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FORMULATION AND EVALUATION OF MATRIX MICROSPHERES FOR SIMULTANEOUS DELIVERY OF CANDESARTAN CILEXETIL AND CAPTOPRIL FOR TREATMENT OF NEPHRITIC SYNDROME

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ABSTRACT

The objective of the present study was to prepare and evaluate matrix microspheres system for simultaneous and sustained release of candesartan cilexetil and captopril for the management of nephritic syndrome, Ethyl cellulose was used as a retardant polymer and IR study showed better compatibility of it with both the drugs, the matrix microspheres were prepared by emulsion solvent evaporation method and the prepared microspheres were characterized for morphology, drug loading and micromeretical properties. The drug release was performed in pH-6.8. The prepared microspheres were spherical in shape and free flowing in nature, the drug loading capacity ranges from 62-86%, the matrix microspheres show extended release up to 6-8 h, thus, the matrix microspheres have a potential for the prolongation and simultaneous release of candesartan cilexetil and captopril for mitigation of nephritic syndrome.

INTRODUCTION: In the present scenario, the diabetes becomes a very common disease and longstanding diabeties mellitus leads in nephritic syndrome which result in the chronic kidney failure or death. The combination of the ACE inhibitors and angiotensin receptor blockers (ARBs), used to treat nephritic syndrome because the combination of the both results in the complete suppression of the Renin Angiotensin System therefore candesartan cilexetil and captopril selected for the treatment of nephritic syndrome. The sustained effect of both the drugs achieved by matrix microspheres in which the ethyl cellulose used as a rate determining constituent [1, 2].

Thus, the objective of the present study includes;

a) Development of sustained release matrix microsphere system containing Candesartan cilexetil and captopril using ethyl cellulose as the retardant polymer which will sustain the release of drugs for a longer period of time to increase the patient compliance.

b) To study the effect of drugs to polymer and solvent ratio on in vitro drug release.

c) To fit the drug release data to various drug release models.

MATERIAL AND METHOD: Captopril was procure from the Lupin Pharmaceutical Ltd., India and candesartan cilexetil was obtained as a gift sample fromVijayshree Chemicals Pvt. Ltd. Ethyl cellulose obtained as a Gift sample from Colorcon Asia Pvt. Ltd., Goa. All other chemicals and reagents used were of the analytical grade.

Method of preparation: The microspheres were prepared by emulsion solvent evaporation method using the formulation as shown in **table 1**. In this method ethyl cellulose was dissolved in acetone and a given amount of the drugs were dispersed in it to make different drugs to polymer ratio of 1:1, 1:2, 1:3 and stirred it for about 15 minutes, then polymer-drugs dispersion poured in to 50 ml of liquid paraffin (light)

containing Tween 80. The whole system stirred for 4 hours at 900 rpm, after stirring, extra liquid paraffin was decanted off and the microspheres formed were collected by filtration and washed them with n-hexane to completely remove the remaining oil and dried at 50°C in vacuum drier for 6 hours and collected for further studies.

TABLE 1: SHOWING DIFFERENT RATIO OF POLYMERS AND SOLVENTS

	Drugs- polymer			Solvent system	
Batch Code	**Captopril**	**Candesartan cilexetil**	**Ethyl cellulose**	**Acetone**	**Ethyl alcohol**
VM1	1	1	1	1	1
VM2	1	1	2	1	1
VM3	1	1	3	1	1
VM4	1	1	3	1	0
VM5	1	1	1	0	1
VM6	1	1	1	2	1

Particle Size Determination: The size of Microspheres was determined by using an optical microscope (Magnus MLX-DX, Olympus, India) fitted with an ocular and stage micrometer. The mean particle size was calculated by measuring 200-300 particles.

Scanning Electron Microscopy: SEM was performed morphological characterization of microspheres using scanning electron microscope (LEO-430, U.K.). Microspheres were mounted directly on to the SEM sample stub using double sided sticking tape and coated with gold palladium film (Thickness 200 nm) under reduced pressure (0.001mmHg).

Estimation of Drug Incorporation Efficiency:- To determine the Incorporation Efficiency 50 mg Microspheres were taken and dissolved in 50 ml of distilled water and stirred for 15 minutesat 1500 rpm , Then the solution was filtered to separate shell fragments and diluted with0.05M^{NaOH}. The estimation of drug was carried out by using UV Spectrophotometer (Shimadzu UV-1700 series) at the λmax of 271 nm and205 nm. The Incorporation Efficiency was calculated by the following equation;

$$\text{Incorporation Efficiency} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100$$

Angle of Repose: Flow characteristic such as Angle of Repose (θ) of the Microspheres, which measures the resistance to particle flow, was determined by fixed funnel method and calculated by the following equation-

$$\tan \theta = h/r$$

Where, h= height of pile, r = radius of the base of pile on the graph paper

% Compressibility: The prepared Microspheres were collected, weighed and poured in to 5 ml of graduated cylinder. This arrangement was tapped 100 times and then measured the volume of the filled Microspheres. It was the ratio of the volume before tapping which was filled in the graduated cylinder and after tapped volume. This was calculated by the use of following formula;

$$\% \text{ Compressibility Index} = (1 - v/Vo) \times 100$$

Where, V_0- volumes of the samples before the standard tapping; V- volumes of the samples after the standard tapping

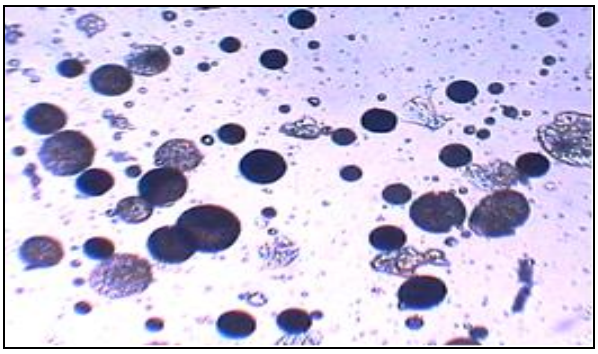
Yield of Microspheres: The prepared Microspheres were collected and weighed. The actual weight of obtained Microspheres divided by the total amount of all non-volatile material that was used for the preparation of the Microspheres multiplied by 100 gives the % yield of Microspheres .This was calculated by the use of following formula;

$$\% \text{ yield} = \frac{\text{Actual weight of the product}}{\text{Total weight of excipients and drug}} \times 100$$

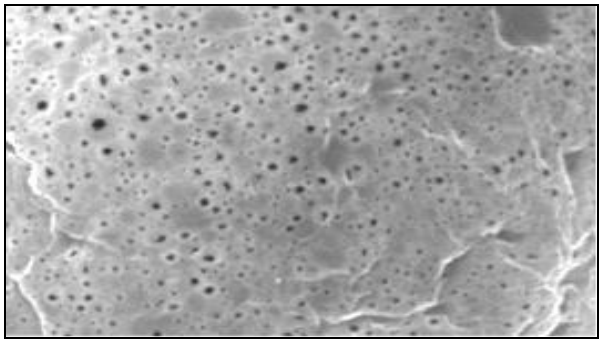
***In-vitro* drug release:** The *in-vitro* release of the microspheres was carried out by the use of the USP rotating basket method at 50 rpm at 37±0.5°C. Dissolution study was carried out in Phosphate buffer (pH -6.8) taking 900 ml for each study. 100 mg of microspheres were placed in the dissolution medium and test samples were taken from the medium at the predetermined time interval over a period of 12 hours

and the samples were analyzed for candesartan cilexetil and captopril content by UV spectroscopy.

RESULTS AND DISCUSSION: The identification and compatibility of drugs with related polymer was studied by the Infra red spectroscopy and the results do not show any interactions. The shape and surface morphology of prepared microspheres was studied by means of scanning electron microscopy, which state that the amount and evaporation rate of the solvent from the solvent system effect the surface morphology and when the amount of acetone increases there is the sign of rupturing and roughness of the surface arises along with the entire morphological characteristics, the structure remain same after 12 hour release of the drug but it becomes bigger and more porous (**Fig. 1**).



(A)



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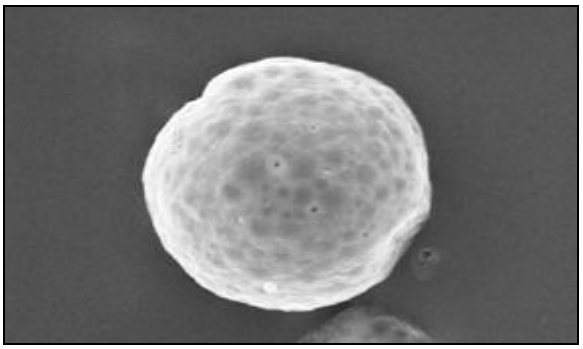


(C)

FIG. 1: (A) OPTICAL MICROGRAPH OF FORMULATION VM4, (B) SURFACE MORPHOLOGY OF FORMULATION- VM3 (C) SEM PHOTOGRAPH OF FORMULATION VM4

The size of the microspheres was studied by optical microscopy method and the size of prepared matrix microspheres was ranges from 72.14±2.86 to 110.67±0.87µm, which shows that the prepared microspheres were smaller and almost uniform in size. The investigation state that the size of the microspheres depends on the amount of the polymer, as the polymer amount increases the size of the prepared microspheres also increases (**Table 2**).

The present investigation state that if the drugs are soluble in the solvent system, it results in high drug entrapment efficiency than that of dispersed in the solvent system. The elimination of the drugs from the prepared microspheres highly dependent on the concentration of the polymer used, as the amount of the polymer increased the entrapment efficiency of the microsphere increased because of the good matrix formation. Drug entrapment was found good and ranges from 62.76±0.33% to 86.34±0.11%. Both the drugs soluble in ethanol ensure high entrapment as well as uniform distribution of drugs through out the matrix (Table 2).

TABLE 2: SHOWING THE SIZE, PERCENTAGE DRUG ENTRAPMENT, ANGLE OF REPOSE, PERCENTAGE YIELD, AND PERCENTAGE COMPRESSIBILITY

Batch Code	Mean particle size (µm)	% drug Entrapment	Angle of repose	% yield	%compressibility
VM1	76.98±2.43	62.76±0.33	16.49±0.81	70.4 ± 2.72	10.68 ± 2.58
VM2	86.34±1.25	68.93±2.75	19.56±0.42	68.50±0.98	12.18 ± 1.09
VM3	98.56±1.61	86.34±0.11	22.74±0.82	65.56 ±1.32	16.65 ± 2.92
VM4	110.67±0.87	81.43±1.57	23.45±0.33	63.5 ± 1.72	16.37 ± 1.77
VM5	72.14±2.86	72.59±0.64	19.32±0.27	76.95 ±3.04	14.08 ± 2.82
VM6	78.97±3.11	76.39±0.76	18.10±0.21	80.43 ±2.83	15.87 ± 4.37

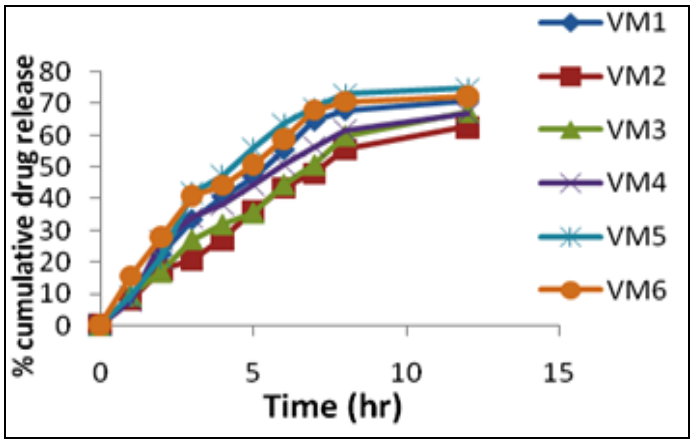
The flow characteristic parameters such as angle of repose of the prepared matrix microspheres was ranges from 16.49 ±0.81 to 23.45 ±0.33, which indicate good flow property of the microspheres, and Percentage compressibility of microspheres ranges from 10.68±2.58% to 16.65±2.92% which also indicate

good flow property of the prepared matrix microspheres (Table 2). The yield of the prepared microspheres was found in the range of 63.5± 1.72 to 80.43 ±2.83% which indicate good yield of the microspheres (Table 2).

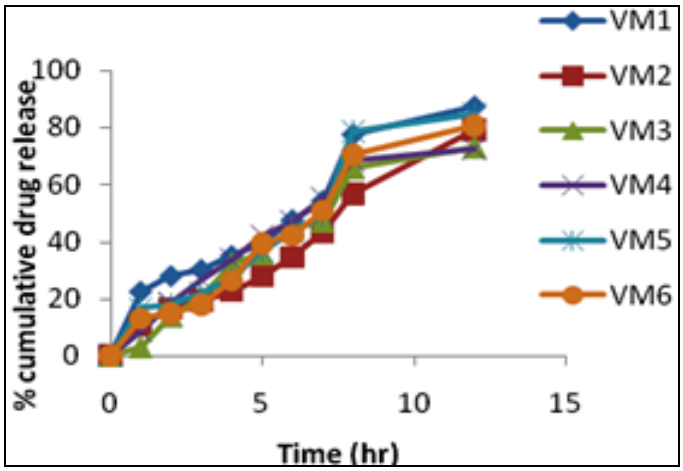
In vitro Drug release study was performed in basket type dissolution apparatus and the effect of the variation of the Polymer drug ratio and solvent ratio on drug release was studied in different physiological solutions and found that increase in the amount of the polymer resulted in the decrease of the drug release rate (**fig. 2**).

Thus, 1:3 ratio was good for the sustaining the release. The release data fitted to different models and the release from the microspheres follow the Matrix model/Higuchi equation based on Fickian model. The investigation also state that the surface porosity of the Ethyl cellulose microspheres play a very important role in drug release since the polymer is non biodegradable and the release of the drug from the microspheres depends on the dissolution and diffusion through pores.

Release Kinetics: Data obtained from in vitro study were fitted to various kinetic equations to find out the mechanism of drug release from the ethyl cellulose microspheres and the investigation state that the release of the drug from matrix microspheres follow Higuchi matrix model (**Table 3**).



(A)



(B)

FIG. 2: (A) CUMULATIVE DRUG RELEASE OF CANDESARTAN CILEXETIL IN pH 6.8, (B) CUMULATIVE DRUG RELEASE OF CAPTOPRIL IN pH 6.8

TABLE 3: KINETICS OF DRUG RELEASE FROM MATRIX MICROSPHERES

Formulation	Kinetic model														
	Zero-order			First order			Higuchi			Peppas and Korsmeyer			Hixon-Crowell		
	R	K_0	RSS	R	K_1	RSS	R	K_H	RSS	R	K_P	RSS	R	K_{HC}	RSS
VM4	0.6082	0.7775	85	0.6751	-0.0082	74	0.9861	2.9810	4	0.9659	3.6243	4	0.6541	-0.0027	77
VM6	0.6194	0.7284	87	0.6584	-0.0077	76	0.9656	2.8091	10	0.9761	2.3707	14	0.6459	-0.0025	80
VM2	0.7055	0.8058	89	0.7431	-0.0086	75	0.9689	3.0683	11	0.9640	2.7935	11	0.7311	-0.0028	79
VM3	0.7487	0.7334	71	0.7744	-0.0078	61	0.9566	2.7749	14	0.9538	1.9980	17	0.7662	-0.0025	64

CONCLUSIONS: Captopril- candesartan cilexetil combination matrix microspheres were prepared successfully using emulsion solvent evaporation method. The polymer- drug and solvent variables give a impact on the size, shape, morphology, entrapment efficiency and drug release. The release of the drugs from the microspheres is diffusion controlled and provides sustaining of both the drugs which results in decrease of side effect, increase of bioavailability and therapeutic action of both combination drugs. Since prepared matrix microspheres are the potential candidate for diabetic nephropathy treatment.

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