Evaluation of Formulation Parameters for Bromelain Microspheres Using Pitaya Mucilage Loaded Topical Gel

Keywords: Osteoarthritis, Bromelain, Dragon fruit mucilage, Natural polymer

ABSTRACT

Arthritis is a general term that means inflammation of the joints. Bromelain, an enzyme has proved for its anti-inflammatory effect and its profound activity in the topical treatment of osteoarthritis. The present study is based on the preparation and evaluation of microspheres using Bromelain and a natural polymer, extracted from the mucilage of dragon fruit using the solvent evaporation method and further various formulations of these microspheres were evaluated for its particle size, micromeritic properties, percent drug release. Further, the optimized formulation of microspheres was fabricated in the form of a topical gel, which was evaluated for its spreadability, swelling index study, in–vitro diffusion studies. Based on this we have concluded that biopolymer used in encapsulating microspheres loaded as topical gel controls the release of drugs for a longer period which will help to avoid the symptomatic relief of post-viral osteoarthritis and also reduces the cost of therapy.
INTRODUCTION:

Osteoarthritis has been often been a nightmare to the majority of the population especially old aged people. The same has also impacts on the physical life of a person once he/she is affected by osteoarthritis. There are varieties of the dosage forms that have been or are being utilized to cure the same. However, still, drug delivery aspects are being deployed to make the therapy more efficient. In the clinical management of arthritis, topical formulations are preferred because of the ease associated with their application [1-3]. Bromelain is a protease enzyme derived from the stems of pineapples Bromelain holds potential therapeutic effect as a treatment of conditions including angina pectoris, bronchitis, sinusitis, surgical trauma, and Osteoarthritis [4].

Natural excipients are very popular, they are inexpensive, non-toxic, and easily available. They have been used by researchers for the development of many dosage forms In the present, study, an attempt was done to explore the potential use of mucilage extracted dragon fruits as a natural polymer in the preparation of microspheres, These natural gums and mucilages are preferred over the synthetic ones because they are biocompatible, cheap, and easily available than the synthetic ones. Also, the natural excipients are preferred on the synthetic and semisynthetic ones because of their lack of toxicity, low cost, soothing action, availability, and non-irritant nature of the excipients [5]. The mucilaginous material in its flesh tissue which envelopes surrounding the seeds of Dragon fruit (Hylocereus species) is used as a biopolymer in the present study.

Microspheres are solid spherical particles ranging in size from 1-1000µm. These are free-flowing powders in which the drug is encapsulated by polymer [6]. Microspheres containing Bromelain were prepared by a solvent evaporation method using various concentrations of pitaya mucilage and hydroxyl propyl methylcellulose.

Skin is one of the most readily accessible organs on the human body for topical drug delivery and constitutes the chief route for topical application. External application of gel at skin offers certain visible advantages like the quick release of the drug directly to the site of action, independent of water solubility of the drug, as compared to creams and ointments. The gel systems may be clear as water or turbid because the ingredients may not be entirely molecularly dispersed (soluble or insoluble) or they may produce aggregates, which disperse
light. So in the present study microspheres are loaded in the form of gel for the topical treatment [7-9].

MATERIALS AND METHODS:

MATERIALS:

Bromelain procured from Vital herbs. Hydroxypropyl methylcellulose, Dichloromethane, Carbapol, Methylparaben, Triethanolamine, Polyethylene glycol, Ethanol were obtained from college laboratory.

METHODS:

Methodology: Extraction of mucilage from dragon fruit

The fresh Dragon fruits were collected and washed with water. They were crushed and soaked in water for 6 hours, boiled for 30 minutes and left to stand for 1 hour to allow the complete release of the mucilage into the water. The mucilage was extracted using a muslin cloth bag to remove the marc from the solution Ethanol (in the volumes of three times to the volume of filtrate) was added to precipitate the mucilage [10]. The mucilage was separated, dried in an oven at 40°C, collected, ground, passed through a # 80 sieve and stored in a desiccator at 30°C & 45% relative humidity till use.

Physicochemical Evaluation of extracted mucilage from dragon fruit:

Physical Description

The mucilage is visually examined for its appearance and odour.

Solubility

A required quantity of mucilage was taken and added to the warm water to check the solubility of mucilage with water. A required quantity of mucilage was taken and checked its solubility in inorganic solvents like methanol and chloroform, dichloromethane.
pH of Mucilage:

Pitaya mucilage sample (5 g) was weighed in triplicate in a beaker, mixed with 20 ml of distilled water, the resulting suspension stirred for 5 minutes and the pH was measured using a calibrated pH meter.

Loss on Drying

Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100˚c or 105˚c, until two consecutive weigh does not differ by more than 0.5mg, cool in desiccators and weigh [11].

\[
\text{Loss on drying (\%) = 100 \left(\frac{Y_i - Y_f}{Y_i}\right)}
\]

Where \(Y_i\) = initial weight of mucilage

\(Y_r\) - final weight after drying.

Micromeritic Properties [12]:

Angle of Repose determination:

This was determined by fixed funnel method by placing the funnel at fixed height of 2 cm and the sample is allowed to flow through funnel to form the heap, now mark the radius of the heap and angle o repose is determined using the following formula

\[
\tan \theta = \frac{H}{R}
\]

Bulk density & Tapped density:

Using a 100ml capacity measuring cylinder and fifty gram of obtained mucilage the bulk and tapped volume of mucilage were determined. Bulk and tapped density of obtained mucilage were calculated using

\[
BD = \frac{50}{BV} \quad \text{&} \quad TD = \frac{50}{TV}
\]

Carr’s Index and Hausner Ratio Determination:

Data values obtained from bulk density and tapped density from BD and TD above were used to calculate the Carr’s index and Hausner ratio.
Carr’s index = Compressibility index = 100 x (TD-BD)

TD Hausner ratio = TD / BD

Pre-formulation studies

Drug-polymer interaction studies by FTIR:

Drug polymer compatibility studies were performed by FTIR (Fourier transform infrared spectroscopy).

Construction of calibration curve

An accurately weighed 100 mg of bromelain is dissolved in pH 6.2 Phosphate buffer and make up the volume up to 100 ml in a volumetric flask (Stock solution: I mg/ml). From this 10 ml of solution was pipette out and make up the volume up to 100 ml (Stock solution: II), 100 µg/ml [13]. The aliquots were prepared by taking 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml and 3ml and dilute to 10ml whose concentration ranging from 5-30 µg/ml and the absorbance was measured at 280 nm by using UV spectrophotometer, against the blank.

Composition and formulation of microspheres by solvent evaporation method:

Polymer (hydroxyl propyl methyl cellulose) was dissolved in 10 ml of Dichloromethane and this solution is slowly introduced into 10ml of various concentrations of mucilage (0.5 %, 1 %, 1.5 %, 2% and 2.5% while being stirred at 700 rpm by a magnetic stirrer at room temperature The solution was stirred for 3 hrs to allow the solvent to evaporate completely and the microspheres were filtered and dried for 1hr at room temperature and stored in desiccators.

Table No. 1: Composition table of Microspheres

<table>
<thead>
<tr>
<th>Code</th>
<th>Drug</th>
<th>HPMC</th>
<th>Pitaya mucilage</th>
<th>Dichloromethane</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100mg</td>
<td>100mg</td>
<td>0.5g</td>
<td>10ml</td>
<td>10ml</td>
</tr>
<tr>
<td>F2</td>
<td>100mg</td>
<td>100mg</td>
<td>1.0g</td>
<td>10ml</td>
<td>10ml</td>
</tr>
<tr>
<td>F3</td>
<td>100mg</td>
<td>100mg</td>
<td>1.5g</td>
<td>10ml</td>
<td>10ml</td>
</tr>
<tr>
<td>F4</td>
<td>100mg</td>
<td>100mg</td>
<td>2.0g</td>
<td>10ml</td>
<td>10ml</td>
</tr>
<tr>
<td>F5</td>
<td>100mg</td>
<td>100mg</td>
<td>0.25g</td>
<td>10ml</td>
<td>10ml</td>
</tr>
</tbody>
</table>
Physicochemical Evaluation

Particle Size and shape:

Particle size and shape of microspheres were evaluated using optical microscopy method.

Entrapment Efficiency

The capture efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The percent encapsulation efficiency is calculated by

\[
\% \text{ Entrapment} = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100
\]

Drug content

Take 100 mg of microspheres and dissolved in ethanol by stirring. Filter the contents by Whatman filter paper and the filtrate is analyzed by UV spectrophotometer for drug content against lank at 280 nm.

\[
\text{Drug content} = \text{concentration of drug} \times \text{volume of the medium} \times \text{dilution factor}.
\]

In-vitro Drug release

The dosage form is placed in the beaker containing the medium and stirred uniformly using stirrer with 900 ml of medium and 300 rpm speed.

Preparation of Bromelain gel containing Microspheres

The best formulation of microspheres was selected to prepare gel. The formulated microspheres were uniformly dispersed in the gel base by mechanical stirring for 30 minutes to get microspheres loaded gel. The prepared gels were packed and stored [14].
Table No. 2: List of Ingredients for Gel

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Prepared microspheres</td>
<td>0.1g</td>
</tr>
<tr>
<td>2.</td>
<td>Carbopol 934</td>
<td>1.5 g</td>
</tr>
<tr>
<td>3.</td>
<td>Propylene glycol</td>
<td>15 ml</td>
</tr>
<tr>
<td>4.</td>
<td>Polyethylene glycol</td>
<td>5 ml</td>
</tr>
<tr>
<td>5.</td>
<td>Ethanol</td>
<td>10 ml</td>
</tr>
<tr>
<td>6.</td>
<td>Triethanolamine</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>7.</td>
<td>Methylparaben</td>
<td>0.08 g</td>
</tr>
<tr>
<td>8.</td>
<td>Distilled water</td>
<td>Qs to 100 ml</td>
</tr>
</tbody>
</table>

Evaluation of Gel:

Appearance of gel

The prepared gels were inspected visually for clarity, colour, and presence of any particle. The test is important regarding patient compliance.

pH

Measurement of pH for all the formulations was done by dissolving one gram of formulation in 100ml distilled water for 2hrs and pH was measured by digital pH meter.

Drug content

One gram of gel was taken and dissolved completely in phosphate buffer of 7.4 pH made up the volume to 50ml by phosphate buffer and withdrawn 1, 2, and 3ml of the stock solution and made up the volume to 10ml with the phosphate buffer of the same pH. Then it was analyzed by validated UV spectrophotometric method at $\lambda_{max}$ of 280nm.

Drug content = concentration of drug X volume of the medium X dilution factor.

Spreadability

The spreadability of the formulations was determined by an apparatus suggested by Multimer, which was fabricated itself in the laboratory and used for slide fixed on the...
wooded block and upper slide with one end tied to a glass slide and other end tied to weight pan. Excess of gel (2gm) was placed between two glass slides and then 100gm weight was placed on slides for 5mins to compress the sample to a uniform thickness. Weight in the increasing order i.e. 20gm, 40gm, 80gm was added to pan [15,16]. The time (sec) required to separate the two slides was taken as a measure of spreadability. It was calculated using the below formula:

\[ S = \frac{ML}{T} \]

\( M \) = Weight tied to the upper slide; \( L \) = Length of glass slide moved.; \( T \) = Time taken (sec).

Shorter time interval to cover the distance of 6.5cm indicates better Spreadability.

![Spreadability Test](image)

**Figure No. 1: Spreadability Test**

**Viscosity**

The viscosity of the gels prepared was determined using Brookfield Viscometer. The sample is filled in the sample holder and a particular spindle No.64 inversed at 50rpm.

**In-vitro diffusion study**

One gm gel equivalent to 100mg of the drug spread uniformly on the surface of cellophane membrane and was fixed to the one end of double side open beaker and the whole assembly was fixed in such a way that the low end of the tube containing gel was just touched the surface of diffusion media i.e. 50ml phosphate buffer of pH of 7.4, and maintained at 37°C [17].
RESULT AND DISCUSSION:

Physiochemical Evaluation of extracted mucilage from dragon fruit:

Visual appearance:

Colour: pale yellow to brown in colour & Odour: Characteristics

Solubility:

The order of solubility of the mucilage in solvents is as follows

Water > ethanol > dichloromethane > methanol > chloroform

pH of Mucilage:

The pH was measured using a calibrated pH meter and the average pH of Mucilage was found to be 5.54, which is nearer to the skin pH.

Loss on Drying

LOD of the mucilage was found to be 10.72 % indicating the hygroscopic nature of the extracted mucilage and thus needs to be stored in an airtight container.

Micromeric Properties

Table No. 3: Micromeric Properties

<table>
<thead>
<tr>
<th>True density (gm/ml)</th>
<th>Bulk density (gm/ml)</th>
<th>Carrs index %</th>
<th>Angle of repose °</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.87</td>
<td>0.72</td>
<td>0.38</td>
<td>27.13</td>
</tr>
</tbody>
</table>

Drug-polymer interaction studies by FTIR:

The following are the FTIR graphs indicating there is no incompatibility between Bromelain, pitaya mucilage, and other excipients.
Figure No. 2: Bromelain + EC + Mucilage

Figure No. 3: Bromelain + Mucilage

Figure No 4: FTIR Spectra of Bromelain + Ethylcellulose + Pitaya Mucilage + Carbopol
Construction of calibration curve:

Calibration curve was constructed for Bromelain, the value of $R^2 = 0.993$ which shows a linear relationship between the concentration and absorbance [18].

![Figure No. 5: Calibration curve of Bromelain](image)

Physicochemical Evaluation of Microspheres:

Microspheres are spherical and the mean particle size is less for F4 formulation. The drug content was increased with the ratio of the mucilage. The drug content was maximum with F4 formulation, which indicates that the increased particle size also favors the drug loading capacity [19]. The increase in entrapment efficiency was achieved by increasing the mucilage ratio, from 0.5% to 2%. With further increase in the ratio of the mucilage from 2% to 2.5%, a decrease in entrapment efficiency was observed.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (µm)</th>
<th>Entrapment efficiency %</th>
<th>Drug content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>107</td>
<td>15.97</td>
<td>44.9</td>
</tr>
<tr>
<td>F2</td>
<td>173.5</td>
<td>27.7</td>
<td>47.8</td>
</tr>
<tr>
<td>F3</td>
<td>180</td>
<td>27.27</td>
<td>52.9</td>
</tr>
<tr>
<td>F4</td>
<td>92</td>
<td>32.43</td>
<td>73.7</td>
</tr>
<tr>
<td>F5</td>
<td>235</td>
<td>7.78</td>
<td>48.4</td>
</tr>
</tbody>
</table>
Drug release studies:

All the formulations exhibited a biphasic release pattern in which about 70 % of the drug was released within 3 hrs (F4) to 7 hrs (F5). This burst release will be quiet effective in achieving the minimum effective concentration whereas the remaining 40 % was released slowly and this may help to maintain the plasma drug concentration within the therapeutic range.

Table No. 5: *In-vitro* drug releases of Bromelain from Microspheres

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Time (Hrs)</th>
<th>% Drug Release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>22.4</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>37.4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>43.4</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>54.4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>72.8</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>77.5</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>85.3</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>94.4</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>98.2</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Based on the evaluation parameters F4 was selected as an optimized formulation containing 2.0 % of pitaya mucilage and hence F4 microspheres were loaded as a topical gel.

**Figure No. 6: In-vitro drug release of Bromelain from microspheres**
Evaluation of prepared topical gel:

The gel was clear and homogenous in consistency and pH is nearer to skin pH and did not produce any irritation when applied on the skin. Viscosity and spreadability were excellent such that the gel can be easily applied on the skin with the ease of spreading.

Table No. 6: Evaluation of prepared topical gel of Bromelain

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Appearance</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>7.3</td>
</tr>
<tr>
<td>3</td>
<td>Spreadability</td>
<td>12.74 g.cm/sec</td>
</tr>
<tr>
<td>4</td>
<td>Viscosity</td>
<td>83 CPS X 10³</td>
</tr>
<tr>
<td>5</td>
<td>Drug content</td>
<td>94.1 %</td>
</tr>
</tbody>
</table>

*In-vitro* drug release studies:

Drug release by diffusion studies showed that percentage release was found to be 83.77 at the end of 10 hrs as shown in the following table. This shows that a more sustained release was observed and represented in the following graph.

Table No 7: *In-vitro* drug release studies of Bromelain from gel.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Time ( Hrs)</th>
<th>% Drug release of Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>19.45</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>30.33</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>39.54</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>45.75</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>58.72</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>74.34</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>71.7</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>78.57</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>83.02</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>83.77</td>
</tr>
</tbody>
</table>

CONCLUSION:

Natural excipients are very popular, they are inexpensive, non-toxic, and easily available. They have been used by researchers for the development of many dosage forms. In the present study, an attempt was done to explore the potential use of mucilage extracted from dragon fruits as a natural polymer in the preparation of microspheres. From the in-vitro diffusion study, we have concluded that gel prepared from natural polymer controls the release of drugs for a longer period which will help to avoid the symptomatic relief of post-viral osteoarthritis and also reduces the cost of therapy.

From the above study, we have concluded that it is possible to design a topical gel loaded microspheres prepared from the natural polymer dragon fruit mucilage having good spreadability. So the topical gel prepared from natural polymer will be a great idea for making an ideal topical preparation. However, in vivo experiments are essential to establish the actual usefulness of these microspheres.

ACKNOWLEDGEMENT:

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Figure No. 7: In-vitro drug release of Bromelain from gel
REFERENCES: