

Microsponges Enriched Gel (MEGs): A Novel Strategy for Ophthalmic Drug Delivery System Containing Ketotifen

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Abstract

Microsponge containing ketotifen drug with three different proportions of ethyl cellulose and drug were obtained successfully using quasi-emulsion solvent diffusion method. These formulations were studied for particle size and physical characterization. These microsponges enriched gel formulation were prepared by using 2 and 3 % w/w of SCMC and studied for viscosity, pH, gel strength, spreadability, bioadhesive force, drug content, in vitro release, HPLC and SEM analysis. The viscosity of microsponges enriched gel was found to be in the range 1299 to 1600 centipoises. The maximum gel strength and mucoadhesive force was found to be up to (8.12 sec) and (32.32 dynes/cm²) respectively. The formulations exhibited spreadability (22.88 gm.cm/sec), The optimized formulations were able to release the drug up to 8 hours.

Keywords: Microsponges Enriched Gel, ketotifen, HPLC, SEM, Controlled Release

INTRODUCTION

Most conventional ophthalmic dosage forms are simplistic. It is usual that water-soluble drugs are delivered through topical administration in an aqueous solution [1], and water-insoluble drugs are administered topically as an ointment or aqueous suspension. The major deficiencies of these conventional dosage forms include poor ocular drug bioavailability, pulse-drug entry after topical administration, systemic exposure because of nasolacrimal duct drainage, and a lack of effective systems for drug delivery to the posterior segment of ocular tissue.

Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage [2], and the high efficiency of the blood-ocular barrier [3]. It is standard for only 1% or less of a topically applied dose to be absorbed across the cornea and thus reach the anterior segment of the eye [4,5]. Pulse entry is a common, and yet highly undesirable, pharmacokinetic characteristic associated with eye drops [6]. The initial high drug concentration found in tears, followed by a rapid decline, poses a potential risk of toxicity, and suggests a requirement for frequent dosing.

Early attempts

A considerable amount of effort has been made in ophthalmic drug delivery since the 1970s. The various approaches attempted in the early stages can be divided into two main categories: bioavailability improvement and controlled release drug delivery. The former was attempted by the methods like Viscosity enhancers [4,7-9], Gels [10], Penetration enhancer [4,11], Prodrugs [4], Liposomes [12-17] and the latter was attempted by various types of inserts and nanoparticles. After initial investigations, some approaches were dropped quickly, whereas others were highly successful and led to marketed products.

Recent developments

An important lesson learned from earlier efforts in ophthalmic drug delivery is the necessity of balancing the technologies of sustained drug release or bioavailability improvement with patient comfort and ease of use. The preferred system would also provide improved bioavailability, site-specific delivery, and/or continuous drug release [2,4,18]. With this in mind, recent research efforts have focused largely on microsponge enriched gel (MEG) systems.

Microsponges are porous microspheres having myriad of interconnected voids of particle size ranging from 5-150 μm . Microsponge Drug Delivery System is a unique technology which provides controlled release of active ingredients [19,20]. It offers numerous advantages over other technologies like reduced side effects, improved stability, increased elegance and enhanced formulation flexibility [21,22]. Microsponges are porous, polymeric microspheres that are used mostly for topical and recently for oral administration. They can be incorporated into conventional dosage forms such as creams, lotions, gels, ointment, tablet and powder and share a broad package of benefits & thus provides formulation flexibility [23-25].

MATERIALS AND METHODS

Preparation of Microsponges:

The microsponges containing drugs were prepared by quasi emulsion solvent diffusion method [26] using different polymer ratio as shown in Table 1. The inner phase, Ethyl cellulose was dissolved in dichloromethane and then added drug to solution under ultrasonication at 35°C and outer phase prepared by dissolving PVA in distilled water at 60°C for 10 min. The inner phase is poured into PVA solution in water. The resultant mixture was stirred by magnetic stirrer for 60 min at 25°C, and filtered to separate the microsponges. The microsponges were dried in an air heated oven at 40 °C for 12 hrs, and weighed to determine the yield [27].

Table 1. optimum values for microsp sponge formulation

Specification	Optimum Value
Drug and polymer ratio	1:1, 1:2, 1:3, 2:1 and 3:1
Amount of drug (mg)	100-300
Poly vinyl alcohol (mg)	100
Inner phase solvent (ml)	Ethyl alcohol
Amount of inner phase solvent	10
Amount of water in outer phase (ml)	100
Temperature of inner phase	25°C
Type of process	Magnetic stirrer and bath sonicator
magnetic stirrer speed	1000 rpm
Sonication temperature and time	25°C and 30 min

Table 2. Composition of microsponges enriched gel containing Ketotefen: Ethyl cellulose

Formulation code	Drug : polymer ratio (w/w)	Production yield (%)	Loading Efficacy (%)	Bulk Density (gmL ⁻¹)	True Density (gmL ⁻¹)	% Porosity	Mean particle size diameter (µm)
Magnetic stirrer							
A1	1:1	51.55	91.30	0.433	0.21	48.49	6.4
A2	2:1	66.67	91.01	0.421	0.29	68.88	7.9
A3	3:1	31.56	87.11	0.418	0.25	60.52	8.9
A4	1:1	51.55	87.04	0.432	0.17	39.35	6.4
A5	1:2	23.88	87.03	0.431	0.21	48.72	11.9
A6	1:3	35.55	87.00	0.432	0.23	53.24	13.6
Bath sonicator							
A7	1:2	63.26	--	--	--	--	--
A8	1:1	61.10	--	--	--	--	--
A9	1.5:1	65.48	--	--	--	--	--

Preparation of microsponges enriched gel (MEG):

Accurately weighed amount of gelling agents was taken and dissolved in water and soaked for overnight. Accurate amount of prepared gelling agent was dispersed slowly in appropriate of the drug containing microsponges with the help of overhead stirrer. Finally add few drops of triethanolamine to adjust the pH. The suitable gelling agent was selected on the basis of compatibility with microsp sponge structure, feel and ease of spreadability.

EVALUATION OF MICROSPONGES ENRICHED GEL (MEG):**Particle size studies:**

Particle size analyses were performed on microsp sponge by optical microscopy (DN-117M, USA). The results are the average of three analyses. The values (*d*50) were expressed for all formulations as mean size range.

Scanning electron microscopy [28]:

The morphology and size of microsponges were observed by scanning electron microscopy. Prepared microsponges were coated with gold and studied by scanning electron microscopy (Phenoworld) under vacuum at room temperature.

Determination of loading efficiency:

The drug content in the microsponges was determined by HPLC method. A sample of drug containing microsponges (10 mg) was dissolved in 100 ml of methanol. The drug content was calculated from the calibration curve and expressed as loading efficiency [28].

$$\text{Loading Efficiency} = \frac{\text{Actual drug content in microsp sponge}}{\text{Theoretical drug content}} \times 100$$

Determination of production yield:

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

$$\text{Production Yield (PY)} = \frac{\text{Practical mass of microsponges}}{\text{Theoretical mass (Polymer + drug)}} \times 100$$

Determination of true Density:

The true density of microparticles is measured using an ultracycrometer under helium gas and is calculated from a mean of repeated determinations.

Characterization of pore structure:

Pore volume and diameter are vital in controlling the intensity and duration of effectiveness of the active ingredient. Pore diameter also affects the migration of active ingredients from microsponges into the vehicle in which the material is dispersed. Mercury intrusion porosimetry can be employed to study effect of pore diameter and volume with rate of drug release from microsponges. Porosity parameters of microsponges such as intrusion-extrusion isotherms pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density can be determined by using mercury intrusion porosimetry.

Diffusion studies

The diffusion medium used was simulated tear fluid pH 7.4. Assembly of diffusion cell for in-vitro diffusion studies the diffusion cell was designed as per the dimension given. Diffusion cell with an effective diffusion area of 3.14 cm² was used for in vitro permeation studies. The diffusion cells

were placed on the magnetic stirrers. The donor compartment consisting of 1% w/w of microsponges enriched gel (MEG) containing ketotifen. The receptor compartment was filled with fluid. Then the cellophane membrane was mounted on the cell carefully so as to avoid the entrapment of air bubble under the chicken membrane. Intimate contact of cellophane membrane was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment. With the help of 1ml pipette 1ml of sample was withdrawn at a time intervals of 30 minutes from sampling port of receptor compartment and same volume was the replaced with receptor fluid solution in order to maintain sink condition. The samples were appropriately diluted and measured by using HPLC method.

Determination of pH

The pH of the microsponges enriched gel was determined using a calibrated pH meter. The readings were taken for average of 3 samples.

Viscosity Studies

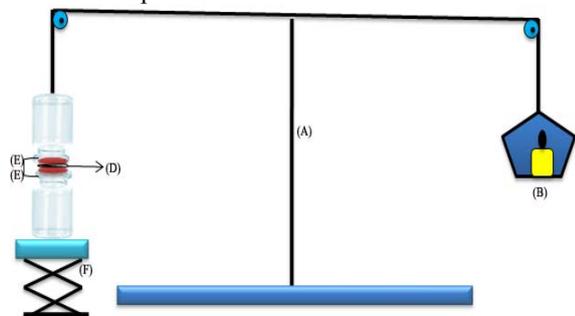
The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosity of microsponges enriched gel and the solution were determined at different angular velocities (0.3, 0.6,12, 20, 30, 40...to 60 rpm) and average of two reading were used to calculate the viscosity.

Determination of mucoadhesive force

The mucoadhesive force of all the optimized batches was determined as follows, a section of the chicken mucosa fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. Microsponges enriched gel was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The chicken mucosa was changed for each measurement [29].

$$\text{Detachment stress (dynes/cm}^2\text{)} = m \text{ g/A}$$

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s²; and A is the area of tissue exposed.

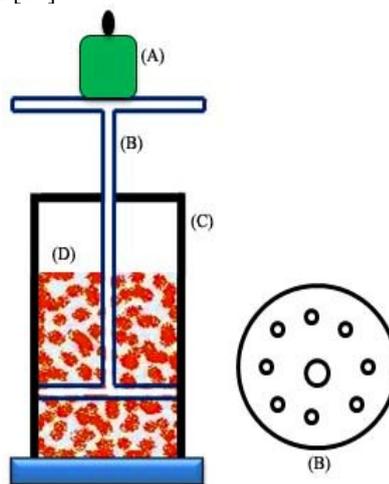


(A) Modified Balance, (B) weight (C) Glass vial (D) Gel (E) Chicken membrane (F) Height adjustable pan

Figure 1: Apparatus used for finding mucoadhesive force

Measurement of Gel Strength

A sample of 50 gm of microsponges enriched gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37⁰ C. The apparatus for measuring gel strength (apparatus as shown in figure 14 weighing 27 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the gels at physiological stimuli was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel [30].



(A) Weights (B) device (C) measuring cylinder (D) gel

Figure 2: Apparatus used for finding gel strength.

Spreadability

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability [29].

$$S = ML/T$$

Where,

- M = weight tide to upper slide (g)
- L = length moved on the glass slide (cm)
- T = time taken (sec)

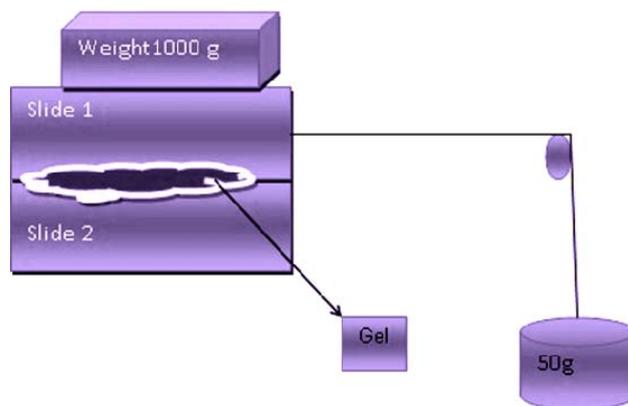


Figure 3: Apparatus used for finding spreadability.

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PROPERTIES OF KETOTIFEN MICROSPONGES ENRICHED GELS.

Measurement of particle size

The microsponges sizes for the formulations are represented in Figure 4. The result shows that the microsponges diameter increases with increasing ratio of drug: polymer. Optical and scanning microscopy showed that particle was spherical in shape. Average particle size of optimized formulations ranged from 7.9 to 8.9 μm .

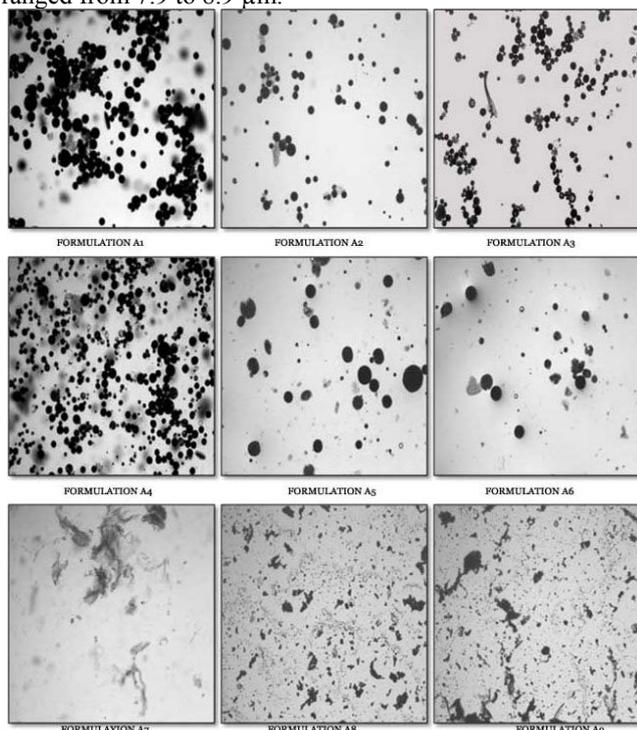


Figure 4: particle size and shape of microsponges enriched gel (optical microscopy)

Viscosity

The viscosity of microsponges enriched gel were determined at different angular velocities (0.3, 0.6, 1.5, 3.0, 6.0, 12, 30 and 60 rpm) by using Sodium Carboxymethyl Cellulose (SCMC) with the concentration of 2% and 3%. As the rpm increased, the viscosity of prepared gel decreased. The formulation of 2% SCMC and 3% SCMC having optimum viscosity for ophthalmic use. The formulation of more than 3% SCMC having higher viscosity which tend to increase the

concentration of polymer and hence not suitable for the administration of ophthalmic preparation.

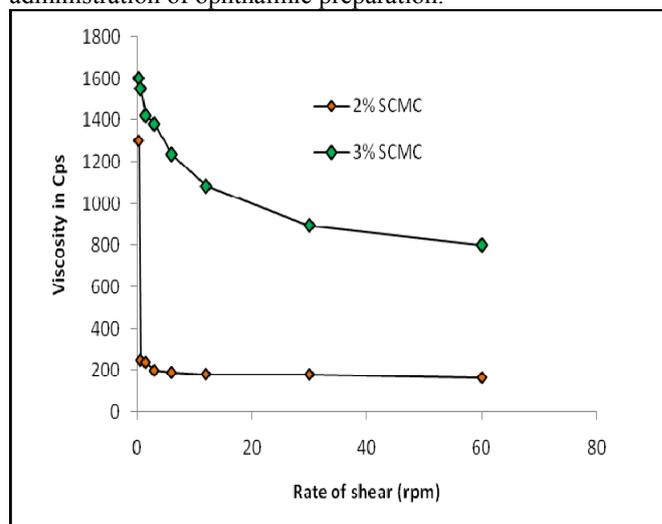


Figure 5: Showing the viscosity optimized formulation Spreadability

The spreadability of formulations were determined by $S=ML/T$. The formulation of 2% SCMC which has spreadability of (22.886 gm.cm/sec.) was found to be more compared to the formulation of 3% SCMC which has spreadability of (16.183 gm.cm/sec). Hence, the spreadability of 3% SCMC is more than 2% SCMC which is suitable for the topical.

Gel Strength

The gel strength of the prepared gel of SCMC was determined by the viscosity of the gel with the time. The formulation of 2% SCMC (4.58 sec.) was found to be more than compared to formulation of 3% SCMC (8.12 sec.) which showed good gel strength.

Mucoadhesive Force

The mucoadhesive force of formulations is determined by $F=mg/A$. The formulation of 2% SCMC (32.32 dynes/cm²) was found to be more compared to 3% SCMC (29.5 dynes/cm²) which showed higher value of mucoadhesive force in the prepared gel concentration.

Scanning electron microscopy

Scanning electron microscopy of the pure benzoyl peroxide and its microsp sponge forms are shown in Fig. 6. It is clear from the figure that microsponges have predominantly spherical shape and contain orifices.

Table 4: Characteristics of Various microsponges enriched gel Formulations

Formulation code	Viscosity (Cps)	pH	% drug content (w/w)	Mucoadhesive force(dynes/cm ²)	Spreadability (gm.cm/sec.)	Gel Strength (seconds)
2% SCMC	1299	6.5	87.99	32.32	22.886	4.58
3% SCMC	1600	6.4	90.45	29.5	16.183	8.12

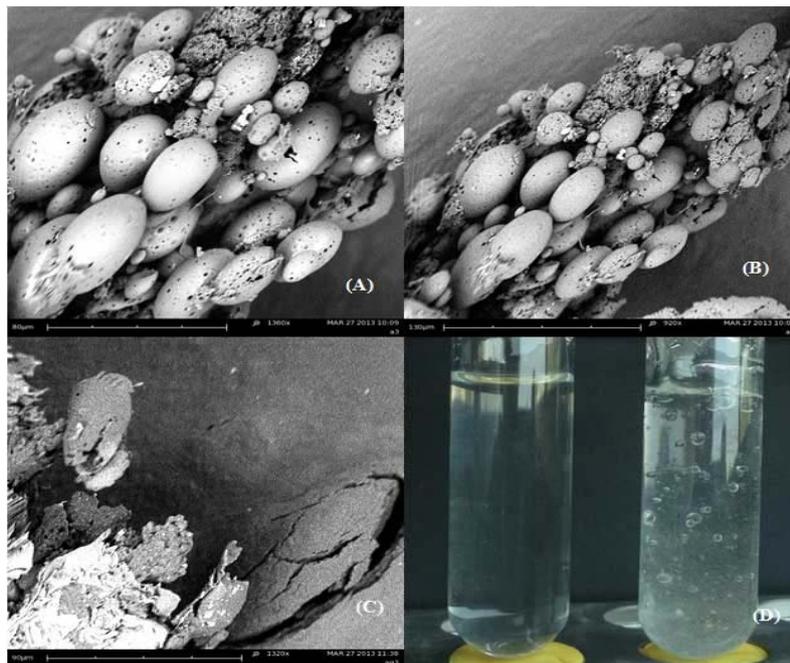


Figure 6. (A and B) SEM photography of microsponges, (C) SEM photography of microsponges enriched gel, (D) Appearance of microsponges and microsponges enriched gel

Chromatographic conditions

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20A injector with 50µL loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of methanol -10mM ammonium acetate buffer (pH 3.0 adjusted with orthophosphoric acid (30:70, v/v)), and detection was made at 298.0nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation.

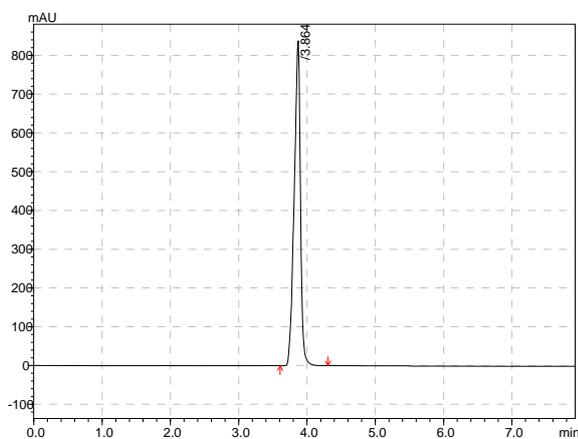


Figure 7. Typical chromatogram of drug sample

In vitro Release Studies:

The percentage of ketotifen diffused through a membrane over a period of 8 hrs from formulation MEG1 and MEG2 was found to be 80.05% and 83.04 % respectively.

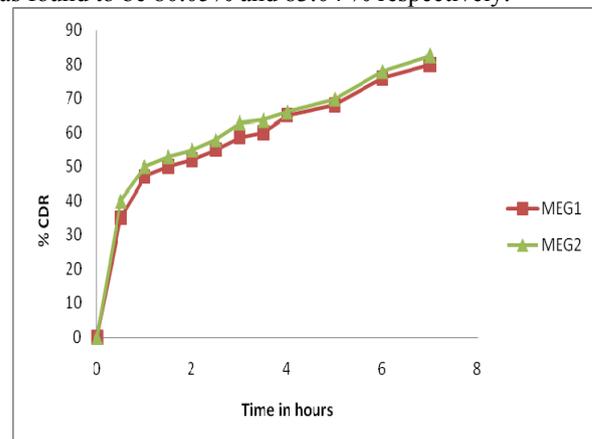


Figure 8. Showing the Diffusion of MEG formulation Drug Release Kinetics

Mechanism the drug release followed diffusion controlled zero order from the microsphere enriched gel, as the value of 'r' for zero order kinetics ranged from 0.99 to 0.991 and also found to be more than that of first order which ranged from 0.98 to 0.982. All the formulations were subjected to PCP DISSO software analysis. The value of 'r' for Higuchi kinetics, which ranged from 0.982 to 0.991. The formulations MEG1 and MEG2 exhibited good *in vitro* release kinetics with fickian type of diffusion mechanism. Data were fitted in to korsmeyer -peppas exponential model where the 'n' values were in the range of 0.056 to 0.057. It was concluding that

formulation MEGs was following predominantly zero order and fickian diffusion mechanism of drug release.

Table 5. Release kinetics of MEGs formulation

Order of process	MEG1	MEG2
Zero order	0.991	0.990
	6.532	6.861
	-0.411	0.169
First order	0.98	0.982
	-0.058	-0.068
	2.09	2.098
Higuchi	0.991	0.982
	6.532	6.861
	-0.411	0.169
korsmeyer	0.95	0.963
	0.057	0.056
	1.334	1.371

CONCLUSION

Microsponges enriched gel novel delivery system has been developed to provide topical delivery of ketotifen. The formulations showed controlled release of drug through skin, indicating better potential of delivery system as compared with ophthalmic solution. If this process can be scaled-up to manufacturing level; this technology has the potential to provide the topical ketotifen microsponges enriched gel with better patient compliance. On the grounds of efficacy and improved patient compliance due to reduced frequency of application, microsphere-based gel formulations will have significantly better role in topical treatment of Allergic infection.

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