Development and characterization of liposomal formulation containing phytosterols and tocopherols with the aim of reducing low-density lipoprotein cholesterol

Asmita Poudel1, George Gachumi1, Zafer Dallal Bashi1, Ildiko Badea1 and Anas El-Aned1
1 College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan

INTRODUCTION

Phytosterols are plant sterols having structural resemblance with cholesterol.

Phytosterols lower LDL-cholesterol in the range of 7-12% (approved by FDA and health Canada).1

Tocopherols (vitamin E) are antioxidants.2

Phytosterols and tocopherols, extracted from canola oil deodorizer distillate (canola waste), can be utilized as pharmaceuticals and nutraceuticals.

HYPOTHESES AND OBJECTIVES

Hypothesis 1

Phytosterols and tocopherols extracted from canola oil deodorizer distillate can be entrapped at high efficiency (above 90%) using optimized liposomal formulation comprised of soy phosphatidylcholine.

Objective 1: To formulate liposomes containing phytosterols and tocopherols using thin layer hydration homogenization, thin layer hydration ultrasonication, and Mozafari methods.

Objective 2: To develop and validate liquid chromatography tandem mass spectrometry (LC-MS/MS) method for comparative evaluation of the entrapment efficiency of liposomes prepared by the above-mentioned techniques.

MATERIALS AND METHOD

1. Formulation of liposomes

Methods

i. Thin layer hydration Homogenization3
ii. Thin layer hydration Ultrasonication4
iii. Mozafari method5

Phosphatidylcholine(PC) was utilized in all three methods and the utilized phase transition temperature was 55 °C.

Particle size and zeta potential characterization were done using Malvern zeta sizer.

2. Development and validation of LC-MS/MS method

MS and LC conditions

a. AB Sciex quadrupole linear ion trap (QTRAP 6500) with APCI positive mode was used (figure 2).

b. UPLC system was Agilent binary pump, Agilent 1290 infinity.

c. Agilent Poroshell, C18 column 2.1 mm × 150 mm, 5μm was utilized.

de. Elution was isocratic with Acetonitrile (0.1%Acetic acid) and methanol (0.1% Acetic acid) at 99:1 ratio.

Method validation was done as per International council for Harmonization (ICH) guidelines6.

RESULTS AND DISCUSSIONS

1. Particle size and zeta potential

The particle size was significantly larger when employing the Mozafari method (260 nm) compared to homogenization (186 nm) and ultrasonication (196 nm) methods. Whereas zeta potential was comparable among all three formulations (-14 mV to -9 mV) as shown in Table 1. Polydispersibility index (PDI) was in the range of 0.29-0.37. All of these values are adequate for colloidal stability.

Table 1: Particle size (nm), Zeta potential (mV), and polydispersibility index (PDI) of liposomes prepared using homogenization, ultrasonication, and Mozafari methods expressed as mean ± standard deviation where * represents statistical significance (p<0.05).

<table>
<thead>
<tr>
<th>Formulation techniques</th>
<th>Average particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenization</td>
<td>186.3±4.4</td>
<td>0.370 ±0.1001</td>
<td>-13.0±5.0</td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>196.2±16.1</td>
<td>0.294±0.084</td>
<td>-14.0±3.4</td>
</tr>
<tr>
<td>Mozafari method</td>
<td>260.0±23.0</td>
<td>0.348±0.087</td>
<td>-9.8±0.3</td>
</tr>
</tbody>
</table>

2. LC-MS/MS method development

LC-MS/MS method was successfully developed and validated according to ICH guideline (the chromatogram is figure 3).

3. Method Validation

Table 2: Linear range and sensitivity of phytosterols and tocopherols (LLOQ represents lower limit of quantitation and LOD represents limit of detection).

<table>
<thead>
<tr>
<th>Compound name</th>
<th>LLOQ (μg/mL)</th>
<th>LOD (μg/mL)</th>
<th>Linear range (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherols</td>
<td>0.25</td>
<td>0.01</td>
<td>0.25-10</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>0.05</td>
<td>0.005</td>
<td>0.05-10</td>
</tr>
</tbody>
</table>

3.1. Particle size and zeta potential

Following validation parameters were assessed:

a. Linearity
b. Sensitivity
c. Accuracy and Precision
d. Matrix effect
e. Dilution integrity
f. Stability

All of these parameters met the ICH acceptance criteria.

3.2. Entrapment efficiency

All three methods showed the intended entrapment efficiency that was greater than 89% as shown in table 3.

Table 3: Entrapment efficiency of liposomal phytosterols and tocopherols formulated using homogenization, ultrasonication, and Mozafari methods expressed as mean ± deviation.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenization</td>
<td>95.6±1.7</td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>91.6±3.9</td>
</tr>
<tr>
<td>Mozafari</td>
<td>89.4±2.7</td>
</tr>
</tbody>
</table>

CONCLUSIONS AND FUTURE WORK

All three formulation strategies showed size and zeta potential suitable for colloidal stability and oral delivery.

Robust analytical method was developed and validated. It was successfully applied to measured entrapment efficiency.

Ongoing work is the assessment of cholesterol lowering efficacy of liposomal phytosterols in animal model.

REFERENCES

6. International council on Harmonization, 2005, 11-12

ACKNOWLEDGEMENT

The authors thank Dr. Amaal Makhhouf for her help in formulation development.

The authors thank Dr. Ellen Wasan and Dr. Kishor Wasan for their valuable suggestions in this work.

The authors thank Ms. Deborah Michel for the training she provided in operating homogenizer and mass spectrometer.