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Research Article

Preparation, Characterization and Evaluation of Antioxidant Flavosomes

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Abstract

The objective of the present study was to prepare flavosomes loaded with kaempferol and curcumin in order to improve the bioavailability of the flavonoids. The flavosomes loaded with kaempferol and curcumin were prepared using bulk co-loading method using lecithin as the lipid molecule. The particle size of the flavosomes was determined using zeta sizer and ranged from 497.3 nm to 167.1 nm. The results indicated that increase the ratio of lipid to extract was able to reduce the particle size of the flavosomes. The flavosomes prepared using 4:1 ratio of lipid to flavonoids were found to be rigid, spherical as seen in SEM with smooth regular surface. Sharp and distinct endothermic peaks in DSC revealed the formulation of stable flavosomes due to molecular interactions between the extract and lecithin. The entrapment efficiency of flavonosome preparation F4 was determined by measurement of the non-entrapped flavonoids and subtracting from the total flavonoids used to obtain the flavonoids entrapped in the formulation. The entrapment efficiency of F4 has been calculated to be 94.12%. The flavonosome F4 was studied for release of the entrapped flavonoids by dialysis method. The amount of flavonoids released at 1, 2, 3, 4, 6, 8, 10 and 12 hours was determined using UV spectrophotometry. After the first hour, the entrapped flavonoids released in a steady manner from the flavonosome depicting and almost complete release (90.52% for kaempferol and 94.67% for curcumin) from the formulation. The flavosomes were found to be stable under the storage conditions. The best formulation with respect to particle size and anti-inflammatory action was F4 that contained 4:1 ratio of lecithin: flavonoids. DPPH radical scavenging assay was used to determine the antioxidant action of the individual flavonoids and the flavonosome formulation. It was found that the formulation F4 was having higher antioxidant activity in comparison to kaempferol and curcumin. The IC₅₀ of kaempferol, curcumin and flavonosome were found to be 228.20 µg/mL, 175.51 µg/mL and 158.19 µg/mL respectively.

Keywords: Flavonosome, Kaempferol, Curcumin, Bulk co-loading, antioxidant

INTRODUCTION

Natural products, including plants, animals and minerals play a significant role in the discovery of new chemical entities for drug discovery and thus form the basis for treatment of human diseases. Research in natural products inclined owing to their large-scale structural diversity and thereby serving as a template for the development of new drugs especially in anticancer, anti-infective, immunosuppression and neurological diseases.^{1,2} Ayurveda often involves complex formulations which are prepared over several days and can contain many herbal and mineral components. e. Dosage ranges for individual non-toxic herbs are generally in the region of 1 to 6 g/day as powders or tinctures, with higher doses often recommended for decoctions.³ Water-soluble phytochemicals such as flavonoids and poly-phenols are poorly absorbed in the body due to their large molecular size which did not allow them to be absorbed by passive diffusion, as well as their poor lipid solubility makes a serious limiting to their pass across the lipid-rich biological membranes, subsequent poor bioavailability. Phytosomes incorporate medicinal plant active ingredients and water-soluble phytochemicals into phospholipids to create lipid-compatible molecular complexes in order to immensely modify their absorption and bioavailability. The important technological advantage of phytosome as a drug carrier is high stability, high carrier capacity, feasibility of incorporation of

phytoconstituent and feasibility of routes of administration. These properties of phytosome enable improvement of drug bioavailability and reduction of the dosing frequency and may resolve the problem of non-adherence to prescribed therapy.⁴

Due to their multiple-ring large molecular size which cannot be absorbed by passive diffusion and poor lipid solubility and miscibility limiting their ability to cross the lipid-rich biological membranes, the bioavailability of water-soluble phytoconstituents (flavonoids, tannins, terpenoids etc.) is decreased.^{5,6} Flavosomes are specialized phytosomes, a type of flavonoid delivery system, which incorporate multiple polyphenolic secondary metabolites found in plants and commonly consumed in the diets of humans. Flavosomes have been shown to have antioxidant, anti-inflammatory, and anticancer properties, as well as modulating cellular enzyme functions. Flavosomes may have beneficial effects on cardiovascular health, aging, and immune system. In this work, we have prepared and optimized flavosomes containing flavonoids kaempferol and curcumin and assessed the change in antioxidant potential of the preparation compared to the individual flavonoids.

MATERIAL AND METHODS

Material

Kaempferol and curcumin was purchased from Yucca enterprises, Mumbai. Various chemicals and reagents used for preliminary phytochemical screening of extracts and other reagents for testing of anti-oxidant activity were purchased from CDH, SD Fine and Oxford Fine Chemicals Pvt Ltd, Mumbai. All the chemicals were used as received without any further processing or purification.

Preformulation Studies⁷

The following preformulation studies were carried out on the curcumin procured from the source. The preformulation studies were carried in order to confirm the purity and identity of the procured curcumin and also to study any possible interaction with the polymeric carrier to be used in the investigation.

Drug excipient compatibility Study

IR spectra of drug and a physical mixture of drugs and lipids were obtained using FT-IR spectrophotometer. The spectra were observed for physical and chemical incompatibility amongst the drug and the lipids under study.

Calibration curve of curcumin⁸

Stock solutions of curcumin containing 100 µg/mL were prepared in methanol and its aliquots were transferred in a series of 10 mL volumetric flasks in varying fractions and their volumes were made with methanol to prepare different standard dilutions (5-25 µg/mL). The solution was scanned using UV-Visible spectrophotometer from 1100 to 200 nm and

the absorption maximum (λ_{max}) was obtained to be 421 nm. The absorption of the standard dilutions was recorded at 421 nm to construct a calibration curve of concentration against absorbance.

Calibration curve of kaempferol⁹

Stock solutions of kaempferol containing 100 µg/mL were prepared in methanol and its aliquots were transferred in a series of 10 mL volumetric flasks in varying fractions and their volumes were made with methanol to prepare different standard dilutions (5-25 µg/mL). The solution was scanned using UV-Visible spectrophotometer from 400 to 200 nm and the absorption maximum (λ_{max}) was obtained to be 365 nm. The absorption of the standard dilutions was recorded at 365 nm to construct a calibration curve of concentration against absorbance.

Preparation of flavonosomes by bulk co-loading method

The specific amount of kaempferol, curcumin were dissolved in 10 mL acetone in an Erlenmeyer flask¹⁰. In another flask required quantity of soya lecithin (Table 1) was dissolved 10 mL of dichloromethane. The two solutions were mixed and the mixture was sonicated for 2h at room temperature. The sonicated mixture was transferred to a round bottom flask and allowed the solvent was allowed to evaporate using rotary vacuum evaporation resulting in the formation of thin film. The thin film was then dissolved using 10 mL of DCM and added dropwise into 40 mL of distilled water under moderate magnetic stirring at room temperature overnight. Consequently, the DCM phase steadily got evaporated with stirring overnight, resulting in the formation of flavonoid-loaded phytosomes.¹¹

Table 1 Batch formula for flavonosome preparation

Formulation Code	Ratio of Lecithin : kaempferol : curcumin	Dichloromethane (mL)
F1	1:0.5:0.5	20
F2	2:0.5:0.5	20
F3	3:0.5:0.5	20
F4	4:0.5:0.5	20
F5	5:0.5:0.5	20
F6	6:0.5:0.5	20

Evaluation of flavonosomes¹²

Visualization

Visualization of flavonosomes was accomplished by utilizing scanning electron microscopy. Scanning electron microscopy has been utilized to decide particle size estimate appropriation and surface morphology of the complex. The samples were sputter-covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive SEM (Hitachi-S 3400N) and watched for morphology at voltage of 15.0 kV.

Particle size and size distribution

The particle size (z-average) and size distribution of the prepared flavonosomes was calculated from the auto correlation function of the intensity of light scattered from the particles expecting a circular type of particles using Malvern Zeta sizer.

Differential scanning calorimetry

The thermograms were obtained for the flavonosomes and lecithin to ensure compatibility. Each sample was heated in the

range of temperature 25°C to 300°C at a heating rate of 5°C per minute. The thermograms were observed for enthalpy changes, appearance/vanishing of peaks, and changes to a peaks onset time, shape, and relative area.

Entrapment Efficiency

The prepared flavonosome formulation (F4) was filtered using syringe filter (0.22µm) and the supernatant was examined using UV spectrophotometry for the amount of non-entrapped kaempferol and curcumin. The entrapment efficiency was determined by the formula¹¹:

$$\text{Entrapment (\%)} = \frac{\text{Total Flavonoids Used} - \text{Nontrapped Flavonoids}}{\text{Total Drug Used}} \times 100$$

In vitro release study

The *in vitro* release of kaempferol and curcumin from the flavonosomes was determined by dialysis method. Flavonosomes equivalent to 1mg of each flavonoid was filled in dialysis bag and the bag was immersed in dissolution medium

comprising of phosphate buffer of pH 7.2, maintained at 37°C and stirred using magnetic stirrer at 50 rpm. At predetermined time intervals, the medium was sampled and the bulk topped-up with fresh medium. The concentration of kaempferol and curcumin in the sample was determined by measuring the absorbance using UV spectrophotometer against the respective solvent blanks.¹³

Stability studies of flavonosome formulation

The prepared flavonosomes were subjected to stability studies at 40±2°C/75±5% RH and 30±2°C/60±5% RH according to the ICH guidelines for a period of 3 months.

In vitro antioxidant study

Using the stable radical DPPH, the hydrogen-donating or radical-scavenging ability of the synthesized compounds was used to quantify their free radical scavenging activity. After being produced in DMSO, the test samples (100 µL, 100-500 µg/mL) were combined with 1.0 mL of DPPH solution and then filled to a final volume of 4 mL with methanol. In a visible spectrophotometer, the absorbance of the resultant solution was measured at 517 nm. As the reference substance, ascorbic acid was employed. Higher free radical scavenging activity was shown by the reaction mixture's lower absorbance. The percentage of free radicals that the sample inhibited was used

to express the radical scavenging activity.¹⁴ This was calculated using the formula

$$\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0} \times 100$$

RESULTS AND DISCUSSION

Preformulation Study of Kaempferol and curcumin

The results of organoleptic characterization and melting point are presented in table 2.

Table 2 Organoleptic properties of Kaempferol

Test	Curcumin	Kaempferol
Color	Pale Yellow	Pale Yellow
Odor	Characteristic	Characteristic
Taste	Bitter	Not determined
Melting Point	181-185°C	274-276°C

Calibration curve of kaempferol

The calibration curve of kaempferol was prepared in methanol using UV-Visible spectrophotometer at 365 nm by plotting the absorbance against concentration (Figure 1a).

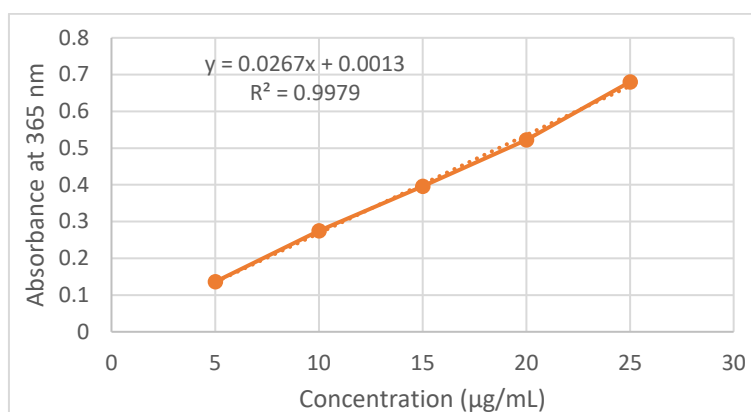


Figure 1a: Calibration curve of kaempferol in methanol

Calibration curve of curcumin

The calibration curve of curcumin was prepared in methanol using UV-Visible spectrophotometer at 421 nm by plotting the absorbance against concentration (Figure 1b).

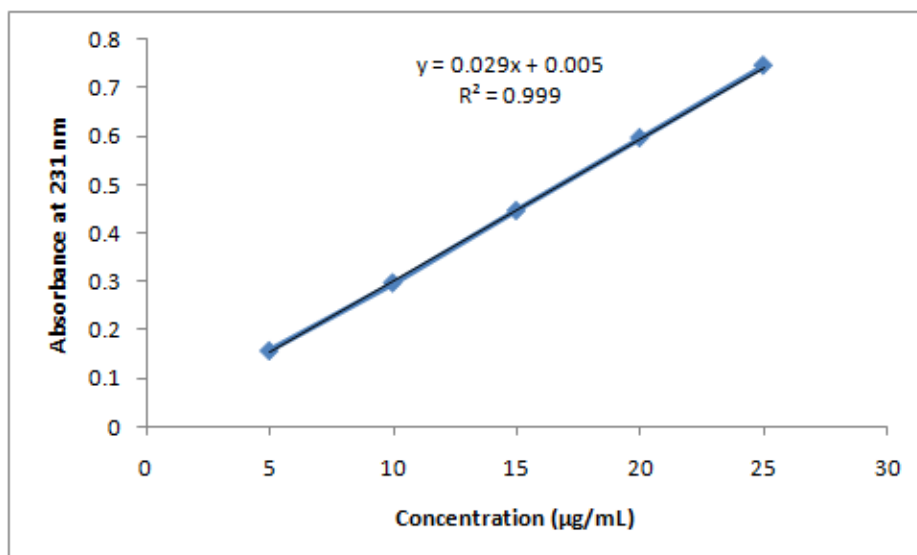


Figure 1b: Calibration curve of curcumin in methanol

FT-IR study

The FT-IR spectrum of kaempferol (Figure 2a), curcumin (Figure 2b) and a physical mixture of kaempferol, curcumin and lecithin (Figure 2c) were obtained and observed for any deletion of the peaks of the pure drug. The spectrum of

kaempferol and curcumin exhibited peaks at 3341 cm^{-1} (OH stretching), 3056 cm^{-1} (CH aromatic stretching), 2923 cm^{-1} (CH_2 stretching), 1647 cm^{-1} (C=O stretching), 1574 cm^{-1} (C=C aromatic stretching), 1441 cm^{-1} (CH_2 bending), 1146 cm^{-1} (C-O stretching). All the peaks were present in the physical mixture indicating a compatibility between the both the components.

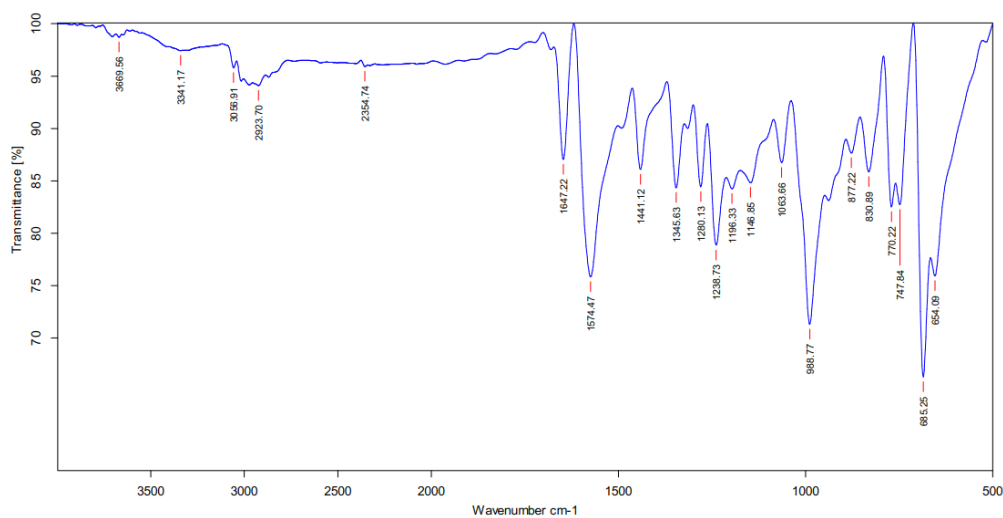


Figure 2a: FT-IR spectrum of Kaempferol

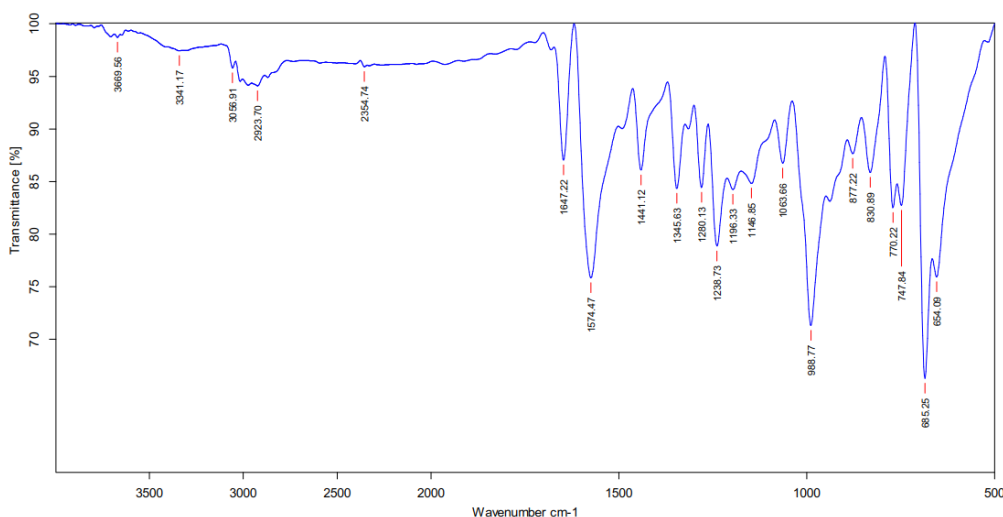


Figure 2b: FT-IR spectrum of Curcumin

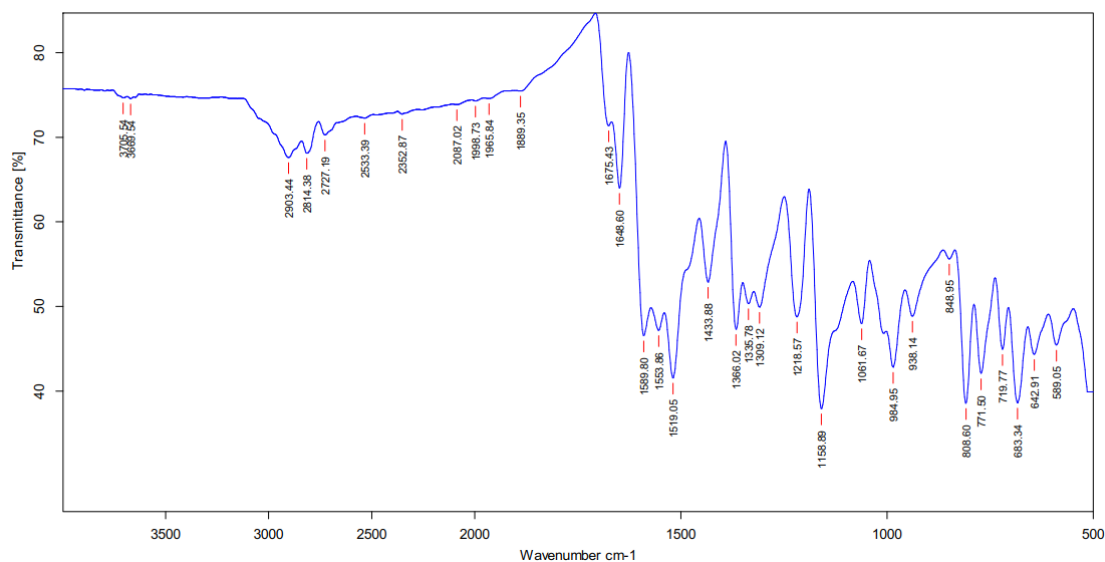


Figure 2c: FT-IR spectrum of physical mixture of kaempferol, lecithin and curcumin

Preparation of flavonosomes

The flavonosomes loaded with kaempferol and curcumin were prepared using bulk co-loading method. In this technique, the flavonoids are dissolved in suitable solvent and the lipid (lecithin) solution is prepared in separate flask. The two solvents are mixed and sonicated resulting in nanosized formation. The solvent is then evaporated to form thin film which is then redispersed in solvent and added to aqueous phase under stirring resulting in preparation of the flavonoid loaded preparation (flavonosome). The organic solvent was then removed by stirring.

Particle size and size distribution

The particle size and size distribution for each batch of flavonosomes was determined using zeta sizer. The formulations ranged from 497.3 nm to 167.1 nm in size with a polydispersity index varying between 0.421 - 0.729 (Table 3).

Table 3 Particle size and size distribution of various batches of phytosomes

Formulation Code	Particle Size (nm)	Polydispersity Index (PDI)
F1	326.5	0.421
F2	291.7	0.574
F3	243.4	0.536
F4	167.1	0.428
F5	243.9	0.729
F6	497.3	0.622

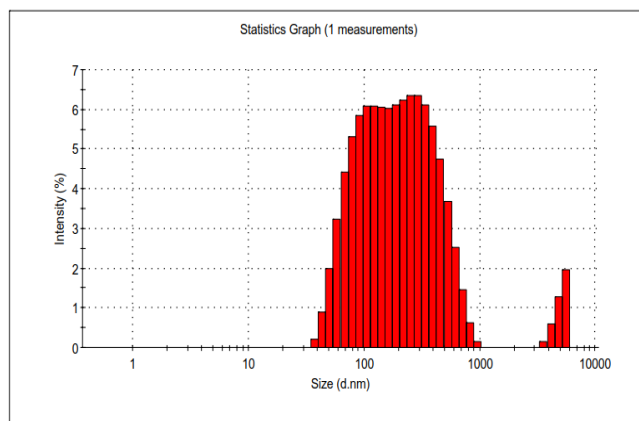


Figure 4: Particle size and size distribution of F4

The results indicated that increase the ratio of lipid to extract was able to reduce the particle size of the flavonosomes. Nevertheless, on increasing the ratio of lipid to extract to more than 4:1 resulted in a paradoxical increase in particle size of the flavonosomes. This could be due to fact that at lower concentration of lecithin, the dispersion could not achieve desired stability and on higher concentration of lecithin aggregation of particles might have occurred.¹⁵ Previously reported where the size of phytosomes loaded with drug extract decreased by increasing the lipid concentration.¹⁶

Surface morphology (visualization)

The flavonosomes prepared using 4:1 ratio of lipid to flavonoids were found to be rigid, spherical as seen in SEM with smooth regular surface (Figure 5).

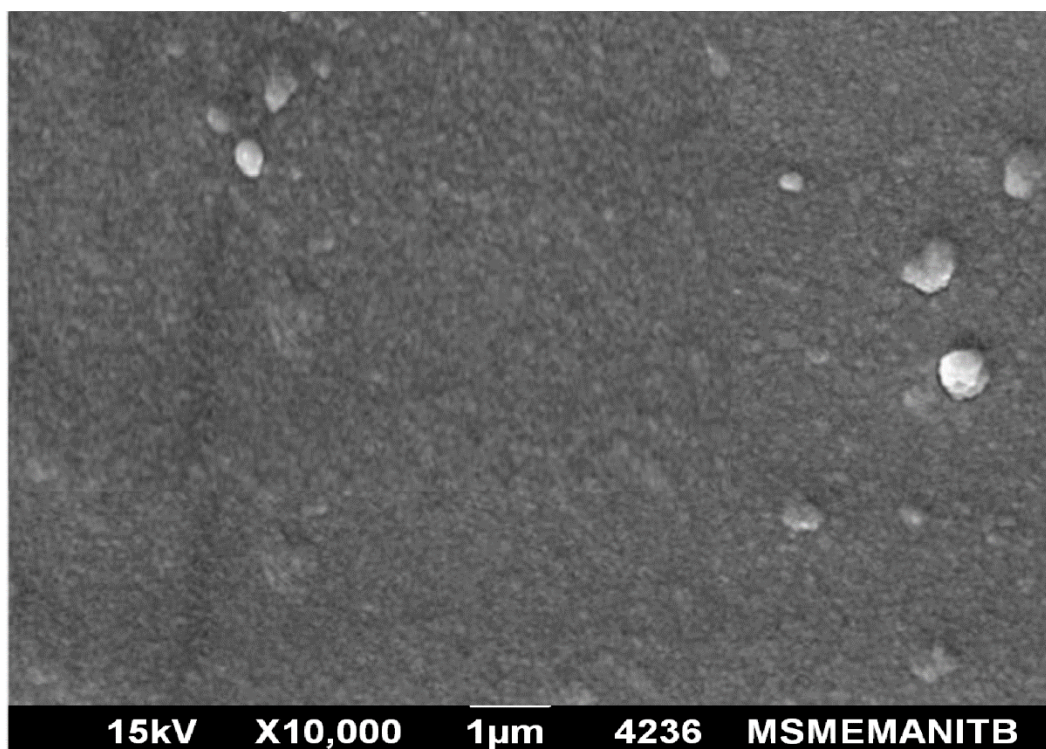


Figure 5: SEM photograph of F4

Differential Scanning Calorimetry

The DSC thermogram of lecithin exhibited sharp endotherm at around 54°C (Figure 6a) suggesting change of state (liquefaction). The change in glass transition temperature

visible by the endotherm shift from 54°C to 252°C (Figure 6b). Hence it could be concluded that a stable flavonosome was formed due to molecular interactions of either van der waals type or hydrogen bonding of the flavonoids and phospholipid distributing the flavonoid molecularly into the phospholipid.

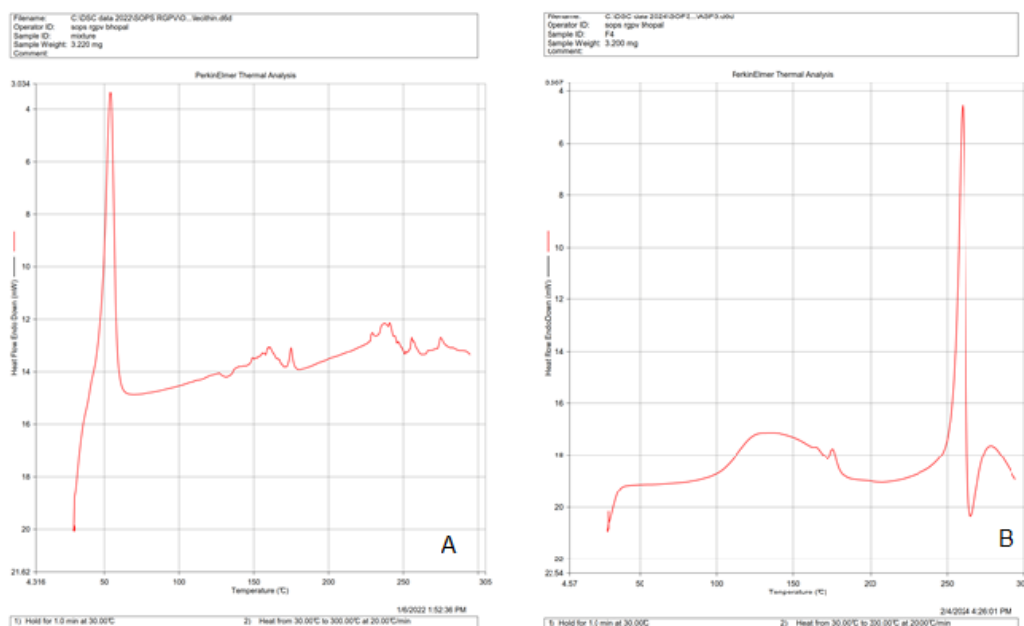


Figure 6: DSC thermogram of (A) Lecithin (B) Falvososome (F4)

Entrapment Efficiency

The entrapment efficiency of flavonosome preparation F4 was determined by measurement of the non-entrapped flavonoids and subtracting from the total flavonoids used to obtain the flavonoids entrapped in the formulation. The entrapment efficiency of F4 has been calculated to be 94.12%.

In vitro release of flavonoids from flavonosome

The flavonosome F4 was studied for release of the entrapped flavonoids by dialysis method. The amount of flavonoids released at 1, 2, 3, 4, 6, 8, 10 and 12 hours was determined using UV spectrophotometry.

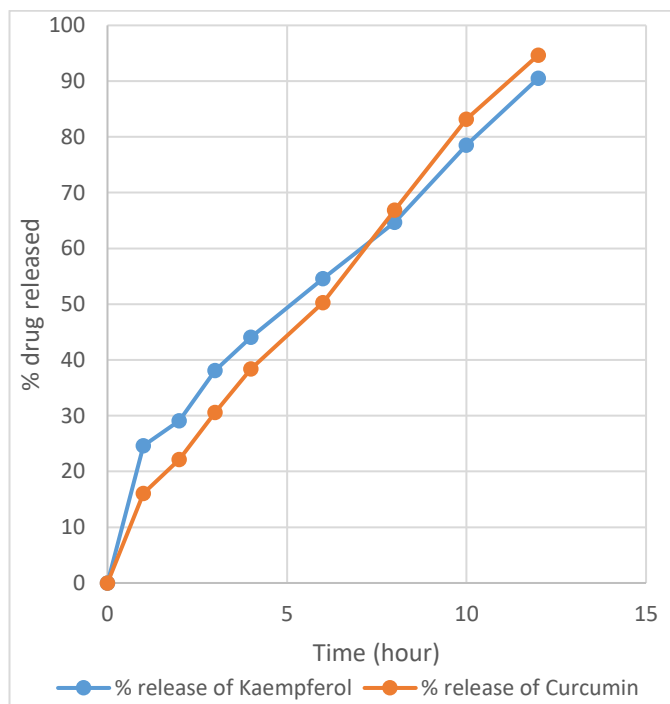


Figure 7: Release profile of flavonoids from flavonosome

The high release of the flavonoids from the flavonosome in the first hour of the study might be due to loosely surface bound flavonoids (Figure 7). After the first hour, the entrapped flavonoids released in a steady manner from the flavonosome

depicting and almost complete release (90.52% for Kaempferol and 94.67% for curcumin) from the formulation. This suggests that the formulation owing to its sustained and controlled release property might be able to improve the bioavailability of the entrapped flavonoids.

Stability study of flavonosome

Following ICH criteria, stability studies were conducted on formulation F4, and after three months, the phytosomes were assessed for changes in particle size. Until the formulation reaches the intended tissue, its stability must be preserved. Lecithin makes the lipid bilayer flexible, which is essential for preserving the physical stability.¹⁷ It was discovered that the mean particle size varies very slightly between room temperature and accelerated temperature settings. As a result, the formulation might be regarded as stable under the storage circumstances.

In vitro antioxidant action of flavonosome

DPPH radical scavenging assay was used to determine the antioxidant action of the individual flavonoids and the flavonosome formulation. The inhibition of DPPH radical was calculated from the absorbance of the test sample measured at 514 nm.

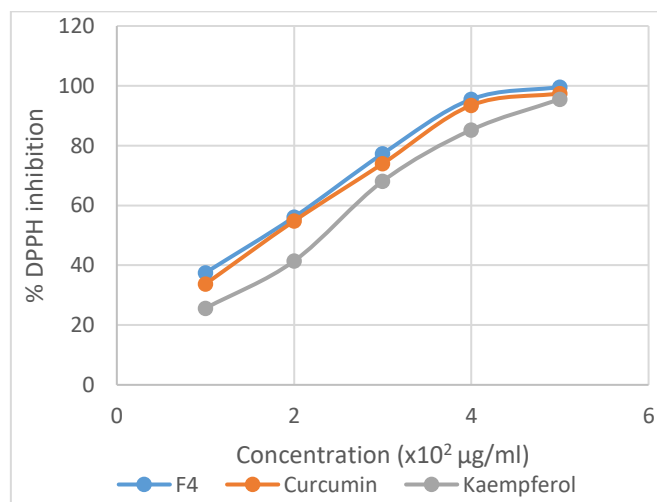


Figure 8: Antioxidant action of F4, curcumin and kaempferol

It was found that the formulation F4 was having higher antioxidant activity in comparison to kaempferol and curcumin. This could be due to the fact that the flavonosome was composed for a mixture of both the flavonoids. The added effect of the flavonoids contributed to the higher antioxidant potential of the flavonosome. The IC₅₀ value of both the individual flavonoids and the flavonosome was calculated from the plot of inhibition against concentration (Figure 8). The IC₅₀ of kaempferol, curcumin and flavonosome were found to be 228.20 µg/mL, 175.51 µg/mL and 158.19 µg/mL respectively.

CONCLUSION

The study presented in this thesis reveals the excellent potential of flavonosome based drug delivery system for improving the bioavailability as well as antioxidant potential of flavonoids. We can conclude that flavonosome based formulation could be a valuable approach to improve the therapeutic efficacy, to reduce dose and improvement in dosage regimen for flavonoids.

REFERENCES

- Lahlou M, "The Success of Natural Products in Drug Discovery" *Pharmacology and Pharmacy* 2013;4:17-31. <https://doi.org/10.4236/pp.2013.43A003>
- Patwardhan B, Vaidya A.D.B and Chorghade M "Ayurveda and Natural Products Drug Discovery" *Current Science* 2004;86(6):789-799.
- Nadkarni KM, Nadkarni AK. *Indian Materia Medica*. Bombay: Popular Prakashan Private Ltd; 1976.
- Kidd PM, Phytosomes, Highly Bioavailable Plant Extracts. Available from: http://www.dockidd.com/pdf/2LinksPhytosomeInd_5_10_04.pdf#stifolia
- Sharma R, Dua JS, Prasad DN, Hira S, Advancement in novel drug delivery system: Niosomes, *Journal of Drug Delivery and Therapeutics*. 2019;9(3-s):995-1001
- Manach C, Scalbert A, Morand C. Polyphenols: Food Sources and Bioavailability. *American Journal of Clinical Nutrition*. 2004; 79:727-747. <https://doi.org/10.1093/ajcn/79.5.727> PMID:15113710
- Khare R, Tripathi D, Lal M, Kondalkar A. Formulation and evaluation of transdermal patches of etodolac for topical application. *Journal of Pharmacology and Biomedicine*. 2023; 7(2): 589-597
- Hazra K, Kumar R, Sarkar BK, Chowdhary YA, Devgan M, Ramaiah M. UV-Visible spectrophotometric estimation of curcumin in nanoformulation. *International Journal of Pharmacognosy* 2015; 2(3):127-130
- Telange DR, Patil AT, Tatode A, Bhojar B. Development and Validation of UV Spectrophotometric Method for the Estimation of Kaempferol in Kaempferol: Hydrogenated Soy PhosphatidylCholine (HSPC) Complex. *Pharmaceutical Methods*. 2014; 5(1):34-38 <https://doi.org/10.5530/phm.2014.1.6>
- Rahman HS, Othman HH, Hammadi NI, Yeap SK, Amin KM, Samad NA, Alitheen NB. Novel Drug Delivery Systems for Loading of Natural Plant Extracts and Their Biomedical Applications. *International Journal of Nanomedicine*. 2020; 15:2439-2483. <https://doi.org/10.2147/IJN.S227805> PMID:32346289 PMCid:PMC7169473
- Karthivashan G, Masarudin MJ, Kura AU, Abas F, Fakurazi S. Optimization, formulation, and characterization of multiflavonoids-loaded flavanosome by bulk or sequential technique. *International Journal of Nanomedicine*. 2016; 11: 3417-3434. <https://doi.org/10.2147/IJN.S112045> PMID:27555765 PMCid:PMC4968855
- Dhase AS, Saboo SS. Preparation and Evaluation of Phytosomes Containing Methanolic Extract of Leaves of Aegle Marmelos (Bael). *International Journal of Pharmtech Research* 2015; 8(6):231-240.
- Mishra B, Pati JC, Mishra R, Saluja MS. L-Threonine decorated paclitaxel poly (l-lactide) nanoparticles: formulation, pharmacokinetic and stability study. *Journal of Population Therapeutics & Clinical Pharmacology*. 2023; 30(16):713-717.
- Mishra N, Jain P, Mishra B. Derivatization of Gallic Acid with amino acids for accentuation of its antioxidant potential. *Journal of Pharmacology and Biomedicine*. 2017; 1(3):94-102.
- Saha P, Singh P, Kathuria H, Chitkara D, Pandey MM. Self-Assembled Lecithin-Chitosan Nanoparticles Improved Rotigotine Nose-to-Brain Delivery and Brain Targeting Efficiency. *Pharmaceutics*. 2023; 15: 851. <https://doi.org/10.3390/pharmaceutics15030851> PMID:36986712 PMCid:PMC10052746
- Solanki H, Malviya K, Soni P, Kumar P, Omray LK. Formulation and evaluation of phytosomes loaded with tabernaemontana divaricata leaf extract. *Journal of Population Therapeutics & Clinical Pharmacology*. 2023; 30(18): 1810-1817.
- Khanna R, Chauhan P. Formulation and evaluation of Annona squamosa hydroalcoholic extract loaded phytosomes. *Journal of Pharmacology and Biomedicine*, 2021; 5(3):342-351