PREPARATION AND CHARACTERIZATION OF RESOMER RG 503 BASED NANOSUSPENSION FOR OPTHALMIC IN SITU GEL OF SULPHACETAMIDE

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ABSTRACT

The purpose of study was to develop Resomer RG 503 based nanosuspension for ophthalmic in situ gel of sulphacetamide using nanoprecipitation method to improve absorption, penetration, retention time at the site of action in treatment of conjunctivitis. The process was optimized by studying the effect of various process parameters on the size of nanosuspension using factorial design approach. Various formulations of PLGA nanoparticles prepared by nanoprecipitation method which affect the properties like particle size, drug release study and entrapment efficiency. Particle size of nanosuspension is controlled by Stabilizer ratio, the Organic/aqueous phase volume ratio. The O/A ratio was exercised as 1:12 in which organic phase composed of mixture of acetone and ethanol in ratio of 5:1. Gellan gum (1.7%) is used in situ gelling polymer. FTIR and DSC study revealed no interaction between Sulphacetamide and excipients. Formulation parameters were optimized by $2^3$ factorial designs. SEM imaging was confirmed the nanosized drug particles. The optimized formulation shows spherical shape and mean particle diameter 107.0 nm. Ophthalmic in situ gel of Sulphacetamide exhibits 88.58% total cumulative release up to 8 h. In conclusion in situ forming gel comprise of resomer RG 503 is a promising tool in treatment of conjunctivitis.

Key words: Sulphacetamide, Resomer RG 503, Nanosuspension, Nanoprecipitation, In-situ gel, Gellan gum
INTRODUCTION

Conjunctivitis is an inflammatory process of the conjunctiva that varies in mild hyperemia to severe purulent discharge. The more common causes of conjunctivitis include viruses, allergies, environmental irritants, contact lenses and chemicals, the more commonly reported infection agents are adenovirus and herpes simplex virus, followed by other viral and bacterial source. Effective management is based on selection of an appropriate antibiotic for suspected bacterial pathogens\(^1\).

An ideal drug therapy achieves effective concentration of drug at the target for a specified period of time in order to minimize general and local side effects. An exciting challenge for developing suitable drug delivery systems targeted for ocular diseases is one of today’s major focuses of pharmaceutical scientists. Conventionally, most ocular diseases or disorders are treated with water-soluble drugs in aqueous solution while water insoluble drugs in ointments or aqueous suspension\(^2\).

Sulphacetamide is sulfonamides antibiotics used to treat pink eye (conjunctivitis)\(^3\). They are bacteriostatic in nature and inhibit bacterial synthesis of dihydrofolic acid by preventing condensation of pteridine with amino benzoic acid through competitive inhibition of the enzyme dihydropteroate synthetized\(^4\). The drug is marketed as ophthalmic solution of its sodium salt, in a USP concentration of 10 % (W/V) under the brand name Blepha®-10. The usual adult dose for conjunctivitis is 1 to 2 drops into the conjunctival sac every 2 to 3 h for 7 to 10 days\(^5\). The drug has an ionization constant of 5.4 and an elimination half-life of 7 to 13 h\(^6\).

The Aim of present study to prepare nanosuspension based ophthalmic in-situ gel of Sulphacetamide in order to improve absorption, penetration, retention time and antibacterial activity at site of action. Resomer RG 503 nanosuspension attractive colloidal system as compare to conventional dosageforms it having increase stability and longer elimination half-life in tear.

MATERIALS AND METHOD

Sulphacetamide was received from Ishita Pharma Ahemdbad, Resomer RG 503 was obtained from Evonic industry Mumbai, gellan gum was obtained from ostrich bioscience Coimbatore, poloxamer 188 obtained from Chemdyes Corporation Ahemdbad, and other chemicals were analytical grade.

Method Preparation of Sulphacetamide Nanosuspension

In this method polymer (PLGA) is dissolved in organic phase contain Acetone: Ethanol in a different ratio. Aqueous phase is prepared using poloxamer 188 in ultrapure water. Organic phase is Drop wise added into aqueous phase with continuous stirring. Mixture is stirred for 4 h at 2400 rpm speed \(^6\).
Optimization formulation using $2^3$ design

$2^3$ design is used to study the effect of variables different variables on the quality determinant parameters of any formulation. Based on the principle of design of experiments, this design was working to study the effect of three independent factors. A $2^3$ factorial design for three factors at two levels each was selected to optimize the varied response variables. The three factors are organic to aqueous ratio(X1), Poloxamer 188(X2) and Gellan gum(X3) and the levels were suitable coded. Particle size (nm), gelling time(s) and Drug release (%) were taken as response variables. In this design three factors are evaluated each at two levels. Experimental trials were performed at 8 possible combinations. Regression polynomials equations for dependent variables were calculated by design expert software version 10.

<table>
<thead>
<tr>
<th>Variables</th>
<th>-1(Low)</th>
<th>+1(High)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic to aqueous phase</td>
<td>1:7</td>
<td>1:12</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>400</td>
<td>600</td>
</tr>
<tr>
<td>Gellan gum</td>
<td>1.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 1: Variables levels for $2^3$ factorial design

Table 2: Formulation of nanosuspension based ophthalmic in situ gel of Sulphacetamide

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
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</thead>
<tbody>
<tr>
<td>Sulphacetamide (%)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Resomer RG 503(mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Poloxamer 188(mg)</td>
<td>400</td>
<td>400</td>
<td>600</td>
<td>600</td>
<td>400</td>
<td>400</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Acetone:Ehanol(ml)</td>
<td>5:1</td>
<td>5:1</td>
<td>5:1</td>
<td>5:1</td>
<td>5:1</td>
<td>5:1</td>
<td>5:1</td>
<td>5:1</td>
</tr>
<tr>
<td>Organic to aqueous phase ratio</td>
<td>1:7</td>
<td>1:12</td>
<td>1:7</td>
<td>1:12</td>
<td>1:7</td>
<td>1:12</td>
<td>1:7</td>
<td>1:12</td>
</tr>
<tr>
<td>Gellangum(%)</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Water(ml)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

EVALUATION PARAMETERS

**Thermal analysis using differential scanning calorimetric**[^7]

[^7]: Additional details or references related to the thermal analysis.
Thermal behavior of the materials was determined using Differential Scanning Calorimetry (DSC 60 Shimadzu Japan). Approximately 5 mg drug and Physical polymer mixture of Sulphacetamide, Resomer RG 503, gellan gum, Poloxamer 188 and their mixtures were accurately weighed into aluminum pans and crimped by aluminum caps with a pinhole. Indium standard was used to calibrate the DSC temperature and enthalpy scale. Nitrogen was used as the purge gas through DSC cell at flow rate of 50 ml per min and 100 ml per min through the cooling unit. The sample (5-10mg) was heated in a hermetically sealed aluminum pans.

**Fourier Transform Infrared Spectrum Analysis**[^8]

Infrared spectrophotometry is a useful analytical technique utilized to check the chemical interaction between the drug and other excipients used in the formulation. Fourier–transform infrared (FT–IR) spectra of moisture free powdered samples of SS take 1-2 mg of solid fine powder of drug and 200-300 mg of dry fine powder of Potassium bromide (KBr) (IR grade) in a mortar and mix well with the help of a spatula. Spectrum measurements were carried out using Potassium bromide (KBr) disk method in the wavelength region of 2000-4000 cm\(^{-1}\) by IR Affinity (Shimadzu Japan).

**pH measurement**

The pH of solution is measure by the digital pH meter. Electrode was immersed in solution and each experiment performed in triplicate.

**Rheological measurement**[^9]

Viscosity of *in situ* gelling solution and *in situ* gel was performed by Brookfield DV-E VISCOMETER. Viscosity of *in situ* gelling solution was measured at 20 rpm using 61 spindle numberfor and *in situ* gel is measured at 64 spindle numbers at 20 rpm. Each experiment performed in triplicate.

**Drug entrapment efficiency**[^10]

A 20 ml portion of freshly prepared nanosuspension was centrifuged at 10000 rpm for 2 h. The amount of unincorporated drug was measured by taking the absorbance of appropriately diluted supernant solution at 258 nm using double beam UV spectrophotometer against blank/control nanosuspension. By subtraction from the initial amount of drug taken, entrapment efficiency was calculated. Each experiment performed in triplicate.

**Drug content**

Drug content was determined by dissolving 10 ml *in situ* gels in water. After suitabledilution absorbance was recorded by using UV/Vis double beam Spectrophotometer at 258 nm. Each experiment performed in triplicate.
In vitro drug release study

In vitro release of the drug from the different \textit{in situ} gel was studied using Franz diffusion cell. The \textit{in situ} gel put on the prehydrated cellophane membrane between donor and compartments. The prehydrated cellophane membrane act as corneal epithelium. The entire surface was in contact with receptor compartment comprised of 10 ml of stimulated tear fluid pH 7.4. The content of the receptor compartment was stirred continuously using magnetic stirrer and temperature was maintained at 37±0.5°C, 1 ml sample was withdrawn from receptor compartment and replaced with fresh stimulated tear artificial fluid pH 7.4. The sample was analyses for drug content using double beam spectrophotometer against stimulated tear fluid pH 7.4. Each experiment performed in triplicate.

Statistical analysis of responses by design expert software

Design Expert 10 software (stat-Ease,USA) was used for the analysis of effect of each variable on the designated response. Qualitative and quantitative contribution of each variable on each of the response was analyzed. The significant response polynomial equation generated by design expert used. Response surface plots were generated to visualize the simultaneous effect of each variable on each response parameters.

Sterility testing

The sterilized solution was removed from the pack and transfer to fluid thioglycolate medium and soyabean-casein digestive medium separately at 30 to 35°C and 20 to 25°C respectively for 7 days. A control evaluation was carried out.

Microbiological studies

Microbiological studies were carried out to determine microbiological activity of drug against microorganism. \textit{Staphylococcus aureus} has been used as a test organism. The optimized formulation was evaluated for microbiological study by Standard pour plate method. During the study strict aseptic condition was maintained. Melted nutrient agar taken in test tube and inoculated with 4-5 loop full of test organism from the provided culture. The inoculated medium was then poured in sterile petridish and allows solidifying. Cups were made on the solidified agar layer with the help of sterile borer at 4 mm diameter. Then volume of the formulations (optimized formulation, control and conventional suspension) containing equivalent amount of drug poured into the cups. The petridish was incubated at 37±0.5°C for 24 h. After incubation the petridish was observed for the zone of inhibition around the ophthalmic solution. The zone of inhibition was observed and recorded.

Stability testing
For any rational design and evaluation of dosage forms, the stability of the active component must be major criterion in determining the acceptance or rejection. For stability studies, the formulations were stored in hermetically closed glass vials and kept at 40 ± 20 °C/ 75 ± 5% RH for 1 month. The samples were evaluated for particle size, zeta potential, and PDI and in-vitro drug release study after 1 month.

**RESULT AND DISCUSSION**

**Drug excipient compatibility study**

The DSC of Sulphacetamide peak was observed at 189.35 °C where in DSC spectra of Sulphacetamide and excipient mixture drug peak was observed at 170.45 °C, hence there is negligible change in drug peak. It was found that all the excipient used in the formulation does not affect the melting point of drug. This was conformed that there was no incompatibility between drug and excipient.

**Infrared spectroscopy**

The IR analysis of pure drug, excipient and physical mixture of all were done on FT-IR 8400 S Shimadzu. It was found that all the prominent functional group picks of NH stretching (3469 cm⁻¹), NH₂ stretching (3383 cm⁻¹), Aromatic CH stretching (3000 cm⁻¹), Aliphatic CH stretching (2910 cm⁻¹), O=C=O stretching (1325 cm⁻¹), C=O stretching (1645 cm⁻¹) and CH₃ out of plane stretching (995 cm⁻¹) were observed in physical mixture. This was conformed that there was no interaction between drug - excipient or incompatibility between drug and excipient.

**%Entrapment efficiency**

Eight different batches were prepared by various polymers and organic to aqueous ratio and drug keep constant for all batches from the preliminary trials. Drug entrapment efficacy of Sulphacetamide nanosuspension for various batches were shown in table No.3. Drug entrapment efficacy of all batches range from 39.01% to 65.18%, the reason for poor entrapment efficacy is may be water soluble drug. High entrapment efficiency is may be due to higher solubility of Resomer RG 503 in organic phase. As per graph Drug entrapment efficacy of Sulphacetamide nanosuspension for F6 having higher entrapment efficiency 65.18%, it also higher organic to aqueous ratio. In F1, F3, F5, F7 batches organic to aqueous ratio in this drug entrapment efficacy lower than other batches drug entrapment efficiency is affected by organic to aqueous ratio.
Particle size

Different polymer concentrations and organic to aqueous phase ratio have shown very major effect on particle size of Sulphacetamide nanosuspension particle size is affected by different variables of polymer and organic to aqueous phase (O/A) ratio, in which polymer ratio is keep constant for all batches but poloxamer 188 and organic to aqueous phase ratio is differ. F2, F4, F6, F8 batches having higher O/A ratio but F4 has good particle size but poor PDI because it have higher amount of stabilizer. Were F2 and F6 having higher organic to aqueous phase ratio with good PDI. In reaming batches were high particle sizes due to lower O/A ratio, so high concentration of O/A ratio decrease the particle size. Were F6 batch having 115.6 nm particle size at higher O/A ratio with lower stabilizer ratio.

Viscosity of in situ gel

Table No.3 shows that different batches having different viscosity because the poloxamer 188 and gellan gum concentration combination is different batches. As shown in table that the F1, F2, batches having lower poloxamer 188 concentration and gellan gum (1.5%) having low viscosity in F3, F4 having higher concentration of poloxamer 188 and lower the concentration of gellan gum, its having higher viscosity in other batches F5, F6, have lower concentration poloxamer 188 and higher concentration of gellan gum decrease in viscosity, F7, F8 batches having higher the poloxamer 188 and gellan gum increase viscosity.

DSC of sulphacetamide

![DSC Graph](image)

**Fig.1: DSC of Sulphacetamide**
DSC OF Mixture (Sulphacetamide + Resomer RG 503 + Pluronic F 68 + Gellan gum)

Fig. 2: DSC of Mixture (Sulphacetamide + Resomer RG 503 + Pluronic F 68 + Gellan gum)

FTIR of Sulphacetamide
Fig. 3: FTIR of Sulphacetamide

FTIR of Mixture (Sulphacetamide + Resomer RG 503 + Pluronic F 68 + Gellan gum)

Fig. 4: FTIR of Mixture (Sulphacetamide + Resomer RG 503 + Pluronic F 68 + Gellan gum)
**Evaluation parameters**

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Particle size (nm)</th>
<th>%Entrapment efficiency</th>
<th>Gelling time (S)</th>
<th>pH of <em>in situ</em> gelling solution</th>
<th>Viscosity of <em>in situ</em> gelling solution (Cps)</th>
<th>Viscosity of <em>in situ</em> gel (Cps)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>529.6</td>
<td>45.28±0.64</td>
<td>52</td>
<td>7.20± 0.10</td>
<td>81± 10.02</td>
<td>2128± 11.35</td>
<td>91.52±0.31</td>
</tr>
<tr>
<td>F2</td>
<td>185.5</td>
<td>55.55±0.71</td>
<td>45</td>
<td>7.04± 0.05</td>
<td>86± 5.51</td>
<td>1255± 10.0</td>
<td>94.55±0.32</td>
</tr>
<tr>
<td>F3</td>
<td>710.4</td>
<td>39.01±0.78</td>
<td>60</td>
<td>7.41± 0.03</td>
<td>102±.86</td>
<td>3550± 4.0</td>
<td>90.58±0.14</td>
</tr>
<tr>
<td>F4</td>
<td>197.6</td>
<td>61.17±0.87</td>
<td>55</td>
<td>6.99±0.10</td>
<td>115± 7.00</td>
<td>2533± 12.4</td>
<td>95.64±0.11</td>
</tr>
<tr>
<td>F5</td>
<td>410.5</td>
<td>53.08±0.97</td>
<td>41</td>
<td>7.29± 0.04</td>
<td>85± 5.57</td>
<td>3646± 10.54</td>
<td>93.45±0.29</td>
</tr>
<tr>
<td>F6</td>
<td>115.6</td>
<td>65.18±0.64</td>
<td>26</td>
<td>7.25± 0.05</td>
<td>81± 7.09</td>
<td>3256± 10.15</td>
<td>97.70± 0.15</td>
</tr>
<tr>
<td>F7</td>
<td>312.4</td>
<td>56.74±0.45</td>
<td>30</td>
<td>7.45± 0.05</td>
<td>135± 5.57</td>
<td>4224± 10.50</td>
<td>95.73±0.23</td>
</tr>
<tr>
<td>F8</td>
<td>149.4</td>
<td>60.68±0.62</td>
<td>38</td>
<td>7.45± 0.05</td>
<td>143± 7.94</td>
<td>5055± 14.47</td>
<td>98.59±0.15</td>
</tr>
</tbody>
</table>

*(mean±SD,n=3)*

Particle size of optimized batch of Resomer RG 503 based nanosuspension of sulphacetamide

**Fig.5: Particle size of Resomer RG 503 based nanosuspension of sulphacetamide**
**In vitro drug release study**

![In vitro drug release study](image)

**Fig.6: In vitro drug release of 2³ design batches F1 to F8**

From the results figure No.2 shows% cumulative drug release of F1 to F8 Batches. *In vitro* drug release study was performed on Franz diffusion cell using Stimulated tear fluid pH 7.4. Drug entrapment efficiency and Particle size have a direct effect on the drug release profile from the eight formulations. It shows increase in drug release with increase in Organic to aqueous phase ratio, so decrease in particle size and increase entrapment efficiency result shows increase in drug release. But in F1,F3 and F5 having higher particle size(529.7nm,710.4nm and 410.5nm) and low drug entrapment efficiency (49.28%, 39.01% and 53.08%) it having lower drug release. Other batches having increasing drug release with increasing entrapment efficiency and decreasing particle size. In which out of all batches F6 was found smallest particle size that is 115.6 nm and higher entrapment efficiency 65.18% with highest drug release 96.54% after 8 h.

**Statistical design of response by design expert software**

Based on results obtained for particle size, gelling time and % Drug release, the response polynomial coefficient were determine in order to evaluate each response.

Correlating Y1 (Particle size), the result of multiple linear regression analysis showed that the coefficients b1 and b3 stand negative effect sign and b2 stand positive sign. The fitted equation relating the Y1 to the transferred factor are shown in following equation

\[
Y1=326.36-164.34X1+16.09X2-79.39X3-4.16X1X2+49.86X2X3-32.16X1X3\ldots\text{eq. (1)}
\]

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Correlating Y2 (Gelling time), the result of multiple linear regression analysis showed that the coefficients b1 and b3 stand negative effect sign and b2 stand positive sign. The fitted equation relating the Y2 to the transferred factor are shown in following equation

\[ Y2 = 42.13 - 1.13X1 + 1.87X2 - 4.88X3 - 4.13X1X2 - 0.12X2X3 - 0.12X1X3 \] ………… eq. (2)

Correlating Y3 (% drug release), the result of multiple linear regression analysis showed that the coefficients b1, b2 and b3 stand positive sign. The fitted equation relating the Y3 to the transferred factor are shown in following equation

\[ Y3 = 72.93 + 12.13X1 + 2.84X2 + 4.91X3 - 2.38X1X2 + 1.56X2X3 - 3.63X1X3 \] …………eq. (3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Predicted value</th>
<th>Experimental value</th>
<th>Relative error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Practical size (nm)</td>
<td>107.193</td>
<td>115.6</td>
<td>-8.407</td>
</tr>
<tr>
<td>Gelling time(s)</td>
<td>32.263</td>
<td>30</td>
<td>2.263</td>
</tr>
<tr>
<td>Drug release (%)</td>
<td>88.5897</td>
<td>96.54</td>
<td>-7.950</td>
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</table>
RESPONSE SURFACE PLOTS

The response surface plots generated using polynomial equations represent quantitative simultaneous effect of any two variables on response parameters taking one as constant, using design expert software.

Fig.7: Response surface plot for particle size
Fig. 8: Response surface plot for gelling time

Fig. 9: Response surface plot for % drug release
Scanning Electron Microscopy (SEM)

Fig.10: Scanning Electron Microscopy (SEM) of nanosuspension for ophthalmic in situ gel of Sulphacetamide

The figure 5.31 showed that SEM image of nanosuspension for ophthalmic in situ gelling solution of optimized batch. The figure shows the spherical shape of particle and crystals of drug and excipients.

Sterility testing

From the sterility testing it was found visually that the soyabean casein digest medium and fluid thioglycolate medium containing sterilized ophthalmic in situ gel was free from turbidity. This conform that absence of microbial growth. From this result conformed the sterility of ophthalmic in situ gel.
In vitro Microbial study

From results it was observed that optimized batch of nanosuspension for ophthalmic in situ gelling solution showed that higher anti-microbial activity as compare to conventional suspension and control. Increase an In vitro microbial study of nanosuspension based ophthalmic in situ gelling solution with increasing microbial activity at site of action.

![Graph showing zone of inhibition for different formulations](image)

**Fig.11: In vitro microbial study of ophthalmic in situ gel**

Stability study of optimized formulation

Stability study was performed on only optimized batch The table 5.21 shows the stability study data of optimized batch at 40 ± 20°C/ 75 ± 5% RH for one month. Results of stability studies indicate that there is no significant difference in mean particle size, zeta potential, pH, Viscosity and in- vitro drug release study of optimized batch after a period of 1month.
CONCLUSION:

From the present study nanosuspension of sulphacetamide was prepared by nanoprecipitation method. Optimized formulation having lower particle size with higher entrapment efficiency and higher drug release for eight hour, when solution is covert in to gel, rate of elimination is decrease and contact time is increase and microbial activity was increase at site of action. so it conclude Resomer RG 503 based nanosuspension for ophthalmic in situ gel of Sulphacetamide is more effective dosage form as compare to conventional eye drops its provided better absorption and longer retention time. Optimized formulation is effective in treatment of conjunctivitis.
REFERENCE

