FORMULATION AND EVALUATION OF EZETIMIBE NANOSUSPENSIONS BY USING PRECIPITATION METHOD

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ABSTRACT
In the present study, an attempt was made to prepare Nanosuspension of Ezetimibe is an anti-hyperlipidemic medication which is used to lower cholesterol levels. Nanosuspension containing the drug were prepared by precipitation method using combinations of polymers such as PVP K-25, Sodium lauryl sulphate (SLS), urea, poloxamer (188), and methanol. Estimation of Ezetimibe was carried out spectrophotometrically at 232nm. The Oral Nanosuspension were evaluated for various physical and biological parameters, drug content uniformity, particle size analysis, zeta potential, in-vitro drug release, short-term stability, drug- excipient interactions (FTIR). IR spectroscopic studies indicated that there are no drug-excipient interactions. The formulations F1 to F9 (containing PVP K-25, Urea, SLS, Poloxamer (188), and Methanol) used different ratio were found to be promising, of that formulation F9 containing Urea showed 99.43% release at the end of 25min & it follows zero order drug release kinetics. These formulations have displayed good Nanosuspension strength.

KEYWORDS: Ezetimibe, Nanosuspension, PVP K-25, SLS, poloxamer (188), Urea and Methanol.
INTRODUCTION
Poor solubility of drug substance has always been a challenging problem faced by pharmaceutical scientists and it is increased now because more than 40% of new chemical entities are poorly water soluble. One of the most persistent problems faced by drugs with poor aqueous solubility is that their oral delivery is frequently associated with implication of low bioavailability and lack of dose proportionality. There are number of technologies like solid dispersion1-2, complexation, co-solvency, use of surfactants, etc., but they lack universal applicability to all drugs. A novel technology that can used to overcome problems associated with this method is nanosuspension, which is based on size reduction mechanism.

In the present research work an attempt was made to improve the solubility and dissolution rate of model drug Ezetimibe. Ezetimibe is a drug that lowers plasma cholesterol levels. It acts by decreasing cholesterol absorption in the small intestine. The aim of the present investigation was formulation and evaluation of ezetimibe nanosuspensions (F1-F9) by using different concentrations of surfactants and to find out the effect of different surfactants on the formulation of ezetimibe nanosuspension. Nanosuspension of Ezetimibe is prepared by precipitation method using poloxamer 188 and PVP K25 as carriers and sodium lauryl sulphate and urea as surfactants. The prepared nanosuspensions were evaluated for Particle size, Zeta potential analysis, SEM, solubility, %yield, drug content, %Entrapment Efficiency, invitro drug release studies were performed. Solubility studies and in-vitro drug release studies states that the formulation F9 containing urea and poloxamer 188 shows higher drug release at the end of 25 minutes and it follows zero order drug release kinetics.

MATERIAL AND METHODS
Ezetimibe is obtained from Aurobindo pharmaceuticals, PVP-K25, SLS, Urea, Poloxamer 188 was obtained from Narmada chemicals, methanol, water, hydrochloric acid and other ingredients were obtained from SD fine chemicals, Mumbai.

Preparation of ezetimibe Nanosuspension by precipitation method
Nanosuspension of ezetimibe was prepared by precipitation method with various carriers and drug. At first the weighed amount of ezetimibe was taken and dispersed into the beaker containing acetone which acts as organic solvent. This drug and acetone solution is termed as organic phase. Now the carriers Urea, PVP was dissolved in water and add surfactant (SLS) to the stabilizer solution and labelled as aqueous phase. This solution was kept on magnetic stirrer for uniform mixing. The organic phase was slowly added drop wise to aqueous phase
and continues the stirring on magnetic stirrer at an rpm of about 800 until complete evaporation of solvent. After 1 hour, the solution was kept in sonicator for about 30 mins. Finally the nanosuspensions was formed.

**Evaluation parameters of Nanosuspension of ezetimibe**

**Drug content uniformity**

10 ml of each formulation was taken and dissolved in 10 ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10 µg/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations were read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at 232 nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

**Entrapment efficacy**: The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at 232 nm using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated.

**The entrapment efficiency (EE %) could be achieved by the following equation**

\[
\%\text{Entrapment efficiency} = \frac{\text{Drug content} \times 100}{\text{Drug added in each formulation}}
\]

**Particle size and shape**

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

**Zeta potential**

There are three ways by which a solid particle (colloid) dispersed in a liquid media can acquire a surface charge. First, by the adsorption of ions present in the solution. Second, by the ionization of functional groups on the particle’s surface. Third, due to the difference in dielectric constant between the particle and the medium. The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the
electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases. The most widely-used theory for calculating zeta potential was developed by Smoluchowski in 1903. The theory is based on electrophoresis and can be expressed as

\[ \mu = \frac{\zeta \varepsilon}{\eta} \]

where (\(\mu\)) is the electrophoretic mobility, (\(\varepsilon\)) is the electric permittivity of the liquid, (\(\eta\)) is the viscosity and (\(\zeta\)) is the zeta potential.

**In vitro drug release study:** In vitro dissolution studies were performed in USP apparatus-II (LAB INDIA DS 8000), employing paddle stirrer at rotation speed of 50 rpm and 200 ml of pH 6.8 phosphate buffer as dissolution medium. Accurately weighed bulk drug and nanosuspensions were dispersed in dissolution medium. The release study was performed at 37 ± 0.5°C. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink condition. The samples were filtered through 0.22 μm membrane filter disc (Millipore Corporation) and analyzed for ezetimibe after appropriate dilution by measuring the absorbance at 232 nm.

**RESULTS AND DISCUSSION**

**Composition of Nanosuspensions of Ezetimibe precipitation method**

<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>
<th>F9</th>
</tr>
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<tbody>
<tr>
<td>EZETIMIBE</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>PVP-K25</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>POLOXAMER 188</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>METHONOL</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td>10</td>
</tr>
<tr>
<td>WATER(ml)</td>
<td>Q.S</td>
<td>Q.S</td>
<td>Q.S</td>
<td>Q.S</td>
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<td>Q.S</td>
<td>Q.S</td>
<td>Q.S</td>
<td>Q.S</td>
</tr>
</tbody>
</table>

Ezetimibe is a BCS class-II drug having low solubility and high permeability. Thus, it is challenging to enhance the solubility of Ezetimibe particles in an aqueous solution.

**Determination of melting point:** The melting point of Ezetimibe was found to be 165°C which was determined by capillary method. Fine powder of ezetimibe was filled in glass capillary tube (previously sealed on one end). The capillary tube is tied to thermometer and the thermometer was placed in fire. The powder at what temperature it will melt was noticed.

**Saturation Solubility:** Saturation solubility was carried out at 25°C using 0.1N HCL, 6.8 phosphate buffer, and purified water. From the above conducted solubility studies in various
buffers we can say that 0.1N HCL has more solubility (3.82 mg/ml) when compared to other buffer solutions.

**Construction of calibration curve:** A simple Spectrophotometric method for estimation of Ezetimibe was developed in 0.1N HCL, which exhibited $\lambda_{\text{max}}$ at 232 nm in Beer’s range of 5-30 $\mu$g/ml as shown in figure.

**Spectrum Curve of Ezetimibe**

![Spectrum Curve of Ezetimibe](image)

**Standard graph of Ezetimibe ($\lambda_{\text{max}}$ 232 nm)**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
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<tr>
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<tr>
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<tr>
<td>10</td>
<td>0.324</td>
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<td>15</td>
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<td>20</td>
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<td>25</td>
<td>0.794</td>
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<tr>
<td>30</td>
<td>0.952</td>
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</table>
**Drug excipient compatibility:** From the drug excipient compatibility studies we observed that there are no interactions between the pure drug (Ezetimibe) and optimized formulation (Ezetimibe+ excipients) which indicates there are no physical changes.

**Drug content:** The drug content of the formulated Nanosuspension was found in the range of 94.41 to 99.67% respectively.

The percentage of drug content of formulation F1 was found to be 94.41%, formulation F2 was found to be 96.06%, formulation F3 was found to be 97.01%, formulation F4 was found to be 98.80%, formulation F5 was found to be 97.79%, formulation F6 was found to be 98.11%, formulation F7 was found to be 97.04%, formulation F8 was found to be 98.64%, and finally formulation F9 was found to be 99.67%.

**Entrapment efficacy:** The entrapment efficacy of the formulated Nanosuspension was found to be in the range of 88.12%-94.46% respectively.

The entrapment efficacy of formulation F1 was found to be 88.12%, formulation F2 was found to be 91.11%, formulation F3 was found to be 93.34%, formulation F4 was found to be 88.64%, formulation F5 was found to be 89.99%, formulation F6 was found to be 93.12%, formulation F7 was found to be 92.09%, formulation F8 was found to be 93.31%, and finally formulation F9 was found to be 94.46%.

**Scanning electron microscopy:** Determination of surface morphology of Ezetimibe nanosuspension of optimized formulation (F9) was carried out by scanning electron microscopy (SEM).

![SEM Image](image-url)
Invitro drug release studies: From the above invitro studies for all the formulations (F1-F9) were performed we can say that of all the formulations it shows that the formulation (F9) containing urea and poloxamer 188 shows best drug release of 99.43% within 25 minutes where as all the other formulations takes about 30 to 45 minutes to release the drug.

Drug release kinetics studies: The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order and equation methods. Based on the regression values it was concluded that the optimized formulation F9 follows zero order kinetics having $R^2$ value 0.909.

In-vitro drug release data of formulation F1 to F9

<table>
<thead>
<tr>
<th>TIME</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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<tr>
<td>5</td>
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<tr>
<td>45</td>
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<td>96.69</td>
<td>98.16</td>
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CONCLUSION

Oral Nanosuspension of Ezetimibe can be prepared by precipitation method using polyvinyl PVP K25, urea, Sodium lauryl sulphate, Poloxamer 188 and methanol. As the amount of polymer increases, the drug release rate increases, whereas Nanosuspension strength
increases. The optimized batch (F9) had a Zeta Potential and average particle size within the acceptable range. IR spectroscopic studies indicated that there are no drug-excipient interactions. The formulations F1 to F3 (containing polyvinyl PVP K25, Poloxamer 188, methanol and water) and F4 to F6 (containing Sodium lauryl sulphate, Poloxamer 188, methanol and water) and F7 to F9 (containing Poloxamer 188, urea, methanol and water) were found to be promising, which showed formulation F9 is 99.43% of drug released respectively within 25 min. The formulation F9 is compared to other formulations the F9 is the best formulation of the released the percentage drug of Nanosuspension. Remaining formulation are drug releasing percentage showing respectively of Nanosuspension of ezetimibe.

REFERENCES


