RESEARCH PAPER

FORMULATION AND EVALUATION OF A NOVEL PHYTOSOME FOR IMPROVED STABILITY AND PHYSICO CHEMICAL CHARACTERISTICS.

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INTRODUCTION

Nanotechnology-based drug delivery systems have been extensively explored to overcome the limitations associated with poorly soluble bioactive compounds. Among these systems, phytosomes have emerged as a promising lipid-based carrier technology capable of enhancing the bioavailability and therapeutic performance of natural compounds. A phytosome based drug delivery system is expected to improve its physicochemical properties and therapeutic effectiveness for managing various skin disease. A phytosome drug delivery may provide sustained and control release for phytoconstituents especially flavonoids. Phytosomes are advanced herbal drug delivery systems developed to improve the absorption and effectiveness of plant-based medicines. The word phytosome is derived from two words “phyto” means “plant”, “some” means “cell like structure”. Phytosomes are formed by binding the active phytoconstituents of medicinal plants with phospholipids, usually phosphatidylcholine. This complex enhances the ability of herbal compounds to cross biological membranes, resulting in better bioavailability and improved therapeutic action.

Many herbal constituents especially flavonoids and polyphenols possess excellent medicinal properties. But it shows poor absorption when administered alone. Phytosome technology overcomes this limitation by creating a molecular complex between the plant extract and phospholipids, it allows the active compounds to be absorbed more efficiently into the body

2. MATERIALS AND METHODS

2.1. Materials

The chemicals used were 2-methyl Anthraquinone, lecithin, cholesterol, Tween 80 and solvents of analytical grade were purchased from SRL.

2.2. Methods

2.2.1. Preparation of Phytosomes

The Phytosomes were prepared using the thin film hydration method with the aid of a magnetic stirrer. Accurately weighed quantities of the cholesterol and phospholipid, typically in a molar ratio 1:6, were dissolved in a suitable organic solvent chloroform to obtain a clear solution. The drug (1:2 ratio of lipids and drug) was dissolved in ethanol. The drug solution was added dropwise to the lipid solution under magnetic stirring at 500 RPM with mild heating (40–60°C) until a uniform thin film was formed. The dried thin film was then hydrated with a measured volume of phosphate buffer (pH 6.8–7.4) containing Tween80 (0.2%), while maintaining the temperature above the lipid transition temperature. The system was continuously stirred using a magnetic stirrer to facilitate hydration and vesicle formation. During this process, the lipid film swelled and peeled off, resulting in the formation of phytosomal vesicles. The resulting suspension was further subjected to sonication Finally, the prepared phytosomal suspension was stored in a sealed

container under refrigerated conditions (4°C) for further characterization and use.

2.2.2. Characterization of the prepared Phytosome

The prepared formulation was studied for their physical characteristics (Particle size, zeta potential, drug content, % yield, % encapsulation efficiency, and drug loading capacity), as well as their analytical (FTIR, XRD) and morphological (SEM) properties.

a. Particle size, PDI and Surface charge (Zeta potential)

Determine using a particle size analyzer (Malvern Zetasizer NanoZS), the particle size of phytosome was measured in triplicates. Disposable cuvettes were used to hold the sample solution, and the principle based is dynamic light scatering.

b. % Yield, % Encapsulation efficiency, and % Drug loading capacity

After centrifugation at 4°C for 20 minutes at 12,000 rpm, the supernatants of the phytosomes were used for the analysis. The free drug's concentration in the resulting supernatant was measured using UV-vis spectrophotometry at 327 nm, based on a previously established standard calibration curve for 2-methyl anthraquinone. Following parameters are calculated using standard formulas.

$$\% \text{ Yield} = \frac{\text{Weight of nancomposite}}{\text{Whole weight of excipients and drug added}} \text{ x } 100$$

$$\% \text{ Encapsulation efficienc} = \frac{\text{Amount of drug present in the nanocomposite}}{\text{The total amount of drug taken}} \times 100$$

$$\% \text{ Drug loading} = \frac{\text{The total amount of drug taken initially} - \text{Amount of free drug}}{\text{Total weight the nanocomposite}} \text{ x } 100$$

c. Determination of drug content

2mg of formulation was accurately weighed, dispersed in ethanol, and allowed to stand for 24 hours. After incubation, 1ml of the sample was taken and the amount of 2-methyl anthraquinone present in the phytosome was measured using UV-vis spectrophotometry at 327 nm.

d. Morphological characterization

Determine using scanning electron microscopy (SEM), the morphology of phytosome was observed. The sample was coated with a 5nm layer of gold via sputtering (Hitachi S-3400N) before the analysis.

2.3. Analytical Characterization

Fourier-transform infrared (FTIR) spectroscopy analysis was carried out using Thermo Scientific Nicolet iS10, X-Ray Diffraction (XRD) analysis was measured at 40kV and 30ma with Cu radiation in the range of 10-70◦ (2θ).

2.4. Drug release assay and kinetics at pH 5.5

Drug release from the formulation was evaluated by dialysis bag technique using buffer pH 5.5 (representing cancer skin pH).100mg of phytosome was dispersed in 200 ml of bath stirred under magnetic stirrer at 50 RPM and at room temperature. 1 ml of aliquots was withdrawn for specific period of time every 15 min for one hour, every one hour for 8h and for every 24h for 3 days) and replaced with same volume of buffer at regular intervals to sustain the equilibrium condition. The drug concentration in the collected samples was quantified using a UV-vis spectrophotometer at 327nm, and the results were fitted with mathematical models to identify the mechanism of drug release.

2.5. Stability assessment of Phytosomes by forced degradation analysis

To evaluate the stability profile of the Phytosomes, forced degradation studies under acidic, alkaline, and oxidative conditions were conducted. The study was performed under ICH guidelines Q1A, Q1B, and Q2B. A measured quantity (10 mg) of all the Phytosomes was exposed to stress conditions by incubating it in 1 N HCl, 1 N NaOH, and 3% H_2O_2 for 3days at room temperature. Following the incubation, the samples were analyzed for drug content (stability) using a UV-vis spectrophotometer at 327nm.

3. RESULTS AND DISCUSSION

3.1. Preparation of Phytosomes

Phytosomes containing lecithin, Tween 80, cholesterol, and 2-methylanthraquinone formed based on the principles of phospholipid-API self-assembly of amphiphilic molecules into stable vesicular system, complexation, and hydrophobic interaction. Lecithin possessing both hydrophilic polar heads and hydrophobic fatty acid tails. 2-methylanthraquinone, a lipid API interacts with the hydrophobic region of lecithin through hydrophobic interactions and van der Waals forces in contrast the polar functional groups may form H-bonding with the phosphate group of phosphatidylcholine. These

bonding may result in the stable drug-phospholipid complex formation that improves the solubility and bioavailability of the poorly water-soluble compound. By inserting itself between the phospholipid chains, Cholesterol stabilizes and strengthens the phospholipid bilayer thereby decreasing membrane fluidity and permeability. This enhances stability and rigidity, reduces drug leakage. Tween 80 prevents particle aggregation and reduces particle size through steric stabilization, leading to the formation of uniformly distributed nanosized vesicles. As it reduces interfacial tension between lipid and aqueous phases, enable the uniform dispersion and efficient encapsulation of 2-methylanthraquinone within the vesicles. During hydration, the amphiphilic components undergo self-assembly spontaneously, and hydrophilic heads arranges toward the aqueous environment and hydrophobic tails twist inward, entrapping 2-methylanthraquinone within the lipid bilayer. This results in the formation of stable spherical phytosomal vesicles with enhanced membrane permeability, improved solubility, sustained drug release, increased stability, and enhanced therapeutic efficiency.

3.2. *In vitro* characterization of the Phytosomes

a. Particle Size and Zeta Potential Analysis of Phytosomes

The phytosomes showed the desired size of 250.1±87.26 nm. The zeta potential was – 26.7±7.07 mV which is near to 30. The zeta potential ±30 to ±60, and is reported to be good value for the stable formulation. With reference to PDI (0.376), it is considered to be optimal as it exhibited a lower value which confirms its stability and homogeneous nature. Hence, the formulation was found to exhibit good stability within the optimal literature values.

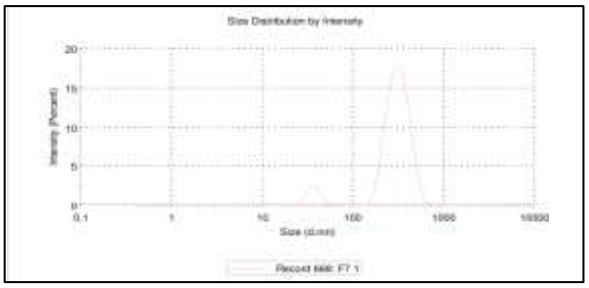

Figure 1: Particle size spectrum of phytosomes

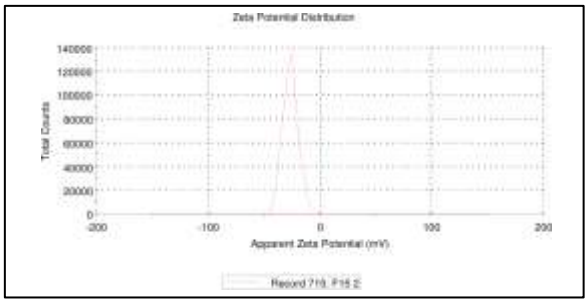

Figure 2: Zeta Potential spectrum of phytosomes

b. % Yield, % Encapsulation efficiency and Drug loading capacity

The prepared phytosome had 45.82±3.89% yield, 89.6±5,01% encapsulation efficiency and 37.08±1.24% drug loading. The encapsulation and drug loading were good enough as per literature, and in correlation with reported studies.

c. Drug content of phytosomes

The formulation showed moderate drug content of 1.954±21.9 mg per 5 mg of phytosomes This is in good correlation with the indirect method of drug loading.

d. Morphology of the phytosomes

Scanning Electron Microscopy analysis of the phytosomal formulation containing lecithin, Tween 80, cholesterol, and 2-methylanthraquinone showed discrete spherical vesicles with smooth surface morphology. Lecithin formed the phospholipid bilayer structure, while 2-methylanthraquinone was entrapped within the hydrophobic lipid region. Cholesterol improved vesicle rigidity and stability by strengthening the phospholipid membrane and reducing drug leakage. Tween 80 reduces particles from clumping, leading to tiny even-sized bubbles floating free. With no sharp crystal shapes showing up, it became clear the 2-methylanthraquinone had slipped fully into the plant-based capsule setup.

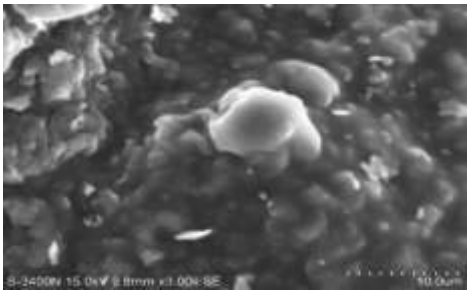

Figure 3: SEM images of phytosomes

3.4. Analytical characterization of phytosomes

a. FT-IR

Peaks showing up close to 3400–3200 cm^{-1} in pure 2-methylanthraquinone point to O–H stretch movements, though signals near 2920 cm^{-1} suggest both aromatic and aliphatic C–H stretches. Around 1660–1600 cm^{-1}, strong signals appear because of the anthraquinone core's linked C=O stretch. Between 1450–1200 cm^{-1}, further signs emerge that match aromatic ring bonds like C=C plus C–O stretches.

Cholesterol FTIR spectrum showed a wide peak at about 3400 cm^{-1} due to O–H stretch. Following that, clear signals appeared close to 2920 and 2850 cm^{-1} from aliphatic C–H vibrations. Peaks near 1460 cm^{-1} corresponds to CH bend. Meanwhile, another peak around 1050 cm^{-1} pointed to C–O stretching. Taken together, these features comply with other studies.

Starting at 3400 cm^{-1}, a wide peak revealed hydroxyl groups present in Tween 80. Around 1730 cm^{-1}, vibration signals showed presence of ester carbonyls. Signals tied to ethers seen between 1100 and 1250 cm^{-1} - this stretch pattern backed up its identity as a nonionic surfactant. The overall profile matched typical features seen in T-80.

To begin with the sample of the lecithin, there are typical indications of phospholipids in the spectrum. Stretches of the aliphatic C-H are apparent at about 2920 cm-1 and 2850 cm-1. Further along at 1735 cm-1, the presence of the ester carbonyl group is evident as a stretch. Between 1230 cm-1 and 1060 cm-1, stretches are present owing to the vibrations of the phosphate group. Combined together these bands prove that the key moiety for the formation of the phytosome is the backbone of phosphatidylcholine.

In the phytosomal formulation 2-methylanthraquinone signal is still visible but broadened, weakened and shifted from its expected position with respect to all the respective functional groups in the 2MAQ. The stretching associated with the carbonyl is indeed slightly shifted and broadened, which confirms that there is interaction occurring between anthraquinone group and the phospholipids. We can observe bands that have broadened between where hydroxyl groups absorb. This confirms that there is some hydrogen bonds formed between the drug and one of the ingredients like the lecithin, cholesterol or Tween 80.

There are no new peaks in the 2-Methylanthraquinone scan, which proves that there is no new chemical entity and it remained stable during the process. However, in the region of phospholipid some signals due to the drug decreased and disappearance was observed, suggesting that 2-methylanthraquinone is encapsulated within the fatty lipid portion. Near where the phospholipids interact with the drug, it starts to combine together; it's as if a fatty particle has attached onto a drug, it can't be leaked.

The FTIR shows the direct connection of 2-methylanthraquinone with lecithin, cholesterol and even Tween 80 forming stable phytosomes. They not come together for encapsulation also bonded via intermolecular Hydrogen bond.

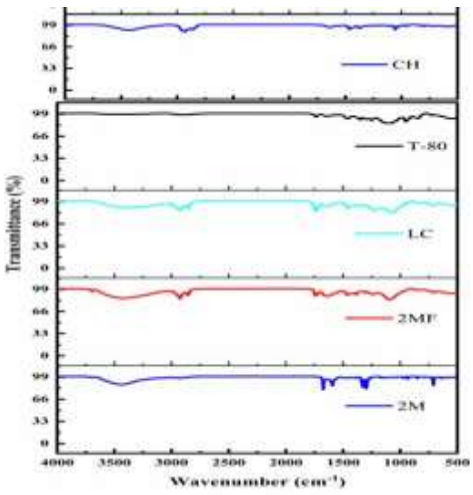

Figure 4: FTIR spectrum of phytosomes

b. X-Ray Diffraction

Pure 2-methylanthraquinone exhibited sharp and intense diffraction peaks at approximately 2θ values of 12°, 17°, 21°, 24°, and 27°, indicating its highly crystalline nature. The presence of multiple narrow peaks confirms the ordered crystal lattice arrangement of the pure anthraquinone compound.

Cholesterol showed characteristic crystalline peaks around 2θ values of 5°, 7°, 15°, and 23°, which are typical of sterol crystalline packing behavior. These sharp reflections indicate the semi-crystalline nature of cholesterol.

Lecithin displayed a broad halo pattern centered around 2θ values of approximately 18°–22°, indicating its predominantly amorphous phospholipid structure. The absence of intense crystalline reflections confirms the disordered molecular arrangement of lecithin.

Tween 80 also exhibited broad diffused peaks near 19°–21° with absence of sharp reflections, suggesting its amorphous and non-crystalline surfactant nature.

In the 2-Methylanthraquinone phytosome formulation, the characteristic crystalline peaks of pure 2-methylanthraquinone were markedly reduced in intensity, broadened, or completely disappeared. The phytosome diffractogram mainly exhibited a broad diffuse halo around 2θ values of 18°–25°, indicating conversion of the crystalline drug into an amorphous or molecularly dispersed state within the phospholipid matrix.

The significant reduction in peak intensity at the characteristic drug diffraction regions confirms successful encapsulation of 2-methylanthraquinone into the lecithin-cholesterol vesicular system. The absence of distinct sharp crystalline peaks in the 2-Methylanthraquinone formulation suggests strong intermolecular interaction between the drug and phospholipid components, preventing recrystallization of the drug.

Additionally, the broadening of peaks and reduction in crystallinity indicate enhanced molecular dispersion and possible hydrogen bonding or hydrophobic interaction among lecithin, cholesterol, Tween 80, and the anthraquinone moiety. Such amorphization is beneficial for improving solubility, dissolution rate, and bioavailability of poorly water-soluble compounds like 2-methylanthraquinone. Finally, the comparative XRD analysis confirms, pure 2-methylanthraquinone possesses crystalline characteristics, lecithin and Tween 80 exhibit predominantly amorphous behavior. The 2-Methylanthraquinone phytosome showed marked reduction in crystallinity, successful molecular encapsulation and phytosome complex formation occurred.

Conversion of drug from crystalline to amorphous state may enhance solubility and therapeutic performance of the formulation.

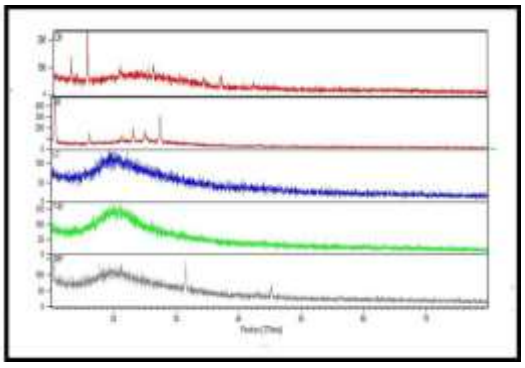

Figure 5: X-ray diffraction pattern of the phytosome and pure compounds illustrating intensity as a function of 2θ (Cu Kα radiation)

3.4. Drug release assay by Dialysis bag technique

The drug release of phytosome was done by dialysis bag technique, at pH 5.2. The drug release from the phytosomes started from the first one hour and there was sustained release of drugs. Nearly 14.43±0.99 % of the drug is released in 24 hours from the formulation and form the pure drug it shows incomplete dissolution of 20.49±1.12% in 5 hours. The formulation exhibited anomalous transport in which both diffusion and erosion mechanisms govern,

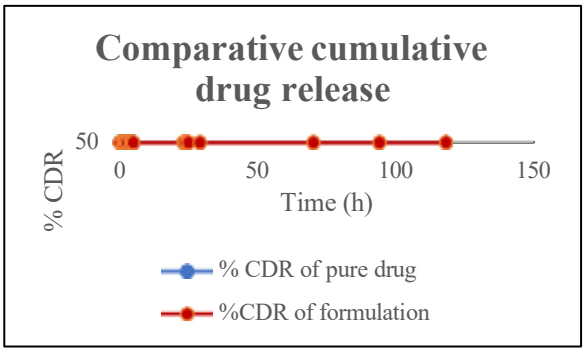

Figure 7: Cumulative drug release profile of Phytosomes and 2-methylanthraquinone

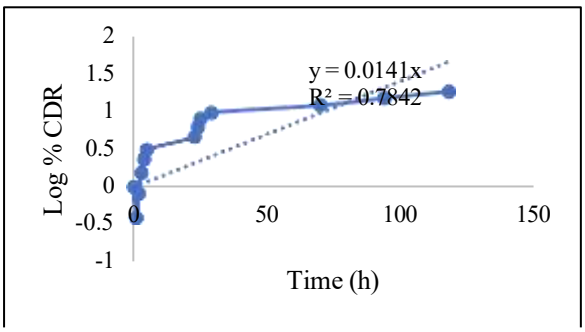

Figure 8: First-order plot of drug release from Phytosomes

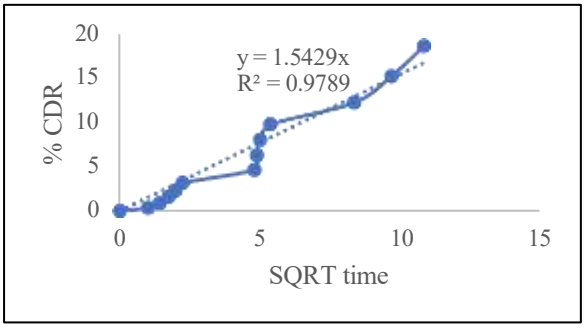

Figure 9: Higuchi model plot of drug release from Phytosomes

3.5. Forced degradation Study of phytosomes

The forced degradation study of 2-methylanthraquinone-loaded phytosomes was performed according to ICH stability guidelines to evaluate the stability-indicating nature of the formulation under various stress conditions including acidic, alkaline, oxidative, thermal, and photolytic degradation. The study demonstrated that the phytosomal formulation possessed appreciable stability with minimal degradation under stressed environments, confirming the protective effect of the phospholipid vesicular system.

Under acidic degradation conditions, the formulation showed slight reduction in drug content due to partial hydrolysis of the anthraquinone structure in acidic medium.

In alkaline degradation studies, comparatively higher degradation was observed because anthraquinone derivatives are more susceptible to alkaline hydrolysis. Nevertheless, the encapsulated phytosomal formulation exhibited lower degradation when compared to pure drug, suggesting stabilization by lecithin and cholesterol components.

Oxidative degradation studies performed using hydrogen peroxide showed moderate degradation of the formulation due to oxidative stress. The phytosomal lipid bilayer restricted direct exposure of the encapsulated drug to oxidative conditions, thereby reducing degradation extent.

Thermal degradation studies indicated that the phytosome formulation remained relatively stable at elevated temperature conditions with only minor reduction in drug content. The improved thermal resistance may be attributed to molecular dispersion of the drug within the phospholipid matrix as confirmed by XRD studies.

Conclusion

In summary, this study was successful in proving that the phytosomal preparation of 2-methylanthraquinone synthesized through the use of lecithin, cholesterol, and Tween 80 improves the physical and chemical stability, solubility, and efficiency of the drug. The phytosomes contained spherical-shaped nanoparticles with an ideal particle size (≈250 nm), appropriate zeta potential (–26.7 mV), high encapsulation efficiency (≈89.6%), and slow drug release in an acidic medium, mimicking the diseased skin environment. The structural investigation of the product demonstrated the existence of strong intermolecular interactions in addition to the drug transition from the crystalline form to the amorphous one increasing the bioavailability of the compound. The results of the morphology investigation confirmed the formation of uniform vesicles with low aggregation level. Forced degradation studies revealed the potential of the phytosome system for protecting the drug against hydrolysis, oxidation, and thermal degradation.

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