Section : Research Paper

Synergistic effect of lemon and orange oils used topically as a liposomal gel to improve skin beauty: Development and Assessment

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ABSTRACT

Background: Nanotechnology is a key part of making cosmetics for skin care. Essential oils are containing many different types of compounds, such as terpenoids, aliphatic chemicals, and phenol derived compounds. Due to their many useful properties, like being virucidal, bactericidal, fungicidal, local anaesthetic, anti-inflammatory, painkiller, and so on, they have been used for a long time. Because they are better for the skin, essential oils are chosen over synthetically manufactured medications made from chemicals. New nano systems like liposomes, niosomes, nano-emulsions, solid lipid nanoparticles, dendrimers, and nanospheres are used to make it easier for essential oils to get into the body through the skin. These nanocarriers allow natural components to be released in a controlled way and also make them more stable. Also, skin care items that are made with nanotechnology look nice. **Study Aim**: The study's goal is to create liposomal gel formulations for skin care that are based on orange and lemon oils. **Study design:** The research objective was achieved by focusing following studies. Firstly, lemon oil and orange oil are used to synthesize the liposomes. Secondly, the liposomes were

converted in to liposomal gel to find an alternative skin care. Thirdly, the characteristics of were liposomal gel studied. **Purpose:** To develop another alternative and herbal option for customers. **Results:** The study predicament that statistical analysis multiple comparisons were made by using Unpaired Student's t-test the statistical significance was considered at *p < 0.05i.e. p value summary *P < 0.01. **Conclusion:** The sensational study indicates that lemon oil and orange oil were successfully entrapped in a lipoosomesto formulate gel.

Keywords: Lemon oil, Orange oil, Entrapped, Liposomes, Gel, Sensational.

INTRODUCTION

Herbaceutical outcomes and herbal based medicaments are a magnificent source of modish therapeutic agents and for the build out of complementary and alternative medicines over traditional drug regimen [1]. Over half of the populace of globe depends on classical medicine for healthcare aid, more than 80% of the less developed countries [2-4]. Orange oil is a markable medicinal plant which defines a wide range of biological activities, and Sensational vesicular drug carrier term was highlighted in 1991 by Gregor Cevc the aqueous core surrounded by a complex of lipid bilayer. Greek word soma clarifies the body [5, 6]. Liposomes have enomerous advantages towards biocompatibility and biodegradability as they are made from natural phospholipids similar to liposome so protect the encapsulated drug from metabolic degradation with excessive permeation of the drugs through the skin [7]. Transfersomes fetch phospholipids, surfactants, solvent, buffering agent, dyes [8, 9]. Transferosomes formulation is the process of administration of active herbaria pharmaceutical dosage form through epidermal route [10]. Cosmetics are a broad category that includes both external and internal goods. They are commonly used to give off a pleasant odour or to alter or correct the look of the body part to which they are applied. The use of cosmetic items has skyrocketed as people become more conscious about their appearance and live longer. Cosmetics are no longer restricted to simple soaps and creams; instead, more complicated goods such as anti-ageing, skin whitening, anti-acne, and soon have taken over the market. It's no surprise that the worldwide cosmetics sector is valued USD 532 billion, with forecasted growth reaching USD 716 billion by 2025 [11]. The cosmetic and skincare industries are a huge and growing part of our society. Cosmetics are defined by the FDA as "items intended to be applied to any part of or to the human body for beautifying, cleansing, increasing attractiveness, or making a striking appearance." The term "cosmeceutical" is derived from the words "cosmetics" and "pharmaceutical medicament." Although these items have both cosmetic and biological effects on the skin, they are promoted as cosmetic or skincare products because they purport to improve the appearance of the skin [12]. Due to consumer demand to improve the appearance of their skin, hair, and nails, cosmeceutical treatments incorporate chemicals that can prevent wrinkles, UV damage, and other ailments. Nano-cosmeceuticals is defined as a cosmetic formulation that uses nanotechnology as a delivery mechanism to improve the performance of bioactive ingredients [13-17].

MATERIALS AND METHODS

Collection of Materials

Lemon oil, Orange Oil, was procured from local drug store, Lucknow, India. Soya lecithin, cholesterol, Carbopol 934, betadine was procured from Transgene Biotech, Lucknow, India.

Method

As per Table 1, Liposomal formulation was prepared. Method of Assembly operation: Soyalecthin and cholesterol was dissolved in chloroform 10ML by using individual beaker by obtained ratio respectively, Orange oil and Lemon oil was also dissolved in a separate beaker by using solvent chloroform. Taken four RBF of 1000ML loaded with respective ratio and coded with S1, S2, S3, and S4 by the 1ml addition of dissolved preparation of Orange oil and Lemon oil individually, and the RBF attached with working instrument (Rotary Vacuum Evaporator) and allowed to rotate for one hour with temperature equilibrium 60^0C. When complete solvent was evaporated than a visualized thin film was succeed at the wall of RBF and the obtained thin film was hydrated by using phosphate buffer pH6.8 [18]. On the basis of observation table 1 the formulations S1 was selected on the basis of thin film around the RBF (figure) for the gel preparation and further studied.

S.No	Cholesterol and Soyalecthin chloroform	Orange oil + Lemon oil	Obtained [Thin film formed on the surface of RBF]
S1	1:1	1:1	√
S2	2:1	1:1	×
S3	2:1	2:1	×
S4	2:2	1:1	×

Table 1. Selection of formulation by the help of Rotary Vacuum Evaporator*via* thin film method

Preparation of Gel:

In this method the2 mg Carbopol 934 was dissolved in 50 ml of deionized waterwith constant stirring and heat for 30 min after heating left for cooling. When gel was stable add the prepared formulation (Figure 2) [19-20].

Drug content:

The uniformity of drug content was determined using a UV spectrophotometric method. 500 mg of gel was dissolved in 50 mL of methanol. The volumetric flask was maintained for 2 hours and then thoroughly mixed in a shaker. The solution was filtered after passing through the filter paper. The drug concentration was determined using spectrophotometry with methanol as a blank [21-24].

Measurement of density:

The bulk density of transferosomes was calculated by dividing its weight by its volume. The final density of a transferosomes was estimated by using an average of three specimens with a volume of 1 ×1 ×1 cm^3[25-28].

Particle size Analysis by Malvern:

The Malvern is a particle size analyzer that uses laser diffraction to measure particle sizes ranging from 0.1 um to 3 mm. For analysis, only a little sample (0.25g) is required, and findings can be obtained in as little as 10 minutes per sample. The fluctuation in angular scattered light intensity is measured using a laser beam passing through a dispersed sample [29-32]. (The scattering angle of small particles is modest, whereas the scattering angle of large particles is huge).

Zeta Potential Analysis by Malvern:

For the measurement of zeta potential, or electrophoretic mobility, Malvern Analytical offers leading zeta potential analyzers. The Zetasizer range offers a simple, fast, and accurate approach to measure zeta potential in a number of cell types, including inexpensive disposable folded capillary cells, in both laboratory and process environments.It improve formulation stability and shelf life while reducing formulation time and expense with zeta potential measurements. One of the fundamental characteristics known to affect stability is zeta potential, which is a measure of the level of electrostatic or charge repulsion/attraction between particles [33-36].

pH of Liposomes:

The pH of the formulated liposomes was checked by using buffer solution pH 6.8. Firstly 1ml of liposomes was weight and kept in 50ml of buffer for 1 hrs. Then with the help of calibrated pH meter the pH of the obtained liposomes were identified [37].

***In vitro* drug release study:**

The release studies will be carried out in 500 ml beaker containing 100 ml of phosphate buffer pH 5.8. The beaker will be assembled on a magnetic stirrer and the medium was equilibrated at 37±50°C. Dialysis membrane will be taken and one end of the membrane was sealed. After separation of non-entrapped transferosomes dispersion was filled in the dialysis membrane and other end will be closed. The dialysis membrane containing the sample was suspended in the medium. Aliquots were withdrawn (1 ml) at specific intervals, filtered and the apparatus was immediately replenished with same quantity of fresh buffer medium pH 5.8. [38-45].

Drug entrapment efficiency Study

The transferosomes were formed and immersed in 100 ml of phosphate buffer with a pH of 7.4 overnight at 25°C. The sample leach ate was then filtered using a 0.2 mm membrane filter after being sonicated for 10 minutes. The concentration of drug in the solution was evaluated using the UV spectrophotometric (Shimadzu, Japan) method, at 228-554nm and the percentage drug entrapment was estimated by:

Entrapment Efficiency= Amount of drug entrapped / Theoretical weight of drug×100

pH of the Gel

pH meter was calibrated by the help of standard buffer solution. About 1 gm of gel mixed with 100 ml of distilled water and store for 2 hours for the measurement of pH.

Microbial Study

The formulated gel was inoculated on the plates of agar media. The plate was controlled by formulated transferosomes gel at 37˚C for 24 hours under incubator. After duration of time period the growth rate checked by visually [45-59].

Stability Study

A stability study is a set of tests used to ensure that a pharmacological product will remain stable over time. The optimum gel formulation was kept at 5°C ± 3°C and 30°C ± 2°C in a well-sealed stoppard glass container. For one month, data was collected by physicalappearance and with digicam microscope at regular intervals.

Statistical analysis

Graph Pad Prism was used to do the statistical analysis. Multiple comparisons were made using Unpaired Student's t-test and ANOVA which was considered significance at $p<0.05$.

RESULT

Lemon oil and orange oil based liposomal gel was formulated by mentioned the above technique and further it was optimized on the basis on gel with hydrophilic. A table 1 shown the loading efficacy of formulation and batch S1 was selected on the basis of optimization because it has highest entrapment among them, which making it ideal for use in gel formulations. In gel preparations, carbopol 934 was frequently used as a gelling agent.

Formation of Transferosomes by Rotatory Vacuum Evaporator method:

As per Table 1, the S1 selection shows the prepared formulation having a thin layer around the round bottom flask and it was hydrated by buffer with constant hand shake for 15 minutes then it was observed under digicam microscope, the tiny vacuoles was visible which provide the conformation regarding the presence of transferosomes as is shown in Figure1.

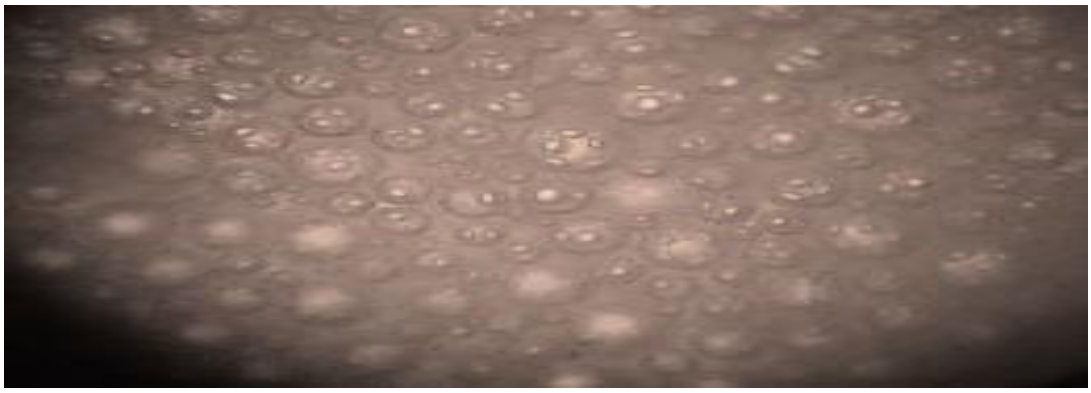


Figure 1: Microscopic image of liposomes at 45x by using Digicam microscope it seems that the prepared formulations were spherical in shape. The lipoosomes was formed successfully at a surface of RBF with a smooth and creamy texture and hydrated by buffer pH 6.8 and microscopically seemed spherical vesicles were formed.

Formation of Transferosomes Gel

Incorporate dcarbopol 934 was assure the formation of curcumin based transferosomes gel which was seemed under projection microscopeas is shown in Figure2.

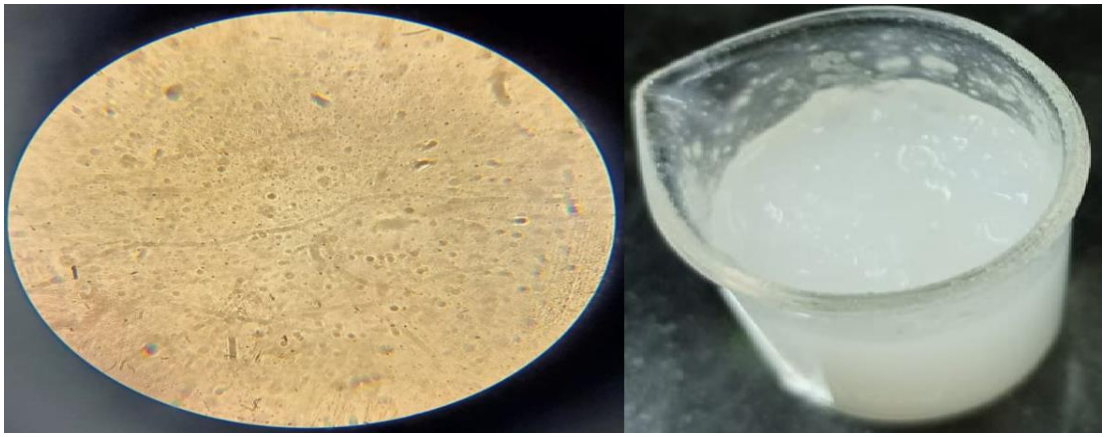
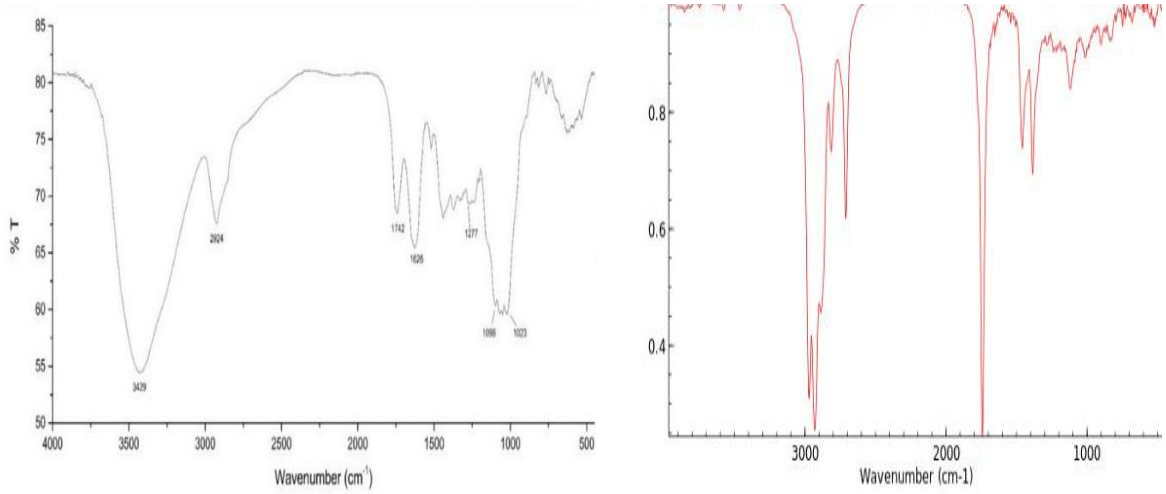


Figure 2: The Macroscopic view of gel was thick and clear, highly viscous appearance and the Microscopic view of gel under projection microscope that shows the vesicles of drug entrapped in a spherical shape.

Fourier Transform Infrared Spectroscopy

FTIR identifies the chemical bonds in a molecule by producing an infrared absorption spectrum. Here the curcumin has a peak around 1500 cm −1 assigned to C=C and a peak around 1300 cm −1 ascribed to C-O in phenol groups present at curcuminas is shown in Figure 3.



[A] **[B]**

Figure 3: FTIR of Orange oil and Lemon oil

Drug content

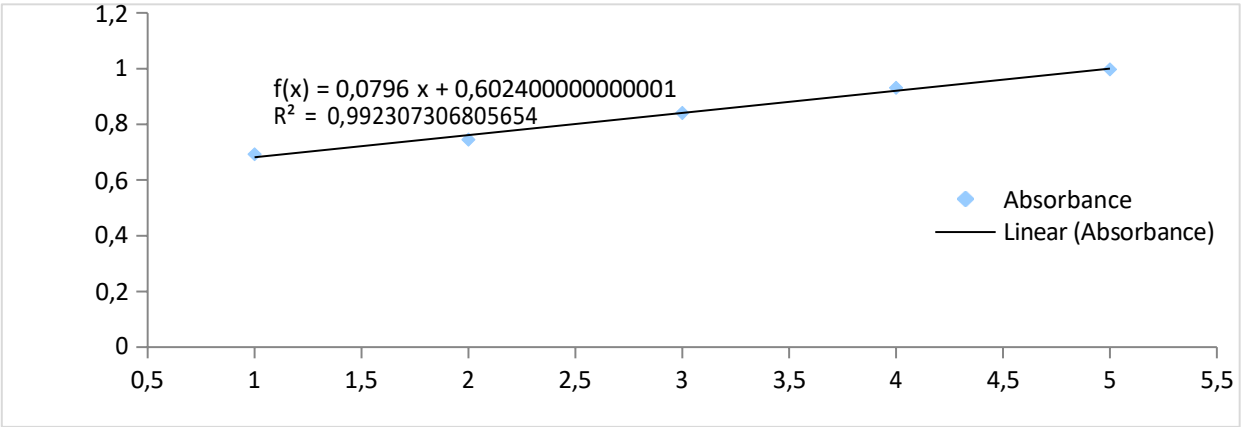


Figure 4: The clear supernatant solution was collected and drug concentration was evaluated using aCalibration curve of in methanol.

Density

The density of formulation at 2% w/v is 0.063 gm/cm3. According to the findings of the density study, the developed formulation has a high degree of porosity, as evidenced by its ultralow density. Additionally, the created preparation's high porosity assures that it has a high adsorption capacity for wound secretion absorption.

Particle size Analysis by Malvern

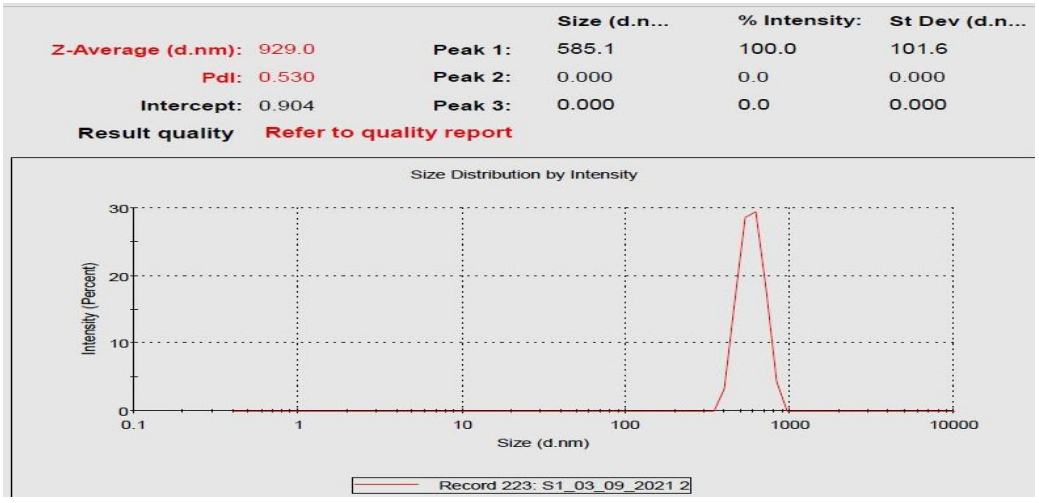


Figure 5: Leposomes appeared as spherical, well identified, unilamellar nanovesicles at peak 585.1 and the numerical value of ideal PDI ranges from 0.0 to 1 which represents perfectly

uniform sample with respect to the particle size and the polymer chains are of same molecular weight, so this graph have PDI: 0.530 which is less than 1 which means the prepared formulation are uniformed with respect to particle size. Result summary, Malvern based on Laser diffraction measure the particle size distribution here, Record 223; S1 represent the liposomes and at 585.1 shows the peak with 100 % intensity, PDI: 0.530.

Zeta Potential

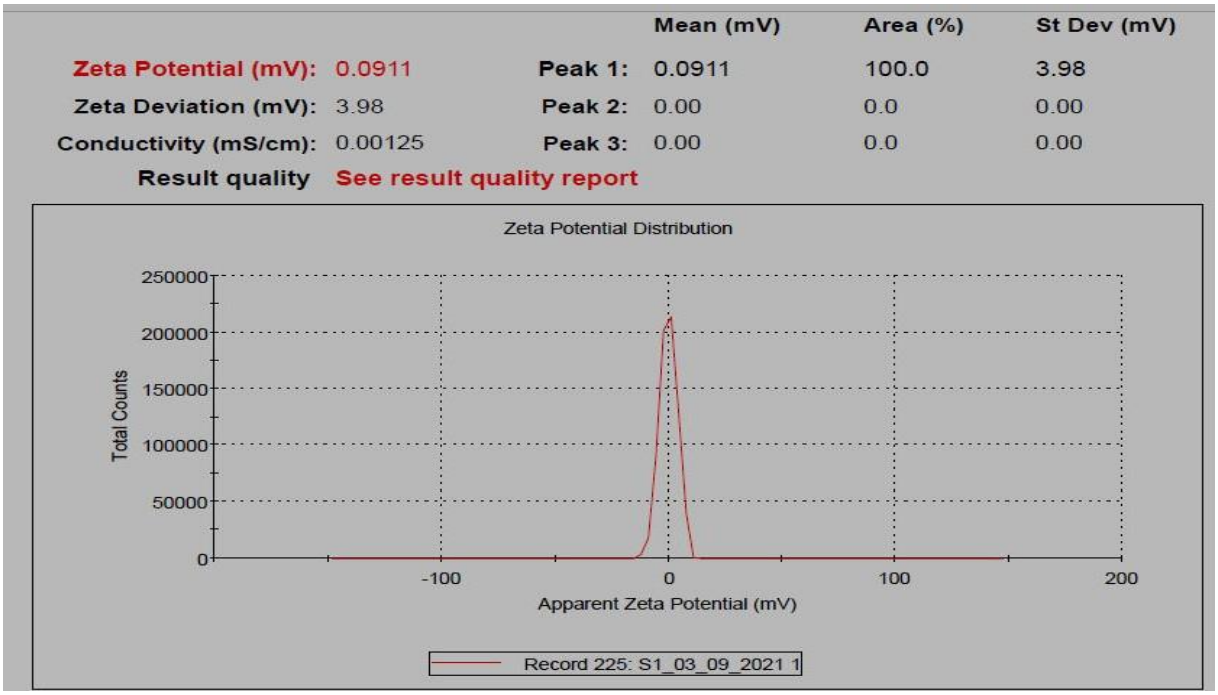


Figure 6: The zeta potential represents the measurement of the surface potential of suspended particles. Particles with a zeta potential greater than ± 30 mV are considered to be stable, because the repulsive force of the same charge can avoid particle aggregation. So The results of liposomes showed that the best formulation was obtained as per mentioned graph the obtained value is ±3.98 mV, which means the formulation has stability because of no aggregation between the charged particles. Result summary Malvern based on Laser diffraction measure the Zeta potential at 0.0911 mV, with 0.00125 ms/cm conductivity and zeta deviation 3.98 mV, and Record 225; S1 represent the liposomes .

pH

The pH of the liposomes was determined successfully with respect to temperature up to 45^0C and it was seemed that pH was similar at different time of interval.

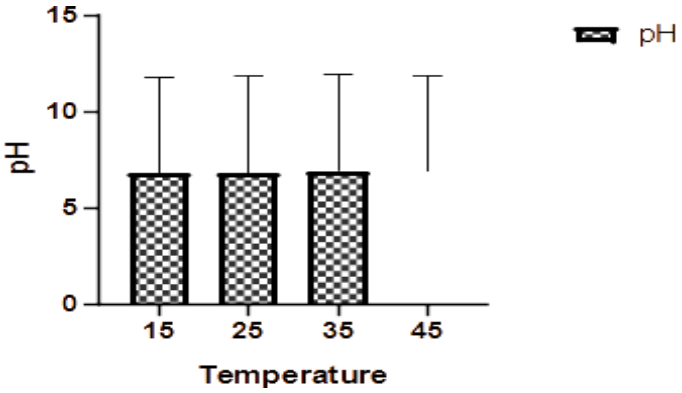


Figure 7: pH of liposomes at different temperature was found 5.8 which is good for topical purpose by applying student "t-test" and all the values are expressed as Mean ± SD calculated by ANOVA summary i.e. p value summary *, (significant diff. among means [P < 0.01].

***In vitro* DR**

In vitro DR of Liposomes was increased by the increase of time period.

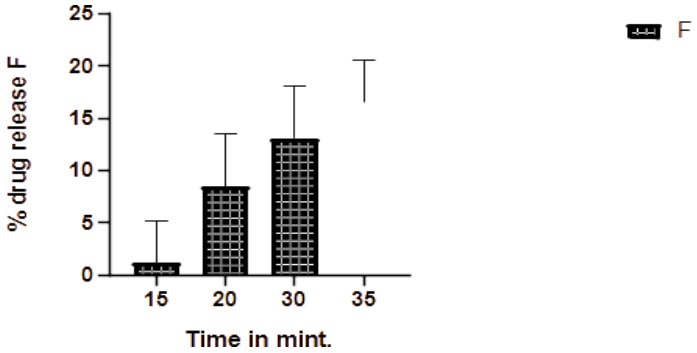


Figure 8: Graph representing *In vitro* DR of liposomes by increasing the rotation time the release property was increased values are expressed as Mean ± SD calculated by ANOVA summary i.e. p value summary *, (significant diff. among means [P < 0.01].

DEE of Leptosomes of formulation

DEE of liposomes shows that the how much amount of drug was entrapped here the result reveals that the maximum amount of drug was entrapped but less than 100 %.

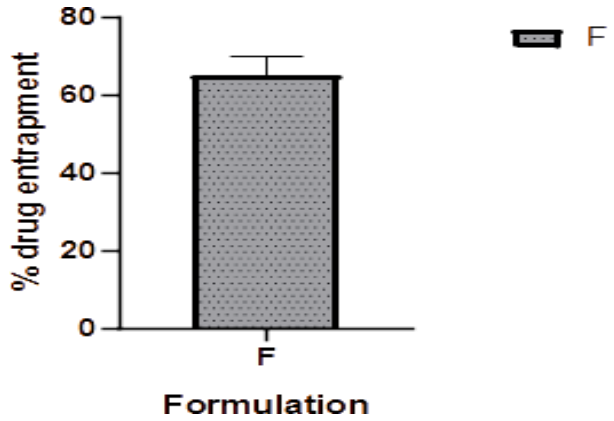


Figure 9: Graph representing DEE of formulation. Values are expressed as Mean ± SD calculated by ANOVA summary i.e. p value summary *, (significant diff. among means [P < 0.01].

pH of Gel

The resultant values reveal that the pH was slightly increased but the pH values approx 5.8 which is good for skin which in not acidic nor basic.

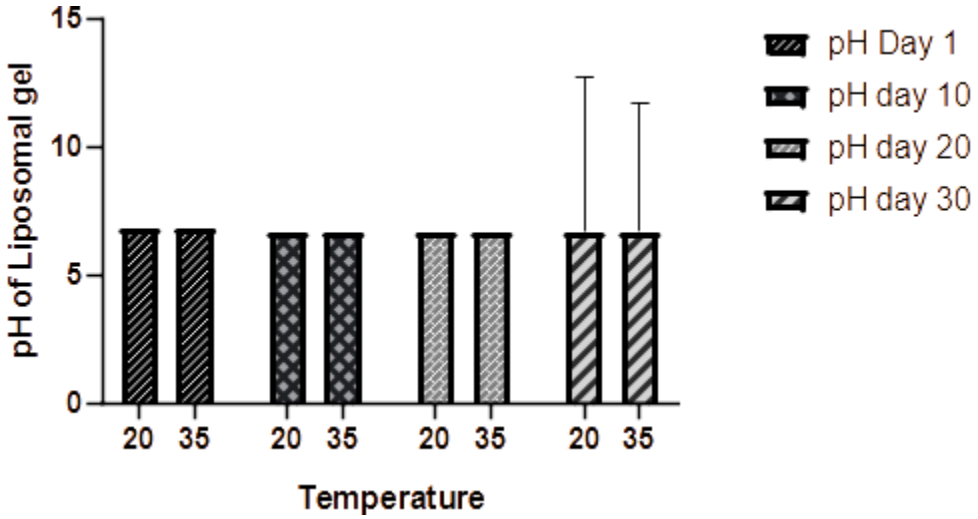
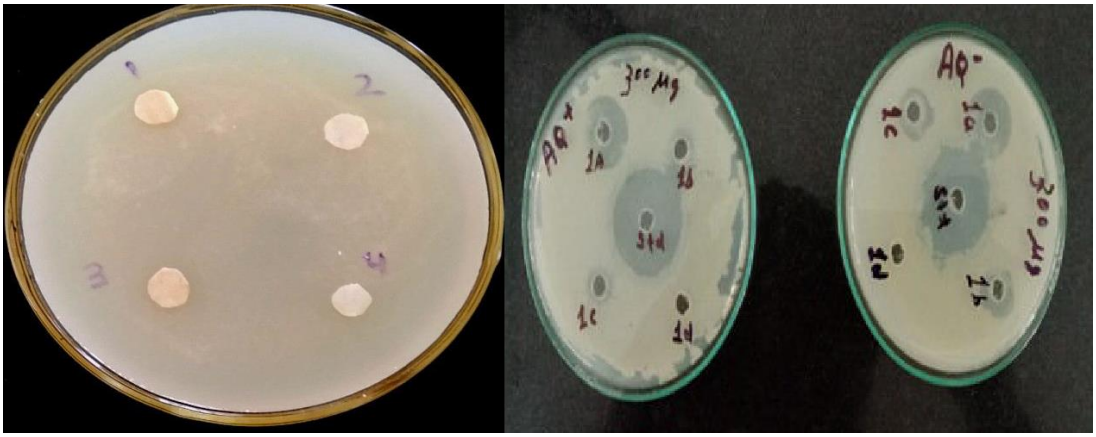


Figure 10: Graph representing the pH was slightly increased of prepared gel at 20ºC-35ºC at different days of interval up to 30th days. All values are expressed as Mean ± SD calculated by ANOVA summary i.e. p value summary *, (significant diff. among means [P < 0.05].

Microbial Study

During microbial test the distilled water, Betadine, and drug extract was compared by gel and the resultant values reveals that gel reject the null hypothesis.



[1] **[2]**

Figure 11: [1] Microbial Study of gel was done by using agar media and result was achieved by using whatsman filter paper [2]Microbial Study of gel was done by using agar media and result was achieved by using B.subtilis (gram positive bacteria), and E.coli (gram negative bacteria).

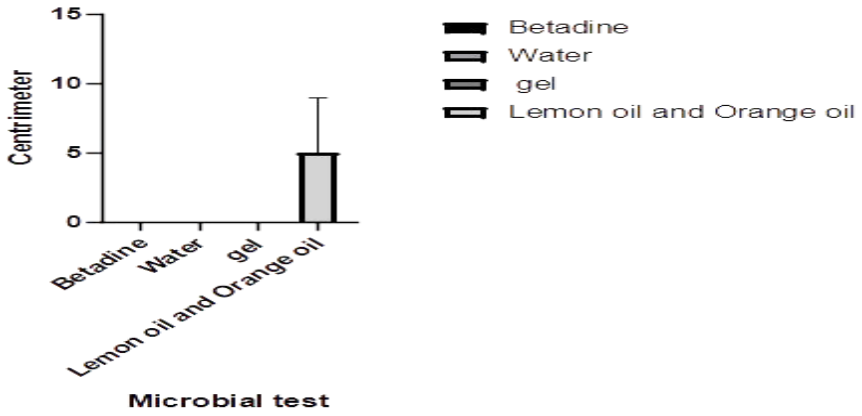


Figure 12: Graph representing the microbial study of gel in this graph the comparative study was done with each other. All values are expressed as Mean ± SD calculated by ANOVA summary i.e. p value summary *, (significant diff. among means [P < 0.01].

Stability study

After one month, the physical appearance of the optimized formulation S1 was found to be unchanged in all of the situations to which it was subjected. Even One month later, the medication content was confirmed to be satisfactory. The formulation was observed under digicam microscope at different time interval there was no noticeable reduction.

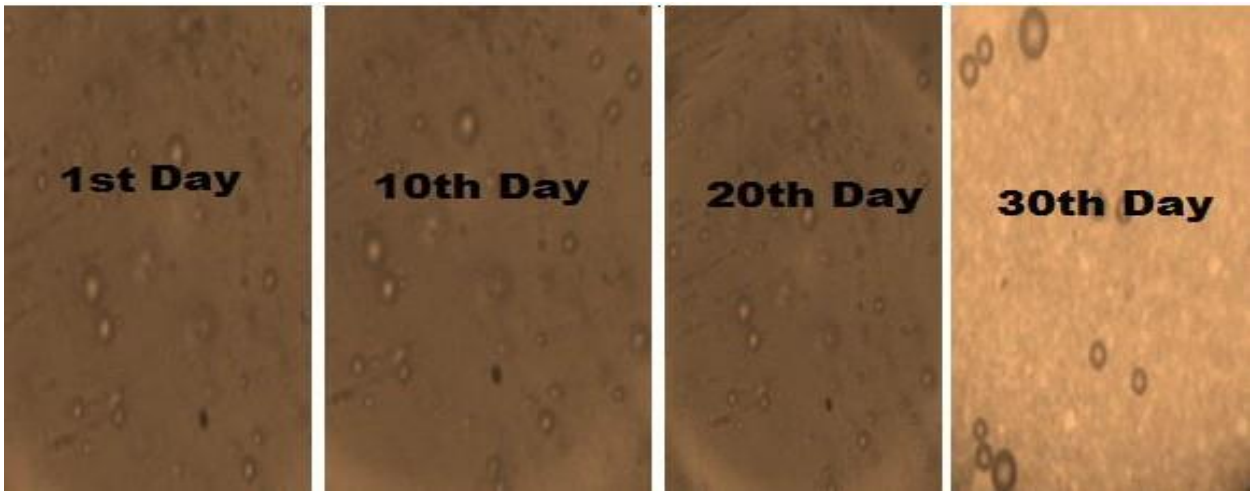


Figure 18: Microscopic image of gel by using Digicam microscope at different time interval for one month which shows no noticeable changes occur during one month of storage which ensures the product stability.

DISCUSSION

Aspects of Formulation

In the most recent research, a based liposomal gel for topical formulation was investigated on a variety of factors. To begin, the formation development, which was prepared by using cholesterol and soyalecthin in chloroform, provides a significant relief in selecting a designerable development of transferosomes because they are phospholipids and have the property of self-assembling in addition to being biodegradable in nature with biocompatibility. This is because they are biocompatible. There are known pharmacological effects associated with the medication, which serves as the primary component of the formulation. After the pH of the liposomes was successfully assessed as per Figure 7 with respect to temperature up to 45°C, and it appeared that pH was consistent at different time intervals, the assurance of formed vesicles of microscopic structure is displayed in Figure 1. At a weight-to-volume ratio of 2%, the density was determined to be 0.063 g/cm^3. Figure 5 depicts the particle size distribution as determined by Malvern using laser diffraction at record 223. The peak was discovered in S1 at 585.1 with 100% intensity and a PDI of 0.530, which is less than 1, indicating that the manufactured liposomes are uniform with regard to particle size. Conductivity was measured at 0.00125 ms/cm, and the zeta deviation was 3.98 mV, as shown in Figure 6. The zeta potential was measured at 0.0911 mV. Particles that have a zeta potential that is more than 30 mV are deemed to be stable by formulation. This is due to the fact that particles with the same charge can resist aggregation thanks to the repulsive force of the same charge. The findings of the liposomes experiment demonstrated that the optimal formulation was accomplished by plotting the value of 3.98 mV on a graph. This indicates that the formulation is stable because there was no aggregation of charged particles during the process. The graph that illustrates the in vitro release of a medicine is presented here in Figure 8. The release quality was improved by increasing the rotation time; this contributed to the overall improvement. Figure 9 illustrates that the formulation has a drug

entrapment efficiency that is lower than one hundred percent. Step then forward towards the nurturing of transferosome gel, which was generated by the addition of gelling agent carbopol, and Figure 2 displays the microscopic view of transferosome gel under a projection microscope with little vesicles of encapsulated drug inside of it. Figure 10 depicts the pH of the produced gel at temperatures ranging from 20 to 35 degrees Celsius at varying intervals of time; Figure 3 depicts the FTIR; and Figure 4 depicts the drug content studies conducted with UV spectroscopy. The microbiological analysis of gel is depicted in Figure 11. This analysis was carried out with agar as the medium, and the results were obtained with the use of Whatsman filter paper. The graph that represented the microbiological investigation of gel with the comparative study was displayed in Figure 12.

CONCLUSION

The present study indicates that liposomes were successfully entrapped in a formulation. The developed formulation was evaluated for pH, particle size, drug entrapment efficiency, percentage drug release, and animal studies. Summarising all the results, we can conclude that nature has provided us with a better healing product than synthetics. So we need to explore herbal systems for more health benefits in human subjects. They are promising carriers for because they have antioxidant properties and they can protect and stable at 4-5ºC for 6 months at least. Solubility was shows in chloroform the pH of the formulated gel was found to be 5 which confirming it is suitable for topical application because in the range of 4.8 to 5.60 which is good recommended pH for the skin. All values are expressed as Mean ± SEM calculated by ANOVA summary i.e. p value summary *, (significant diff. among them). The therapeutic potential of transferosomes gel of curcumin explores the proposed research in the management of wound healing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this article.

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