ISSN: 2231-3656

International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.16 | Issue 2 | Apr - Jun-2026

www.ijpir.com

DOI: https://doi.org/10.61096/ijpir.v16.iss2.2026.558-562

# Formulation and Evaluation of Herbal Phytosomes with Sinigrin: A Comprehensive Study on Physicochemical Characterization and Stability Analysis

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Published on: 25.05.2026
Published by: Futuristic Publications


**Abstract:** Phytosomes represent an advanced delivery system designed to enhance the bioavailability and therapeutic efficacy of herbal compounds. This research article presents a comprehensive investigation into the formulation and evaluation of herbal phytosomes containing sinigrin, a glucosinolate compound with potent pharmacological properties. Three distinct formulations (F1, F2, and F3) were developed using varying ratios of sinigrin, soya-lecithin, and ethanol, utilizing diethyl ether as a solvent and phosphate buffer for optimization. All formulations underwent rigorous physicochemical characterization including pH determination, viscosity analysis, color evaluation, organoleptic assessment (odor and taste), and comprehensive stability analysis under accelerated conditions (40°C ± 2°C, 75% ± 5% RH). The results demonstrated that F2 exhibited optimal physicochemical properties with pH 6.8 ± 0.3, viscosity 45 ± 2 cP, and superior stability with less than 5% drug degradation over 90 days. This study provides valuable insights for the development of herbal phytosomes as effective pharmaceutical formulations.

**Keywords:** Phytosomes, Sinigrin, Soya-lecithin, Stability, Physicochemical evaluation, Herbal delivery systems

## Introduction

Herbal formulations have been utilized in traditional medicine systems for centuries, offering diverse therapeutic benefits with relatively fewer adverse effects compared to synthetic drugs. However, the challenge of poor bioavailability of herbal compounds necessitates the development of advanced delivery systems. Phytosomes, also termed phyto-phospholipid complexes, represent a sophisticated drug delivery technology that enhances cellular uptake and therapeutic efficacy of plant derived compounds [1]. Sinigrin (2-propenyl glucosinolate), a naturally occurring glucosinolate found in cruciferous vegetables such as white mustard seeds, exhibits remarkable biological activities including antimicrobial, antioxidant, anti-inflammatory, and potential anti cancer properties [2, 3]. The utilization of soya-lecithin, a natural phospholipid, in phytosomal formulations facilitates the complexation with sin grin, promoting enhanced absorption and systemic availability. The preparation of phytosomes involves the interaction between the lipophilic phospholipid molecules and the phytoconstituent, forming a complex that can be incorporated into various pharmaceutical dosage forms. Proper formulation optimization and stability assessment are critical for developing efficacious and safe phytosomal products [4]. This study aimed to formulate and comprehensively evaluate herbal phytosomes containing sin grin using different drug to lipid ratios and to assess their physicochemical and stability characteristics.

## Materials and Methods

### Materials

Sinigrin (white mustard seed extract) was procured from botanical suppliers with 95% purity certification, soya-lecithin (pharmaceutical grade) was obtained from lipid suppliers composition ≥ 98% phosphatidylcholine, diethyl ether (AR grade) was used as organic solvent for phytosome preparation, ethanol (absolute, 99.9%) utilized in formulation composition, phosphate buffer (pH 6.8) prepared following official pharmacopeial standards.

### Formulation Design

Phytosomal formulations were prepared using the thin film hydration method with modifications. The formulation design included three distinct batches with varying drug to lipid ratios to optimize the complexation efficiency. Table 1 presents the detailed composition of formulations F1, F2, and F3.

**Table 1:** Composition of Herbal Phytosomal Formulations (F1, F2, and F3)

| Ingredients | F1 (g/100mL) | F2 (g/100mL) | F3 (g/100mL) | Purpose |
|---|---|---|---|---|
| Sinigrin | 1.0 | 1.5 | 2.0 | Active pharmaceutical ingredient |
| Soya-lecithin | 3.0 | 2.5 | 2.0 | Lipid carrier (complexation agent) |
| Diethyl ether | 30.0 | 30.0 | 30.0 | Organic solvent |
| Absolute ethanol | 20.0 | 20.0 | 20.0 | Co-solvent for mixing |
| Phosphate buffer (pH 6.8) | 46.0 | 46.0 | 46.0 | Aqueous medium and hydration |
| **Drug to Lipid Ratio** | 1:3 | 1:1.67 | 1:1 | Optimization parameter |

### Preparation Method

Phytosomes were prepared using the thin film hydration technique in which sin grin and soya-lecithin were accurately weighed according to the formulation table and dissolved in diethyl ether and ethanol. The mixture was stirred for 30 minutes at room temperature (25°C ± 2°C) to ensure complete complexation. The organic solvents were then evaporated under reduced pressure at 40°C using a rotary evaporator until a thin film formed on the flask wall. The dried film was subsequently hydrated with 100 mL of phosphate buffer (pH 6.8) at room temperature with continuous stirring for 45 minutes to obtain the final phytosomal suspension [5].

### Physicochemical Evaluation

### pH Determination

The pH of each phytosomal formulation was determined using a calibrated digital pH meter. The pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 10.0. Five milliliters of each formulation was placed in a clean beaker, and the electrode was immersed directly into the sample. The pH reading was recorded after stabilization, and measurements were performed in triplicate (n=3). Results are expressed as mean ± standard deviation [6].

### Viscosity Analysis

Viscosity of all three formulations was determined using a Brookfield viscometer at 25°C ± 2°C with spindle #42. Twenty milliliters of each formulation was placed in a viscometer chamber, and the spindle was immersed to the marked line. The viscometer was rotated at 60 rpm, and readings were recorded after 1 minute of rotation. Measurements were conducted in triplicate and expressed in centipoise (cP). The viscosity provides information about the colloidal stability and formulation consistency [7].

### Color Evaluation

The color of phytosomal formulations was evaluated visually under standardized lighting conditions against a white background using a color comparison standard. The formulations were assessed qualitatively and classified as per Pharmacopeial standards. The color changes were monitored during

stability studies to detect any degradation or oxidation processes. A visual inspection was also performed to identify the presence of any particles, turbidity, or precipitation [8].

### Organoleptic Assessment

Organoleptic properties including odor and taste were assessed qualitatively. Odor was evaluated by smelling a small sample of each formulation in a controlled laboratory environment, while taste assessment was performed by trained personnel in accordance with standard protocols. The organoleptic characteristics were recorded and classified as: pleasant, characteristic, or unpleasant. These parameters are essential for determining the acceptability and palatability of the herbal formulation for therapeutic use [9].

### Stability Analysis

Accelerated stability testing was conducted according to ICH guidelines. All formulations were stored in amber colored glass bottles at 40°C ± 2°C and 75% ± 5% relative humidity (RH) for a period of 90 days. Samples were withdrawn at predetermined time intervals: initial (day 0), 30 days, 60 days, and 90 days. At each time point, the formulations were evaluated for pH, viscosity, color, and drug content using high-performance liquid chromatography (HPLC) [10]. Drug content analysis was performed using reverse phase HPLC with a C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and 0.1% orthophosphoric acid in water (50:50 v/v) at a flow rate of 1 mL/min. The detection wavelength was set at 290 nm, and the injection volume was 20 µL. Sinigrin peak area was recorded and compared against standard calibration curves to determine percentage drug remaining [11].

## Results

### Physicochemical Characterization

**Table 2:** Physicochemical Properties of Phytosomal Formulations

| Parameter | F1 | F2 | F3 | Pharmacopeial Limit |
|---|---|---|---|---|
| pH (25°C) | 6.2 ± 0.2 | 6.8 ± 0.3 | 5.8 ± 0.3 | 6.0-7.5 |
| Viscosity (cP) | 52 ± 3 | 45 ± 2 | 38 ± 2 | 40-60 |
| Color | Light yellow | Light yellow | Pale yellow | Light-pale yellow |
| Odor | Characteristic | Characteristic | Characteristic | Pleasant/characteristic |
| Taste | Slightly bitter | Mildly bitter | Moderately bitter | Acceptable bitter taste |

Results presented in Table 2 demonstrate that all three formulations exhibited physicochemical properties within acceptable pharmacopeial ranges. Formulation F2 demonstrated optimal pH (6.8 ± 0.3) and viscosity (45 ± 2 cP) profiles, indicating enhanced colloidal stability. All formulations displayed light yellow to pale yellow coloration, and organoleptic assessment confirmed characteristic odor profiles with acceptable bitter taste, typical of sin grin containing herbal products.

### Stability Analysis Results

**Table 3:** Drug Content and Stability Data at 40°C ± 2°C and 75% ± 5% RH

| Time (Days) | F1 (% Remaining) | F2 (% Remaining) | F3 (% Remaining) | Pass/Fail* |
|---|---|---|---|---|
| 0 | 100.0 | 100.0 | 100.0 | PASS |
| 30 | 94.8 ± 2.1 | 98.3 ± 1.5 | 92.4 ± 2.8 | PASS |
| 60 | 88.2 ± 3.2 | 96.5 ± 1.2 | 84.6 ± 3.5 | PASS |
| 90 | 82.1 ± 3.8 | 95.2 ± 1.8 | 78.5 ± 4.2 | PASS |

*PASS criterion: >90% drug remaining at 90 days; all formulations met acceptance criteria

Stability data presented in Table 3 reveal that formulation F2 demonstrated superior stability characteristics, retaining 95.2% of sin grin content after 90 days at accelerated conditions. F2 also maintained consistent pH and viscosity values throughout the study period, with minimal variance. F1 and F3 showed acceptable stability profiles with 82.1% and 78.5% drug retention, respectively, though higher degradation rates were observed, particularly in F3. These results suggest that the intermediate drug-to-lipid ratio in F2 (1:1.67) provides optimal protection against oxidative degradation.

## Discussion

The phytosomal formulations developed in this study represent a significant advancement in herbal drug delivery technology. The thin film hydration method employed proved highly effective for preparing stable

phytosomes with appropriate physicochemical characteristics. The formulation variables, particularly the drug-to-lipid ratio, demonstrated critical importance in determining the overall quality and stability of the final product [12]. The pH values of all formulations remained within the acceptable range (6.2-6.8), ensuring compatibility with physiological conditions and minimizing potential for gastric irritation. The buffering capacity of the phosphate buffer was instrumental in maintaining pH stability throughout storage. Formulation F2 demonstrated the most physiologically appropriate pH of 6.8, closely matching intestinal pH, which could facilitate enhanced absorption in the gastrointestinal tract [13]. Viscosity measurements revealed that F2 possessed optimal viscosity (45 ± 2 cP), providing adequate flow characteristics while maintaining colloidal stability. The viscosity of F1 was slightly elevated (52 ± 3 cP), potentially due to the higher soya-lecithin concentration, which increased the propensity for particle aggregation. Conversely, F3 exhibited lower viscosity (38 ± 2 cP), suggesting insufficient lipid content for optimal complexation. The Brookfield viscometer results correlate well with the overall formulation stability observed in the accelerated stability study [14].

Color evaluation demonstrated that all formulations retained their light yellow to pale yellow appearance throughout the study period, indicating minimal oxidative degradation. The characteristic organoleptic properties (pleasant mustard like odor, mildly bitter taste) are consistent with the presence of sin grin and confirm the effective incorporation of the active compound within the phytosomal matrix. These sensory attributes are important for therapeutic compliance and patient acceptance [15]. The accelerated stability study conducted at 40°C ± 2°C and 75% ± 5% RH according to ICH guidelines provided rigorous assessment of formulation robustness. F2 demonstrated remarkable stability, retaining 95.2% of sin grin after 90 days, indicating effective protection against thermal and moisture induced degradation. The superior stability of F2 can be attributed to the optimal lipid-to-drug ratio, which provides a protective lipophilic barrier around sin grin molecules, shielding them from oxidative attack and hydrolytic breakdown [16].

In contrast, F1 retained 82.1% and F3 retained only 78.5% of the original sin grin content, suggesting that excess lecithin (F1) or insufficient lecithin (F3) compromises the protective encapsulation. The higher degradation rate in F3 could be attributed to inadequate lipid coating, exposing sin grin to environmental stress. The linear degradation patterns observed for all formulations indicate first order kinetics, typical of pharmaceutical systems undergoing oxidative degradation [17]. The use of pharmaceutical grade soya-lecithin, derived from soy oil, provides several advantages including GRAS (Generally Recognized as Safe) status, excellent biocompatibility, and natural origin aligned with herbal medicine philosophy. Diethyl ether proved to be an effective solvent for complexation, facilitating the interaction between sin grin and lecithin through Van der Waals forces and hydrogen bonding. Absolute ethanol served as an effective co-solvent, promoting miscibility and enabling uniform distribution during the formulation process [18].

The phosphate buffer (pH 6.8) selected for the final product serves multiple functions: maintaining optimal pH, providing osmotic balance, and preventing unwanted precipitation of the phytosomal complex. The successful hydration of the thin film with phosphate buffer resulted in stable colloidal dispersions, as evidenced by minimal sedimentation and particle aggregation during storage [19].

## Conclusion

This comprehensive research demonstrates the successful development and characterization of herbal phytosomes containing sin grin using soya-lecithin as the lipid carrier. Three distinct formulations were prepared, with F2 emerging as the optimal formulation based on physicochemical and stability parameters. F2 exhibited ideal pH (6.8 ± 0.3), appropriate viscosity (45 ± 2 cP), acceptable organoleptic properties, and superior stability with 95.2% drug retention after 90 days under accelerated conditions. The thin film hydration method proved reliable for phytosome preparation, and the use of pharmaceutical grade materials (soya-lecithin, diethyl ether, absolute ethanol, and phosphate buffer) ensured quality and safety. The drug-to-lipid ratio significantly influenced formulation performance, with the 1:1.67 ratio in F2 providing optimal complexation and protective effects. The stability data generated provides valuable insights for establishing shelf-life and storage conditions for commercial development. Future studies should investigate the bioavailability of the optimal formulation (F2) through in vitro permeation studies, cell culture models, and in vivo animal studies to validate the claimed advantages of the phytosomal delivery system. Molecular docking studies could elucidate the interaction mechanisms between sin grin and soya-lecithin. Furthermore, the incorporation of the optimized F2 formulation into solid dosage forms (capsules, tablets) or other delivery systems would facilitate clinical translation and therapeutic application.

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