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Sustained Transdermal Release of Lignans Facilitated by Sophorolipid based Transferosomal Hydrogel for Cosmetic Application

N. Jayarama Naik[2], Isha Abhyankar[1], Priti Darne[1], Asmita Prabhune[1] and Basavaraj Madhusudhan[2]*

[1]Division of Biochemical Sciences, National Chemical Laboratory, Pune, India
[2]Department of Food Technology, Davangere University, Davangere, Karnataka, India

**Corresponding author*

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A B S T R A C T

The current study focuses on designing biodegradable transferosomal hydrogel using lignans and bio surfactant for transdermal applications. In the study, the lignans concentrate (LC) was extracted from flaxseeds and used to prepare vesicular transferosomes/transferosomal hydrogel by thin film hydration technique for drug delivery. The resultant formulations were characterized using light microscopy, DLS, Zeta potential, entrapment efficiency (EE %) and stability. *In vitro* skin permeation studies were also performed. The synthesized transferosomes were spherical in shape. The entrapment efficiency (%EE) of transferosomes with synthetic surfactant (SST) was 38.54% while the efficiency obtained by bio-surfactant transferosomes (BST) was 45.87%. Upon optimization, BST exhibited improved %EE (75.81 %). The particle sizes, zeta potential and PDI of BST were 213.4 nm, -30.6 mV, 0.316 and of SST210.5 nm, −23.62 mV, 0.349, respectively. The transferosomes follow Higuchi model whereas transferosomes hydrogel follow the First order kinetics. The transferosomes were stable over a month at 4°C and exhibited similar transdermal permeation as fresh samples. The Hydrophile-Lipophile Balance (HLB) of SPL was in the order of 13 to 15making BST a better alternative to synthetic surfactants. Thus it can be concluded that the transferosomal hydrogels infused with sophorolipid could be used as carriers of LC with promising permeation characteristics for transdermal and cosmetic applications.

Introduction

Pre-mature ageing of skin is one of the important challenges of 21 century owing to increase in beauty consciousness amongst society. Currently, skin is exposed to various physio-chemical agents like UV radiation, chemicals, pesticides and insecticides which results in ageing (Katiyar, 2015). Such exposure leads toageing of the dermis thereby affecting the chemical structure of skin proteins. Exposure also results in generation of reactive oxygen species which is the preliminary reason for ageing. Moreover, the adaptive ability of the skin to such adverse stimuli is drastically reduced resulting in pre-

mature ageing (Poeggeler *et al.,* 1993). To overcome these problems, extensive research is being conducted to develop new strategies to prevent pre mature ageing of skin.

Plant extracts are known to be rich source of anti-oxidant molecules and thereby assist preventing premature ageing of skin. Plant extracts include polyphenols, triterpenes, flavonoids, catechins, alkaloids, curcuminoids, resveratrol, caffeic acid, quercetin groups which help in absorbing the UV light, scavenging the ROS, reducing metal ions, modulating protein phosphorylation and inhibiting lipid peroxidation, thus reducing the risk of wrinkle formation of skin (Katiyar, 2016). Flaxseeds contain high amount of polyphenolic compounds in the form of lignan. The major constituent of lignan is secoisolariciresinoldiglucoside (SDG), which possesses ability to scavenge the free radicals and also inhibit lipid peroxidation (Bekhit *et al.,* 2017). In this regard, the current work involves use of lignans for transdermal applications.

Recently, many photo protective agents and free radical scavengers (Synthetic) are being welcomed for topical application. Transdermal delivery systems are emerging as an interesting field owing to its varied applications. The primary step for drug delivery system is permeation of the drug into the skin surfaces (Marwah *et al.,* 2016). Different mechanisms are used for this. One of them is transferosomes that can be defined as artificial drug carriers resembling the cell structure. They are complex aggregates with high adaptability and stress responsiveness. Structurally they contain an outer lipid bilayer encompassing an aqueous core. Transferosomes are synthesized using phospholipid which forms the lipid bilayer and surfactant that helps in increasing the elasticity and permeability of the molecule (Suvarna *et al.,* 2016; Sharma *et al.,* 2015). Sophorolipid (SL) is a glycolipid biosurfactant synthesized by *Candida bombicola* and is an attractive alternative for chemical surfactant. SL being an amphiphilic molecule helps to increase the bio availability of compound of interest (Dubey *et al.,* 2014).

Thus, the present study aims to synthesize transferosomes using lignans concentrate from flaxseed and SL, and also exploring their release profile in vitro for transdermal applications. The transferosomes thus obtained will be biocompatible, ecofriendly and contribute towards high end-value products for cosmetic application.

Materials and Methods

Chemicals

All the media components, chemicals and solvents of analytical grade were procured from HiMedia India. Lignan standard (SDG) was obtained from Sigma Aldrich (USA),

Extraction of flaxseed lignans

The extraction of lignan from flaxseed was performed using method by Ramsay *et al.,* (2017) with slight modification. For the experiment, flaxseeds were washed and subjected to a dehulling process using KisanKrishi Yantra Udyogdehuller at Grain Science and Technology Department, CFTRI, Mysore, India. The hull fraction was extracted with n-hexane to remove fats. The defatted hull fraction was sieved and mixed with 400 ml of distilled water and 2 M aqueous sodium hydroxide. This mixture was incubated for 1h at 20ºC under shaking conditions. The fraction was acidified to pH 3and centrifuged at5000 rpm for 10 min. The resulting supernatant was collected. Extraction was done using ethanol at room temperature. After extraction the solution was centrifuged at

10000 rpm for 5 min. The pellet was discarded and the supernatant was subjected to rotary evaporation. The final lignans concentrate (LC) was then lyophilised and stored until further use (Zhang *et al.,* 2007).

Transferosome preparation

Transferosomes were prepared using soybean phosphotidylcholine, lignans concentrate and sophorolipid. The components in varying concentrations were dissolved in solvent system comprising of choloroform: ethanol (1:1) with overnight incubation at room temperature. After incubation, the hydrated film formed was suspended in phosphate buffer of pH5.5. The resulting mixture was kept at 150 rpm for 1 hour at room temperature. The transferosomes thus formed were subjected to sonication (bath sonicator) for 20 min at room temperature (Marwah *et al.,* 2016).

Preparation of transferosomal hydrogel

Transferosomal hydrogels were synthesized using thin film hydration technique. Of the various gelling agents, carbopol 940 was used for this study. Carbopol 940(1%) was dissolved in distilled water using magnetic stirrer for 12 hours. The transferosomes (30 ml) were then added to the carbopol mixture and stirred at 8000 rpm for 3 hours at pH 6(Sultana and Krishna, 2015; Shaji and Lal,2014).

Characterization

HPLC analysis

HPLC was performed using UFLC SHIMADZU instrument with UV Detector (SPD-20A).C_{18}Column with dimensions of 150x3 mm with 5 micron pore size was used [8]. The solvent system consisted of 0.05% trifluoroacetic acid (solvent A) and 0.05% trifluoroacetic acid in acetonitrile (solvent B) with a flow rate of 0.4 mL min^{-1}. Gradient solvent system was used as 90% A for 5 min, decreasing to 60% over next 15 min, returning to 90% for 10 min and isocratic at 90% A for 5 min. The wavelength used was 280 nm (Marwah *et al.,* 2016).

Transmission electron microscopy

TEM procedure was followed similar to that of Durrani *et al.,* (2013). Sample preparation was done using freshly prepared transferosomal dispersions (10 times diluted). Sample was drop coatedunto carbon coated copper TEM grid. The grid was dried and stained with 2% uranyl acetate. TEM analysis was performed on Hitachi H-7500 at room temperature under varied magnifications.

Dynamic light scattering and zeta potential analysis of transferosome

The particle distribution profile and the stability of the transferosomes were analyzed using DLS and Zeta potential. The analysis was performed on Zetasizer ZS (Brookhaven Instruments crop.) at room temperature. The experiment was performed in triplicates (Singh *et al.,* 2014).

Percentage entrapment efficiency (%EE)of transferosome

Lignan loaded transferosomes were evaluated for % EE by using centrifugation method (Shaji and Lal, 2014). 2 mL of LC-loaded transferosomes were centrifuged at 10,000 rpm for 40 minutes using the high-speed cold centrifuge. The supernatant was filtered with 0.45µ filter and used for determining the untrapped LC using HPLC. The precipitate was treated with 80% ethanol (1mL) (Durrani *et al.,* 2013) and suspended in phosphate buffer (pH 5.5) to release the entrapped LC. The content was centrifuged at10,000rpm for

15 min and subjected to HPLC (Ali *et al.*, 2015). %EE is calculated by:

% EE of LC =

$$\frac{\text{Total amount of LC- unentrapped LC}}{\text{Total amount of LC}} * 100$$

In-vitro release of LC

In vitro studies of transferosomes were carried out using cellophane membrane [9]. The apparatus consisted of donor and receptor compartment. In the receptor compartment 60 mL of phosphate buffer (pH 5.5) was added and agitated at 100 rpm (37 ± 0.5° C). The LC transferosomes (1 ml) were added to the donor compartment. An aliquot of 0.5 ml was withdrawn at specific time intervals, simultaneously replacing it with equal volume of diffusion medium. The cumulative amount of LC permeated across the cellophane membrane during the process was calculated using HPLC and graph was plotted as time vs. concentration for quantitative estimation (Biswas *et al.*, 2016).

In vitro skin permeation studies for transferosomal hydrogel

LC permeation study was performed on goat skin using franz diffusion cell. Fresh goat skin was collected from local slaughter house. Hairs were removed and the skin was thoroughly washed. Skin was hydrated with normal saline. The fat tissue layer of skin was removed and preserved in isopropyl alcohol at 0-4^{0}C. For the study, the skin was horizontally mounted in the receiver compartment with the stratum corneum side facing the donor compartment of diffusion cell. The receptor compartment was filled with 50ml of phosphate buffer (pH 5.5) maintained at 37± 0.5°C and kept under magnetic stirrer at 100rpm. Transferosomal hydrogel (approximately: 3mg LC) was applied on the skin. At specific time intervals, 1 ml aliquot of the receptor medium was withdrawn and immediately replaced by an equal volume of phosphate buffer (pH 5.5). The samples were analyzed by using HPLC (Malakar *et al.*, 2012).

Stability of LC-loaded transferosomes

Stability studies of the LC-loaded transferosomes were carried out to evaluate their aggregation and leaching out during storage, the method followed here is as reported by Ali *et al.*, (2015). The prepared transferosomal vesicles were stored at different temperature4±1°C, 25±1°C (room temperature), and 37±1°C for 1 month. The physical stability of the prepared vesicles was evaluated by % EE measurement. Samples from each transferosomal formulation (2 mL) were periodically withdrawn and analyzed using HPLC. The physical appearance of LC-loaded transferosomes was examined by visual observation for sedimentation (Ghule *et al.*, 2015).

Results and Discussion

Extraction of flaxseed lignans

The extraction of lignan was performed using the method reported by Ghule *et al.*, (2015). In the present study, the SDG content obtained was 23.28 mg/g along with other constituents. Through this method comparatively higher amount of SDG was extracted. The other reported values of SDG content are in the range (11.9–25.9 mg/g) along with p-coumaric acid glucoside (1.2–8.5 mg/g), and ferulic acid glucoside (1.6–5.0 mg/g) (Hao and Beta, 2012).

HPLC analysis

The LC extracted from flaxseeds was subjected to HPLC. Commercially available SDG was used as standard for comparison having retention time of 27.241min [Figure 1(a) and 1(b)].

It can be concluded from the graph that retention time and the peaks obtained for standard and the LC is similar. Slight change in the retention time can be attributed to the solvent systems used (Hao and Beta, 2012).

Transferosomal hydrogels

Transferosome hydrogels were prepared using Soybean phosphotidyl choline, LC and sophorolipid. Synthetic surfactants were also used for comparison.

The table 1 summarizes the different concentrations of SL and SDG used for the preparation of hydrogels (Sharma *et al.*, 2015).

From the above data it can be inferred that the maximum entrapment is observed using BST4 and SST4 as (45.87%) and (38.54%) respectively.

The entrapment efficiency obtained using biosurfactant is more than synthetic surfactant.

The high % EE of BST_4can be attributed to SL, which enhance the elasticity and flexibility of the transferosomes thereby enabling higher encapsulation of drug (Abdelbary, 2016; Agrawal, 2017).

This can be due to amphiphilic property of SL which is reported by Singh *et al.*, (2014) and Darne *et al.*, (2016) regarding enhancement in bio availability of curcumin.

Morphology of transferosomes

TEM images revealed the shape of transferosomes as spherical. The formulation appeared as multi-lamellar vesicles with no aggregation (Figure 2).

Transferosome size and charge

From DLS, variations of particle size for both BST and SST were noticed (Table 2). BST based formulations exhibited slight increase in size (210.5 to 328.1nm) compared to SST based transferosomes (218.6 to 291.3 nm). Increased transferosomes size can be attributed to the influence of surfactants.

The HLB (hydrophilic lipophilic balance) of surfactants may lead to change in size of individual transferosome vesicle, which can lead to the increase in surface free energy. Any increase in surface free energy might cause the fusion of lipid bilayers [23]. As the BST4 and SST4 have shown better values they were chosen for further evaluation.

The zeta potential of transferosomes is summarized in Table 2. The potential was between -22.86 AND -30.06 mV for BST based transferosomes indicating marginal rise in comparison to SST transferosomes (-23.80 to -28.76 mV).

The charges over the formulations were sufficient enough to avoid any aggregation of vesicles imparting stability [24]. The negatively charged transferosomes would be advantageous to improve the permeation through skin barriers during transdermal delivery. As maximum entrapment was observed in BST4 and SST 4, these concentrations were used for analysis. The DLS and Zeta potential data is summarized in table 2.

In-vitro release of LC through cellophane membrane

The release study of LC from transferosomes was performed using cellophane membrane. From the figure (3 a), release of SDG showed sudden increase in the release profile at around 30 min.

Table.1 Optimization parameters of hydrogel

Names	SPC	SL	Conc. of LC (mg/ml)	Vol. of SDG(ml)	% EE
BST_1	95	5	1	5	34.70
BST_2	90	10	1	5	36.93
BST_3	85	15	1	5	32.70
BST_4	80	20	1	5	45.87
BST_5	75	25	1	5	34.19
SST_1	95	5	1	5	33.04
SST_2	90	10	1	5	35.61
SST_3	85	15	1	5	37.26
SST_4	80	20	1	5	38.54
SST_5	75	25	1	5	38.18

Table.2 Size and zeta potential of synthesized transferosomes

Sample	Size (nm)	Zeta(mV)	Polydispersity
BST4	210.5 to 328.1	-22.86 to -30.06	0.316 to 0.380
SST4	218.6 to 291.3	-23.80 to -28.76	0.327 to 0.358

Fig.1a HPLC chromatogram of a flaxseed Standard (280 nm)

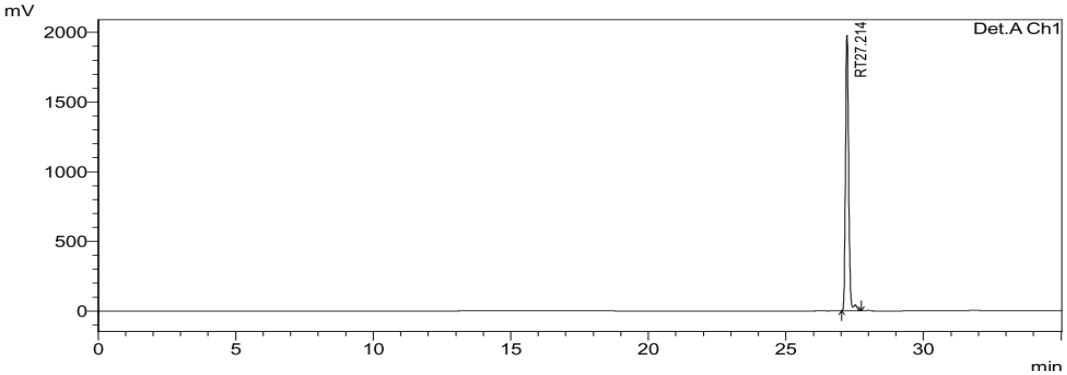


Fig.1b HPLC chromatogram of flaxseed LC (280 nm)

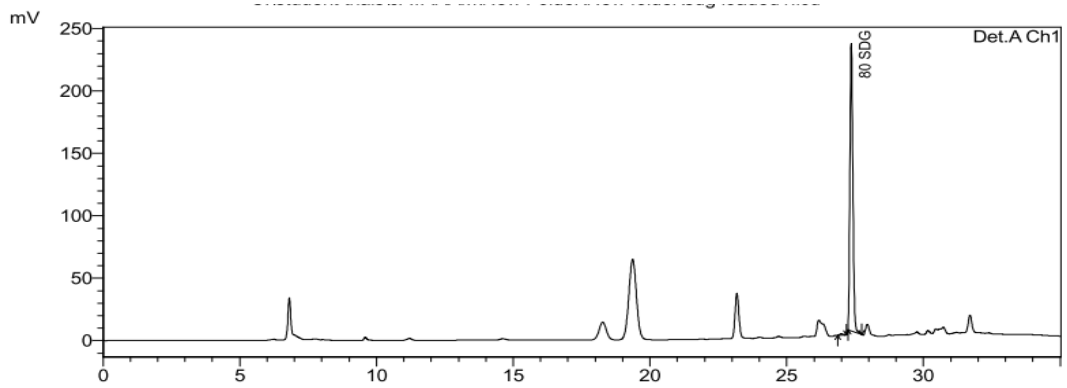


Fig.2 TEM images of transferosomes

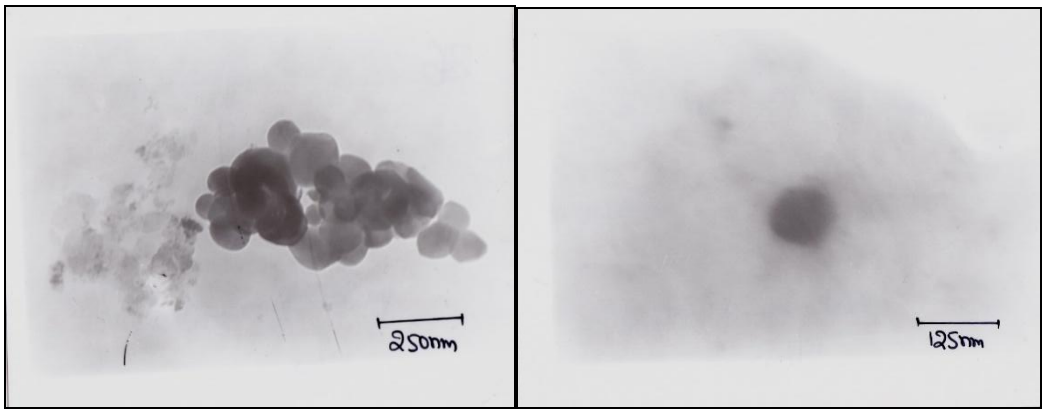


Fig.3 *In-vitro* release profile of (a) transferosome suspension and (b) transferosome hydrogel

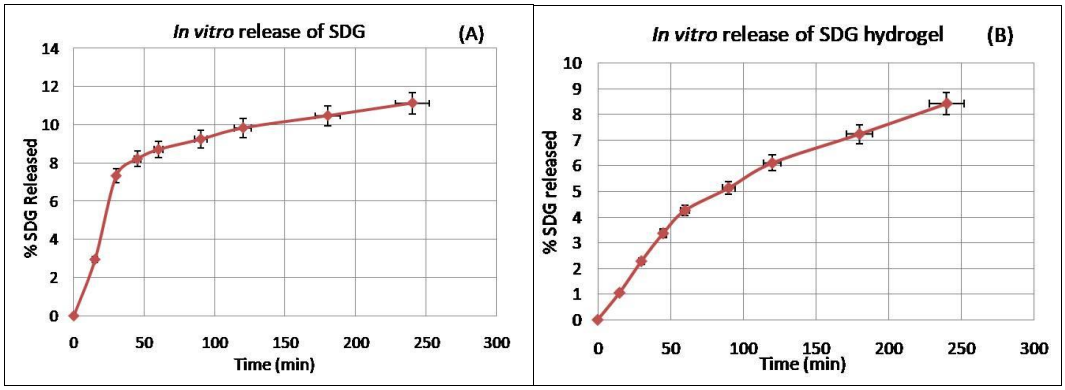
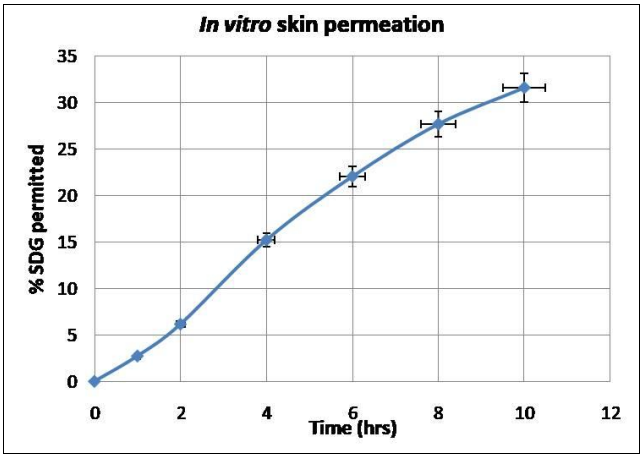


Fig.4 *In-vitro* release profile of LC from LC-loaded transferosomal gel in franz diffusion cell



After 30 min, linear increase was observed in the release pattern up to 4 hours. 10- 12 % of SDG is released after 4 hours. The graph represents a biphasic pattern [23]. Since sustained release is a desired feature for any cosmetic application, this sudden release of LC in less than 30 min might not serve the purpose of transdermal application. Hence,

the reason to synthesize hydrogel whose LC release pattern is represented in figure (3 b). This hydrogel releases LC gradually over the period of 4 hr. This sustained release is more preferred for delivery application.

In vitro skin permeation studies

In-vitro skin permeation studies were performed using goat skin by Franz diffusion cell and the gradual permeation of the LC-loaded transferosomes gel is shown graphically in Figure 4. The trend of the permeation was slower in the beginning (< 2 h) and continued to increase with the time (> 4 h). The increased permeation of transferosomal hydrogel may be due to the higher viscosity.

Stability study

Stability is an important criteria for nano-formulations used for drug delivery. The stability of transferosomes was studied at different storage temperature. The results indicated that there was no aggregation after refrigeration at 4°C. Whereas transferosomes stored at 25°C and 37°C exhibited slight decrease in their % EE. The leaching of LC at these temperatures may be due to the changes in lipid bilayer of transferosomes.

In conclusion, the present study successfully designed the transferosomal hydrogel using lignans concentrate extracted from flaxseeds and SL. The *In vitro* release profiles of the transferosomes in suspension and their hydrogel as certain the potential use for transdermal applications in cosmetics.

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