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FORMULATION, CHARACTERIZATION AND *IN-VITRO* EVALUATION OF STIGMASTEROL-LOADED PHYTOSOMES FOR ENHANCED DRUG DELIVERY

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Abstract:
Stigmasterol is a naturally occurring phytosterol possessing anti-inflammatory, antioxidant, anti-osteoarthritic, and cardioprotective properties. However, its therapeutic application is limited due to poor aqueous solubility and low oral bioavailability. The present study aimed to formulate and evaluate Stigmasterol-loaded phytosomes using phosphatidylcholine to improve its physicochemical properties and dissolution characteristics. Preliminary formulations were prepared by anti-solvent precipitation, solvent evaporation, and rotary evaporation methods. Among these methods, rotary evaporation produced phytosomes with superior particle size, entrapment efficiency, and drug loading and was selected for further optimization. Three formulations (SP1, SP2, and SP3) with different drug-to-phospholipid ratios were prepared and evaluated. The optimized formulation SP2 exhibited a particle size of 486.58 ± 5.19 nm, percentage yield of 87.04 ± 1.86%, entrapment efficiency of 88.79 ± 1.30%, and drug loading of 34.00 ± 5.70%. FTIR and DSC studies confirmed compatibility between stigmasterol and phosphatidylcholine, while SEM analysis revealed spherical vesicular morphology. The optimized phytosomal formulation demonstrated significantly enhanced in vitro drug release (94.63 ± 1.78%) compared with pure stigmasterol (58.62 ± 1.68%) after 180 minutes. These findings indicate that phytosome technology is an effective approach for improving the dissolution and potential bioavailability of stigmasterol.

Keywords: *Stigmasterol, Phytosome, Phosphatidylcholine, Entrapment efficiency, Drug release, Osteoarthritis.*

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1. INTRODUCTION:

Stigmasterol is a naturally occurring phytosterol found in numerous medicinal plants and edible oils[1]. It possesses anti-inflammatory, antioxidant, hypoglycemic, anticancer, and cholesterol-lowering activities[2]. Despite its pharmacological potential, its clinical utility remains limited due to poor aqueous solubility and low bioavailability[3]. Phytosome technology represents an advanced lipid-based delivery system capable of enhancing membrane permeability, drug solubility, and gastrointestinal absorption of poorly soluble phytoconstituents[4].

Phytosomes are molecular complexes formed between phytoconstituents and phospholipids, usually phosphatidylcholine[5]. Complexation improves lipophilicity and facilitates transport across biological membranes[6-10]. Therefore, the present study was undertaken to formulate and evaluate stigmasterol-loaded phytosomes to improve its dissolution characteristics and therapeutic potential[11-15].

2. MATERIALS AND METHODS:

Materials

Stigmasterol, phosphatidylcholine (soylecithin), ethanol, methanol, dichloromethane, phosphate buffer saline, potassium bromide, sodium hydroxide and analytical-grade reagents were used.

Preparation of Phytosomes

Preliminary batches were prepared by: Anti-solvent precipitation method, Solvent evaporation method and Rotary evaporation method[16].
Based on evaluation results, the rotary evaporation technique was selected for optimization.

Optimized Formulations

Three formulations were prepared with different molar ratios as shown in table 1

Table 1 Final Batches of Phytosome of Stigmasterol

S.No	Phytosomes	Molar ratios	
		Stigmasterol	Soy lecithin
1	SP1	1	1
2	SP2	1	2
3	SP3	2	1

Evaluation Parameters

FTIR Analysis

Optimized phytosome formulations of stigmasterol SP2, had their FTIR spectra recorded in an FTIR instrument (Model/Make: IFS 25, Bruker, Germany), with PC-based software controlling instrument operation and data processing[17]. SP2 was mixed with IR grade KBr in a ratio of 1:100. Each mixture was compressed into a pellet. Both the pellet samples were analyzed in triplicates with plain KBr pellets as blank. The information on infrared transmittance was gathered using wave numbers between 4500 cm-1 region and 500 cm-1 region[18]. The functional groups present in the sample were identified by comparing the spectral data with a reference.

Differential Scanning Calorimetry Studies

Using a differential scanning calorimeter (Mettler-Toledo India Pvt. Ltd., Mumbai, India), aluminium pans were used to record the thermogram of the optimised phytosome formulations[19]. The dry samples (2.00, 10.00 ±5 mg) were weighed separately, hermetically fastened in aluminium pans, and heated between 30 °C and 360 °C at a rate of 10 °C/min. Cleaning nitrogen stream moving at a rate of 40 mL/min created the perfect atmosphere[20].

Scanning Electron microscope (SEM) study

The surface appearance and form of the optimised phytosome of stigmasterol was examined using scanning electron microscopy (Hitachi Ltd., S-3400N type II model, Tokyo, Japan). Dry samples were placed separately on an electron microscopy metal stub and coated with gold using a particle-sputter method [21]. After that, samples were scanned, and by randomly inspecting the stub at various amplifications, digital photos of the formulations were taken [22].

Detection of Zeta Potential

The surface charge of the microspheres was determined using ZS-90 Zetasizer® (Malvern Instruments, UK) on the basis of dynamic light scattering technique[23]. The measurements were carried out in an aqueous solution of KCl 0.1N. All measurements were performed in triplicates at 25 °C with a detection angle of 90°. The measured values were corrected to a standard reference for temperature of 25° C. The results are the means of triplicate experiments.

In-Vitro Drug Release Study

The release profile of stigmasterol from the phytosomal formulation was evaluated using the dialysis membrane diffusion method. A pre-treated dialysis membrane (MWCO 12,000–14,000 Da) was secured to form a diffusion bag, into which a known quantity of phytosomal dispersion was introduced [24]. The sealed membrane was immersed in 100 mL of phosphate buffer (pH 6.8 or 7.4) maintained at 37 ± 0.5°C under continuous stirring at 100 rpm. At predetermined intervals, aliquots of dissolution medium were withdrawn and replaced with fresh buffer to maintain sink conditions. The collected samples were analyzed spectrophotometrically at a wavelength of 246nm and cumulative percentage drug release was calculated and

plotted against time [25].

3. RESULTS AND DISCUSSION:

Selection of Preparation Method

Among the three methods evaluated, rotary evaporation produced phytosomes with superior characteristics including reduced particle size and improved entrapment efficiency. Therefore, this technique was selected for optimization.

Evaluation of Optimized Formulation

The optimized formulation SP2 exhibited the most desirable physicochemical characteristics as shown in table 2

Table 2 Evaluation of final Batches of Stigmasterol Phytosomes

Parameter	SP2
Particle size (nm)	486.58 ± 5.19
Percentage yield (%)	87.04 ± 1.86
Entrapment efficiency (%)	88.79 ± 1.30
Drug loading (%)	34.00 ± 5.70

The high entrapment efficiency indicates successful complexation of stigmasterol with soya lecithin. The nanosized vesicles provide a larger surface area that may contribute to improved dissolution and absorption.

FTIR Analysis

FTIR spectra indicated the preservation of characteristic functional groups of stigmasterol and soyalecithin. No significant peak shifts or disappearance of major peaks were observed, confirming the absence of chemical incompatibility and successful formation of the phytosomal complex as shown in fig 1

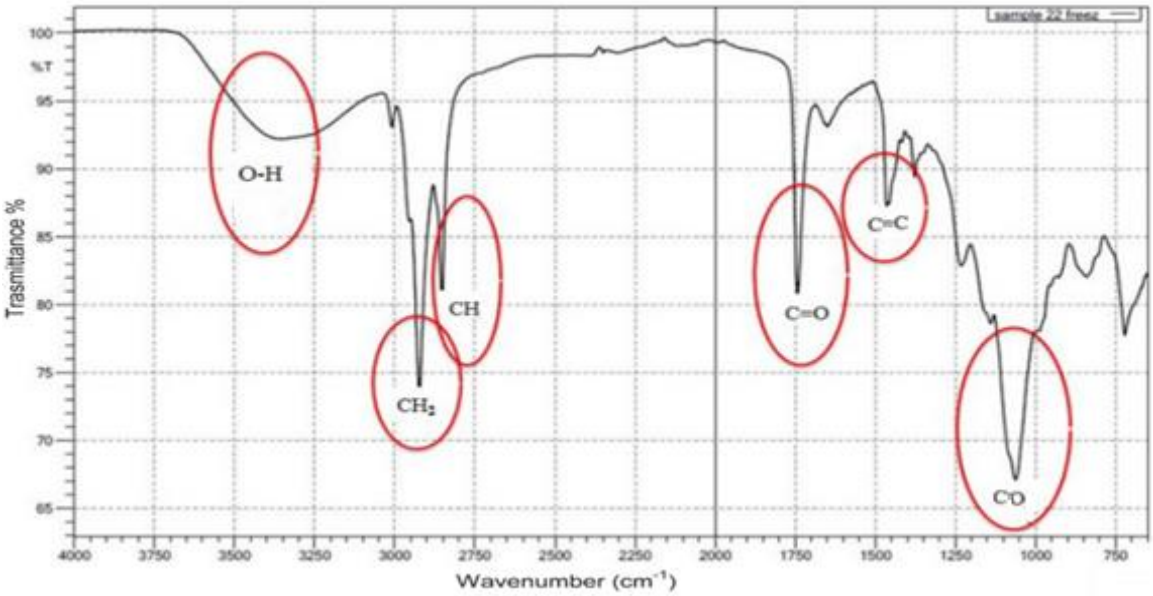

Fig 1 FTIR spectrum of Optimized Stigmasterol phytosomes SP2 formulation

DSC Analysis

DSC thermograms showed compatibility between stigmasterol and phosphatidylcholine. Reduction in drug crystallinity suggested successful incorporation of stigmasterol into the phospholipid matrix as shown in fig 2

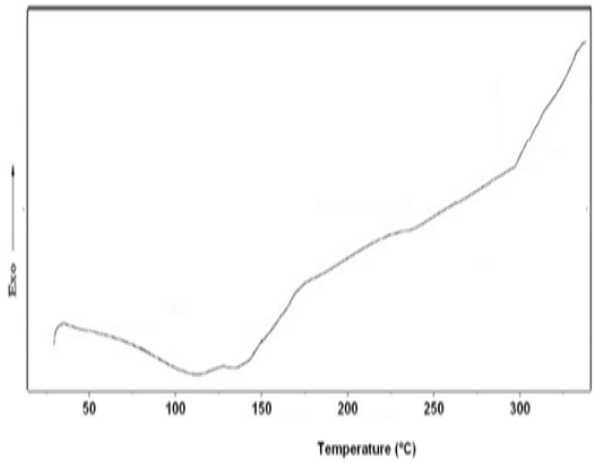

Fig 2 DSC thermogram of Optimized Stigmasterol phytosomes SP2 formulation

SEM Analysis

SEM images revealed spherical vesicular structures with relatively uniform morphology. The smooth surface appearance confirmed successful phytosome formation as shown in fig 3

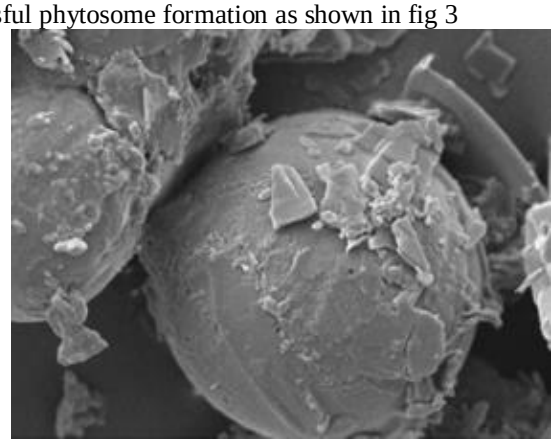

Fig 3 SEM analysis of optimized Stigmasterol phytosomes SP2 formulation

Zeta Potential

The optimized formulation exhibited satisfactory zeta potential values, indicating good physical stability and reduced tendency to aggregate as shown in table 3.

Table 3 Zeta potential analysis of the optimized phytosome formulations

S.No	Formulation code	Zeta potential
1	SP1	-46.8±4.34 mV
2	SP2	-48.6± 1.82 mV
3	SP3	-41.1 ± 3.75 mV

In Vitro Drug Release

The phytosomal formulation showed markedly improved dissolution compared with pure stigmasterol as shown in table 4

Table 4 Comparative In Vitro Drug Release Profile of Pure Stigmasterol and Optimized Stigmasterol Phytosome

Time (min)	Pure Stigmasterol (% CDR)	Phytosome (% CDR)
15	8.46 ± 0.54	21.66 ± 0.78
30	14.68 ± 0.69	38.86 ± 1.12
45	21.75 ± 0.83	52.54 ± 1.35
60	28.61 ± 1.04	65.49 ± 1.50
90	38.84 ± 1.26	79.96 ± 1.43
120	47.56 ± 1.45	88.26 ± 1.67
180	58.62 ± 1.68	94.63 ± 1.78

The optimized phytosomal formulation released approximately 95% drug within 180 minutes, whereas pure stigmasterol released only 58.62%, demonstrating the superiority of the phytosomal delivery system.

4. CONCLUSION:

Stigmasterol-loaded phytosomes were successfully prepared and optimized using the rotary evaporation technique. The optimized formulation SP2 demonstrated excellent physicochemical characteristics, high entrapment efficiency, and significantly enhanced drug release compared with pure stigmasterol. FTIR, DSC, SEM, and zeta potential studies confirmed successful phytosome formation and formulation stability. The results suggest that phytosome technology is a promising strategy to overcome the limitations associated with stigmasterol, particularly poor aqueous solubility and low bioavailability. Further in vivo and pharmacokinetic studies are recommended to

establish its therapeutic effectiveness in osteoarthritis and other inflammatory disorders.

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