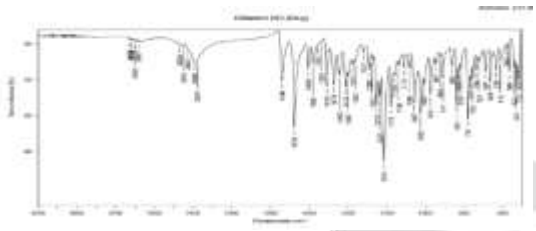
Formulation Code	%Yield	Angle of Repose (θ)	%Drug Entrapment Efficiency	Particle Size (µm)
F1	79.35%	26.56± 0.16	75.00 %	127.80
F2	82.70%	28.12±0.12	90.50%	146.56
F3	75.67%	27.13±.0.05	82.60%	158.00
F4	71.00%	28.34±0.32	79.00%	130.8
F5	79.90%	29.14±0.19	81.80%	138.00
F6	78.07%	29.03±0.94	84.80%	150.75
F7	77.00%	28.37±0.38	80.06%	131.00
F8	71.00%	27.54±0.14	81.60%	139.00
F9	77.15%	25.13±0.07	79.80%	146.00

DEGREE OF SWELLING

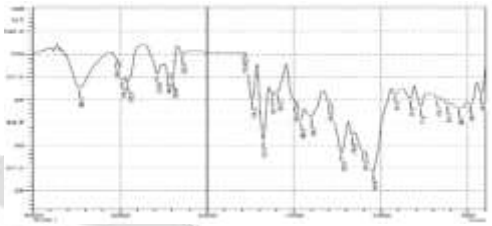
It can be concluded from the data shown in that with an increase in hydrophilic polymer concentration, the degree of swelling also increases. Thus, we can say that amount of hydrophilic polymer directly affects the degree of swelling.



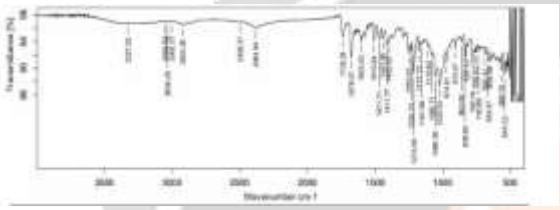
FTIR ANALYSIS :-Drug polymer compatibility studies were carried out using Fourier Transform Infrared spectroscopy to establish any possible interaction of Diltizam Hydrochloride with the Ethyl cellulose , HPMC K4M and Gelatin Polymers used in the formulation. The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug. The results indicated that the principle peaks obtained from the combinations were almost similar to that of pure drug without any significant change in their position, indicating no chemical interaction between (drug) Diltizam Hydrochloride and (polymers) Ethyl cellulose, HPMC K4M and Gelatin.



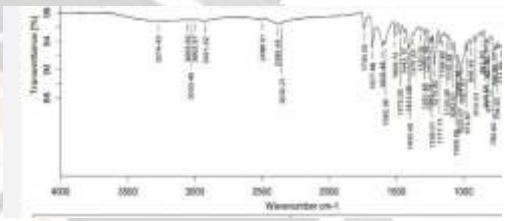
IR spectra of Diltiazem hydrochloride



IR spectra of Diltiazem hydrochloride + Ethyl cellulose



IR spectra of Diltiazem hydrochloride +HPMC K4M



IR spectra of Diltiazem hydrochloride + Gelatin

Table-4: FT-IR Characteristic peak of Pure Drug and excipients

S. No	Functional group	IR Observed peaks (cm^{-1})				
		IR range	Diltiazem Hcl	Drug + Ethyl Cellulose	Drug+ HPMC K4M	Drug + Gelatin
1.	N-H	3100-3010	3054.61	3055.63	3054.62	3054.86
2.	C=O	1685-1666	1677.78	1677.86	1678.57	1677.88
3.	N-O	1550-1500	1509.45	1509.72	1510.64	1509.65
4.	C-N	1250-1020	1236.48	1236.61	1236.75	1236.59

***IN-VITRO* DRUG DISSOLUTION STUDIES**

The initial burst effect was considerably reduced with increase in polymer concentration. The fact that increases in the polymer concentration resulted in better incorporation efficiency could be the reason for the observed decrease in burst effect, since the amount of surface associated drug decreases with an increase in entrapment efficiency. The formulations F2 & F9 containing EC/Gelatin 1:2 and 1:3 ratios respectively showed the maximum release 96.327 % and 92.673 %, respectively, compared to other formulations at 12 h. The results obtained in the *in-vitro* drug dissolution studies are tabulated in **table 5.**

Table-5: in-vitro drug release after 12 hour

Formulation code	Cumulative % drug release
F1	90.124
F2	96.327
F3	92.716
F4	88.592
F5	90.321
F6	91.940
F7	89.852
F8	91.400
F9	92.673

CONCLUSION:

The present work described a study on formulation and evaluation of controlled released microspheres of Diltiazem HCl by using different polymers (Ethyl cellulose, HPMC K4M, and gelatin) emulsifying agent as Span 80. From the result it is found that all the formulations shows excellent sustained property. The microspheres of all the formulations were more or less spherical in nature. From dissolution study of above formulations, it was concluded that the formulation containing high amount of polymers gave high controllrd effect i.e. 12 hrs. The results of this investigation indicate that non-aqueous solvent evaporation technique can be successfully employed to fabricate Diltiazem Hydrochloride -loaded Microspheres.FT-IR spectra of the physical mixture revealed that the drug is compatible with the polymers used. It revealed a distinct biphasic release pattern that may be desirable for oral drug delivery, since a therapeutic loading dose can be provided initially and the sustained drug release could maintain the therapeutic drug level.

REFERENCES:-

1. Kadam N. R., Suvarna V. Microspheres: A brief review. Asian j. biomed. pharm. sci.2015; 5(47): 13-19.
2. Virmani T and Gupta J: Pharmaceutical application of microspheres: An approach for the treatment of various diseases. Int. J. Pharm. Sci. Res. 2017; 8(8): 3252-60.
3. Ramteke K.H, Jadhav V.B, Dhole S.N. Microspheres: as carriers used for novel drug delivery system. IOSR J Pharmacy. July 2012; 2(4): 2250-3013.
4. Sharma N, Purwar N. and Gupta PC: Microspheres as Drug Carriers for Controlled Drug Delivery: A Review. Int J Pharm Sci Res 2015; 6(11): 4579-87.
5. D'Souza SS, Deluca PP. Development of a dialysis *in-vitro* release method for biodegradable microspheres. Aaps Pharm Sci Tech. 2005; 6(2): E323-8.
6. Kamalakkannan V, Kumaran KA, Kannan C, Bhama S, Kumar RS. Effects of Permeability Characteristics of Different Polymethacrylates on the Pharmaceutical Characteristics of Diltiazem Hcl-Loaded Microspheres. Indian J Res in Pharmacy and Biotechnology. 2013 Jul 1; 1(4): 504.
7. Basak SC, Kumar KS, Ramalingam M. Design and release characteristics of sustained release tablet containing metformin HCl. Revista Brasileira de Ciências Farmacêuticas. 2008 ;44(3): 477-83.
8. www.wikipedia.org/wiki/Hypertension.
9. Kokiwar PR, Gupta SS, Durge PM. Prevalence of hypertension in a rural community of central India. J Assoc Physicians India. 2012; 60(6): 26-9.
10. Kaur P, Rao SR, Radhakrishnan E, Rajasekar D, Gupte MD. Prevalence, awareness, treatment, control and risk factors for hypertension in a rural population in South India. International journal of public health. 2012; 57(1): 87-94.
11. Mungati et al. Factors affecting diagnosis and management of hypertension in Mazowe District of Mashonal and Central Province in Zimbabwe: 2012. BMC Cardiovascular Disorders 2014;14: 102.
12. Singh M, Kotwal A, Mittal C, Babu SR, Bharti S, Ram CV. Prevalence and correlates of hypertension in a semi-rural population of Southern India. Journal of human hypertension. 2018; 32(1): 66-74.
13. Pharmacotherapy-A Pathophysiologic Approach, Joseph T. Diprio, Robert L. Talbert Essentials of Medical Pharmacology- KD Tripathi
14. https://en.wikipedia.org/wiki/Diltiazem.
15. Kumar M and coworkers. Formulation and characterization of a floating microsphere of glimepiride by using solvent evaporation technique. Int. J. Pharm. Res. Sch. 2021:10 (1).
16. Kulkarni n and coworkers . Formulation and evaluation of gastro-retentive floating microspheres: a systematic review**. Int. J. Pharm. Sci. Res.** 2020; 11(1): 1000-13.
17. Dr. M Ravi and coworkers. Design and Characterization of Diltiazem Hydrochloride Sustained Release Microspheres. Int. J. Eng. Res. 2021:9 (9).
18. Bolourchian N, Bahjat M. Design and *In Vitro* Evaluation of Eudragit-Based Extended Release Diltiazem Microspheres for Once- and Twice-Daily Administration: The Effect of Coating on Drug Release Behavior. Turk. J. Pharm. Sci .2019;16(3):340-7.

19. Gupta MK , Swarnkar SK. Preformulation studies of diltiazem hydrochloride from tableted microspheres. J. Drug Deliv. Ther. 2018; 8(1):64-9
20. Prasanthi D, Deepika Y, Devi SR. Formulation and evaluation of linagliptin mucoadhesive microspheres. Int. Res. J. Pharm.. 2018; 9(5): 11-7.
21. Kalidindi DV. Microspheres of diltiazem hydrochloride by ionotropic gelation technique. Int. J. Pharm. Sci. Res. March 2017; 8(3): 1413-9.
22. Betala S, Varma MM, Abbulu K. Formulation and evaluation of sustained release microspheres of proprano lol. World J. Pharm. Pharm. Sci. 2017; 6(11): 1497-507.
23. Dr. V. Uma maheshwar rao and coworkers. Formulation and evaluation of diclofenac microspheres for sustained drug delivery.Int. J.Pharm. Anal. Res. 2017: 6(2) 354-62.
24. Chilukala S, BonthA VK, Pragada RR. Formulation development of floating microspheres of cefditoren pivoxel by 3^2 factorial design and *in-vitro* characterization. Asian J. Pharm. 2016; 9(5): 14-22.
25. Patel KS, Patel MB. Preparation and evaluation of chitosan microspheres containing nicorandil. Int. J. Pharm. Investig. 2014; 4: 32-7.