INTRODUCTION

Many active core components such as antioxidants, flavors, perfumes are lipophilic substances available in liquid form. Microencapsulation of such lipophilic substances is a means of formulating a liquid component in a solid dosage form thereby facilitating its handling, enhancing its stability and/or sustaining its release. Such microencapsulated components can then be incorporated in cosmetic, dermatological or feed supplements. The present research work focuses on formulation of Microspheres of Vitamin E (model lipophilic drug) using a combination of two natural polymers viz: Pectin and sodium Alginate such that these microspheres would be free flowing, hard and intact in dry state but on incorporation in a cosmetic topical gel would swell to their optimum capacity. During application these microspheres would rupture when rubbed between the palms, thereby releasing vitamin E. The technique used for formation of microspheres was Ionotropic gelation. The microspheres were prepared using pectin alone and pectin and alginate together in different ratios such as 5:0.5, 5:1.0, 5:1.5, 5:2.0 and 5:2.5. Of all the microspheres prepared it was found that those microspheres with pectin and alginate in a ratio of 5:1.5 respectively confirmed best to the desired objective and hence these were evaluated further for percent yield, average particle size, shape, surface topography, water content and swelling index. Thus from the study it can be concluded that a combination of Pectin & alginate in the ratio (5:1.5) has wide potential for formulating microspheres for cosmetic application.

Keywords: Pectin Alginate Microspheres, Lipophilic drugs, Ionotropic gelation, cosmetic application.

Vitamin E loaded pectin alginate microspheres for cosmetic application

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ABSTRACT

Microencapsulation is a technology of packaging core materials (solids, liquids or gases) within a suitable polymeric membrane which may be of natural or synthetic origin resulting in products that find wide application in Pharmaceutical, Cosmetic and Food Industry. Many active core components such as antioxidants, flavors, perfumes are lipophilic substances available in liquid form. Microencapsulation of such lipophilic substances is a means of formulating a liquid component in a solid dosage form thereby facilitating its handling, enhancing its stability and/or sustaining its release. Such microencapsulated components can then be incorporated in cosmetic, dermatological or feed supplements. The present research work focuses on formulation of Microspheres of Vitamin E (model lipophilic drug) using a combination of two natural polymers viz: Pectin and sodium Alginate such that these microspheres would be free flowing, hard and intact in dry state but on incorporation in a cosmetic topical gel would swell to their optimum capacity. During application these microspheres would rupture when rubbed between the palms, thereby releasing vitamin E. The technique used for formation of microspheres was Ionotropic gelation. The microspheres were prepared using pectin alone and pectin and alginate together in different ratios such as 5:0.5, 5:1.0, 5:1.5, 5:2.0 and 5:2.5. Of all the microspheres prepared it was found that those microspheres with pectin and alginate in a ratio of 5:1.5 respectively confirmed best to the desired objective and hence these were evaluated further for percent yield, average particle size, shape, surface topography, water content and swelling index. Thus from the study it can be concluded that a combination of Pectin & alginate in the ratio (5:1.5) has wide potential for formulating microspheres for cosmetic application.

Keywords: Pectin Alginate Microspheres, Lipophilic drugs, Ionotropic gelation, cosmetic application.

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tio of Pectin: alginate was optimized to achieve the desired objective. The selected microspheres were evaluated for percent yield, water content, swelling index, average particle size, shape, surface topography, and other micromeretic properties.

**MATERIALS AND METHODS**

Vitamin E (dl - α- tocopherol acetate) was purchased from Sigma Aldrich, Bangalore. Tween 80, Calcium chloride, Pectin, Sodium alginate, were procured from Research lab, Pune. Allantoin, Carbopol 940, Triethanolamine, brilliant blue CI No. 42090, F17 IDACOL, Apple green FCF were purchased from Loba chemicals, Pune. Aloe Vera gel was purchased from Ayurved Rasashala, Pune. All other reagents used were of A.R. grade.

**Formulation of Pectin/Pectin-Alginate Microspheres using Ionotropic Gelation Technique**

Microspheres containing Vitamin E were prepared using pectin alone and pectin alginate in different ratios as depicted (Table 1). The formulation process consists of preparing a 5% w/v solution of Pectin in distilled water containing 10% v/v of glycerol as dispersing aid. Solution of sodium alginate in distilled water was prepared depending upon the formulation to be made. The two solutions were then mixed under continuous agitation to form a homogenous solution of the two polymers. 0.1% w/v of Tween 80 and Brilliant blue dye (C.I. No. 42090) was added till a deep blue colour homogenous mixture (Mix I) was obtained. 1% w/v Vitamin E was then added dropwise to the above mixture to obtain (Mix II). The homogenous mixture was subjected to further homogenization for a period of 1 hr on RQ 127 homogenizer to produce o/w emulsion. The stable emulsion was then dropped with (0.6mm × 25mm) 23 G needle into a 5% w/v calcium chloride solution. The microspheres obtained were further hardened by cross linking with epichlorhydrin for half an hour. The microcapsules were then thoroughly washed with ice cold water and air dried for 24 hours. A schematic representation of the process is depicted (Figure 1).

**Characterisation of Microspheres**

The microspheres prepared were then characterized for the following parameters.

1. **Particle Size, Shape and surface**

The obtained microspheres were evaluated for particle size using optical microscopy. The size of minimum 100 particles was measured using vernier callipers to determine the average particle size. The shape and surface morphology of micro spheres was determined using Scanning Electron microscopy (SEM) analysis using (Model JEOL-6360A, Japan). The samples for SEM were prepared by lightly sprinkling the microspheres on a double adhesive tape, which was stuck on to an aluminum stub. The stubs were then coated with gold to a thickness of 300 Å using a sputter coater and viewed under Scanning Electron Microscope. SEM Photomicrographs of suitable magnification were obtained.

2. **Estimation of % loading efficiency**

% Loading efficiency = Total % Vitamin E – Free % Vitamin E

(a) **Determination of Total % Vitamin E**

Vitamin E content in the microcapsules was determined using HPLC (Model: Thermofinnigan). The Chromatographic conditions maintained were as follows: Mobile phase: Methanol, Column : 250 mm × 4.6 mm, Peerless Basic C18, 5 micron Flow rate : 1.5 ml/min, Temperature : Ambient, Detector : UV at 284nm, Inj. Vol.: 100µl, Run time : 20 min

**Standard preparation:** 17.5 mg of Vitamin E std was dissolved in 25 ml methanol (0.7mg/ml)

**Sample Preparation:**

To 250 mg of microspheres, 5 ml distilled water was added and microspheres were crushed using mortar and pestle. 25 methanol was added to the solution and refluxed for 30 min. The solution was extracted with 2 x 25 ml portions of n-hexane and the combined hexane layer was collected over anhydrous sodium sulphate and evaporated to 5 ml on water bath. It was diluted to 25 ml with methanol and subjected to HPLC analysis. The determinations were made in triplicate.

(b) **Determination of Free % Vitamin E (Surface Content)**

50 mg of prepared microcapsules were weighed and dispersed in 50ml of methanol. This dispersion was agitated for 10 mins and then filtered. The filtrate was then subjected to HPLC analysis to estimate the content of vitamin E. The amount of vitamin E present was calculated as follows:

mg/gm of sample area/standard area x conc. of Std. Soln in mg x 25 x1/sample weight in mg

3. **Bulk Density, Tap density & Carr’s Index**

The bulk and tapped densities were measured as a measure of packability of the microspheres. Around 10gms of microspheres were taken in a 25 ml measuring cylinder and the volume was recorded as bulk Volume. The bulk density was then calculated as weight of Microspheres/bulk volume. The cylinder was tapped 100 times to determine the tapped volume and the tapped density. The Carr’s Index was then calculated as follows:

\[\text{Carr’s Index} = \frac{\text{Tapped Density} – \text{Bulk density}}{\text{Tapped Density}}\]

4. **Angle of repose**

The flow properties were investigated by measuring the angle of repose of Vitamin E loaded microspheres by fixed-base cone method. Around 10 gms of Microspheres were loaded into a dropping conical funnel and allowed to flow so as to form a pile. The radius of the pile of microspheres and the height of the cone was measured. The angle of repose was calculated as follows:

\[\text{Angle of Repose} = \tan^{-1} \left( \frac{h}{r} \right)\]

Where h = height and r = radius of the pile.
5. % yield
The % efficiency of a process was estimated as follows:
% Yield = (Theoretical yield – Practical Yield/Theoretical yield) X 100

6. Moisture Content
a) Neutralization of Methanol
Sufficient anhydrous methanol (about 20ml) was added to the titration vessel and titrated to the amperometric end point with the Karl Fischer agent to neutralize the water present in methanol.

b) Standardisation of Karl Fischer Reagent
10 µl (10mg) of distilled water was added to the titration vessel and titrated to the amperometric end point with the Karl Fischer reagent. The above titration was repeated thrice and the average factor for the Karl Fischer reagent was calculated by the following formula: For Distilled Water,
Factor = 10 mg/ Burette reading of Karl Fischer Reagent

c) Determination of Water content of the Sample
The prescribed amount of the substance to be examined was quickly transferred to the titration vessel after weighing and titrated to the amperometric end point with the Karl Fischer reagent. The water content of the sample was calculated by the following formula:
Water content = Burette reading of Karl Fischer reagent x Factor x 100/Weight of the sample in mg

7. Swelling Index
100 mg of microspheres were placed in water and allowed to swell up to constant weight. The microspheres were removed, blotted with filter paper and weighed. The degree of swelling (α) was then calculated from the formula.
\[ \alpha = \frac{W_g - W_o}{W_o} \]
Where Wo is the initial weight of the microspheres and Wg is the weight of the microspheres at equilibrium swelling in purified water.

Formulation of Topical Gel loaded with Vitamin E Microspheres
Of all the different formulations of pectin/pectin alginate microspheres studied only PA3 formulation was investigated further for incorporation in a topical gel. A General purpose Topical Gel was prepared (Table 2). The topical gel loaded with microspheres was investigated further to check the ability of microcapsules to rupture when the gel was rubbed between the hands prior to application.

RESULTS
The results obtained after characterization of the selected microspheres are depicted in table 3. The SEM photomicrographs of pectin alginate microspheres are depicted in figure 2.
Table 1: Composition of Pectin/Pectin-Alginate Microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>P</th>
<th>PA1</th>
<th>PA2</th>
<th>PA3</th>
<th>PA4</th>
<th>PA5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1.00%</td>
<td>1.00%</td>
<td>1.00%</td>
<td>1.00%</td>
<td>1.00%</td>
<td>1.00%</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
</tr>
<tr>
<td>Pectin</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Sodium Alginate</td>
<td>——</td>
<td>0.5%</td>
<td>1.0%</td>
<td>1.5%</td>
<td>2.0%</td>
<td>2.5%</td>
</tr>
<tr>
<td>Purified water q.s</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Brilliant blue C.I. No. 42090 q.s. was added to each formulation P: Pectin, PA: Pectin Alginate

Table 2: Composition of Topical Gel loaded with Microspheres

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E loaded pectin alginate (PA3) Microspheres</td>
<td>Around 500</td>
</tr>
<tr>
<td>Aloe Vera gel</td>
<td>50%</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.5%</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>0.3%</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.015%</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>q.s pH to 7.0</td>
</tr>
<tr>
<td>F17 Idacol Apple green FCF</td>
<td>q.s</td>
</tr>
<tr>
<td>Purified water</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3: Evaluation of Pectin/Pectin Alginate Microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>P</th>
<th>PA1</th>
<th>PA2</th>
<th>PA3</th>
<th>PA4</th>
<th>PA5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation Parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Average particle size (mm)</td>
<td>0.98</td>
<td>0.94</td>
<td>0.95</td>
<td>0.95</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td>Free % Vit E</td>
<td>0.03</td>
<td>0.031</td>
<td>0.035</td>
<td>0.036</td>
<td>0.034</td>
<td>0.032</td>
</tr>
<tr>
<td>% Encapsulation efficiency</td>
<td>80.8</td>
<td>81.4</td>
<td>84.0</td>
<td>82.1</td>
<td>79.5</td>
<td>80.3</td>
</tr>
<tr>
<td>Bulk density g/ml</td>
<td>0.50</td>
<td>0.44</td>
<td>0.41</td>
<td>0.42</td>
<td>0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>Carr’s Index</td>
<td>6.66</td>
<td>9.10</td>
<td>15.68</td>
<td>12.81</td>
<td>13.30</td>
<td>14.29</td>
</tr>
<tr>
<td>Angle of repose</td>
<td>21.4°</td>
<td>22.1°</td>
<td>23.6°</td>
<td>24.3 °</td>
<td>21.6 °</td>
<td>24.3°</td>
</tr>
<tr>
<td>% yield</td>
<td>91.50</td>
<td>92.10</td>
<td>93.10</td>
<td>92.50</td>
<td>90.80</td>
<td>92.30</td>
</tr>
<tr>
<td>Moisture content</td>
<td>4.67</td>
<td>4.69</td>
<td>3.28</td>
<td>4.45</td>
<td>3.25</td>
<td>3.88</td>
</tr>
<tr>
<td>Swelling index</td>
<td>1.01</td>
<td>1.11</td>
<td>1.26</td>
<td>1.38</td>
<td>1.47</td>
<td>1.55</td>
</tr>
<tr>
<td>Appearance</td>
<td>Disc shaped</td>
<td>Cube shaped near Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td>Spherical</td>
<td></td>
</tr>
</tbody>
</table>

Pectin solution
Blue Dye
Vitamin E
Mix I
Homogenized for 1 hr.
Mix II (RQ 127 homogenizer, Remi Motors O/W emulsion
Dropped with (0.6mm× 25mm) 23 G needle
5% w/v Calcium chloride solution for half an hour
Microspheres obtained
Cross linked further with Epichlorhydrin for half an hour
Washed with cold water & air dried for 24 hrs.
*Present only in Pectin Alginate Microspheres

Fig 1: Formulation of Pectin/Pectin Alginate Microspheres

Fig 2: SEM Photomicrographs of Pectin/Pectin Alginate Microspheres
tration from 0.5% w/v to 2.5% w/v it was found that the shape of the microcapsules changed from disc shape to spherical shape till the concentration of sodium alginate was increased to 1.5% w/v. After this increase in the concentration of sodium alginate did not significantly affect the shape of the microcapsules. The other parameters that were considered for evaluation such as % yield, % encapsulation efficiency, and micromeretic studies did not show any significant change with addition of sodium alginate or increase in the concentration of sodium alginate. Thus from the experiments done and the results obtained it can be concluded that Pectin alginate microspheres in the ratio 5:1.5 are most suitable for incorporation into a gel base. These microspheres may be loaded with vitamin E or any other antioxidants or with any fragrance in order to enhance stability of the contents and improve the effectiveness of the formulation.

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