FORMULATION AND EVALUATION OF SUSTAINED RELEASE MICROSPHERES OF ACETAZOLAMIDE BY SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

A short half-life carbonic anhydrase Inhibitor such as Acetazolamide was developed as sustained release microcapsules to reduce the frequency of drug administration, ease of dose adjustment and improve patient compliance. The formulated sustained release microcapsules of Acetazolamide were prepared by solvent evaporation techniques using Ethyl cellulose and HPMC as polymer and particle size, encapsulation efficiencies and *invitro* releases of the fabricated microcapsules were evaluated. The final results showed that the encapsulation efficiencies were desired for all the formulations. Particle sizes of the microcapsules were influenced by the concentration of Ethyl cellulose and HPMC. From the results of the *in vitro* study shows that the desired release rate is achieved by the combination of Ethyl cellulose and HPMC.

Key words: Acetazolamide, Sustained release, Ethyl cellulose and HPMC.

INTRODUCTION

Microspheres are carriers for Control Release and can be defined as solid spherical monolithic free flowing particles having size range between 1-1000 micrometers; typical size is 1 -500 micrometers [1]. Usually the localized effect of drug is sustained by microspheres. Microencapsulation is a process by which relatively thin coating is applied on, the small particle of solids droplets of liquid and dispersion[2]. Acetazolamide is a carbonic anhydrase inhibitor and it is widely used in the treatment of glaucoma, epilepsy and also used as diuretics. The drug has a relatively short half-life (3-4 hr) and usually administered 3 – 4 times daily in the form of an immediate release formulation[3]. Dosage regimen reduction and patient compliance improvement is done by sustained release formulations. Micro encapsulation by solvent evaporation techniques is widely used to formulate sustained release of a drug for a better clinical benefit. Water insoluble polymers like ethyl cellulose and HPMC are used as encapsulation matrix using this technique. The purpose of this study was to formulate and evaluate microspheres of Acetazolamide[4].

Drug Profile[5]

Acetazolamide

Chemical Name: 2-acetylamino-1, 3, 4-thiadiazole-5-sulfonamide

Chemical formula: $C_4H_6N_4O_3S_2$

Molecular weight: 222.245

Solubility:

1.Readily Soluble in 1N sodium bicarbonate solution

2. Soluble in DMSO (DIMETHYL SULFOXIDE)

3. Slightly soluble in water, methanol and ethanol.

Melting point: 258- 259˚C

Mechanism of action:

Direct inhibition of carbonic anhydrase in CNS influence the anticonvulsant activity of acetazolamide which decreases carbon dioxide tension in the pulmonary alveoli, thus increasing arterial oxygen tension. The diuretic effect depends on the inhibition of carbonic anhydrase, causing a reduction in the bioavailability of hydrogen ions for active transport in the renal tubule lumen, leads to alkalinity of urine and an increase in the excretion of bicarbonate, sodium, potassium, and water.

MATERIALS AND METHODS:

Acetazolamide and Ethyl cellulose was procured from Intermed laboratories, Chennai. Hydroxypropyl methyl cellulose, Ethanol, span 80, Liquid paraffin and Benzene was procured from Scientific lab, Chennai.

PREPARATION OF MICROSPHERES[6,7]:

Acetazolamide microspheres were prepared by solvent evaporation technique. Various proportions of polymers like ethyl cellulose and HPMC were dissolved in Ethanol. Acetazolamide was powdered and dispersed in polymer solution. This solution was added slowly to a jacketed flask containing 100ml of liquid paraffin and 2% w/w span 80 under constant stirring (1000 RPM). After evaporation of Ethanol, the microspheres formed were collected by filtration in vacuum, washed 3-4 times with 50ml of benzene each and dried at room temperature for one day.

TABLE.NO.1 FORMULATION TABLE

S. NO	INGREDIENTS	F-1	F-2	F-3	F-4
1.	Acetazolamide (gm)	1	1	1	1
2.	Ethyl cellulose (gm)	0.5	1	1	1.5
3.	Hydroxy propyl methyl cellulose (gm)	0.5	0.5	1	1
4.	Ethanol(ml)	10	10	15	20
5.	Span 80	0.2 %	0.2 %	0.2 %	0.2 %
6.	Liquid paraffin(ml)	100	100	100	100

CALIBRATION CURVE OF ACETAZOLAMIDE [8]:

Standard stock solution of acetazolamide was prepared by dissolving accurately weighed 100 mg of Acetazolamide in phosphate buffer of pH 7.4 in a 100 ml volumetric flask. The volume was then made up to the mark by using phosphate buffer, so as to get a solution of 100µg/ml. From the standard stock solution (100µg/ml) aliquots of 2, 4, 6, 8 and 10.0 ml were transferred to a series of 100 ml volumetric flasks and final volume was made with phosphate buffer, so as to get drug concentrations of 2 to 10.0 µg/ml respectively. The absorbance of these drug solutions was estimated at 265 nm. Calibration curve was constructed by using triplicate values.

SCANNING ELECTRON MICROSCOPY (SEM)

Particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface was determined by scanning electron microscopy. SEM is the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. The SEM analysis of microspheres of Acetazolamide in ethyl cellulose and HPMC showed spherical

COMPATIBILITY STUDIES [9]:

Compatibility studies were performed using FTIR (Fourier transform infrared spectrophotometer). The spectrum of pure drug, polymers and mixture of drug and excipients were studied. The compatibility studies were done by using spectra of each formulation correlates with peak of drug spectrum.

EVALUATION[10]

PARTICLE SIZE: The particle size of the ACETAZOLAMIDE microspheres was first evaluated using an optical microscope fitted with a calibrated eyepiece micrometer under a magnification of 40X. The average particle size was determined by usingdiameters of about 100 microspheres.

PERCENTAGE YIELD:

The efficiency of any method is calculated by percentage practical yield, and it helps in selection of appropriate method of production. Practical yield was calculated as the weight of Acetazolamide microspheres obtained from each batch in relation to the amount of drug and polymer used in the preparation.

Percentage yield was calculated using the formula:

$$\%\text{Yield} = \frac{\text{Weight of microspheres obtained after solvent evaporation}}{\text{Amount of drug and polymer used}} \times 100$$

shape, smooth texture and no burst of microsphere material.

Percentage drug entrapment efficiency:
Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula.

$$\text{Entrapment efficiency} = \frac{\text{Practical drug percentage content}}{\text{Theoretical drug content}} \times 100$$

Theoretical drug content was determined by calculation assuming that the entire ACETAZOLAMIDE present in the polymer solution used gets entrapped in microspheres, and no loss occurs at any stage of preparation of Acetazolamide microspheres. Practical drug content was analyzed by using the following procedure Weighed amount of Acetazolamide microspheres equivalent to 100 mg of Acetazolamide was dissolved in 100 ml of Phosphate buffer. This solution was kept overnight for the complete dissolution of the Acetazolamide in Phosphate buffer. The filtered solution further diluted to make a conc of 10 µg/ml solution. The absorbance of the solutions was measured at 265 nm using double beam UV-Visible spectrophotometer against phosphate buffer as blank and the percentage of drug present in the sample was calculated. The procedure was repeated three times and standard deviation was calculated.

***IN VITRO* DRUG RELEASE STUDIES[11]:**
The drug dissolution studies of microspheres were carried out using 900ml of Phosphate buffer of pH 7.4 using the rotating basket method. Weighed microspheres with equivalent to 50 mg of drug were filled in capsules. Then these microspheres were placed in the basket of dissolution apparatus with rotation speed of 100 rpm and thermostatically controlled at 37±0.5°C. The sample solution was withdrawn at a suitable interval from the dissolution vessel and analyzed spectrophotometrically at 265 nm.

RESULTS AND DISCUSSION:

TABLE NO: 1. CALIBRATION CURVE

CONCENTRATION (µg/ml)	**ABSORBANCE (nm)**
0	0
2	0.055
4	0.095
6	0.157
8	0.214
10	0.278

TABLE.NO.2 PARTICLE SIZE ANALYSIS

S.NO	FORMULATION	RANGE OF PARTICLE SIZE(µm)	AVERAGE PARTICLE SIZE(µm)
1.	F1	2.5– 30.0	20.2
2.	F2	30.4– 50.8	40.0
3.	F3	53.2– 64.6	55.5
4.	F4	66.3– 70.5	69.5

TABLE.NO.3 PERCENTAGE YIELD

S.NO	**FORMULATION**	**PERCENTAGE YIELD**
1.	F1	90.12±0.03
2.	F2	88.02±0.08
3.	F3	89.31±0.6
4.	F4	85.64±0.4

TABLE NO: 4 ENTRAPMENT EFFICIENCY

FORMULATION	**ENTRAPMENT EFFICIENCY (%)**
F1	68.86±0.05
F2	74.65±0.78
F3	80.12±0.59
F4	88.51±0.21

TABLE.NO.4*IN VITRO* DRUG RELEASE

TIME IN HOURS	F1	F2	F3	F4
0	0	0	0	0
1	20.24±0.12	18.11± 0.05	15.05±1.21	12.26±1.14
2	29.51±1.18	20.24±0.12	21.10±1.56	14.62±0.46
3	30.06±1.01	31.42±1.23	26.15±0.05	16.82±0.19
4	36.75±1.21	35.57±0.15	33.66±0.58	20.69±0.35
5	43.89±0.15	40.67±0.96	36.75±0.10	23.35±0.42
6	52.93±0.62	49.98±0.29	40.32±0.20	27.17±0.58
7	65.12±0.76	54.22±034	45.25±0.22	29.83±0.11
8	77.24±0.81	64.21±0.99	48.15±1.60	32.58±0.22
9	81.31±0.96	73.59±0.52	50.16±0.18	37.62±0.15
10	90.55±0.82	80.92±1.32	61.83±0.22	41.35±0.26

KINETICS OF DRUG RELEASE:TABLE.NO.4 KINETICS OF DRUG RELEASE

TIME	CPR	logCPR	logT	SQRT	%DRUG RETAINED	Log % DRUG RETAINED
1	18.11	1.258	0	1	81.89	1.913
2	20.24	1.306	0.301	1.414	79.76	1.901
3	31.42	1.497	0.477	1.732	68.58	1.823
4	35.57	1.551	0.602	2	64.43	1.809
5	40.67	1.609	0.699	2.236	59.33	1.773
6	49.98	1.699	0.779	2.449	50.02	1.699

FIG NO 1: Structure of Acetazolamide

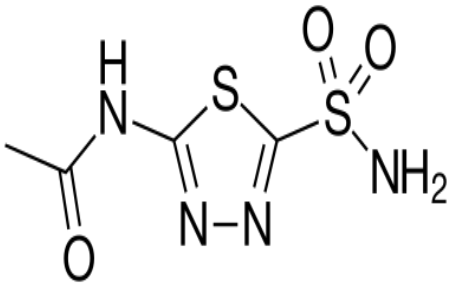
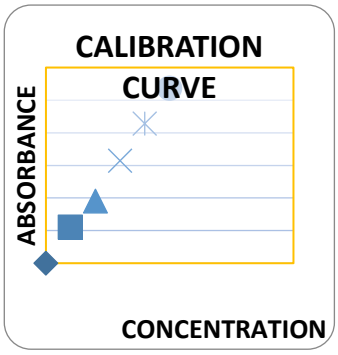


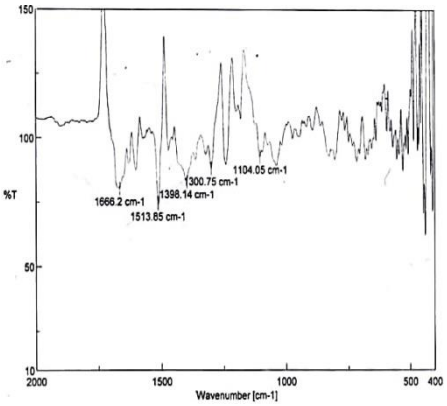
FIG NO2:Calibration curve



FTIR (Fourier transform infrared spectrophotometer):

FIG NO 3: ACETAZOLAMIDE:

FIG NO 4: FORMULATION 1(DRUG + EXCIPIENTS)



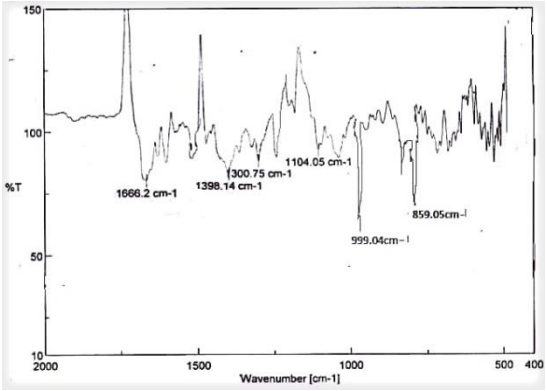


FIG NO 5: SCANNING ELECTRON MICROSCOPY (SEM):

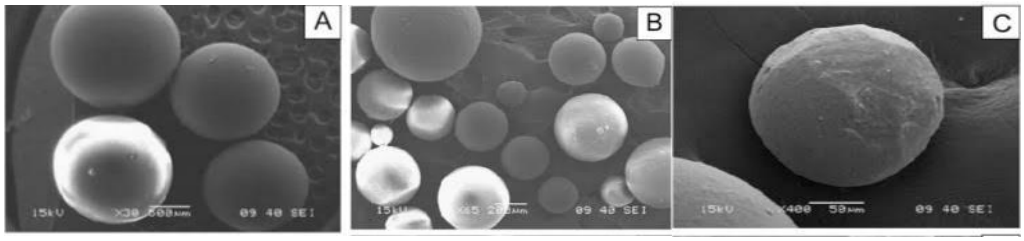
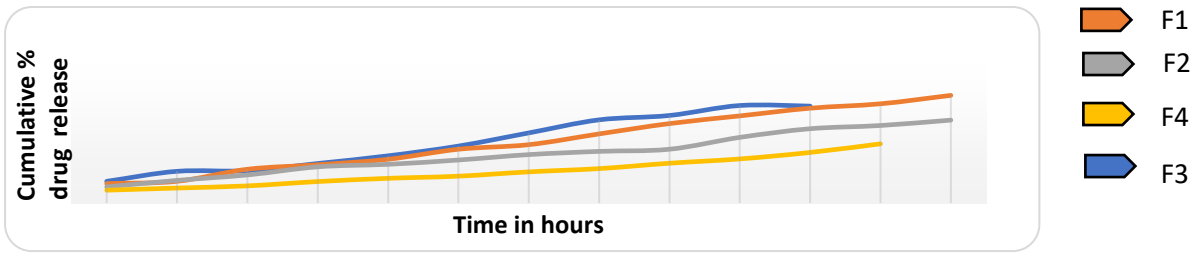


FIG NO6:*IN VITRO* DRUG RELEASE STUDIES



CONCLUSION:

It can be concluded that as the polymer ratio increased the particle size, the percentage yield and percentage entrapment efficiency also get increased. The percentage cumulative amount of drug released at the end of 13 hours was 95.95±0.09 for the formulation F2 which was optimized to be the best formulation. By analyzing the kinetic of the drug profile, it was found that the drug was released by fickian diffusion.

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