DICLOFENAC LOADED CROSSLINKED SODIUM ALGINATE AND GELLAN GUM MICROSPHERES: DEVELOPMENT AND IN VITRO RELEASE PROFILE

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ABSTRACT

Diclofenac loaded microspheres were prepared using sodium alginate and gellan gum and were cross-linked by maleic anhydride, aluminium chloride or calcium. DSC study shows there was no interaction between drug and excipients. The resulting microspheres were evaluated for surface morphology analysis by SEM, entrapment efficiency, percentage yield, particle size analysis, in-vitro release study and swelling index. Entrapment was fair in all the formulations while the maximum entrapment (98.1%) was recorded in formulation cross-linked by aluminium chloride and their average particle size were 140 to 150 µm. From this experiment, it is observed that the formulation with cross-linked by aluminium chloride is the better formulation among others due to good release profile (51.56% over 6 hr), entrapment efficiency and surface morphology behaviour.

KEYWORDS: Microspheres, Diclofenac, Gellan gum.

INTRODUCTION

Microspheres are free flowing spherical particles with an average size range 1 to 1000 µm.[1] It is defined as a monolithic sphere with therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles or as a structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at molecular or macroscopic level.[2] The particles of the microspheres are simple matrices of polymer in which the drug is dispersed and is released from the matrix by a first order process. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue.
in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. They are novel drug delivery systems formulated to achieve a sustained therapy of drugs to prolong the residence time of the drug in the plasma.

The objective of this scientific research was to successfully formulate sustained formulations of candidate drug diclofenac sodium as microspheres to prolong the residence time of the drug in the plasma. Diclofenac was chosen for this sustained therapy because of its gastro-irritative property which provides the rationale to be used as sustained delivery prolonging the residence time of the drug by slower release of the drug minimising its gastro-irritative property. Apart from its gastro-irritative property, diclofenac has a short biological half-life of 2 hours and is administered in a dose of 150 mg 2-3 times a day. Diclofenac sodium is poorly soluble in water and has acidic pH (1-3) but is rapidly soluble in alkaline pH(5-8). Thus it is an ideal candidate for development which could result in prolonged clinical efficacy, reduced frequency of administration and less side effects.

Diclofenac is mainly a NSAID, hence its sole use is to reduce inflammation. It also works as an analgesic or pain reliever. It also is effective to treat the symptoms associated to inflammatory disorders like arthritis, osteoarthritis, rheumatoid arthritis, polymyositis, dermatomyositis, temporomandibular joint (TMJ) pain, spondyl arthritis, spondylitis, ankylosing spondylitis. Diclofenac is also used to relieve pain from tooth ache, gout, acute migraines and post operative and post traumatic pain.

Diclofenac sodium is designed as sustained delivery in form of microspheres by the use of biodegradable polymers of sodium alginate and gellan gum. Both sodium alginate and gellan gum has the ability to undergo ionotropic gelation in aqueous solution in presence of multivalent cations like Ca$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Al$^{3+}$ etc. Cross linking occurs by formation of covalent bonds between two or more molecules. The cross linking agents contain two or more reactive ends to chemically attach with functional groups. This phenomenon is known as cross-linking. This scientific research uses maleic anhydride, calcium chloride and aluminium chloride for cross-linking with the microspheres formed by sodium alginate and gellan gum.

**MATERIALS AND METHODS**

**Materials required:** Diclofenac sodium was obtained from Yarrow Chem. Mumbai; Maleic Anhydride, Aluminium Chloride, anhydrous Calcium Chloride and Sodium alginate were
Study of physical interaction between drug and excipients
Differential Scanning Calorimetry (DSC) thermograms were taken by scanning the samples of (i) pure drug (diclofenac sodium), (ii) pure excipients (gellan gum and sodium alginate) (iii) formulation (microsphere) using DSC (Pyris Diamond TG/DTA, PerkinElmer, SINGAPORE) in nitrogen atmosphere (150ml/min). Platinum crucibles were used with alpha alumina powder as reference.

METHOD OF PREPARATION OF MICROSPHERES

The diclofenac sodium-locaded microsphers were prepared by incorporating in a polymer matrix of sodium alginate and gellan gum. The polymer matrix was cross-linked with various combinations of cross-linking agents, like maleic anhydride, aluminium chloride and calcium chloride. The ingredients were taken as per the formula given in table-1. Sodium alginate, 500mg, was dissolved in 40ml of warm distilled water with the help of a magnetic stirrer. Gellan gum, 500mg, was dissolved in 40ml of pre-warmed (50°C) distilled water with a magnetic stirrer. Finally both the solutions were mixed with stirring at 300rpm until the polymer mixture become homogeneous and bubble-free. In separate beaker diclofenac sodium, 500ml, in 20ml distilled water was dissolved and the solution was mixed with the polymer mix solution (80ml) by stirring with a magnetic stirrer. The drug-polymer mixture solution was added dropwise with a pipette in an aqueous solution (70ml) of cross-linking agents. After 20 minutes of curing, the microspheres were strained, washed thoroughly with distilled water to remove any trace of crosslinking agent from the surface of the spheres. The microspheres were collected on a petri-dish and air dried under room temperature overnight.

Table 1: Compositions of different formulations of Diclofenac microsphere.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Sodium Alginate (%w/v)</th>
<th>Gellan Gum (%w/v)</th>
<th>Diclofenac Sodium (%w/v)</th>
<th>Maleic Anhydride (%w/v)</th>
<th>Aluminium Chloride (%w/v)</th>
<th>Calcium Chloride (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>F3</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>F4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>F5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>F6</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>F7</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Surface morphology analysis\cite{11}

Surface morphology is the test to check the surfaces of the microspheres to examine the pores and predict the types of release the particle is supposed to exhibit. Analysis was done by scanning electron microscope (SEM). The microspheres were gold coated and mounted on a brass stub using double sided adhesive tape and under vaccum in an iron sputter with thin layer of gold (3-5 nm) for 75seconds and at 18 KV to make them electrically conductive and their morphology examined.

Particle size determination\cite{11, 12}

The size of the particles was measured with the help of an optical microscope. An ocular micrometer was previously attached and the particles were placed on a slide and their size was measured. After collection of data, the data was divided in size ranges and the frequency was calculated by this formula:

\[
\text{frequency (\%)} = \frac{\text{Number of particles in each size range}}{\text{Total number of particles}} \times 100
\]

Determination of percentage yield\cite{11, 13}

The amount of polymers and the drug incorporated was accurately weighed and recorded. This was taken as the theoretical weight. The total amount of microspheres obtained from each formulation by weighing accurately using a digital balance and recorded. This was taken as the practical weight. Percent yield was calculated by taking the ratio of theoretical and practical weight and is given by the following formula.

The percent yield was calculated by the following formula:

\[
\text{Percent yield} = \frac{\text{Weight of microspheres recovered finally}}{\text{Total weight of drug and polymers taken initially}} \times 100
\]

Drug entrapment efficiency\cite{12, 13}

Drug entrapment efficiency is the amount of drug physically entrapped by the polymer in the microspheres. From the formulations, 100 mg was accurately weighed and triturated adding few drops of water to form a paste. Finally it was diluted to 10 ml. The solution was filtered, and checked for absorbance in the UV-Vis spectrophotometer at 275 nm. Entrapped drug is calculated by this formula:

\[
\text{Entrapment efficiency (\%)} = \frac{\text{Actual drug content in microsphere}}{\text{Theoretical drug content in microspheres}} \times 100
\]
Swelling behaviour\textsuperscript{[12, 13]}

Swelling behaviour was observed in dissolution apparatus type 1 in phosphate buffer 6.8 at a temperature of 37 ± 1°C under 50 rpm. 100 mg of the formulated microspheres were placed in the basket and rotated for a fixed time period. The microspheres were removed after the fixed time period and weighed after drying the surface on a tissue paper.

Swelling index is given by:

\[
Swelling\ index = \frac{Weight\ of\ microspheres\ after\ swelling - Dry\ weight\ of\ microspheres}{Dry\ weight\ of\ microspheres} \times 100
\]

In vitro release studies\textsuperscript{[14]}

In vitro release studies were carried out in USP dissolution apparatus type 1 which is the rotating basket type. The microspheres were placed in the basket and immersed in the dissolution media containing 500 ml of phosphate buffer 6.8. The basket was rotated at 50 rpm and temperature was set at 37 ± 0.5°C. At intervals of 15 minutes, 5 ml aliquots were withdrawn and replenished by 5 ml fresh media. The samples were diluted ten times by phosphate buffer of pH 6.8. The diluted samples were checked for absorbance using UV-Vis spectrophotometer at 275 nm.

RESULTS AND DISCUSSION

Differential Scanning Calorimetry

The pre formulation study of drug-excipient interaction was carried out by DSC, which showed no interactions of the drugs and excipients. The melting point of the pure Diclofenac sodium was found from the peaks of DSC thermogram. The results are shown on fig. 1.

![DSC curve of comparison of above three curve.](image)
**In vitro release studies**

*In vitro* release studies results are shown fig. 2. *In vitro* release studies were carried out for the formulations of microspheres containing diclofenac sodium as drug and gellan gum and sodium alginate as polymer, cross-linked with cross-linking solutions of maleic anhydride, aluminium chloride and calcium chloride in various ratios in phosphate buffer 6.8 for 6 hours (viz table 1 and fig 2). Drug released immediately from few formulations while a few formulations showed a lag phase in the release pattern. Formulations F2, F4 and F7 showed significant lag phase up to 45 minutes while the remaining formulations released immediately. The probable cause of immediate release at the beginning might be due to surface drug that was deposited during drying of the microcapsules. The lag phase indicates that better cross-linking and drug entrapment resulting in sustained activity.

Microspheres prepared with 1% maleic anhydride [F1] was found to sustain the drug release for a period of 4.5 hours, while drug release of the formulations were found to be moderate for formulations having cross-linked with only 1% calcium chloride and combination of 1% maleic anhydride and 1% calcium chloride [F3 and F6]. Better cross-linking was observed in the formulations cross-linked with 1% aluminium chloride, combination of 1% aluminium chloride and 1% calcium chloride, combination of 1% maleic anhydride and 1% aluminium chloride and combination of 1% calcium chloride and 1% maleic anhydride and 1% aluminium chloride [F2, F5, F7 and F4].

Maleic anhydride alone was insufficient as a cross-linking agent having sustained action for a short period [F1], but combining maleic anhydride with aluminium chloride and calcium chloride improved the results. Approximately 50% of drug was released by the formulation cross-linked by aluminium chloride [F2] over a period of 6 hours while formulations cross-linked by combinations was found to release approximately 60% over a period of 6 hours [F7, F5, F4] and approximately 70% of the drug was released by the formulation cross-linked by the calcium chloride and 65% of the drug was released for the formulation cross-linked by maleic anhydride and calcium chloride.

Thus it can be safely said that the drug diclofenac sodium exhibited sustained activity by cross-linking and esterification with aluminium chloride [F2] while esterification was poor for formulation cross-linked by maleic anhydride [F1] and moderate for calcium chloride cross-linked formulation [F3].
Fig 2: Release profile of F1 to F7 of Diclofenac microsphere.

Yield and Entrapment Efficiency

The percent yield and percent entrapped is shown in table 2. Percent yield was found to be the highest in formulation cross-linked with aluminium chloride [F2]. The yield of microspheres improved with the incorporation of aluminium chloride in formulations containing maleic anhydride and calcium chloride. Nearly 100% yield was found in aluminium chloride cross-linked microspheres [F2] and 75% for maleic anhydride cross-linked microspheres [F1] and 80% in calcium chloride cross-linked microspheres [F3]. Incorporation of aluminium chloride in formulations F1 and F3 as cross-linking agent increased the percent yield of the microspheres, while yield was reduced in formulation that did not use aluminium chloride as cross linking agent [F6]. The incorporation of all the three cross-linking agents in formulations resulted in a fair and moderate percent yield.

Drug entrapment in the formulations was found to be fairly good, the maximum entrapment being exhibited by the formulation cross-linked by aluminium chloride [F2]. The formulation cross-linked by the combination of all three cross-linking agents also showed good entrapment efficiency. The probable reason for the high entrapment efficiency of formulation F2 might be due to cross-linking reaction of aluminium chloride and diclofenac sodium. Due to strong bonding of the drug and polymer and counter-ion poymers, entrapment of the drug in the polymer matrix is high.
Table 2: Percentage Entrapped efficiency & yield of F1 to F7.

<table>
<thead>
<tr>
<th>Code</th>
<th>% Entrapped</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>87.6</td>
<td>75.3</td>
</tr>
<tr>
<td>F2</td>
<td>98.1</td>
<td>100.7</td>
</tr>
<tr>
<td>F3</td>
<td>92.3</td>
<td>80.0</td>
</tr>
<tr>
<td>F4</td>
<td>81.4</td>
<td>83.3</td>
</tr>
<tr>
<td>F5</td>
<td>87.2</td>
<td>93.3</td>
</tr>
<tr>
<td>F6</td>
<td>91.2</td>
<td>78.7</td>
</tr>
<tr>
<td>F7</td>
<td>93.5</td>
<td>87.3</td>
</tr>
</tbody>
</table>

Swelling Indices

Swelling behaviour of the formulations was recorded and data as plotted is shown in fig 3. From fig, it is evident that maximum swelling property is exhibited by the formulation cross-linked by maleic anhydride and calcium chloride [F6] and least swelling by formulation cross-linked by aluminium chloride [F2]. It is also noticeable that the percentage of swelling increased gradually with increasing cross-linking agent concentrations. Swelling of formulations cross-linked by aluminium chloride and maleic anhydride [F4] is greater than individual swelling percentages of aluminium chloride and maleic anhydride. The same is exhibited for combined cross-linked formulation of maleic anhydride and calcium chloride [F6]. However the swelling rate was found to exhibit a decrease in the formulation cross-linked by maleic anhydride, aluminium chloride and calcium chloride. This might be due to the hard gel strength and rigid bonding of the cross-linked polymers.

![Fig 3: Swelling behaviour of microsphere containing diclofenac.](image-url)
Microscopic Analysis

Microscopic analysis is plotted as a graph and presented in fig 4. Microscopic analysis of diclofenac sodium microspheres reveals that the size range of diclofenac sodium microspheres lies between 60 $\mu$m to 180 $\mu$m. Microspheres cross-linked by 1% w/v aluminium chloride [F2] exhibited maximum frequency of size in 140 $\mu$m to 150 $\mu$m. Microspheres cross-linked by 1% aluminium chloride and calcium chloride [F5] exhibited maximum frequency of size range in 120 $\mu$m to 130 $\mu$m. Microspheres cross-linked by 1% maleic anhydride, aluminium chloride and calcium chloride exhibited maximum size range in 150 $\mu$m to 160 $\mu$m and 170 $\mu$m to 180 $\mu$m. From this analysis, it can be inferred that frequency becomes maximum from size range 120 $\mu$m to 180 $\mu$m as the ratio of counter-ion increases.

![Microscopic Analysis Graph](image)

Fig 4: Comparative particle size distribution of various gellan gum/ sodium alginate microsphere.

SEM Study

SEM study image of F2 and F7 is shown in fig 5. From the SEM analysis of two formulations F2 and F7; F2 being the microspheres cross-linked by only 1% aluminium chloride and F7 being the microspheres cross-linked by 1% maleic anhydride, 1% aluminium chloride and 1% calcium chloride, it is evident that the size of the microspheres largely depends on the concentration of the cross-linking agent polymer added for formulating microspheres. The size of the microspheres increases and improves on increasing the cross-linking agent polymer in formulations.
CONCLUSION

This project was conducted to exploit the activity of diclofenac sodium as a model drug by attempting to prolong its release for a longer period of time. After the formulation of microspheres, they were evaluated to estimate their particle size distribution, swelling, yield and entrapment and release. From this experiment, we have come to know that the formulation with aluminium chloride as a cross linker or F2 is the better formulation among the rest of the formulations. Swelling of F2 was least while the entrapment and yield percent was the maximum. A 99% of yield and entrapment efficiency was recorded by the formulation F2. Release profile of the formulations revealed the sustained design of the drug, particularly formulation F2 releasing approximately 50% over 6 hours. It was also observed that on increasing the cross-linking agent solutions, the release rates were also improved, ranging within 55% to 60% approximately. Size analysis revealed that the maximum frequency of microspheres lied in the range between 120 μm to 180 μm as counter-ion ratio increases. Therefore, long term stability study and clinical trial is required for future development of this dosage form.

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