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FORMULATION AND *IN VITRO* CHARACTERIZATION OF TRETINOIN PHARMACOSOMES FOR THE TREATMENT OF ACNE

MALAVI S[1,2]*, DISOUZA J[3], KUMBHAR P[3], SHINDE T[3], MANJAPPA A[3], KATARIA U[4] AND DWIVEDI J[1,5]

1: Pacific Academy of Higher Education and Research, Udaypur, Rajasthan, India

2: Department of Pharmaceutics, Genesis Institute of Pharmacy, Sonyachi Shiroli, Tal: Radhanagari, Dist: Kolhapur Maharashtra 416212, India

3: Tatyasaheb Kore College of Pharmacy, Warananagar, Tal: Panhala, Dist: Kolhapur Maharashtra, 416113, India

4: Geetanjali Institute of Pharmacy, Geetanjali University, Udaipur Rajasthan, 313001

5: Pacific College of Pharmacy, Pacific University, Udaipur, India

***Corresponding Author: Dr. Shobharaj Malavi: E Mail: shobhraj2005@gmail.com**

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ABSTRACT

Tretinoin (TRT) is principally used in the management of different kinds of acne. However, the applications of TRT are restricted due to its poor aqueous solubility, stability and associated side effects. Therefore, the main objective of the present research was to develop and characterize TRT pharmacosomes with improved solubility and stability, and reduced side effects in the treatment of acne. The pharmacosomes of TRT was prepared with hydroxylated Soyalecithin (HS) using the conventional solvent evaporation technique at different ratio of TRT to HS. The pharmacosomes were optimized based on the % yield and drug content, and optimized pharmacosomes were further characterized for Fourier transforms infrared spectroscopy (FTIR), saturated solubility, particle size and zeta potential, and *in vitro* dissolution study. The batch F2 of pharmacosomes displayed maximum % yield (79.22±0.77%) and TRT content (90±2.3%) when compared to other batches. The FTIR analysis confirmed the reaction between the carboxyl group of TRT and the polar moiety of HS. The pharmacosomes displayed significantly (p<0.05) increased (50-folds) solubility of

TRT when compared to plain TRT in phosphate buffer solution (PBS) of pH 7.2. The optimized batch of TRT pharmacosomes showed a particle size of 152±7nm with PDI of 0.3693±0.009 and zeta potential of -3.3mV. Furthermore, *in vitro* dissolution study demonstrated remarkable release of TRT from pharmacosomes (97.6±7.2%) than plain TRT solution (39.5±4.5%) after 12hr in PBS of pH 7.2. Thus, the pharmacosomes could be a promising strategy for the delivery of TRT with enhanced solubility and therapeutic efficacy.

Keywords: Tretinoin, Hydroxylated soyalecithin, Pharmacosomes, Acne, Saturated solubility, *In vitro* dissolution study

INTRODUCTION

Acne vulgaris (acne) is a chief chronic infectious disease of the skin affecting different body parts such as neck, upper chest, and oily glands rich back of subjects. More than 23 million Indians are expected to affect by acne by the end of 2026 **[1, 2]**.

A vitamin A derivative tretinoin (TRT) is used as a first-line treatment strategy in the management of acne **[3]**. TRT acts primarily by reversing abnormal follicular keratinization via interaction with nuclear retinoic acid receptors. Topical TRT has been widely used for the treatment of mild to moderate inflammatory lesions **[4]**. However, poor aqueous solubility and associated local side effects such as dryness, erythema, burning, peeling, etc of TRT have restricted its applications in the treatment of acne. In addition, TRT is extremely unstable in presence of heat, light, and air that entails the use of an elevated dose of TRT causing adverse effects thereby resulting in discontinuation of TRT therapy **[5]**. Therefore, there is a necessity to develop a novel formulation that can defeat the aforementioned flaws.

The different kinds of novel approaches such as liposomes, niosomes, emulgel, nanoemulsion, micelles gel and pharmacosomes have been designed for the topical delivery of the drug **[6-9]**. Among the diverse approaches, pharmacosomes found to be potent approach because it overcomes the problems associated with liposomes, transferosomes, and niosomes. Pharmacosomes are amphiphilic lipid vesicular systems in which drug are conjugated with phospholipid. The drugs with an active hydrogen atom in polar moiety (-COOH, -OH, $-NH_2$, etc.) can be chemically linked to a phospholipid at an equimolar or another molar ratio. These pharmacosomes show improved solubility, absorption, permeation, stability, and modified drug release, and reduced side effects of drugs **[10-14]**. Moreover, pharmacosomes can be used orally or topically **[15-17]**. Furthermore, the system is used as a promising vehicle for the delivery of both

hydrophilic and hydrophobic types of drugs.

In the present study, we aimed to develop TRT pharmacosomes using hydroxylated Soyalecithin (HS) with improved solubility, absorption and stability for efficient treatment of acne.

MATERIALS AND METHODS

Materials

Tretinoin (TRT) was purchased from Shakti Chemicals Pvt. Ltd., Mumbai. Soya lecithin (modified) was supplied as gift sample by Vippy industries LTD., Dewas. Ethanol and chloroform were procured from Molychem, Mumbai, India. All other analytical reagent grade chemicals were used as such without any further processing.

Preparation of TRT pharmacosomes (TRTP)

Pharmacosomes of TRT was prepared by the conventional evaporation technique. Briefly, TRT and hydroxylated Soyalecithin (HS) were dissolved in ethanol at different molar ratios (1:1, 1:2, and 2:1). Then the mixture was refluxed in a round bottom flask for 1 hr and solvent was allowed to evaporate under vacuum at 40ºC in a rotary vacuum evaporator. Finally, the collected dried residues placed overnight in a vacuum dessicator to remove residual solvent and then subjected to characterization.

Characterization of TRTP

Percentage yield

The pharmacosomes were dried and weighed. The total weight of drug and ingredients used for the preparation of pharmacosomes was also recorded. Finally, the % yield of pharmacosomes was calculated by using the following equation,

$$\%\ Yield = \frac{\text{Actual weight of pharmacosomes}}{\text{Total weight}} \times 100$$

FTIR analysis

The prepared TRTP was analyzed by FTIR. The FTIR spectra of TRT and TRTP were recorded using an FTIR spectrophotometer (Bruker) over the wavenumber of 4000 to 650 cm-[1].

Drug content

The TRT content in TRTP was measured by weighting TRTP equivalent to 20mg of TRT. Then, it was added into a volumetric flask containing 50mL ethanol. The flask was stirred at room temperature for 20 minutes. Finally, the samples were filtered through a syringe filter and analyzed for TRT content using UV-Vis spectrophotometer 120 (Agilent Technology Carry 60 UV-vis) at 350nm against ethanol as a blank solution.

Saturated Solubility

The saturated solubility of TRT in optimized TRTP was assessed in PBS of pH 7.2. Briefly, the excess amount TRTP was placed in a volumetric flask containing 5mL of PBS pH 7.2 and the mixture was

stirred rotary shaker for 24hr at room temperature. After completion of 24hr, the filtrate obtained by filtration was diluted and subjected for analysis using a UV-visible spectrophotometer at 350nm.

Particle size analysis

The mean particle size and zeta potential of optimized TRTP were investigated using Malvern ZS Zetasizer (Malvern Instruments Ltd.) by dispersing 5mg of TRTP in to 5mL of PBS (pH 7.2) at room temperature (25°C). The measurements were carried out in triplicate.

Dissolution study

In vitro dissolution study of optimized TRTP was performed by a USP type 2 (rotating paddle) apparatus using PBS of pH 7.2 and compared with plain TRT solution. Briefly, plain TRT (5mg) and TRTP equivalent to 5mg of TRT was added into dissolution flask containing 500mL of PBS (pH 7.2) and maintained at 50 rpm and 32 ± 0.5 °C. 2mL of dissolution medium was removed at a predetermined time (1, 2, 4, 6, 8, 10, and 12 h) for analysis. At the same time, a 2mL fresh dissolution medium was transferred back to the apparatus. The test samples withdrawn were analyzed, following centrifugation and suitable dilutions with release medium, at 350 nm using a UV-visible spectrophotometer. The release profile of TRT from each formulation was carried out in triplicate. Cumulative % TRT release was calculated and plotted against time **[18-20]**.

Statistical analysis

Data are mentioned as the mean ± standard deviation of three independent experiments. The statistical analysis was performed by using GraphPad Prism software version 5 (GraphPad Software, Inc., La Jolla, CA, USA). The results obtained were analyzed by one-way ANOVA. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS:

Characterization of TRTP

Percentage yield

The % yield of pharmacosomes batches prepared at different TRT to HS ratios is presented in **Table 1**. The batch F2 prepared at a 1:2 ratio of TRT to HS displayed maximum product yield (79.22±0.77%) when compared to three other batches.

FTIR analysis

The complex formation between TRT and HS (TRT-HS) was confirmed by using FTIR. The FTIR spectra of TRT and TRT-HS are shown in Figure 1. In the FTIR spectra of plain TRT **(Figure 1A)**, the peaks at 2315 and 1651cm^{-1} are corresponding to the C=O stretch H bending (aromatic). Besides, the characteristic peak at 951cm^{-1} is attributed to the trans vinyl (CH=CH) groups of TRT. In the FTIR spectra of the TRT-HS complex **(Figure 1B)**, we observed a shift

in the C=O stretch H bending of TRT from 1651cm^{-1} to 1737cm^{-1} revealing the participation of this functional group in the interaction (TRT-HS complex formation).

Drug content

The TRT content in three batches (F1, F2, and F3) prepared at (TRT to HS ratio of 1:1, 1:2 and 2:1) are shown in **Table 1**. The batch F2 displayed maximum TRT content (90±7.6) and therefore selected as optimized batch and used for further characterization.

Saturated solubility

The aqueous solubility of TRT in TRTP was found to be significantly (p<0.05) increased (0.2374mg/mL) in PBS of pH 7.2. Thus, the solubility of TRT was increased by 50-folds following pharmacosomes preparation when compared to the aqueous solubility of plain TRT (0.00477mg/mL). The increase in the aqueous solubility of TRT following pharmacosomes could be due to the formation of an amphiphilic complex.

Particle size analysis

The particle size of the optimized batch of pharmacosomes after hydration with PBS pH 7.2 was determined using a Malvern ZS Zetasizer. The mean particle size of pharmacosomes was found to be 152±7 nm with polydispersity index (PDI) of 0.3693±0.009 **(Figure 2A)**. The zeta potential of pharmacosomes was found to be -3.3mV **(Figure 2B)**. It is expected that the obtained vesicles with this size range can be permeated through the skin and thereby result in better absorption of TRT **[21]**.

Dissolution study

The *in vitro* dissolution of TRT from the formulations was studied by using a USP type 2 dissolution apparatus. The *in vitro* release profile of TRT from optimized batch of pharmacosomes and plain TRT solution is depicted in **Figure 3**. The pharmacosomes displayed significantly (≤0.05) higher TRT release (97.6±7.2%) when compared to TRT release from plain TRT solution (39.5 ± 4.5%) after 12hr in PBS of pH 7.2. Thus, increased release of TRT from pharmacosomes could be attributed to the enhanced solubility of TRT following pharmacosomes.

Table 1: % Yield and % TRT content of formulated batches of pharmacosomes

Formulation Batch	TRT:HS Ratio	% Yield	% TRT Content
F1	1:1	43.56±1.72	85±6.5
F2	1:2	79.22±0.77	90±7.6
F3	2:1	54.23±1.34	69±4.8

Values are mean ± SD (n=3)

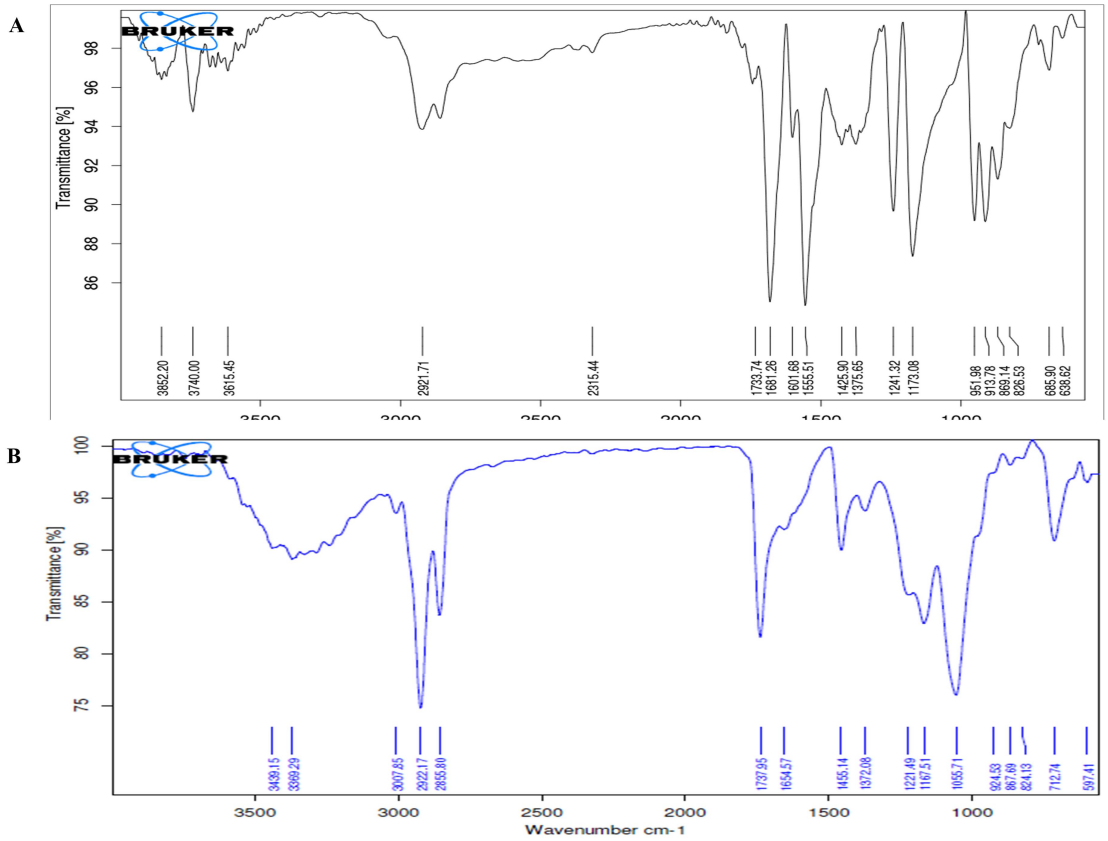

Figure 1: FTIR spectras of (A) Plain TRT, (B) Pharmacosomes

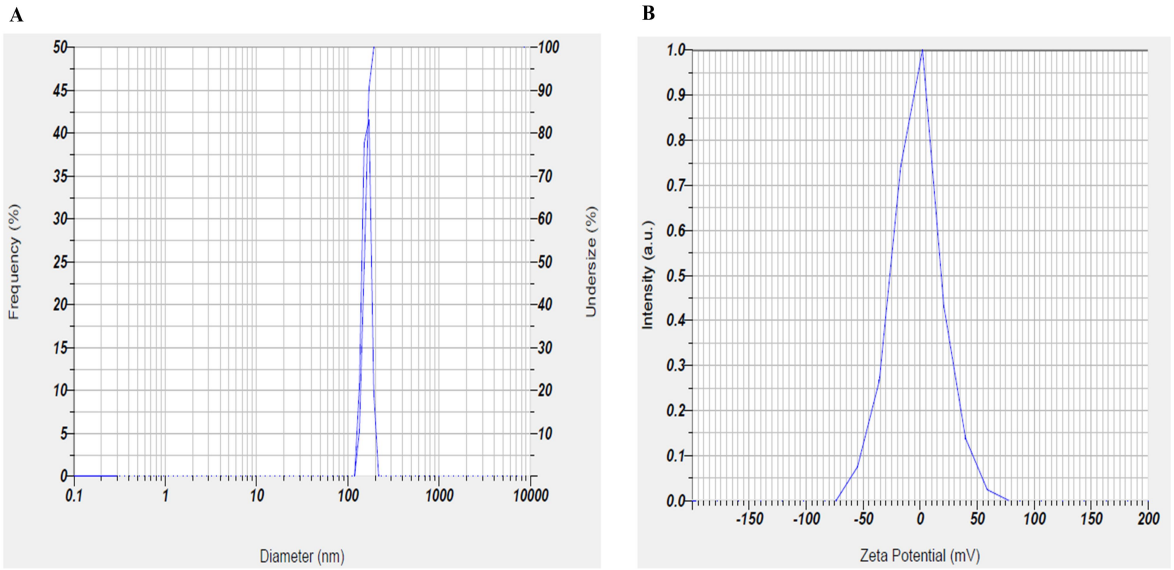

Figure 2: (A) Particle size, and (B) Zeta potential of optimized batch of pharmacosomes

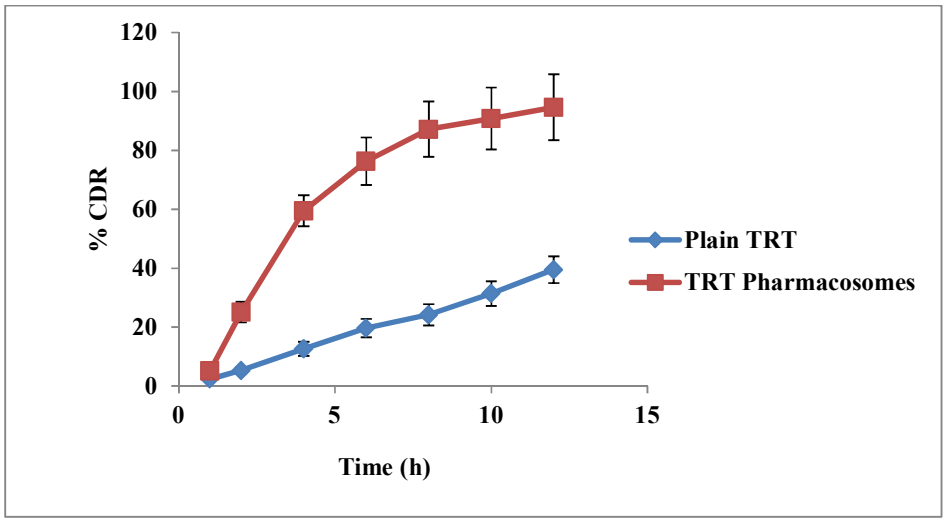

Figure 3: % Cumulative drug release of formulations

CONCLUSION

In the current investigation, pharmacosomes of TRT with HS prepared by a conventional solvent evaporation technique which showed high % yield and TRT content at TRT to HS ratio of 1:2 and therefore selected as optimized formulation. The FTIR analysis of pharmacosomes revealed the interaction between TRT and HS. Moreover, the solubility of TRT was found to be enhanced remarkably following pharmacosomes. The particle size of pharmacosomes was found in nm which is suitable for better topical absorption of TRT. Furthermore, the pharmacosomes exhibited more release of TRT when compared to TRT release from plain TRT solution. Thus, the present strategy could be promising for the efficient treatment of acne using TRT. However, further detailed investigations are needed to validate these obtained *in vitro* results.

ABBREVIATIONS

TRT: Tretinoin; **HS:** Hydroxylated soyalecithin; **FTIR:** Fourier transforms infrared spectroscopy; **PBS:** Phosphate buffer solution; **TRTP:** TRT pharmacosomes; **PDI:** Polydispersity index; **CDR:** Cumulative drug release

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CONFLICT OF INTERESTS

The authors declare that they have no any conflict of interests.

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