PREPARATION AND EVALUATION OF MICROSPONGE LOADED CONTROLLED RELEASE TOPICAL GEL OF ACYCLOVIR SODIUM

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ABSTRACT
Microsponge containing acyclovir sodium as active constituent with four different formulations by changing the proportions of drug (acyclovir sodium), polymer (ethyl cellulose), emulsifier (Poly vinyl alcohol) were obtained successfully using emulsion solvent diffusion method. These formulations were studied for particle size and physical characterization. Scanning electron microscopy (SEM) images showed the microsponges porous and spherical in shape. The physical characterization showed that microsponge formulation coded by M2 showed a better loading efficiency and production yield. This microsponge formulation was prepared as gel in carbopol and studied for pH, viscosity, spreadability, drug content, in-vitro release. The microsponge formulation gel, F1 showed viscosity 206.72 ps, spreadability of 11.75 g cm/s and drug content of 92.37%. The microsponge acyclovir sodium gel formulations showed an appropriate drug release profile. From the various release kinetic models the F1 formulation was found to be optimized. F1 released 50.85% of drug at 8 hours. Diffusion exponent (n) value of F1 formulation was found to be 0.912 suggesting that the Ficks law of diffusion was not followed. The F1 formulation followed Zero order kinetics in its in vitro drug release.

Key words: Microspounge, Solvent diffusion method, Spreadability, Scanning electron microscopy.

INTRODUCTION
Now a day the major challenge to the pharmaceutical industry is to control the delivery rate of active pharmaceutical ingredient to a pre-determined site in human body. So researcher focused on designing different controlled release drug delivery systems to improve efficacy and patient compliance (Vyas S and Khar RK, 2002). Topical formulations are most useful drug delivery systems for both local and systemic treatment. Controlled release of drugs onto the epidermis with assurance that the drug remains primarily localized and does not enter the systemic circulation in significant amounts is an area of research that has only recently been addressed with success. No efficient vehicles have been developed for controlled and localized delivery of drugs into the stratum corneum and underlying skin layers and not beyond the epidermis (Storm JE et al., 1990). 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. Other drawbacks of topical
formulations are uncontrolled evaporation of active ingredient, unpleasant odour and potential incompatibility of drugs with the vehicles. Thus the need exists for system to maximize amount of time that an active ingredient is present either on skin surface or within the epidermis, while minimizing its transdermal penetration into the body. The microsponge delivery system fulfils these requirements (Viral Shaha et al., 2010; Nacht S et al., 1992).

Microsponge delivery system comprised of a polymeric bead having network of pores with an active ingredient held within was developed to provide controlled release of the active ingredients whose final target is skin itself. The system was employed for the improvement of performance of topically applied drugs. 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. Depending upon the size the total pore length may range up to 10 ft and pore volume up to 1 ml/g. Microsponges are porous microspheres having interconnected voids of particle size range 5-300μm (D’souza JI et al., 2008).

Acyclovir, the first agent to be licensed for the treatment of herpes simplex virus infections, is most widely used drug for infections such as cutaneous herpes, genital herpes, chicken pox, varicella zoster infections and herpes keratitis. So sodium salt of acyclovir was selected for the present work.

MATERIALS AND METHODS

Acyclovir was supplied as gift sample by A to Z pharmaceuticals, Chennai. Carbopol- 940 was supplied by Shree Chemical Ltd. Ahmedabad. Ethyl cellulose(EC), poly vinyl alcohol (PVA) are purchased from HiMedia labs, Mumbai, Dichloro methane and Tri-ethanol amine are purchased from bross chemicals, Tirupathi.

Compatibility studies (Orlu M et al., 2006)

Pure drug (acyclovir sodium) and polymer (ethyl cellulose) and their physical mixture were examined by Fourier Transform Infrared (FT-IR) spectra. The spectra were recorded in a Thermo-IR 200 FTIR spectrophotometer. Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the range of 400-4000 cm⁻¹ at the spectral resolution of 20 cm⁻¹.

Preparation of acyclovir sodium microsponges (Comoglu T et al., 2003)

Four batches of microsponges coded by M₁, M₂, M₃, M₄ utilizing different proportions of ethyl cellulose (EC) and poly vinyl alcohol (PVA) were prepared by emulsion solvent diffusion method. briefly, the dispersed phase consist 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(table No.1) 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 40⁰C for 24 hours. The dried microsponges were stored in vacuum desiccators to ensure the removal of residual content.

Characterization of microsponges

Scanning electron microscopy (D’souza et al., 2008)

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.

Determination of loading efficiency (D’souza et al., 2008)

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.

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

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.

\[
\text{Production yield} = \left( \frac{\text{Practical mass}}{\text{Theorical mass}} \right) \times 100
\]

Size analysis of microsponges

The mean diameter of 100 dried microsponges was determined by optical microscopy (Metzer, India).
The optical microscope was fitted with a stage micrometer by which the size of microsponges could be determined.

**Preparation of microsponge loaded carbopol gels**

Gel forming polymer was soaked in water for 2 hours and then dispersed by agitation at approximately 600 rpm with the aid of magnetic stirrer to get a smooth dispersion. The dispersion was allowed to stand for 15 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>
<thead>
<tr>
<th>S.No</th>
<th>Formulation code</th>
<th>Acyclovir sodium (mg)</th>
<th>Polyvinyl alcohol (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>Dichloro methane (mL)</th>
<th>Distilled water (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M₁</td>
<td>100</td>
<td>300</td>
<td>200</td>
<td>20</td>
<td>150</td>
</tr>
<tr>
<td>2</td>
<td>M₂</td>
<td>100</td>
<td>300</td>
<td>300</td>
<td>20</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>M₃</td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>20</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>M₄</td>
<td>100</td>
<td>200</td>
<td>200</td>
<td>20</td>
<td>150</td>
</tr>
</tbody>
</table>

**Evaluation of microsponge loaded gels of acyclovir sodium**

**Drug content studies** (Kedor-Hackmann et al., 2006)

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 absorbance of the solution was measured spectrophotometrically at 253 nm using placebo gel as reference.

**Spreadability** (Rao, N.G.R et al., 2009)

The spreadability of the gel formulation was determined, by measuring diameter of 1g gel between horizontal plates (20 x20cm²) after 1 min. The standardized weight tied on the upper plate was 125 g. The spreadability was calculated by using the following formula.

\[ S = \frac{m \cdot l}{t} \]

Value “S” is spreadability, m is the weight tied to the upper slides, “l” is the length of glass slide, and “t” is the time taken.

**Determination of pH** (Loganathan, V et al., 2001)

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.

**Viscosity measurement** (Loganathan, V et al., 2001)

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** (Doijad et al., 2006)

Modified frenz 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.

Evaluation of drug release kinetics
To investigate the mechanism of drug release from the microsponge loaded gels of acyclovir sodium, the release data was analysed using Zero order, Higuchi, Korsmeyer-Peppas.

Table 3. Mathematical equations for the models used to describe the drug release

<table>
<thead>
<tr>
<th>S. No</th>
<th>Model</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zero order</td>
<td>( Q_t = Q_0 + K_0 t )</td>
</tr>
<tr>
<td>2</td>
<td>Higuchi</td>
<td>( Q_t = Q_0 + K_h t^{1/2} )</td>
</tr>
<tr>
<td>3</td>
<td>Korsmeyer-Peppas</td>
<td>( Q_t = K_{kp} t^n )</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Compatibility studies
FTIR spectra of pure Acyclovir sodium, Ethyl cellulose and physical mixture were obtained. Primary peaks of Acyclovir sodium at 3450.89 (NH-stretching) cm\(^{-1}\), 3333.54 (OH-stretching) cm\(^{-1}\), 1216.09 (C=O-stretching) cm\(^{-1}\) were observed. It clearly indicates that the significance interaction between the drug and polymer were not observed.

Characterization of microsponges

Production yield
The production yields of Acyclovir sodium microsponge formulations are given in Table 4. 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 Acyclovir sodium microsponge formulations are given in Table 4. The loading efficiency calculated for all microsponges 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 M\(_2\) 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 (M\(_1\), (M\(_4\)). Optimum size is obtained by taking polymer and emulsifier at equal proportions (M\(_2\)). Lesser size is obtained by taking lesser proportion of emulsifier than polymer (M\(_3\)).

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. SEM images showed the microsponges porous and spherical in shape. SEM photographs are given below.

Table 4. Characterization of microsponges

<table>
<thead>
<tr>
<th>S. No</th>
<th>Formulation code</th>
<th>Loading efficiency (%)</th>
<th>Production yield (%)</th>
<th>Mean Particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M(_1)</td>
<td>90.56</td>
<td>62.45</td>
<td>41.8</td>
</tr>
<tr>
<td>2</td>
<td>M(_2)</td>
<td>94.23</td>
<td>75.43</td>
<td>36.7</td>
</tr>
<tr>
<td>3</td>
<td>M(_3)</td>
<td>89.25</td>
<td>78.52</td>
<td>30.4</td>
</tr>
<tr>
<td>4</td>
<td>M(_4)</td>
<td>92.59</td>
<td>65.37</td>
<td>44.2</td>
</tr>
</tbody>
</table>

Table 5. Physical parameters of microsponge loaded topical gels of Acyclovir sodium

<table>
<thead>
<tr>
<th>S. No</th>
<th>Formulation code</th>
<th>% Drug content</th>
<th>Spreadability (gm.cm/sec)</th>
<th>Viscosity (p)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F(_0)</td>
<td>98.54</td>
<td>12.5</td>
<td>210.4</td>
<td>6.83</td>
</tr>
<tr>
<td>2</td>
<td>F(_1)</td>
<td>92.37</td>
<td>11.75</td>
<td>206.72</td>
<td>6.82</td>
</tr>
<tr>
<td>3</td>
<td>F(_2)</td>
<td>91.48</td>
<td>11.25</td>
<td>207.91</td>
<td>6.87</td>
</tr>
<tr>
<td>4</td>
<td>F(_3)</td>
<td>91.07</td>
<td>11.17</td>
<td>205.27</td>
<td>6.85</td>
</tr>
</tbody>
</table>

Table 6. Release kinetics of optimized formulation

<table>
<thead>
<tr>
<th></th>
<th>ZERO ORDER PLOT</th>
<th>HIGUCHI PLOT</th>
<th>KORSEMEYER</th>
<th>INFEERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R(^2)</td>
<td>0.997</td>
<td>0.909</td>
<td>0.859</td>
<td>Zero order kinetics</td>
</tr>
<tr>
<td>M</td>
<td>6.73</td>
<td>19.27</td>
<td>1.489</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. SEM photographs of acyclovir sodium microsponges

Figure 2. *In-vitro* drug release of acyclovir sodium gel formulations in phosphate buffer pH 6.8 (Zero order drug release)

\[ y = 6.7345x + 0.3831 \]
\[ R^2 = 0.9973 \]

Figure 3. *In vitro* release profile of Acyclovir sodium gel formulations in Higuchi plot

\[ y = 19.271x - 8.9379 \]
\[ R^2 = 0.9094 \]
Physical parameters of gels

The formulations showed the spreadability in the range of 11.17-12.5g cm/s, viscosity of 205.27 -210.4 ps, pH of 6.82- 6.87 and drug content of 84.27 to 88.67 %respectively. Spreadability value is higher for acyclovir sodium loaded gel than the gels loaded by acyclovir sodium microsponges. Viscosity value is higher for acyclovir sodium loaded gel than the gels loaded by acyclovir sodium microsponges. pH values of all formulations are within the limits. The drug content of the formulations showed that the drug was uniformly distributed in the gels. This is given in table No. 5.

In vitro diffusion studies and kinetic approach

At 8th hour the drug release of all formulations in ascending order is F0>F3>F2>F1. Highest release is from F0 as it is free drug loaded i.e, 91.75%. Among microspone loaded formulations F3 is having highest drug release at 8th hour, i.e. 54.69%. This may be due to lower viscosity and higher content of permeation enhancer. Remaining formulations F1, F2 showed drug release at 8 hours 50.85% and 54.69 respectively. Drug release profile has been depicted in Figure 2.

From the various release kinetic models the F1 formulation was found to be optimized. In F2 and F3 formulations the drug release was rapid after the 8 hrs. Korsmeyer-Peppas model best described the sustained release of optimized F1 formula and the diffusion exponent (n) value was found to be 0.912 suggesting that the Ficks law of diffusion was not followed. The F1 formulation followed Zero order kinetics in its in vitro drug release.

CONCLUSION

A controlled release topical drug delivery system of acyclovir sodium developed as a microspone loaded gel offer solubilising matrix for the drug, served as a local 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 microspone to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence, smaller microsponges. Microspone formulation M2 showed a good physical parameter study and was used for formulating into gel, incorporated in the carbopol. At 8th hour the drug release of all formulations in ascending order is F0>F3>F2>F1. F1 showed drug release 50.85% at 8 hours, followed Zero order kinetics and non fickian diffusion.

REFERENCES


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