Poly Electrolyte Complex of Chitosan Alginate for Local Drug Delivery

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Abstract: PEC film for local delivery of antifungal agent, clotrimazole which; reduces the frequency of application of formulation, improve patient compliance, reduce amount of drug administered, reduces irritation related to cream base formulation, adhere to the skin for the require duration. Solution of sodium alginate was added drop wise to chitosan solution under constant stirring, formed coacervates pour and dry to form film in Petri dish. Chitosan alginate ratio of 1:1.5 showed complete reactions (viscosity of supernatant closer to solvent viscosity) and having a good swelling property in both distilled water and buffer (pH 5). PEC film of chitosan and sodium alginate film can be used for sustained drug delivery of potent anti microbial and antifungal drugs or in more sophisticated means by formulating it as a transdermal patch

Keywords: Polyelectrolyte, Chitosan, Alginate, Local drug delivery

Introduction
In recent years biodegradable polymeric systems have gained importance in the designing of surgical device, artificial organs, materials for orthopedic application, drug delivery system with different route of administration, carrier for immobilization of enzyme and cells, as a biosensors and as ocular inserts. These polymers are classified as either synthetic or natural. Natural polysaccharides based polymers represents major class of biomaterials, which includes agarose, alginate, carrageenan and chitosan. Skin having a large surface area it is the one of the most common used site for the drug delivery. Polyelectrolyte Complex film is the film prepared out of the complexation of the two opposite charge polymers, here we were used chitosan in 2% lactic acid¹ as a cation and sodium alginate as anion.²,³,⁴ Both polymers are biodegradable and obtained from the natural origin and having a good bioadhesions⁵, which is necessary for the more retention over the skin. Chitosan can as such act as antibacterial and anti fungal activity⁶,⁷,⁸. Clotrimazole is used widely for treatment of cutaneous candidiasis, pityriasis, mild to moderate ringworm of glaborous skin and vulvo vaginal candidiasis. Major problems regarding cream base formulation of clotrimazole are; twice a day application and loss of the formulation in daily activities may occur.

The aim of present study was to design a formulation of PEC film for transdermal delivery of antifungal agent, clotrimazole which reduces the frequency of application of formulation, improve patient compliance, reduce amount of drug administered, reduces irritation related to cream base formulation, adhere to the skin for the require duration.

Material and method
Chitosan 221 was obtained form the Siber Hegner (Mumbai, India); sodium alginate powder and solvents used were of SD fine chemicals (Mumbai, India). Clotrimazole was received as gift sample from the Helios Pharmaceuticals Ltd (Kadi, Gujarat, India).

Preparation of Film
Coacervation between chitosan and alginate was rapid, but the rate may be controlled with the addition of water miscible organic solvents. Acetone was used a solvent moderator.¹⁰ Suspensions of fine, uniformly dispersed coacervates were produced by a drop wise addition of chitosan solution (solvent: 1: 1 v/v of 2% acetic acid and acetone) into sodium alginate solution in water under
rapid agitation. After this, the viscous polymeric coacervate was obtained to which propylene glycol was added as plasticizer. Accurately weighed quantity of drug, clotrimazole was added and stirred gently for 2 min to get uniform dispersion. The resultant coacervate gel was kept a side for sometime to remove air bubble and then it was casted into plastic petri dish of 5 cm diameter. Petri dish was kept into vacuum oven at 60°C for 24 hours drying. The completely dry film was stored in airtight container at room temperature.

**Formulation Conditions**

To get fine and uniform coacervate of chitosan-alginate, various process parameters like type of water miscible solvent addition, stirrer, speed of stirrer, time of reaction, temperature of drying, drying time, concentration of plasticizer, chitosan-alginate ration and drug to polymer ration were optimized based on quality of the complex coacervates obtained.

Various batches of complex coacervate were prepared by varying the parameters under study, like for selection of stirrer coacervates were prepared using both magnetic and three-blade stirrer. For selection of speed of batches were prepared at high (4000), medium (2000) and low (1000) speeds. Three batches were reacted for different time of 10, 20 and 30 min. Drying time and temperature was optimized by taking drying three batches at room temperature, at 60°C and at 70°C respectively. Different concentration of propylene glycol as plasticizer was studied i.e. 0.3%, 0.5% and 0.7%. Finally batches of films were prepared with different ratio of chitosan to alginate i.e. 1:1, 1:1.5, 1:2 and 2:1. Viscosity of the supernatant obtained after complexation of two polymers was optimized based on quality of the complex coacervates obtained.

To get fine and uniform coacervate of chitosan-alginate, various process parameters like type of water miscible solvent addition, stirrer, speed of stirrer, time of reaction, temperature of drying, drying time, concentration of plasticizer, chitosan-alginate ration and drug to polymer ration were optimized based on quality of the complex coacervates obtained.

**Preparation of Film**

For preparation of good film, it is desirable that the polyelectrolyte complex coacervate suspension, which is

**Tensile Strength**

The mechanical properties of chitosan alginate films were evaluated using a texture analyzer (Instron Universal Model) equipped with a 500 gm load cell. Film strip in 10 mm X 10 mm of dimension and free from air bubbles or physical imperfections, was cut from two clamps positioned at a distance of 1 cm. During measurement, the film was pulled by top clamp at a rate of 10mm/minutes. The force and elongation were measured when the films broke. Measurements were run four times for each film. The tensile strength and elongation at break were calculated as below:

\[
\text{Tensile strength (kg/mm}^2\text{)} = \frac{\text{Breaking force (kg)}}{\text{Cross-sectional area of sample (mm}^2\text{)}}
\]

\[
\text{Elongation at break ( % )} = \frac{\text{Increase in length at breaking point (mm)}}{\text{Original length (mm)}} \times 100\%
\]

**Content Uniformity**

To ensure uniform distribution of clotrimazole in film, a content uniformity test was performed. Sample represent different regions within film of 1X1 cm² section of the optimized chitosan alginate film were cut from four different locations and were crushed and dipped to solvent system of 9:1 of acetate buffer and PEG 400 for 24 hours at room temperature. Content was filter out and filtrate was analyzed for drug content by UV spectrophotometrically.

\[
\text{Content Uniformity} = \frac{\text{Actual amount of drug in film}}{\text{Theoretical amount of drug present in film}} \times 100\%
\]

**In Vitro Drug Diffusion**

*In vitro* drug diffusion, from chitosan alginate film was studied using modified franz diffusion cell with 2.52 cm² diffusion area and 20ml receptor volume. 1:9 ratio of PEG 400 to acetate buffer (pH 5) was used to maintain sink conditions in the receptor compartment. Human cadaver skin was used as diffusion membrane. At different time interval aliquots were withdrawn and replaced with fresh buffer. Absorbance was measured.

**Comparative diffusion studies**

For comparative diffusion studies diffusion of final film containing drug to polymer ratio of 1:4 and cream base marketed formulation was carried out using Franz diffusion cell. Accurately weighed quantity of cream that was equivalent to drug contained in diffusion area of film was taken for study.

For this rat skin was used as membrane. Male S D Rat was anesthetized and an area of 3 cm2 was clean shaved on dorsal side. Skin was carefully cut and washed with phosphate buffer pH-7.4 before use.

**Result and discussion**

**Preparation of Film**

For preparation of good film, it is desirable that the polyelectrolyte complex coacervate suspension, which is
cast to form the film, must be fine and uniform. The reaction rate of chitosan to alginate was very high, which can be controlled by addition water miscible solvent like acetone and methanol. Mixture of methanol and acetone was tried in different ratio with 2% lactic acid in preparation of chitosan solution respectively. i.e. 30:50, 50:50 and 30:70. Out of which acetone: lactic acid (2%) in 50:50 showed good and fine coacervate. Further more to obtained high quality of complex coervarvate slow drop wise addition of chitosan solution at high-speed three-blade stirrer (4000 rpm) for 30 minutes at room temperature was carried out. Propylene glycol in 0.5% was used to obtained flexible film and for good peeling of film.

**Polymer-To-Polymer Ratio**
Optimization of chitosan alginate ratio for preparation of complex coacervates was carried out based on the swelling ratio of prepared film in distilled water and acetate buffer solution, as the swelling state of the polymer was reported to be crucial for its release behavior and based on viscosity data of supernatant of complex coacervates to show complete reaction between chitosan and alginate. The viscosity of supernatant shown in table 1 shows that coacervates of 1:1.5 ratio of chitosan to alginate viscosity was nearly same of the solvent system used which indicates the complete reaction of the two polymers and thus the ratio was optimizes.

The swelling data obtained for different chitosan-alginate ratio in distilled water and acetate buffer pH 5 to PEG 400 (9:1) are shown in Fig 1, 2 respectively. Comparative swelling data (fig 1, 2) shows that for all the polymer ratios swelling continuously increased up to 8 hours and then slowly reached equilibrium level. Maximum swelling was observed in 1:2 of CH: SA ratio, while least swelling in 2:1 of CH: SA ratio. This indicates that increase in alginate concentration causes higher swelling and vice versa. From fig 1, 2 it can be concluded that swelling was more in case of distilled water as compared to acetate buffer. That indicates swelling was lower in acidic pH compared to neutral.

Tensile strength and % elongation data shown in table 2, which indicates that as the chitosan concentration increases in film, Tensile strength and % elongation increases, while increase in sodium alginate decreases Ts and % elongation.

From compiled data of table 1 and 2 it was decided to use polymer drug ratio of 1:1.5 which shows complete reaction of two polymer, with good swelling and mechanical property for further work.

**Polymer To Drug Ratio**
The drug to polymer ratio was optimized on the basis of swelling as well as from the drug diffusion through the film. Diffused drug analyzed by UV Spectrophotometry at 228.6 nm.

**Table 3** showed the summarized data of the drug loaded PEC film, which shows that swelling capacity for various drug to polymer ratio increases gradually up to 8 hours, after which it attains saturation. As drug concentration increases, the swelling was decreased, which indicates that the drug has a reducing effect on swelling properties of film. Maximum swelling was observed of 3.346 ± 0.107 in 1:5 ratio and minimum swelling of 2.575 ± 0.304 for 1:2 drugs to polymer ratio.

Maximum drug release (table 3 & fig 3) in 24 hours was observed (66.58±0.46) in 1:2 drug: polymer ratio, while minimum (28.36±0.21) in 1:5 drug: polymer ratio. Thus higher the concentration of polymer, drug release decreased. But 1:4 ratio was selected as further more increase in concentration of polymer part not showed much difference in release behavior and it shows good mechanical strength compared to 1:5 of drug to polymer ratio.

### Evaluation of Film
Data of Table 3 shows that swelling index of film was found to be 3.216 ± 0.125, which shows that the film can be utilize for the purpose of controlled drug release. The data of tensile strength and % elongation (table 3) shows decrease in mechanical strength of film, as the drug part was increase in drug to polymer ratio i.e. drug indirectly affects the mechanical properties of film. Content uniformity of film was found to be 98.88 ± 2.38 % w/w, which confirms uniform and reproducible distribution of drug within entire film.

#### In vitro drug diffusion
Comparative diffusion studies from semi permeable membrane for different drug: polymer ratio batches (Fig. 3) showed that up to 2 hours there was gradual increase in the drug diffusion probably due to film swelling. After 2 hours drug release slowed down due to diffusion-controlled mechanism. That drug release from the film was initially affected by the swelling of the film and hence it can be considered the rate-limiting step, affecting the drug release form the film.

### Comparative of formulation by vitro drug diffusion
Diffusion data of 24 hrs of clotrimazole shows that film formulation gives most controlled and sustained drug release compared to other formulations. Cream formulation gives fastest drug release. Sustained drug release from film formulation is due to drug entrapment into network like structure of complex coacervates.

### Conclusion
Polylelectrolyte complex film of chitosan and sodium alginate film can be used for sustained drug delivery of potent anti microbial and antifungal drugs by transdermal drug delivery. The combination of appropriate drug and chitosan can help to recover topical infections. This polylelectrolyte complex film can be modified to use in more sophisticated means by formulating it as a transdermal patch as it uses the bioadhesive and biodegradable non toxic polymers.
Acknowledgement
We acknowledge SiberHegner (Mumbai, India) and Halios Pharmaceutical (kadi, Gujarat, India) for providing chitosan and drug for the above work.

Table 1: Supernatant viscosity data

<table>
<thead>
<tr>
<th>Ratio of chitosan: alginate</th>
<th>Viscosity of supernatant (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>0.9935</td>
</tr>
<tr>
<td>1:1</td>
<td>1.2119</td>
</tr>
<tr>
<td>1:2</td>
<td>1.2710</td>
</tr>
<tr>
<td>1:1.5</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

Table 2: Summarized data of optimization of chitosan to alginate ratio

<table>
<thead>
<tr>
<th>Ratio CH*: SA*</th>
<th>Thickness mm ± SD</th>
<th>Tensile strength Kg/mm² ±SD</th>
<th>%Elongation ±SD</th>
<th>Swelling ratio ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>0.11±0.01</td>
<td>0.195±0.029</td>
<td>68±2</td>
<td>1.985±0.349</td>
</tr>
<tr>
<td>1:1.5</td>
<td>0.10±0.02</td>
<td>0.182±0.017</td>
<td>72±3</td>
<td>3.925±0.465</td>
</tr>
<tr>
<td>1:2</td>
<td>0.11±0.01</td>
<td>0.162±0.022</td>
<td>78±2</td>
<td>3.619±0.398</td>
</tr>
<tr>
<td>2:1</td>
<td>0.11±0.02</td>
<td>0.250±0.036</td>
<td>89±4</td>
<td>1.268±0.285</td>
</tr>
</tbody>
</table>

* CH-Chitosan, * SA-Sodium Alginate

Table 3: Summarized data for drug loaded film

<table>
<thead>
<tr>
<th>Drug:polymer Ratio</th>
<th>Thickness mm ± SD</th>
<th>Tensile strength Kg/mm² ±SD</th>
<th>%Elongation ±SD</th>
<th>Swelling ratio ±SD</th>
<th>% Drug Diffuse ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>0.11 ±0.01</td>
<td>0.186 ±0.19</td>
<td>76±1</td>
<td>2.575 ±0.304</td>
<td>66.58 ±0.46</td>
</tr>
<tr>
<td>1:3</td>
<td>0.11 ±0.02</td>
<td>0.168 ±0.05</td>
<td>70±2</td>
<td>3.022 ±0.151</td>
<td>42.31 ±0.82</td>
</tr>
<tr>
<td>1:4</td>
<td>0.11 ±0.02</td>
<td>0.154 ±0.12</td>
<td>66±3</td>
<td>3.216 ±0.125</td>
<td>30.19 ±0.36</td>
</tr>
<tr>
<td>1:5</td>
<td>0.12 ±0.01</td>
<td>0.134 ±0.036</td>
<td>53±4</td>
<td>3.346 ±0.107</td>
<td>28.36 ±0.21</td>
</tr>
</tbody>
</table>
Figure 1: Swelling ratio of film in distilled water

![Swelling ratio of film in distilled water](image1.png)

Figure 2: Swelling capacity of film in 9:1 of acetate buffer pH 5 and PEG 400

![Swelling capacity of film in 9:1 of acetate buffer pH 5 and PEG 400](image2.png)

Figure 3: *In vitro* drug diffusion across semi permeable membrane

![In vitro drug diffusion across semi permeable membrane](image3.png)
Figure 4: Comparison of In vitro drug diffusion through rat skin

References

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