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DEVELOPMENT & CHARACTERIZATION OF EMTRICITABINE LOADED MICROSPONGES

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ABSTRACT

The rationale of this study was to develop, optimize and characterize microsponge drug delivery system for antiviral delivery. Antiviral compound, emtricitabine was selected as a model drug. The microsponges were prepared by emulsion solvent diffusion method and optimize for various formulation parameters. The formulation (F1- F6) were characterized in terms of particle size, drug entrapment efficiency (DEE), scanning electron microscopy (SEM), differential scanning calorimetry (DSC) and zeta potential measurement. The drug release from the microsponge loaded gel was studied by modified Franz diffusion cell. F2 released 50.85% of drug at 8 hours. Diffusion exponent (n) value of F2 formulation was found to be 0.912 suggesting that the Ficks law of diffusion was not followed. The F2 formulation followed Zero order kinetics in its in vitro drug release. This study provides future insights for developing delayed release microsponge based gels for treatment of skin infections and disorders.

K**eywords:** Microsponge, Solvent diffusion method, Emtricitabine, Scanning electron microscopy, Differential scanning calorimetry.

INTRODUCTION

Controlled drug delivery technique presents front line part of today's developed technique, in this includes many scientific approaches, serving for individual care. The drug deliverance technique having abundant advantages than existing conventional type of dosage, it involves enhanced effectiveness, minimized poisoning, enhanced consumer conformity also ease. This type of drug deliverance technique utilizes micro molecules, for caring drugs. As the varieties of forms for dosage are invented like microparticle as well as nanoparticles shown more significance. [1-3]

An ideal and advanced oral drug delivery system is that, which exactly controls speed, time as well as site of release of medicament separately of normal physiological variables such as gastrointestinal tract pH, digestive condition of the gastrointestinal tract, peristalsis movement and circadian rhythm.[4] Advance in polymer science and technology outcome in pick up the pace research and developmental activity in the design of drug delivery devices. [5-6]

Application of topical drugs suffers many problems such as ointments, which are often aesthetically unappealing, greasiness, stickiness etc. that often results into lack of patient compliance. These vehicles

require high concentrations of active agents for effective therapy because of their low efficiency of delivery system, resulting into irritation and allergic reactions in significant users. [1-2]

Microsponge delivery system encompass polymeric bead having network of pores with an active ingredient held within was developed to offer controlled release of the active ingredients whose final target is skin itself. Microsponges are porous microspheres having interconnected voids of particle size range 5-300μm The system was employed for the improvement of performance of topically applied drugs. [7-8]

The incorporation of the active substance at its maximum thermodynamic activity in an optimized vehicle facilitates the reduction of the resistance to the diffusion of the stratum corneum. Microsponges consisting of noncollapsible structures with porous surface through which active ingredients are released in a controlled manner. [9-10]

Emtricitabine, widely utilize is a nucleoside reverse transcriptase inhibitor (NRTI) for the prevention and treatment of HIV infection in adults and children. Emtricitabine exhibits clinical activity against the hepatitis B virus (HBV), but is not approved by the FDA for the treatment of HBV infection.[11] Emtricitabine is an analogue of cytidine. It works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. By interfering with this process, which is central to the replication of HIV, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness.

MATERIALS AND METHODS

Emtricitabine was obtained as gift sample by Glenmark pharmaceuticals. Carbopol- 940, Ethyl cellulose, poly vinyl alcohol (PVA) Dichloro methane and Tri- ethanol amine are purchased from HiMedia labs, Mumbai.

Preparation of Emtricitabine sodium microsponges

Batches of microsponges coded by F1, F2, F3, F4, F5 and F6 utilizing different proportions of ethyl cellulose (EC) and poly vinyl alcohol (PVA) were prepared by employing emulsion solvent diffusion method. briefly, the dispersed phase consists of acyclovir sodium(100mg) and requisite quantity of ethyl cellulose (table No. 1) dissolved in 20ml of dichloromethane was slowly added to a definite amount of poly vinyl alcohol in 150ml of aqueous continuous phase. The reaction mixture was stirred at 1000rpm for two hours on a mechanical stirrer. The microsponges were collected by filtration and dried in oven at

400 for 24 hours. The dried microsponges were stored in vacuum desiccators to ensure the removal of residual content. [9, 10,12]

Table.1. Formula for Emtricitabine loaded microsponge [13-14]

S.No	Formulation code	Emtricitabine (mg)	Polyvinyl alcohol (mg)	Dichloro methane (mL)	Ethyl cellulose (mg)	Disilled water (mL)
1	F1	200	200	20	100	150
2	F2	200	250	20	200	150
3	F3	200	300	20	300	150
4	F4	200	250	20	100	150
5	F5	200	300	20	200	150
6	F6	200	300	20	300	150

Preparation of microsponge loaded carbapol gels

Gel forming polymer was soaked in water for 2 hours and then dispersed by agitation at approximately 800 rpm by magnetic stirrer to get a smooth dispersion. The dispersion was allowed to stand for 15-20 min to expel entrained air. To this aqueous solution of triethanolamine (2 % v/v) was added with slow agitation. At this stage microsponges and permeation enhancers were incorporated into the prepared base as ethanolic solution.

Table 2. Formula for Emtricitabine loaded topical gel (100 ml)

S.NO.	Ingredients	Placebo	FM1	FM2	FM3
1	Emtricitabine (mg)	200	-	-	-
2	Microsponges (mg)		Equivalent to 100mg of drug	Equivalent to 100mg of drug	Equivalent to 100mg of drug
3	Ethanol (mL)	20	20	20	20
4	Triethanolamine (mL)	2.0	2.0	2.0	2.0
5	Carbapol (mg)	1	1	1	1
6	Propylene glycol (ml)	-	-	10	-
7	DMSO (mL)	-	-	-	10
8	Distilled water (mL)	Q.S	Q.S	Q.S	Q.S

Characterization of microsponges

Scanning electron microscopy

For morphology and surface topography, prepared microsponges can be coated with gold– palladium under an argon atmosphere at room temperature and then the surface morphology of the microsponges can be studied by scanning electron microscopy (SEM). SEM of a fractured microsponge particle can also be taken to illustrate its ultra-structure. [11]

Determination of loading efficiency

A sample of dried microsponges equivalent to 10 mg was taken in to mortar and pestle and add little amount of phosphate buffer of pH 6.8 and allowed to stand for 24 hours. Then transfer content in to 100 ml volumetric flask and make up volume to 100 ml with phosphate buffer of pH 6.8. The solution was filtered through whatmann's filter paper. From the resulting solution take 1 ml in to 100 ml volumetric flask and then make up volume to 100 ml with phosphate buffer of pH 6.8. Drug content was determined by UV spectrophotometer at 253nm. The entrapment was calculated by using following formula. [12-15]

The loading efficiency (%) of the microsponges can be calculated according to the following equation:

$$\text{Loading efficiency} = \left(\frac{\text{Actual drug in microsponges}}{\text{Theoretical drug concentration}}\right) 100$$

Production yield

The production yield of the microsponges can be determined by calculating accurately the initial weight of the raw materials and the last weight of the microsponge obtained.

$$\text{Production yield} = \left(\frac{\text{Practicalmass}}{\text{The oritical mass}}\right)100$$

Size analysis of microsponges

The mean diameter of 100 dried microsponges was determined by Zeta potential measurement.[16]

Evaluation of microsponge gels containing Emtricitabine

Drug content studies

1.0 g of each gel formulations were taken in 100 ml volumetric flask containing 20 ml of phosphate buffer (pH 6.8) and stirred for 30 minutes and allowed to stand for 24 hours in case of microsponge loaded gel formulations. The volume was made up to 100mL and 1mL of the above solution was further diluted to 50mL with phosphate buffer (pH 6.8). The resultant solution was filtered through membrane filter (0.45µm). The absorbance of the solution was measured spectrophotometrically at 253 nm using placebo gel as reference. [17]

Spreadability

The spreadability of the gel formulation was determined, by measuring diameter of lg gel between horizontal plates (20 x20cm2) after 1 min. The standardized weight tied on the upper plate was 125g.[12-13]

Determination of pH

2.5 g gel was accurately weighed and dispersed in 25 ml of purified water. The pH of the dispersion was measured using pH meter, which was calibrated before each use with buffered solution at 4.0, 7.0 and 9.0. [18]

Viscosity measurement

Viscosity of different formulations was determined using Brookfield viscometer with spindle No. 6 at 10 rpm at temperature 37±0.5°C.

In vitro diffusion studies

Modified franz diffusion cells were used in the in-vitro diffusion studies. The egg membrane was mounted between the compartments of the diffusion cell. In this study, 200 ml of phosphate buffer (pH 6.8) solution was used as receptor medium. The receptor medium was maintained at 37±0.5°C and stirred magnetically at 500 rpm. 1 ml of sample were withdrawn from the receptor compartment at predetermined time interval for 8 hours' period, and replaced by same volume of fresh pre-warmed phosphate buffer (pH 6.8) solution. to maintain constant volume. The amounts of acyclovir in the samples were assayed spectrophotometrically at 253 nm against appropriate blank. [10,19]

Characterization of microsponges

Production yield

The production yields of Emtricitabine microsponge formulations; production yield determined for all microsponges ranged from 62.45- 75.43 %. From the production yields of Acyclovir sodium microsponge formulation, it was indicated that increasing the drug: polymer ratio increased the production yield.

Loading efficiency

The loading efficiency of Emtricitabine microsponge formulations ranged from 89.25 to 94.23 %. Loading efficiency is varied by changing the proportions of drug, polymer, and emulsifier. Higher loading efficiency is achieved with the formulation consists of drug, PVA, EC the ratio of 1:3:3 coded by F2 which is selected for the gel preparation.

Particle size

Particle size of microsponges is varied along with the change in the ratio of polymer (ethyl cellulose) and emulsifier (PVA). By keeping polymer concentration constant, particle size is increased by decreasing the emulsifier (F1-F6). Optimum size is obtained by taking polymer and emulsifier at equal proportions (F5). Lesser size is obtained by taking lesser proportion of emulsifier than polymer (F3).

Scanning electron microscopy (SEM)

Microsponge formulation with least particle size and optimum loading efficiency were investigated by SEM to find out surface morphology. The representative SEM photographs of the microsponges are shown in Fig.1.

Table.3 Evaluation of microsponges

S. No.	Formulation code	Loading efficiency (%)	Production yield (%)	Mean Partical size (µm)	Zeta potential (mV)*	Poly- dispersity index*
1	F1	89.25	62.45	42.8	+17.7 ± 0.81	0.234 ± 0.006
2	F2	97.35	65.37	41.2	+23.9 ± 0.63	0.254 ± 0.004
3	F3	94.23	83.66	31.2	+14.1 ± 0.67	0.780 ± 0.074
4	F4	96.30	86.30	27.9	+15.9 ± 0.71	0.342 ± 0.098
5	F5	92.59	78.52	31.4	+27.8 ± 0.56	0.245 ± 0.009
6	F6	90.56	75.43	38.7	+29.8 ± 0.42	0.345 ± 0.012

Table. 4. **Physical parameters of microsponge loaded topical gel**

S. No.	Formulation code	% Drug content	Spreadability (gm.cm/ sec)	Viscosity (p)	pH
1	Placebo	99.24	12.5	210.4	6.87
2	FM1	96.37	11.75	202.91	6.82
3	FM2	93.48	11.25	208.12	6.83
4	FM3	94.07	11.17	204.27	6.80

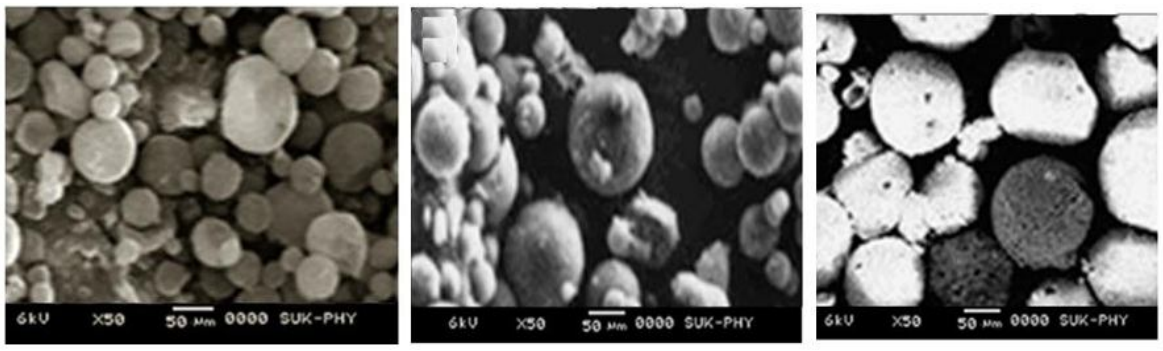

Fig. 1. SEM image of the Microsponge formulation (F2)

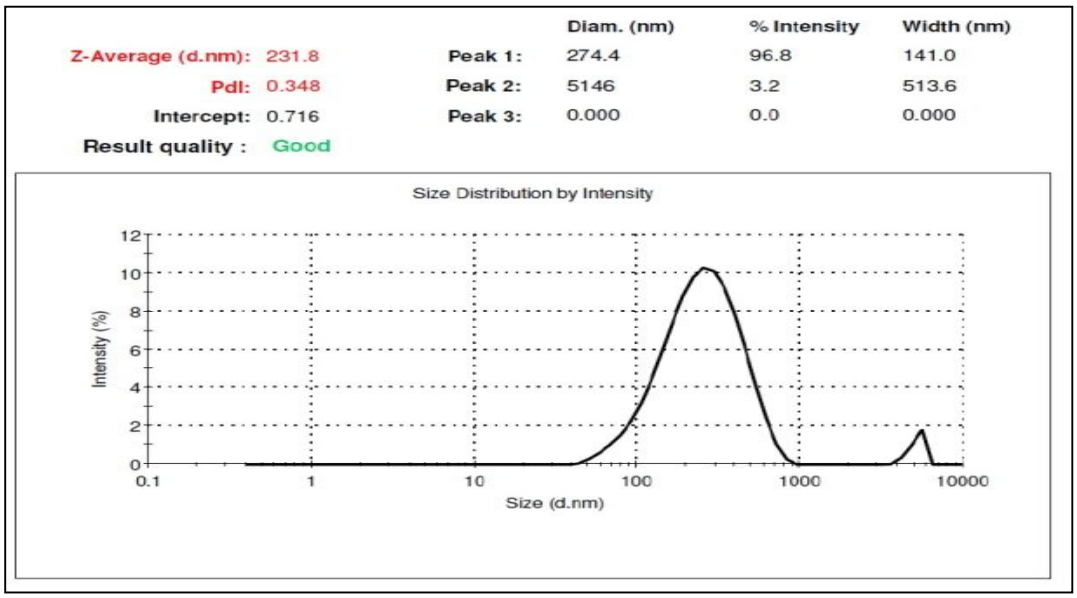

Fig.2. Particle size of formulation F2

*****Each value is average of three separate determinations ±SD

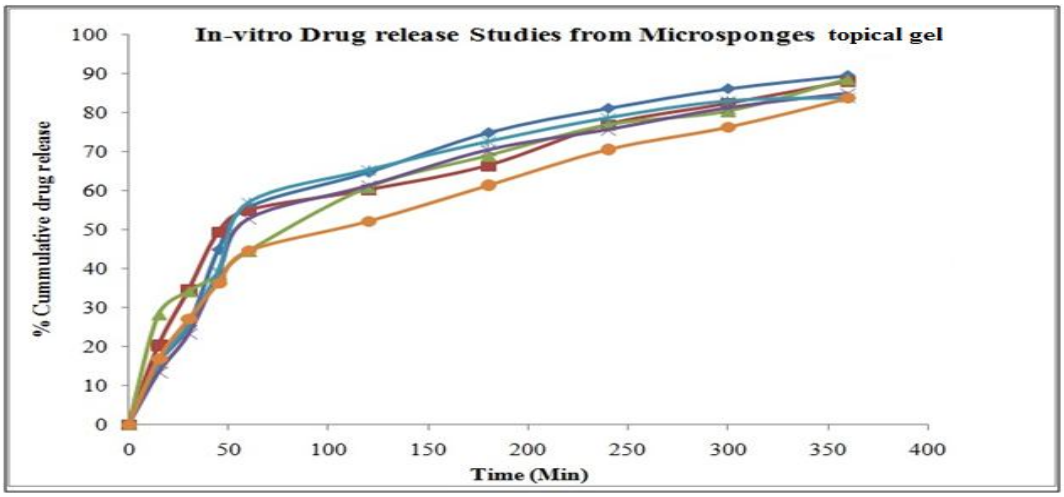

Fig 3. *In-vitro* drug release profiles of Emtricitabine loaded microsponge gel formulations

The drug release profiles of the Emtricitabine microsponge gel formulations are shown in fig. 3. Drug release from Emtricitabine microsponge gel was found to range from 81.32 % to 89.34 % from all formulations.

From the results it was found that, as concentration of polymer increases, percentage of drug released decreases. The initial high drug release could be due to two reasons: first, the drug near or on the surface of the microsponges and second, well known porous nature of microsponges, the pores providing a channel for release of the drug.

CONCLUSION

The controlled release topical drug delivery system of Emtricitabine was developed as a microsponge loaded gel offer solubilising matrix for the drug, served as depot for controlled drug release and provided a rate limiting matrix barrier for modulation of drug release. The emulsion solvent diffusion method used for the preparation of the microsponges was simple, reproducible, and rapid. Furthermore, it was observed that as drug/polymer ratio increases, the particle size is decreased. This is probably due to the fact that at higher relative drug content, the amount of polymer available per microsponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence, smaller microsponges. 81.32 % to 89.34 % from all formulations FM2 followed Zero order kinetics and non fickian diffusion. Microsponge formulation FM2 showed a good physical parameter study and was used for formulating into gel, incorporated in the carbopol.

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