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

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INTRODUCTION

- Phytosterols are plant sterols having structural resemblance with cholesterol.
- Phytosterols **lowers 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.

Challenges	Solution- Liposomes
1.Lipophilicity	1.Liposome entraps lipophilic compounds.
2.Oxidation mediated by heat and light.	2. Liposomes can prevent oxidation of bioactives.

- Further, liposomes can **enhance cholesterol lowering efficacy** of phytosterols.
- **The aim of this study is to develop liposomal formulation containing phytosterols and tocopherols with the aim of reducing LDL-cholesterol (Figure 1)**

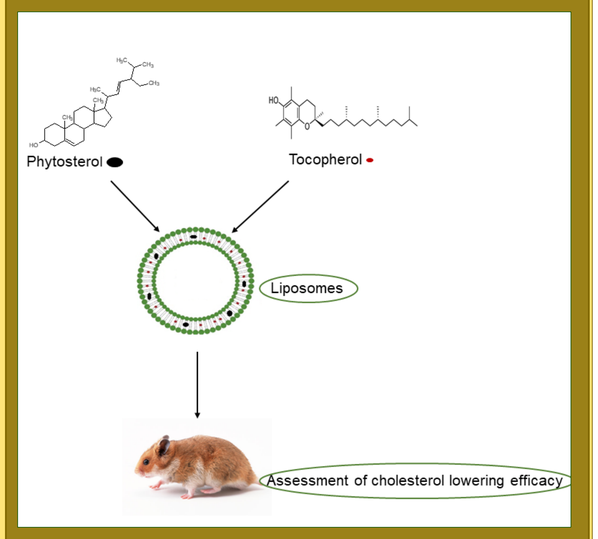

Figure 1: Research summary showing liposomal formulation of phytosterols and tocopherols. Cholesterol lowering efficacy will be assessed in animal model.

HYPOTHESES AND OBJECTIVES

Hypothesis I

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 Homogenization[3]

ii) Thin layer hydration Ultrasonication[4]

iii) Mozafari method[5]

- 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

- AB Sciex quadrupole linear ion trap (QTRAP 6500) with APCI positive mode was used (**figure 2**).
- UPLC system was Agilent binary pump, Agilent 1290 infinity.
- Agilent Poroshell, C18 column 2.1 mm × 150 mm, 5µm was utilized.
- 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) guidelines[6].

Figure 2. Liquid chromatographic system coupled with mass spectrometer

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 ($p<0.05$).

Formulation techniques	Average particle size (nm)	PDI	Zeta potential (mV)
Homogenization	186.3±4.4	0.370 ±0.001	-13.0±5.0
Ultrasonication	196.2±16.1	0.294± 0.084	-14.0±3.4
Mozafari method	260.0±23.0*	0.348±0.087	-9.8±0.3

2. LC-MS/MS method development

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

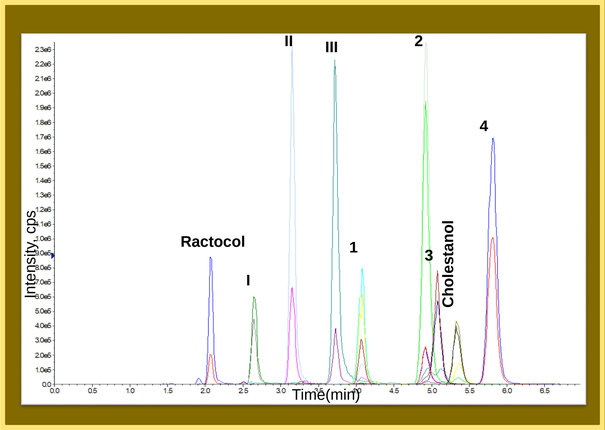

Figure 3. LC-MS/MS spectra of phytosterols and tocopherols. **1**-brassicasterol, **2**-campesterol, **3**-stigmasterol, **4**-β-sitosterol, I- δ , II- β/γ, III-α tocopherols. Cholestanol and ractocol were used as internal standard for phytosterols and tocopherol respectively.

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).

Compound name	LLOQ (µg/mL)	LOD (µg/mL)	Linear range (µg/mL)
Tocopherols	0.25	0.01	0.25-10
Phytosterols	0.05	0.005	0.05-10

- **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. 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.

Methods	Entrapment efficiency (EE %)					
	Brass	Camp	Sito	Alpha	Gamma	Delta
Homo-genization	95.8±1.7	94.1±2.0	94.8±3.0	91.6±2.4	90.5±2.8	91.5±3.5
Ultra sonication	91.4±2.4	92.2±3.3	90.1±1.8	91.2±2.0	89.8±3.0	90.0±2.2
Mozafari	89.4±2.7	93.7±6.0	93.0±6.0	92.3±7.5	97.4±1.8	95.2±1.4

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 measure entrapment efficiency.
- Ongoing work is the assessment of cholesterol lowering efficacy of liposomal phytosterols in animal model.

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