

Development and Characterization of Vitamin C Phytosomes Using the Thin Film Hydration Method

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ABSTRACT

Vitamin C, a hydrophilic compound widely found in citrus containing fruits and vegetable, predominantly in skin care and cosmetic preparations due to its potent anti-oxidant, anti-inflammatory and UV protectant potential. However, its highly crystalline and hydrophilic nature hampers its absorption and skin permeation. The aim of this study was to develop and characterize Vitamin C loaded phytosomes. The phytosomes were prepared using thin-film hydration method using a rotary evaporator with different drug-lipid ratio. Vitamin C loaded phytosomal formulations were evaluated for particle size, polydispersity index (PDI), zeta potential, %EE (entrapment efficiency), in-vitro drug release and antioxidant activity. The formulation F2 (drug: lipid ratio of 1:1.5) exhibited optimal physicochemical characterization with particle size of 293.8 ± 8.6 nm, PDI of 0.268 ± 0.015 and zeta potential of -32.6 ± 2.1 mV. Formulation F2 also exhibited high % EE of 87.2%, and appreciable drug release of 91.5% at 60 min and significant DPPH radical scavenging activity. SEM analysis confirmed formation of spherical to oval particle shape while DSC and XRD analyses verified successful encapsulation of Vitamin C within the lipid matrix in F2.

Keywords: Vitamin C, phytosomes, encapsulation, antioxidant, lipid complex

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1. Introduction

L-ascorbic acid, commonly referred as Vitamin C (VC), is one of the essential hydrophilic micronutrients typically found in fruits and vegetables rich with strong antioxidant properties, supports collagen biosynthesis, tissue repair and improves immune functioning (1–3). VC also acts as co-factor for synthesis of several enzyme and other physiologically important nutrients (4). In addition, VC exhibits strong melanin inhibitory and ultraviolet (UV) rays induced oxidative and photodamage activity (4–6). However, the pharmaceutical and cosmetics applicability of VC is restricted due to its highly hydrophilic nature, inadequate membrane permeability, high chemical instability and reactivity in unfavorable environmental conditions such as light, oxygen, heat, and pH, collectively affecting the chemical, physical and biological stability (7–9). While the thermolabile nature of VC serves additional difficulties during conventional formulation that involves direct heat exposure, affecting the long term stability (4,10). These limitations collectively demand, development of appropriate delivery

systems that can maintain and improve the physicochemical stability and applicability of VC in pharmaceutical and cosmetic preparations (11). Phytosomes, A lipid-based drug delivery system developed by complexation of active agent and phospholipid (a major component of cell membrane) in suitable stoichiometric ratio non-covalent interactions (12–14). Phospholipids are amphiphilic in nature which consists of a positively charged head and two neutrally charged tails, making itself miscible in water as well as lipid (13,15). In phytosomes, the active molecule are anchored with the polar head of phospholipid while tails encapsulate the molecule forming a lipid vesicle, which can modify the physicochemical properties of the active compound and increase its affinity for lipidic environments (5,12,15,16). Such complexation improves the incorporation of hydrophilic active molecules and potentially facilitate their interaction with biological membranes, ultimately enhancing the bioavailability of such poorly absorbed compounds (7,12).

The present study attempted to develop and characterize VC loaded phytosome. Phytosomes were prepared by complexation of VC with hydrogenated soy phosphatidylcholine (HSPC) in different stoichiometric ratio by thin film hydration method. The study focused to study the influence of different VC stoichiometric ratios on the physicochemical and functional characteristics of the resulting phytosomes.

2. Material and Method

2.1. Materials

The chemicals and reagents employed in current study were of the analytical grade and used without further purification. Hydrogenated soya phosphatidylcholine (H-SPC) was obtained as gift sample from VAV lipids Life Science, Pvt. Ltd., Mumbai, India. Vitamin C (L-ascorbic acid), cholesterol, ethanol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and other required chemicals were procured from MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik, Maharashtra, India.

2.2. Experimental procedure

Vitamin C loaded phytosomes were prepared by the thin-film hydration technique with the aid of a rotary evaporator (with slight modifications). VC and H-SPC were dissolved in ethanol and rotated at 75 rpm for 2 hours to facilitate complex formation. Subsequently, an ethanolic solution of cholesterol (20% w/w of H-SPC) was added, and continued rotation for 1 hour. The residual solvent was evaporated under reduced pressure and temperature above lipid phase transition temperature (70 mbar, >51°C) until a uniform thin film was observed on the inner walls of round bottom flask. The resulting film was hydrated with phosphate-buffered saline solution (PBS, pH 7.4) under rotation for an hour, followed by sonication for 5 min to obtain a uniform phytosomal dispersion (13,16–19). Prepared VC phytosomes formulations were stored in amber colored glass bottles. The formulation composition of VC loaded phytosomes are presented in Table 1.

Table. 1: Composition of Vitamin C Phytosomal Formulations

Batch	Molar Ratio (Vitamin C: H-SPC)	Vitamin C (mg)	H-SPC (mg)	Cholesterol (mg)
F1	1:1	100	447.4	89.5
F2	1:1.5	100	671.1	134.2
F3	1:3	100	134.2	268.4
F4	1.5:1	100	298.4	59.7
F5	3:1	100	149.1	29.8

Note: Stoichiometric ratio was identified based on molar mass of active component and lipid. The volumes of ethanol & PBS solution were adjusted according to total solid content of the formulation to achieve uniform dissolution and to ensure adequate hydration of the lipid film, the quantity of solvent ranged from 15 to 30 ml.

3. Characterization of prepared phytosomes

3.1 pH Determination

The pH of phytosomal formulations was measured using calibrated digital pH meter. The samples were diluted with ethanol (1:10) and pH of each formulation batch was recorded in triplicates.

3.2 Particle size, Polydispersity index (PDI) and Zeta potential analysis

Particle size, zeta potential (net surface charge), and polydispersity index (PDI) of the prepared VC phytosome vesicles were determined by Dynamic Light Scattering (DLS) method using Zetasizer, Malvern Instrument, India (13,16,18,20). All measurements were recorded in triplicate and results are expressed as mean standard deviation ($\pm$ SD), $n = 3$.

3.3 Percentage Entrapment Efficiency (%EE)

The amount of entrapped VC within the phytosomes vesicle was determined by indirect ultracentrifugation method. A 10 mL of Phytosomes dispersion was centrifuged at 40,000 rpm for 50 minutes at room temperature. Followed by centrifugation, the supernatant layer containing free VC was separated (un-entrapped) and analyzed by a UV-Visible spectrophotometer at 265 nm (15,18). The free VC was calculated by using calibration equation (VC in ethanol): $y = 0.0389x$ ($R^2 = 0.9979$). The amount of drug entrapped and % EE was estimated by following formulas:

Entrapped Drug

= Total Drug added

– Free drug in supernatant layer (1)

$$\%EE = \frac{\text{Entrapped Drug}}{\text{Total drug added}} \times 100 \dots\dots\dots (2)$$

3.4 In-vitro release

The release profile of VC phytosomes formulation was investigated by *in-vitro drug* release study by using modified Franz diffusion cell. Cellulose acetate dialysis membrane (molecular weight of 12,000 Da) pre-soaked in phosphate buffer saline solution (PBS) pH 7.4 for 24 h, was utilized as diffusion membrane. Approximately 1 mL of each phytosomes formulation was placed into dialysis membrane and both ends were securely tied to form a sealed bag. The dialysis membrane bag was cautiously mounted in the donor compartment. While the receptor compartment contained 15 mL of PBS (pH 7.4) as a receptor medium, maintained at $37 \pm 0.5^\circ\text{C}$ with continuous stirring at 100 rpm. At predetermined intervals (0, 5, 10, 15, 20, 30, 40, 50, and 60 min), 1 mL of aliquots were withdrawn from receptor compartment followed by immediate

replacement with equal volume of fresh PBS, pH 7.4 to maintain sink. Amount of VC released at each time point was determined using UV-Vis spectrophotometer at 265 nm (13,18).

3.5 Antioxidant Activity

The antioxidant potential of prepared formulations was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH solution (0.1 mM stock solution) was used throughout the experiment. Each phytosomes formulations was mixed with DPPH solution and incubated for 30 minutes at room temperature in dark condition and absorbance was recorded at 517 nm using a UV-Visible spectrophotometer. The DPPH solution was used as a control while free vitamin C solution served as reference (21). The percentage free radical scavenging activity was determined by following formula:

$$\% \text{ Free Radical Scavenging} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) * 100$$

3.6 Scanning Electron Microscopy (SEM):

The surface morphology and structural integrity of optimized formulation (F2) was examined by SEM analysis (Nova Nano-SEM, Model: NPEP-303). The sample was analyzed at accelerating voltage of 15.00 kV along with working distance of 5.0 mm, and magnifications at 30,000 X and 10,000 X). The images were captured at a pressure of 1.33×10^{-4} Pa using a Through Lens Detector (TLD) (18,22).

3.7 Differential Scanning Calorimetry:

The heat flow and thermal behavior and possible interactions associated with a between VC and other lipid components was studied via DSC. The DSC thermograms of selected formulation was recorded using DSC instrument (Mettler system). Sample was heated from 40–300 °C, with heating rate of 10 °C/min under nitrogen atmosphere (22,23).

3.8 X-ray Diffraction (XRD):

The crystalline characteristics of formulation of the selected phytosomal formulation was analyzed by XRD to evaluate the changes in physical state of the drug, lipid and selected phytosomal formulation. The XRD patterns were recorded using Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$), scanned in the range of 5° to 50° at diffraction angle of 2 θ (22,24).

4. Results:

4.1. Physicochemical Characterization of VC Loaded Phytosomes

4.1.1 pH

All VC phytosome formulations featured a pH in between 4.8 (± 0.1) and 5.6 (± 0.2). The pH of F2 was (5.0 ± 0.1), which is within the range of physiological skin pH.

4.1.2 Particle size, Zeta potential & Polydispersity Index

Table 2 displays the surface morphology of the VC phytosome formulations with respect to particle size, zeta potential, and polydispersity index (PDI).

The particle size ranged from 293.8 (± 10.6) to 421.4 (± 18.7) nm while the zeta potential values varied from –15.8 (± 1.1) to –32.6 (± 1.8) mV. The PDI was found in between 0.268 $\pm$ 0.018 and 0.468 $\pm$ 0.027.

4.1.3 Entrapment Efficiency

The %EE of the VC phytosome formulations is represented in Table 2. The %EE ranged from 46.7% (± 1.6) to 87.2% (± 1.9). Among all, F2 with drug-lipid ratio of 1:1.5 demonstrated the minimum %EE (87.2 $\pm$ 1.9%), followed by F3 (79.3 $\pm$ 2.0%), F1 (72.6 $\pm$ 1.8%), F4 (64.4 $\pm$ 1.7%), and F5 (46.7 $\pm$ 1.6%). These results indicate that the entrapment of VC within the phytosomal vesicles could be significantly affected by the drug-phospholipid ratio.

4.1.4 Percentage Entrapment Efficiency (%EE)

The %EE of VC phytosomes is represented in table 2 and figure 1. EE ranged from 46.7 $\pm$ 1.6 % to 87.2 $\pm$ 1.9 %. Among all, F2 with drug-lipid ratio of 1:1.5 demonstrated the maximum EE of 87.2 $\pm$ 1.9%, followed by F3 (79.3 $\pm$ 2.0%), F1 (72.6 $\pm$ 1.8%), F4 (64.4 $\pm$ 1.7%), and F5 (46.7 $\pm$ 1.6%). These results indicate that the entrapment of VC in lipid vesicles were found to be higher in formulations containing either equal of increasing concentration of lipid when compared to those with lower lipid concentration.

Table 2: Physicochemical Characteristics and pH of VC phytosomes formulations

Formulation Code (VC:H-SPC)	pH	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)
F1 (1:1)	5.2 $\pm$ 0.1	328.5 $\pm$ 12.4	0.342 $\pm$ 0.021	–21.4 $\pm$ 1.8	72.6 $\pm$ 1.8
F2 (1:1.5)	5.0 $\pm$ 0.1	293.8 $\pm$ 8.6	0.268 $\pm$ 0.015	–32.6 $\pm$ 2.1	87.2 $\pm$ 1.5
F3 (1:3)	4.8 $\pm$ 0.1	310.2 $\pm$ 10.5	0.289 $\pm$ 0.018	–25.3 $\pm$ 1.6	79.3 $\pm$ 2.2
F4 (1.5:1)	5.4 $\pm$ 0.1	376.1 $\pm$ 15.2	0.410 $\pm$ 0.025	–18.7 $\pm$ 2.3	64.4 $\pm$ 2.6
F5 (3:1)	5.6 $\pm$ 0.2	421.4 $\pm$ 18.7	0.472 $\pm$ 0.027	–12.1 $\pm$ 2.5	46.7 $\pm$ 3.1

	0.		0.0		
	2		31		

(pH, % EE & % DL, particle size, zeta potential, PDI were performed in triplicate and results are expressed as Mean $\pm$ SD, n = 3)

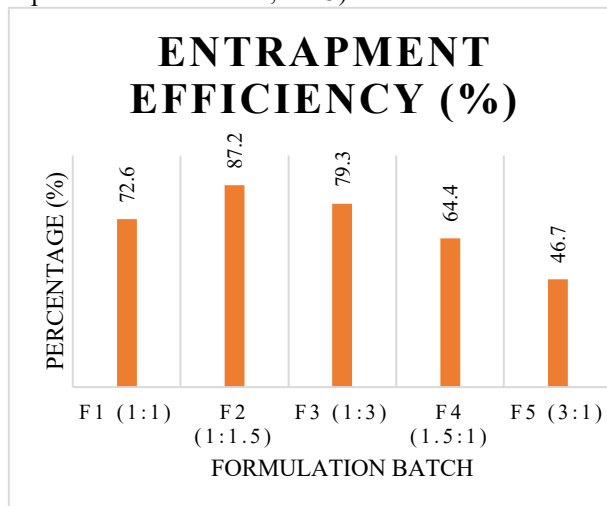


Figure 1: Graphical representation of %EE of VC Phytosome

4.2 In-vitro drug release

All VC phytosomal formulations demonstrated a consistent increase in CDR with an increasing incubation time. The total drug release at 60 minutes was 68.2% for F1, 91.5% for F2, 76.3% for F3, 61.7% for F4, and 43.9% for F5. F2 exhibited the highest release profile of 91.5% at 60 minutes. While least drug release of 43.9% at 60 minutes was observed in F5. Figure 2 shows the drug release characteristics of the phytosomal formulations. % CDR was conducted as single-run experiment and data is represented as single determination

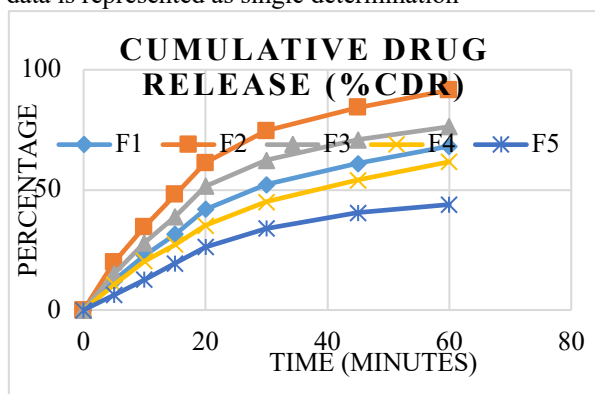


Figure 2: Comparative representation cumulative drug release (%CDR) of VC phytosome formulations (F1-F5) over 60 minutes.

4.3 DPPH Antioxidant Assay

The DPPH assay was used to study the antioxidant activity of phytosome formulations (F1-F5). Figure 3 shows that the VC phytosomes free radical scavenging activities ranged from 48.64% to 72.33%. The maximum antioxidant capacity was shown by F2 (72.33%), which was followed by F3 (65.13%), F1 (58.48%), F4 (53.81%), and F5 (48.64%) while the control sample of free VC

showed 79.14% of antioxidant activity. While free VC (reference) exhibited the 79.14 % of radical-scavenging activity under the same experimental conditions.

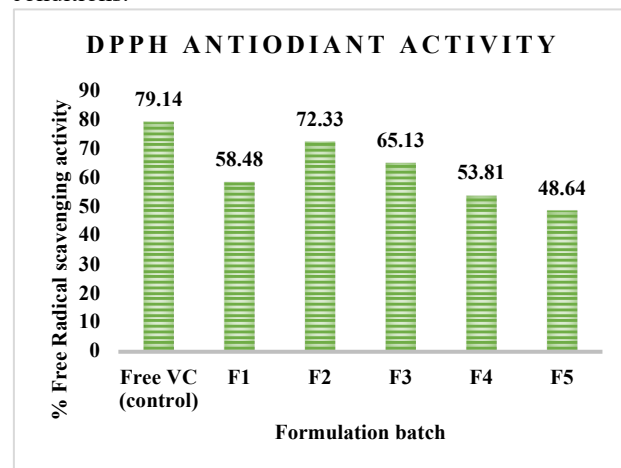


Figure 3: DPPH free radical scavenging antioxidant activity

Among all formulations (F1 to F5), F2 with VC:HSPC ratio of 1:1.5, exhibited most favorable physicochemical characteristics such as smallest particle size (293.8 ± 10.6 nm), lowest PDI (0.268 ± 0.018), and most negative zeta potential (-32.6 ± 1.8 mV). F2 also demonstrated most significance entrapment efficiency (87.2%), followed by maximum drug release (76.3%) and free radical scavenging antioxidant capacity (72.33). Hence, F2 was considered as optimal formulation and further subjected to scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) analysis.

4.3 Scanning Electron Microscopy

SEM was used to analyse the surface morphology of the optimized VC phytosome formulation (F2); typical microscopy images of SEM are shown in figure 4. The phytosomes were generally spherical to oval in shape with a smooth surface. The particles exhibited noticeable agglomeration and were dispersed widely. The SEM micrographs showed a range of particle sizes from 1.03 to 3.6 μ m at 30,000X.

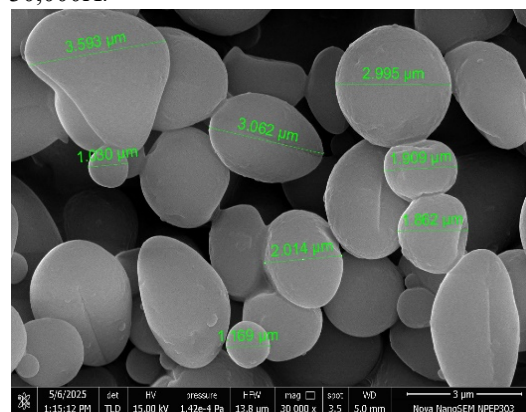


Figure 4: SEM image of phytosomes formulation (30,000X) of F2

4.3 Differential Scanning Calorimetry:

The DSC thermogram of F2, optimized VC-phytosome formulation is given in figure 5. A broad endothermic peak is observed at 62.76°C, with onset temperature of 45.45°C and an endset temperature of 72.07°C.

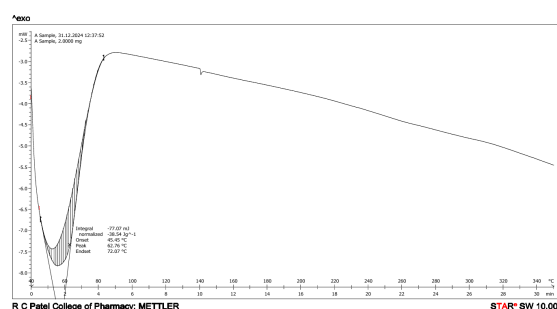


Figure 5: DSC thermogram of VC Phytosomes (F2)
4.3 X-ray Diffraction (XRD):

Figure 6 shows the X-ray powder diffraction (XRD) pattern observed in optimized formulation (F2). The XRD diffractogram, demonstrated multiple sharp, broad and diffuse diffractions peaks predominantly at 2θ range, approximately from 18° to 30° with only one characteristic peak observed at 2θ ≈ 21.25°.

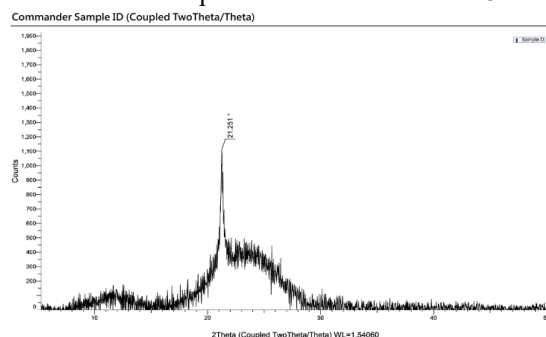


Figure 6: XRD pattern of VC Phytosomes (F2)
5. Discussion:

The present study aimed at the preparation and investigation of Vitamin C loaded phytosomes by thin film hydration method. The goal of the study was to identify the influence of different agent-to-lipid ratio on physicochemical and functional characteristics of the phytosome vesicles (F1-F5). The variation in active agent-lipid ratio reflected a clear difference in physicochemical properties of prepared formulations, F2 with ratio of 1:1.5, exhibited favorable characteristics amongst all formulation. Mayer LD et al. also demonstrated that influence of drug-lipid ratio can significantly affect various formulation properties in doxorubicin liposomes (25).

The VC loaded phytosomes formulations demonstrated a pH values ranging from 4.8 ± 0.1 to 5.6 ± 0.2 , while the pH of F2 was found to be 5.0 ± 0.1 . This mild-acidic pH of F2 is suitable for topical application, as this pH is generally considered to be compatible with the natural acidic nature of skin. The particle size of formulations (F1-F2) was observed in the range of $293.8 (\pm 8.6)$ to $421.4 (\pm 18.7)$ nm and the smallest particle size of 293.8 ± 8.6 nm was shown by F2 while the largest was observed

in F5 421.4 ± 18.7 nm. The change in particle size of phytosomes vesicle suggests that the VC:HSPC ratio can influence the arrangement and organization of phospholipid system in phytosomal formulation during preparation. Similar effects of the drug-to-phospholipid ratio on phytosomal particle size have been reported before (16,22,25).

PDI values of the VC phytosomes was observed in the range of $0.268 (\pm 0.015)$ to $0.472 (\pm 0.031)$. Highest PDI of $0.472 (\pm 0.031)$ was exhibited by F5, indicating greater heterogeneity in phytosomal dispersion while F2 exhibited the lowest PDI value of $0.268 (\pm 0.015)$, indicating a relatively narrower particle-size distribution (15,22).

The zeta potential values of the formulations varied from $-12.1 (\pm 2.5)$ to $-32.6 (\pm 2.1)$ mV. F2 exhibited the highest negative surface charge $-32.6 (\pm 2.1)$ mV, whereas F5 showed the lowest zeta potential $-12.1 (\pm 2.5)$ mV. The negative surface charges indicate an appreciable physical stability of phytosome vesicles, generally associated with the phosphate (polar groups) of phospholipid as it enhances the electrostatic repulsion between dispersed particles in formulation followed by reduced tendency to aggregate. Such negative zeta potentials have been reported in various phospholipid-based formulations, including silymarin phytosomes with a zeta potential of approximately -30.8 mV by (13,14,16).

The %EE of VC phytosomes formulation ranged from $46.7 \pm 1.6\%$ to $87.2 \pm 1.9\%$. Among all, F2 with drug-lipid ratio of 1:1.5 demonstrated the maximum EE of $87.2 \pm 1.9\%$, followed by F3 ($79.3 \pm 2.0\%$), F1 ($72.6 \pm 1.8\%$), F4 ($64.4 \pm 1.7\%$), and F5 ($46.7 \pm 1.6\%$). These results indicate that the entrapment of VC in lipid vesicles were found to be higher in formulations containing either equal of increasing concentration of lipid when compared to those with lower lipid concentration. Hung Z. et al. reported that the Ascorbic acid-phospholipid phytosomes developed at molar ratio of 1:1 showed a high drug-lipid complexing index of $98.52 \pm 0.03\%$ (26). This result supports that an ideal drug-lipid molar ratio is required to show effective complexation to achieve a favorable phytosomal system.

The antioxidant activity of VC found to be retained after complexation with HSPC into phytosomal matrix. The highest free radical scavenging activity of 72.33% was demonstrated by F2, slightly lower when compared to free VC (reference sample) with antioxidant capacity of 79.14%. This antioxidant activity observed for F2 could be related to its favorable formulation characteristics and higher incorporation of VC which could promote the availability of the active compound to interact with DPPH radicals. The lower activity of F4 and F5 may have resulted due to their lower entrapment efficiency and less favorable formulation characteristics.

The formulation, F2 has been determined to exhibit the most favorable overall characteristics with smallest particle size (293.8 ± 8.6 nm), the lowest PDI (0.268 ± 0.015) and the greatest magnitude of negative zeta potential (-32.6 ± 2.1 mV). F2 also exhibited the highest EE, CDR and free radical antioxidant capacity compared to other prepared VC phytosomes formulations (F1, F3, F4 & F5). This result suggests that the VC:HSPC ratio of 1:1.5 may be favorable drug-lipid ratio for phytosome vesicles formation, resulting in a uniform and stable phytosomal dispersion. Thus, based on overall characterizations, F2 was considered as the optimal formulation batch and further subjected to advanced characterization such as SEM, DSC, and XRD analysis.

The particles observed in the SEM micrographic images of the optimized F2 formulation are predominantly oval to spherical in shape with relatively smooth surfaces and edges, while particle size ranged from 1.03 to 3.6 μ m. The particles appear distinct, without any visible crystallinity or sharp edges, indicating that VC could have been efficiently complexed with the phospholipid matrix (H-SPC). Similar spherical, oval and smooth morphologies have been reported in literature for phytosomes prepared with phospholipids, including vasaka and other phytoconstituent–phospholipid complexes (27).

The thermal event recorded in DSC thermogram of optimized VC phytosomes formulation displayed a broad endothermic peak at 62.76 °C with an onset temperature of 45.45 °C and endset temperature of 72.07 °C. This reported onset temperature of 45.45 °C is probably associated with the melting point of H-SPC (28). While, the broad endothermic peak at 62.76 °C supports the formation physical interaction between VC and H-SPC, indicating the subsequent phase transition which is generally related to the dissociation of VC-HSPC complex. While, absence of a sharp endothermic peak of pure VC (~190°C) in thermogram of current formulation is an indicative sign of the decreasing crystallinity of VC (23,29). This disappearance of peak suggests that VC may be molecularly dispersed in the H-SPC matrix, resulting in successful complexation.

The XRD pattern of F2 demonstrated a marked reduction in the characteristic crystalline peaks of VC, with predominance of a broad diffuse halo and only one prominent peak at $2\theta = 21.25^\circ$. the studies reported that the pure VC exhibits several distinct diffraction peaks, including reflections around 10.6 – 10.7° , 17.5° , 21.3° , 25.4° , and 27.4 – 28.2° (24,30,31). The prominent single sharp residual peak observed at 21.25° in F2 may indicate partial loss in crystallinity of VC. This substantial disappearance of reported peaks in F2 suggests disruption of the crystalline lattice of VC and a transition toward a partially amorphous state

following its incorporation into the HSPC-based phytosomal system. Similar reduction in drug-specific crystalline peaks has been reported for phospholipid-based systems and is generally associated with molecular dispersion and drug–phospholipid interactions. Thus, the XRD findings provide supportive evidence for the formation of the VC–HSPC phytosomal system and are consistent with the altered thermal behavior observed in the DSC analysis.

6. Conclusion:

The study successfully developed and characterized Vitamin C encapsulated phytosomes. The findings of the current investigation confirm the feasibility of phytosomal vesicles to incorporate VC, a crystalline and hydrophilic agent into lipid bilayer leading to improve its physical properties. All formulation batches exhibited variations in terms of physicochemical properties, entrapment efficiency, in-vitro drug release and antioxidant activity based on the stoichiometric ratio between VC and HSPC. Amongst the developed formulation batches (F1 to F5), F2 offered a favorable stoichiometric ratio between active agent and lipid (1:1.5). The physicochemical characterization of F2 formulation ensures successful encapsulation of VC within the lipid matrix, with suitable surface morphology, particle size contributing to the colloidal stability. However, the structural analysis (XRD) and thermal parameter (DSC), indicate that crystalline VC has been partially evolved into an amorphous state due to complexation with lipid bilayer, this may be beneficial for enhancing penetration through the skin membrane. Collectively, F2 phytosomes formulation can be considered as an efficient delivery system for dermal and cosmetics preparation with further studies. In order to verify the clinical safety, efficacy, and appropriateness of this VC-loaded phytosomal formulation, additional stability studies, pre-clinical and clinical research needs to be undertaken.

7. Future consideration:

Based on the promising results observed in VC-loaded phytosomes, particularly F2, it can be further investigated for *ex-vivo* and *in-vivo* studies to confirm the dermal permeability and safety profile. The optimized VC loaded phytosomes can be further optimized into suitable cosmetics preparation such as cream, ointment or gel followed by its thorough evaluation including shelf life, suitable packaging conditions, and formulation robustness a long-term and accelerated stability studies can be conducted according to ICH guidelines for cosmetics applicability.

8. Authors contribution:

Current investigation aimed to develop VC loaded phytosomes, both authors contributed equally in conduct of the research work and preparation of the manuscript. The first author (Patil Sonali), was involved in literature review, development of

research concept, conduction of experimental work, results tracking and findings of the experimental work, and compiled all the data and drafting of the manuscript. Dr. S. Chhajed contributed to the conceptual framework, monitoring and verification the experimental methods and findings throughout the research, assisted in the manuscript's intellectual editing.

9. Competing Interests:

The authors declare there is no conflicts of interest.

9. Funding:

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