Optimization of caseinate-coated simvastatin-zein nanoparticles: improved bioavailability and modified release characteristics

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Abstract: The current study focuses on utilization of the natural biocompatible polymer zein to formulate simvastatin (SMV) nanoparticles coated with caseinate, to improve solubility and hence bioavailability, and in addition, to modify SMV-release characteristics. This formulation can be utilized for oral or possible depot parenteral applications. Fifteen formulations were prepared by liquid–liquid phase separation method, according to the Box–Behnken design, to optimize formulation variables. Sodium caseinate was used as an electrostERIC stabilizer. The factors studied were: percentage of SMV in the SMV-zein mixture (X1), ethanol concentration (X2), and caseinate concentration (X3). The selected dependent variables were mean particle size (Y1), SMV encapsulation efficiency (Y2), and cumulative percentage of drug permeated after 1 hour (Y3). The diffusion of SMV from the prepared nanoparticles specified by the design was carried out using an automated Franz diffusion cell apparatus. The optimized SMV-zein formula was investigated for in vivo pharmacokinetic parameters compared with an oral SMV suspension. The optimized nanosized SMV-zein formula showed a 131 nm mean particle size and 89% encapsulation efficiency. In vitro permeation studies displayed delayed permeation characteristics, with about 42% and 85% of SMV cumulative amount released after 12 and 48 hours, respectively. Bioavailability estimation in rats revealed an augmentation in SMV bioavailability from the optimized SMV-zein formulation, by fourfold relative to SMV suspension. Formulation of caseinate-coated SMV-zein nanoparticles improves the pharmacokinetic profile and bioavailability of SMV. Accordingly, improved hypolipidemic activities for longer duration could be achieved. In addition, the reduced dosage rate of SMV-zein nanoparticles improves patient tolerability and compliance.

Keywords: Box–Behnken design, simvastatin, zein, sodium caseinate

Introduction

Many promising antihyperlipidemic agents have disadvantageous physicochemical properties that lead to poor bioavailability.1 One basic requirement for the successful use of any drug is that it should have sufficient bioavailability to achieve effective therapy at low doses, to minimize the side effects after oral administration.2 So it is essential that the drug should be adequately and consistently absorbed. Dyslipidemia, a disorder in lipoprotein metabolism, is one of the key risk factors for cardiovascular diseases. Researchers showed that effective treatment of dyslipidemia reduces the rate of morbidity and mortality.3–5

Simvastatin (SMV) is an antihyperlipidemic drug (statin). Previous studies showed that statins have possible anti-inflammatory characteristics unrelated to their lipid-lowering activity.6,7 Therefore, statins may have a potential effect in a broad range of inflammatory conditions. SMV is a Class II (according to the
Materials and methods

Materials

SMV was kindly supplied from SAJA Pharmaceutical Company (Jeddah, Saudi Arabia). Zein, sodium caseinate, and all other chemicals were obtained from Sigma-Aldrich Corp (St Louis, MO, USA).
Table 2 Experimental runs and their observed dependent variables

<table>
<thead>
<tr>
<th>Run</th>
<th>Factors</th>
<th>Dependent variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁</td>
<td>X₂</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
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<tr>
<td>2</td>
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<td>80</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>80</td>
</tr>
</tbody>
</table>

Notes: Factors studied were percentage of SMV in the SMV-zein mixture (X₁), ethanol concentration (X₂), and caseinate concentration (X₃). The selected dependent variables were mean particle size (Y₁), SMV encapsulation efficiency (Y₂), and cumulative percentage of drug permeated after 1 hour (Y₃).

Determination of SMV encapsulation efficiency

The total drug in the prepared nanoparticles was determined by dissolving SMV-zein nanoparticles in 70% w/v ethanol solution. The solution was then filtered through 0.22 µm filters and assayed for SMV content by high-performance liquid chromatography (HPLC). SMV encapsulation efficiency was determined by Equation 1.

\[
\text{Encapsulation efficiency (w/w%)} = \frac{\text{Amount of SMV in the nanoparticles}}{\text{Weight of SMV initially added}} \times 100
\]

In vitro diffusion study

The diffusion of SMV from the prepared nanoparticles was studied by dissolving SMV-zein nanoparticles in 70% w/v ethanol solution. The solution was then filtered through 0.22 µm filters and assayed for SMV content by high-performance liquid chromatography (HPLC).

Examination of nanoparticle morphology

The morphology of the optimized caseinate-coated SMV-zein nanoparticles was examined by using field emission scanning electron microscopy (SEM) (Jeol JSM-7600F). The SMV-zein nanoparticles were vacuum-dried at room temperature prior to examination under SEM.

In vivo application of optimized SMV-zein nanoparticles

Male Wistar rats weighing 200–250 g were used in this study. The animal use was approved by the local Institutional Review Board for Preclinical and Clinical Research, ensuring that the care and use of animals conformed to the European Union (EU) Legislation for the protection of animals used for scientific purposes. The animals were divided into three groups. The first group was orally administered plain zein nanoparticles (negative control). The second group was orally administered SMV suspension (positive control) at a dose of 5 mg/kg body weight. The third group was orally administered optimized formula of caseinate-coated SMV-zein nanoparticles at the same dose, 5 mg/kg. Pharmacokinetic parameters, namely maximum plasma concentration (Cₘₚₐₚ), time point of maximum plasma concentration, elimination rate constant, half-life, mean absorption time, and area under the plasma concentration–time curve were calculated based on serum SMV concentrations.

Results and discussions

Formulation and characterization of caseinate-coated SMV-zein nanoparticles

The growing prevalence of dyslipidemia necessitates various strategies to prevent this problem. Improvement of the efficiency and accuracy of currently used treatments is one of the strategies applied. This study aimed to develop caseinate-coated SMV-zein nanoparticles, to obtain the optimum formula, and to control preparation factors. Table 1 summarizes the experimental runs, their factor combinations, and the levels of each factor used in the study as well as the dependent variable constraints. According to a Box–Behnken experimental design, the factor combinations yielded various values of observed mean dependent variables. The observed dependent variables were Y₁,Y₂, and Y₃, as shown in Table 2. The results for Y₃ were in the range from 134 to 195 nm for runs 9 and 2, respectively.

Standardized Pareto charts were utilized to identify the significant (P<0.05) studied factors and their interaction on certain dependent variables as shown in Figure 1. A positive sign illustrated a direct relationship of the variable with the dependent variable. On the other hand, a negative sign showed inverse relationship. The data showed a range for Y₃ from 55.46% (run 7) to 97.05% (run 12). In the case of Y₁, results were in the range of 15.5% to 51.2% for run 2 and 3, respectively. The P-values resulted from analysis of variance (ANOVA) and were 0.006, 0.016, and 0.006 for...
the dependent variables $Y_1$, $Y_2$, and $Y_3$, respectively. These results indicated a significant effect of the independent factors ($X_1$ and $X_2$) on the dependent variables as shown in Table 3. On the other hand, $X_3$ was nonsignificant for its influences on the investigated dependent variables. Caseinate concentration, expressed as $X_4$, was a significant factor for the critical characteristics of the prepared nanoparticles during the preliminary screening design experiments (data not shown). The screening study was performed according to Plackett Burmann design, as a component of a seven factor design. This variable was significant for its effects on the volume weighted particle size and size distribution and the resultant zeta potential. However, it was nonsignificant for drug content and release attributes. Hence, it was included in the current optimization study for its effect on particle size. Unfortunately, the experiments factorial combinations showed the nonsignificant effect of $X_4$ on the investigated dependent variables. This demonstrates the importance of the risk analysis before attempting to optimize the formulation.

The interaction of the factors and their quantitative effects on the dependent variables were generated in mathematical regression Equations 1, 2, and 3, for the observed dependent variables $Y_1$, $Y_2$, and $Y_3$, respectively. Theoretical values of the dependent variables, and $Y_1$, $Y_2$, and $Y_3$ were obtained by the substitution of $X_1$–$X_4$ values in Equations 2, 3, and 4.

$$Y_1 = 611.497 + 3.593X_1 - 7.681X_2 - 11.820X_3$$
$$+ 0.001X_1^2 - 0.029X_2X_3 - 0.007X_1X_3$$
$$+ 0.0225417X_2^2 + 0.14X_2X_3 + 0.011X_3^2$$

$$Y_2 = 225.417 + 1.761X_1 - 3.447X_2 - 0.688X_3$$
$$+ 0.002X_1^2 - 0.035X_2X_3 + 0.006X_3$$
$$+ 0.008X_2^2 + 0.077X_2X_3 - 0.075X_3^2$$

$$Y_3 = 100.181 + 0.226X_1 - 1.324X_2 + 1.322X_3$$
$$+ 0.0185X_1^2 + 0.0198X_2X_3 - 0.021X_3$$
$$+ 0.0004X_2^2 + 0.009X_2X_3 + 0.001X_3^2$$

The dependent variable $Y_3$, cumulative percentage of drug permeated after 1 hour, was selected as a result of

### Table 3 Estimated effects and associated $P$-values for all three dependent variables

<table>
<thead>
<tr>
<th>Factor</th>
<th>$Y_1$ Estimated effect</th>
<th>$P$-value</th>
<th>$Y_2$ Estimated effect</th>
<th>$P$-value</th>
<th>$Y_3$ Estimated effect</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>42.325</td>
<td>0.006*</td>
<td>-25.213</td>
<td>0.016*</td>
<td>-5.8</td>
<td>0.243</td>
</tr>
<tr>
<td>$X_2$</td>
<td>13.25</td>
<td>0.204</td>
<td>-0.815</td>
<td>0.912</td>
<td>-20.5</td>
<td>0.006*</td>
</tr>
<tr>
<td>$X_3$</td>
<td>0.575</td>
<td>0.952</td>
<td>-6.138</td>
<td>0.423</td>
<td>0.9</td>
<td>0.846</td>
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<tr>
<td>$X_4$</td>
<td>0.658</td>
<td>0.963</td>
<td>1.856</td>
<td>0.865</td>
<td>0.865</td>
<td>0.070</td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>-11.5</td>
<td>0.411</td>
<td>-13.95</td>
<td>0.220</td>
<td>-14.817</td>
<td>0.259</td>
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<tr>
<td>$X_1X_3$</td>
<td>-2.85</td>
<td>0.833</td>
<td>2.595</td>
<td>0.805</td>
<td>-8.4</td>
<td>0.234</td>
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<tr>
<td>$X_2^2$</td>
<td>4.508</td>
<td>0.75</td>
<td>1.691</td>
<td>0.877</td>
<td>0.083</td>
<td>0.990</td>
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<tr>
<td>$X_3^2$</td>
<td>28</td>
<td>0.081</td>
<td>15.5</td>
<td>0.18</td>
<td>-1.8</td>
<td>0.783</td>
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<tr>
<td>$X_4^2$</td>
<td>2.158</td>
<td>0.878</td>
<td>-15.034</td>
<td>0.207</td>
<td>0.183</td>
<td>0.978</td>
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</table>

**Notes:** *Significant ($P<0.05$) effect of factors on individual dependent variables. Factors studied were percentage of SMV in the SMV-zein mixture ($X_1$), ethanol concentration ($X_2$), and caseinate concentration ($X_3$). The selected dependent variables were mean particle size ($Y_1$), SMV encapsulation efficiency ($Y_2$), and cumulative percentage of drug permeated after 1 hour ($Y_3$).*
the sustained release nature of the formulated SMV-zein nanoparticles. The initial release (burst effect) of sustained-release formulations can be viewed in two perspectives, either as negative or desirable consequence, according to the detailed review by Huang and Brazel. In case of the SMV-zein nanoparticles, because of the hydrophobic nature of both SMV (drug) and zein (polymer), it is desirable to obtain high initial rates of delivery to initiate SMV therapy.

The response surface and contours of the estimated response surface plots (Figure 2) reveal the dependent and independent variable relationships. The optimized caseinate-coated SMV-zein nanoparticle formula was prepared according to the predicted $X_1$, $X_2$, and $X_3$ levels, to measure the obtained dependent variables and for comparison with the calculated values. The observed, predicted values and the residuals for the optimized formula are shown in Table 4.

The GIT absorption efficiency of nanoparticles is strongly affected by the particle size. Larger particles (about 500 nm in size) can be taken by phagocytic-uptake, while for the smaller size nanoparticles, the main route of entry is through fluid phase endocytosis. The external structural properties of the optimized formula were characterized by SEM technique. SEM image (Figure 3) of the optimized formula showed spherical particles, with uniform size distribution. The mean particle size was 131 nm, as measured by the nanosizer described in the “Materials and methods”. SMV nanoparticles were discrete entities and compactly arranged, as a result of the process of centrifugation, and without

**Figure 2** The effects of independent variables and their combined effects on $Y_1$, $Y_2$, and $Y_3$ represented as three-dimensional (3D) response surface plots (left side) and contours of estimated response surface plots (right side).

**Notes:** Factors studied were percentage of SMV in the SMV-zein mixture ($X_1$), ethanol concentration ($X_2$), and caseinate concentration ($X_3$). The selected dependent variables were mean particle size ($Y_1$), SMV encapsulation efficiency ($Y_2$), and cumulative percentage of drug permeated after 1 hour ($Y_3$).
Table 4 Predicted, observed values and the residuals for the optimized sMV-zein nanoparticles

<table>
<thead>
<tr>
<th>Optimized formulation</th>
<th>Factors</th>
<th>Level</th>
<th>Dependent variables</th>
<th>Predicted</th>
<th>Observed</th>
<th>Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X&lt;sub&gt;1&lt;/sub&gt;</td>
<td>10.0005%</td>
<td>Y&lt;sub&gt;1&lt;/sub&gt;</td>
<td>134.0</td>
<td>131.4</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>X&lt;sub&gt;2&lt;/sub&gt;</td>
<td>70%</td>
<td>Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>91.057</td>
<td>89.21</td>
<td>1.847</td>
</tr>
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<td></td>
<td>X&lt;sub&gt;3&lt;/sub&gt;</td>
<td>39.767%</td>
<td>Y&lt;sub&gt;3&lt;/sub&gt;</td>
<td>44.371</td>
<td>42.20</td>
<td>2.171</td>
</tr>
</tbody>
</table>

Abbreviation: SMV, simvastatin.

Notes: Factors studied were percentage of SMV in the SMV-zein mixture (X<sub>1</sub>), ethanol concentration (X<sub>2</sub>), and caseinate concentration (X<sub>3</sub>). The selected dependent variables were mean particle size (Y<sub>1</sub>), SMV encapsulation efficiency (Y<sub>2</sub>), and cumulative percentage of drug permeated after 1 hour (Y<sub>3</sub>).

In vitro membrane permeation of optimized nanoparticles

Figure 4 depicts the percentage of SMV permeated from the optimized caseinate-coated SMV-zein nanoparticles. Results revealed a biphasic sustained-release pattern during the 48-hour release study. The initial (burst effect) stage is usually attributed to the fast release of drug entrapped near the surface of the nanoparticles. After the initial stage, SMV release was characterized by a slow-release stage. After 12 hours, about 43% of SMV in zein nanoparticles was released. About 85% of the entrapped SMV was released after 48 hours.

The slow release pattern of SMV from nanoparticles after the initial stage could be attributed to deeper entrapped SMV in the core matrix of zein nanoparticles. SMV in the core of the nanoparticles has a longer diffusion path to reach the surface compared with SMV entrapped near the surface. The release of SMV from the core matrix zein nanoparticles is controlled by the parameters affecting drug-release kinetics from the polymeric matrix. These parameters are: drug dissolution/diffusion rate, the rate of water uptake, and the size of the particles and their matrix erosion/degradation rate. In addition, the hydrophobic nature of zein augments the delay of water penetration and could retard the diffusion of the poorly water soluble SMV into the release medium. On the other hand, SMV zein nanoparticles release showed improved release characteristics compared with previously reported zein microspheres containing the poorly water soluble drug ivermectin, as a result of the increased ratio of surface area-to-particle volume.

In vivo application of optimized SMV-zein nanoparticles

The pharmacokinetic study of the optimized SMV-zein nanoparticles showed lower initial plasma concentration relative to oral suspension for the first 2 hours, possibly because SMV was released slowly from the caseinate-coated zein nanoparticles for an extended period of time. After 2 hours, the plasma concentration of SMV released from the nanoparticles began to rise in relation to oral suspension. The optimized SMV-zein showed a higher C<sub>max</sub> and a relatively higher area under plasma-time curve compared with SMV suspension, as shown in Figure 5. The C<sub>max</sub> of SMV was 23.57 and 14.52 µg/L for the optimized SMV-zein nanoparticles and SMV suspension, respectively (Figure 5). The results also revealed that the nanoparticles could significantly modify the pharmacokinetic profile and can increase the bioavailability of SMV by more than fourfold in comparison with the oral suspension.

These results indicated that formulation of SMV as caseinate-coated zein nanoparticles formulation enhanced its dissolution and absorption characteristics across the GIT.
membrane. This could be attributed to the fact that preparation of SMV, a poorly water soluble drug, as nanoparticles formulation enhanced, not only solubility of SMV but also, tissue permeability and GIT paracellular absorption of the nanoparticles. Also, the nanoparticles were introducing a drug as fine dispersion compared with coarse particles in the case of oral suspension, and hence, an increased surface area with reduced diffusion path length. In addition, the presence of caseinate as a coating for SMV-zein nanoparticles provided a higher adhesion surface contact between nanoparticles and the absorption site, which enhanced the permeation of SMV through the GIT membrane. These results showed that a distinct advantage of nanoparticle formulation might be to improve SMV performance and bioavailability in vivo.

Conclusion

The formulation of systematically optimized caseinate-coated SMV-zein nanoparticles significantly improved the pharmacokinetic profile and bioavailability of SMV by more than fourfold in comparison with SMV oral suspension. These results indicate that formulation of SMV as caseinate-coated zein nanoparticles enhanced the dissolution characters and sustained the release of SMV. As a result, hypolipidemic activities could be improved for longer time and with lower dosing frequency, which could improve patient tolerability and compliance.

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Disclosure

The authors report no conflicts of interest in this work.

References