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DEVELOPMENT AND EVALUATION OF LIPOSOMAL CREAM FOR ENHANCEMENT OF PERFUME

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Abstract: **Liposomes are aqueous-cored spherical vesicles composed of phospholipids that can be used as a delivery vehicle. The properties of liposomes are being used in cosmetics such as moisturizers, hair shampoos, creams, lotions, and other goods, resulting in a cosmetic revolution. Essential oils have perfumery properties and their vivid fragrances make them use on a large scale for various perfume in cosmetics, and their constituents have antibacterial effects, but their inherent volatility, which makes them vulnerable to air, light, and temperature, is a limiting factor. As a result, they can be enclosed into vesicles like liposomes for action and preservation. That technique can also prevent their volatile nature. The aim of study was to prepare liposomes with encapsulated essential oil and further, adding them as a suspension to cream so as to formulate liposomal cream**

Keywords- **Liposomes, essential oil, cream, formulation, evaluation**

INTRODUCTION: Novel vesicular drug delivery systems:

Novel vesicular drug delivery systems aim to deliver the drug at a rate directed by need of body during the period of treatment, and channel the active entity to the site of action. Biologic origin of these vesicles was first reported in 1965 by ÂBinghamÊ and has been given the name ÂBingham bodiesÊ. A number of novel vesicular drug delivery systems have been emerged encompassing various routes of administration, to achieve targeted and controlled drug delivery. [1]

Liposomes:

Liposomes are spherical vesicles with a core aqueous portion that is surrounded by one or more bilayer membranes (Lamella) that are commonly found in aquatic environments. Amphiphilic lipids collide with the aqueous layers to produce these vesicles. They range in size from 15 nanometers to several micrometers. Liposomes have expanded their use from medicine delivery to cosmetics in the last 30 years, and they are now the most extensively used cosmetic delivery technology.

Liposomes can be used as a delivery mechanism because of their unusual structure, which allows hydrophilic compounds to pass through their enclosed aqueous part and lipophilic substances to pass through the nonpolar tails of the bilayer section. [2]

Liposomes are biodegradable with less toxicity and capable of entrapping hydrophilic and lipophilic medicines. Liposomes may transport medications or other macromolecules into human and animal systems, making site specific drug administration easier, such as to tumor tissues, and are thus of enormous interest to the pharmaceutical and cosmetic industries. [3]

MATERIALS AND METHODS: The materials and methods use for the preparation of liposomes and liposomal cream were as follows:-

Apparatus: Rotary Vacuum evaporator

Chemicals: Gift sample of Soy lecithin (Phospholipon 90 H) from Lipoid Germany, Cholesterol (LR Grade), Rose essential oil from Veda oils.

Methods: Pre formulation studies of oil and excipients:-

A) Confirmation of Oil: Sample of oil received from seller was confirmed by doing identification test:-

Appearance: The oil was visually assessed, color odor of oil was determined and reported.

Refractive Index determination: Refractive Index Measurement or Refractometer is the method and instrument of measuring substances refractive index and assess their composition or purity. Abbe's refractometer was used to measure the refractive index of the given sample. Using a particular monochromatic light source, the apparatus was calibrated with water as the oil. Micrometer screw was adjusted, to focus the boundary between the bright and dark regions. Refractometer scale was adjusted to place the cross wire of the telescope exactly on the boundary between the bright and dark regions. Index of refraction was read using telescope scale and result was reported.

pH: The pH of the oil was measured using pH paper. The one end of oil pH paper was dipped into oil and then after couple of seconds, the color change of paper was observed and compared with the standard color chart provided by pH paper kit and result were reported. Solubility of oil: Essential oils are sparingly soluble in water, so their solubility was determined in organic solvent in which they generally soluble. The soil solubility was then determined in organic solvent such as Chloroform, methane, ethanol and ether.

B) Spectrophotometric Estimation of Rose oil (geraniol): Spectrophotometric estimation of oil was carried out in methanol as a solvent.

Geraniol being the components which is responsible for fragrance hence, it was selected to be a single components from rose oil [4]

C) FTIR study: The FTIR study was done to identify the excipient and sample of oil and for compatibility of excipient with oil.

FTIR study of Excipients: Potassium Bromide was dried in Hot air oven at 105 0C for 2hr. Potassium bromide approximately 500 mg triturated in mortar and pestle. The sample of soy lecithin and cholesterol 50 mg was added and triturated separately during each process until it forms uniform mixture. This mixture was filled in sample cavity. Then the mixture of sample of soy lecithin and cholesterol was scanned in FTIR in range 400-4000 cm-1 to get spectrum of both. [5]

FTIR of Geraniol: A Cary 630 FTIR (Agilent Technologies Pty Ltd., Mulgrave, Australia) interfaced with an ATR (attenuated total reflectance) sampling accessory with a single bounce diamond crystal was used to get the FTIR spectra of geraniol. In the absorbance mode, spectra were accumulated from 4000 cm-1 to

1000 cm-1 using 64 scans with a spectral resolution of 4 cm-1before sample measurement, a reference (air background spectrum) was scanned under the identical experimental conditions. Resolution Pro FTIR spectroscopy software was used to process the spectra (version 5.2.0, Agilent Technologies Pty Ltd., Mulgrave, Australia). The sample spectrum is acquired by placing a little drop of essential oil sample on the diamond ATR crystal's surface and FTIR spectra of geraniol was obtained.

Compatibility study of Oil with excipients: FTIR for oil and mixture of oil with excipients: A Cary 630 FTIR (Agilent Technologies Pty Ltd., Mulgrave, Australia) interfaced with an ATR (attenuated total reflectance) sampling accessory with a single bounce diamond crystal was used to get the FTIR spectra. In the absorbance mode, spectra were accumulated from 4000 cm-1 to 1000 cm-1 using 64 scans with a spectral resolution of 4 cm-1 .Before each sample measurement, a reference (air background spectrum) was scanned under the identical experimental conditions. Resolution Pro FTIR spectroscopy software was used to process the spectra (version 5.2.0, Agilent Technologies Pty Ltd., Mulgrave, Australia). The sample spectrum is acquired by placing little drop of oil and excipients mixture on the diamond ATR crystal's surface and FTIR spectra of sample was obtained and results were reported. [6]

Formulation of Liposomes: Liposomes formulation were prepared by rotary vacuum evaporator method using, soy lecithin and cholesterol dissolved in Chloroform and Ethanol in the ratio of (9:1). And was transferred to rotary flask and kept under vacuum pressure at 50 ^{0}C thus depositing a thin layer of the solid mixture which was further hydrated with 10 ml of Saline Phosphate buffer solution of pH 7.4. By shaking and was transferred into beaker for further studies. Formulation of liposomes were done using various concentration of soy lecithin and cholesterol [7]. And selection was done on the basis of observations made during experiment. Below tables are shown with optimization of excipients.

Table showing concentration of Soy lecithin used for formulation of Liposomes

Batch No	Soy Lecithin amount in mg	Cholesterol amount in mg
F1	100	100
F2	200	100
F3	300	100
F4	400	100
F5	500	100
F6	1000	100

Table showing variable amount of Cholesterol used for formulation of liposomes

Batch No	Soy Lecithin amount in mg	Cholesterol amount in mg
F1	400	25
F2	400	50
F3	400	75
F4	400	100
F5	400	125
F6	400	150
F7	400	175
F8	400	200

The Optimized quantity of soy lecithin and cholesterol was fixed at 400 mg for soy lecithin and 150 mg of cholesterol and formulation of liposomal essential oil was done for next step.

Incorporation of Essential oil into optimized liposomes formulation

Batch No	Soy Lecithin in mg	Cholesterol in mg	Oil in ml
F1	400	150	0.4
F2	400	150	0.6
F3	400	150	0.8
F4	400	150	1.0
F5	400	150	1.5
F6	400	150	2.5

The oil was optimized and encapsulation efficiency was determined which then helped to select the oil in ml to be added in the formulation.

$$\text{Encapsulation efficiency}\left(\text{EE}\right)=\frac{\text{amount of total loaded drug}}{\text{total amount of drug}}\times 100$$

The encapsulation efficiency of formulation F4 F5 and F6 was found to be satisfactory hence it was selected as a formulation of liposomal essential oil to be added in formulation of liposomal cream.

Formulation of Cream:

Beeswax-Borax or (W/O) type of cream:

Bees wax and mineral oil was melted together keeping their temperature at 70 ^{0}C. Borax was dissolve in water and temperature of that solution was maintained to 70 ^{0}C. Now water phase of borax in water was added to oil phase with rapid stirring. After addition of water agitation was done while slowly cooling down the temperature of cream prepared. The liposomal suspension was added when temperature dropped to 50 ^{0}C. Cream was filled into the container and evaluation was done for each batch.

Formulation of Cream using variable amount of given ingredients

Ingredient's	Batch F1 (gm)	Batch F2 (gm)	Batch F3 (gm)	Batch F4 (gm)
White Bees Wax	20	20	15	10
Liquid Paraffin	50	40	20	15
Distilled Water	28.8	39.06	63.58	73.06
Borax	0.7	0.94	1.42	1.94

The above formulation was evaluated and on the basis of the parameters the formulation no F4 showed satisfactory result hence, it was selected to which the liposomal essential oil suspension was added.

Formulation of Liposomal cream:

The optimized quantity of bees-wax borax cream was done and the liposomal cream was prepared by addition of liposomal essential oil quantity around 10 ml in w/o cream prepared.

Method of Evaluation:

A). Liposomes

Simple Microscope: Liposomes were analyzed by simple microscope under 10x aperture and result are reported.

Determination of Particle Size: Particle size measurements were carried at 25 ºC by scattering light technique scattering angle as kept at 90 ^{0} using instrument Malvern Zetasizer Nano Z®. A dynamic light scattering instrument (Malvern Instruments Inc., Malvern, UK) was used to measure zeta potential of the liposomal formulation. The sample was analyzed at 25 °C and results were reported and discussed.

Determination of Shape and Surface morphology: The ESEM experiments were conducted using (Hitachi S-3700N). The sample was placed on stage and monitored in real time (hydration/dehydration step) in the ESEM chamber while the Peltier stage was used to control the temperature (Emott AG, Zurich, Switzerland). The chamber pressure and sample temperature were set at 6.45 Torr and 4°C, respectively. The relative humidity reached 100% in these conditions. The sample was kept in these "initial conditions" for roughly 2 minutes before an image of interest (initial state–wet state) was chosen. The chamber pressure was then gradually increased to the dehydration condition (P 4–2.65 Torr), while the sample temperature was maintained at 9°C. The photographs were then taken and results were reported. [8]

B). Liposomal Cream

Organoleptic Properties: The determination of organoleptic property was carried out.

Physical appearance: The physical appearance of the cream was observed and reported.

Homogeneity: Homogeneity and texture were tested by pressing a small quantity of the formulated cream the thumb and index finger. The consistency of the formulations and presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. Immediate skin feel (including stiffness, grittiness, and greasiness) was also evaluated.

Spread ability: The spread ability of cream was determined by, a small amount of prepared cream was taken and then it was applied on to skin and with slow stress it was spread on to skin and determination was done by checking whether the cream was easily spreadable and left a residue after washing with flowing water for 10 seconds.

Rheological Studies: Rheological characters of cream was determined using Brookfield viscometer. The measurements were performed using spindle no-4. Viscosity parameters were collected at different rpm with 1 minute equilibration time at every rpm. Different torque values were obtained at respective spindle speeds for an up-cuve and down-curve. Rate of shear and shearing stress were calculated by using formula. Rheogram was constructed to determine flow of formulation by plotting shear stress on x-axis and shear rate on y-axis and results were reported .[9[

RESULT:

Preformulation studies

Identification of Oil

Organoleptic Property of the oil

Color – Transparent to pale yellow

Odor - Aromatic

Taste – Balsamic sharp

Appearance – concentrated liquid

Refractive Index- 1.486

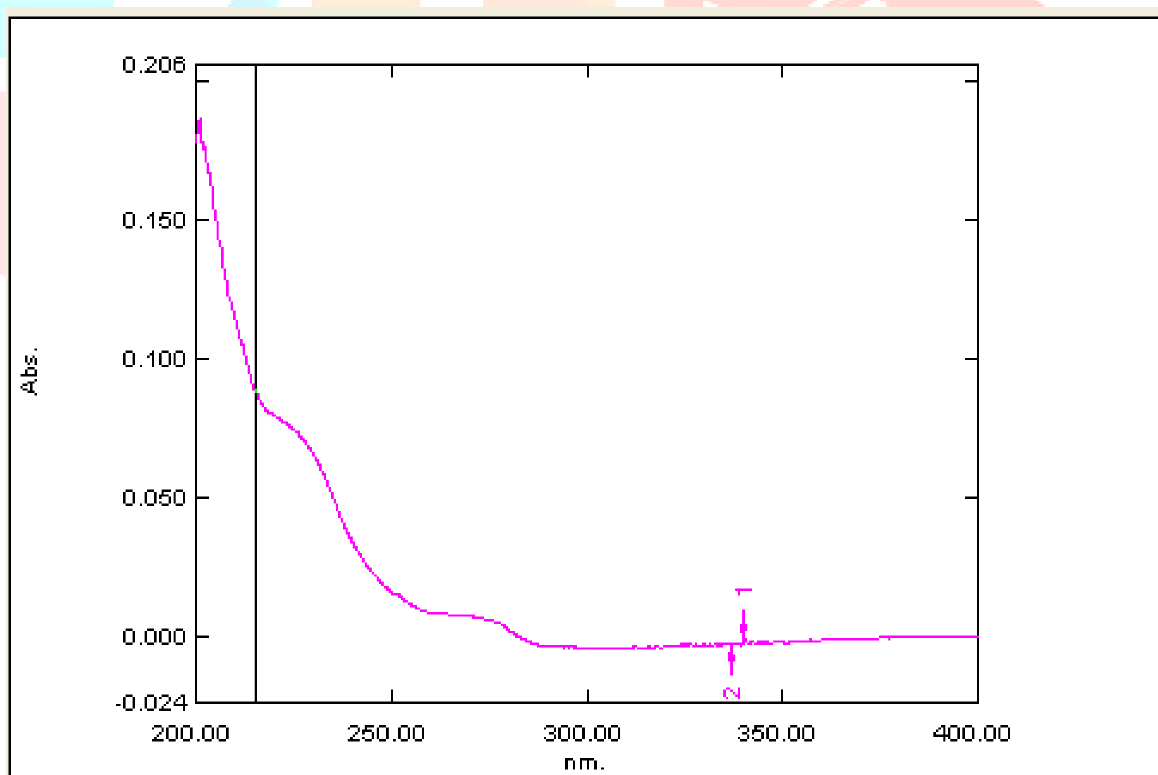
pH: 6.5

Solubility- Oil was soluble in chloroform, ethanol, methanol, and ether and insoluble in water.

Rose oil has same properties as mentioned above in literature survey and sample analysis provided by seller.

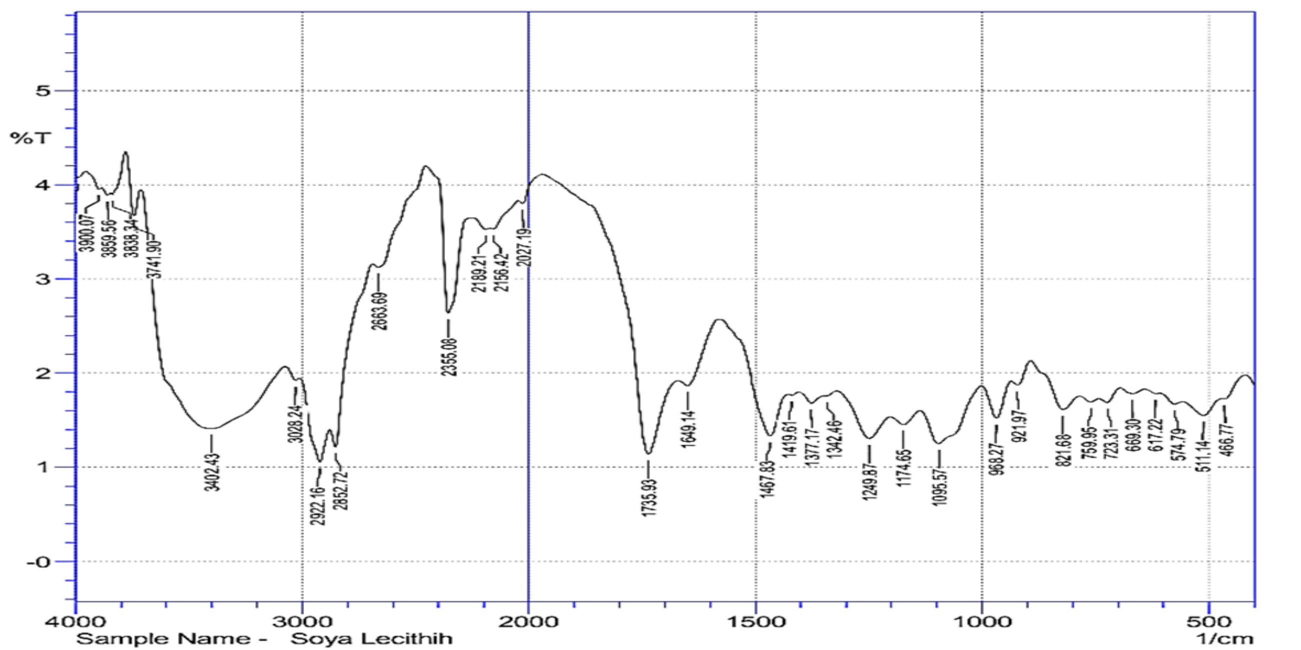
Determination of maximum absorbing wavelength:

The maximum absorbing wavelength of geraniol in methanol as a solvent was found to be 215 nm which is reported in the literature survey.



Graph showing λ max of Rose oil (Geraniol) on UV spectrum in Methanol Solvent

FTIR Study: A) FTIR of Soy lecithin

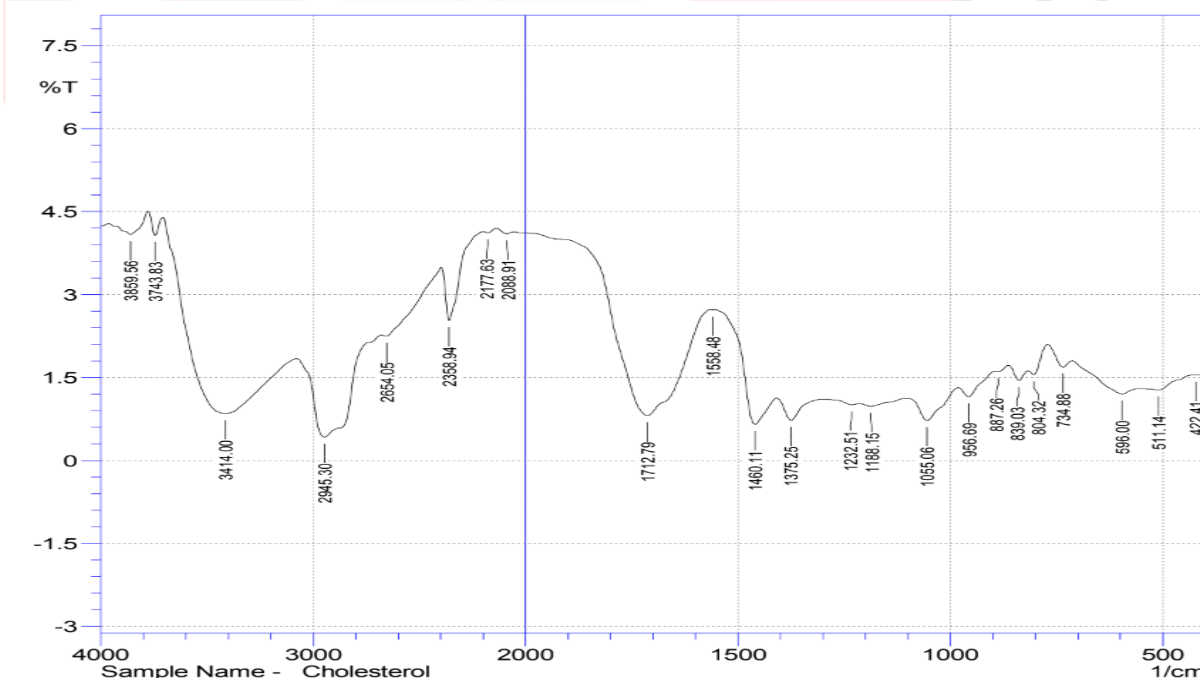


Graph Showing FTIR spectrum of Soy lecithin

The FTIR spectra obtained of soy lecithin shown in above figure was similar to the standard spectrum of soy lecithin.

B) FTIR of Cholesterol

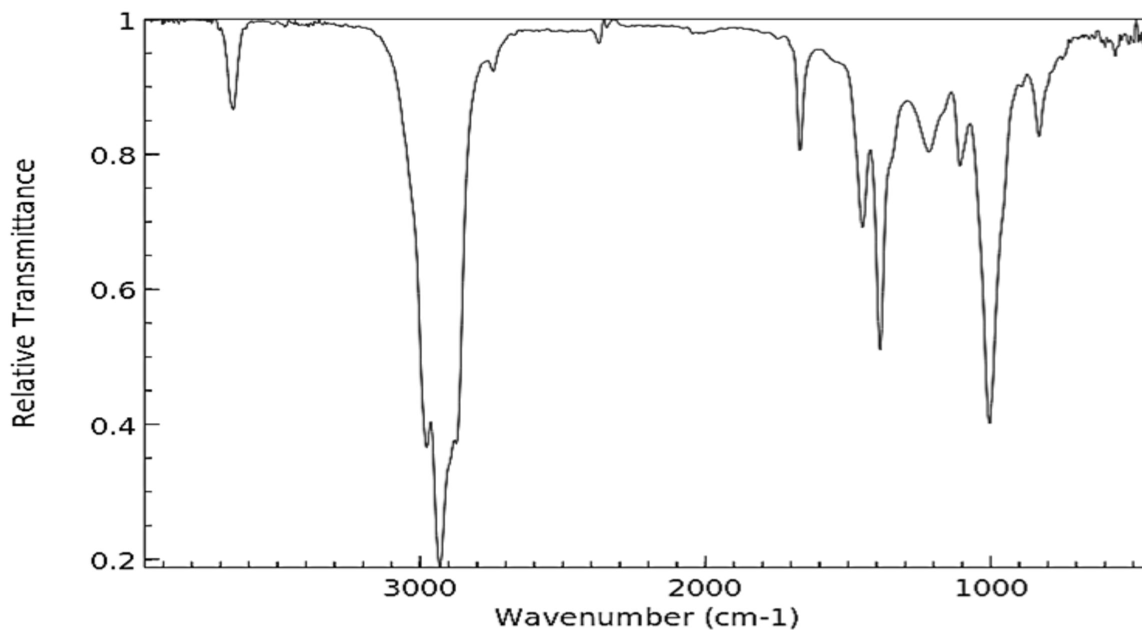
Graph showing FTIR spectra of cholesterol



The FTIR spectra obtained of cholesterol shown in above figure was similar to the standard spectrum of cholesterol.

C) FTIR of Geraniol :

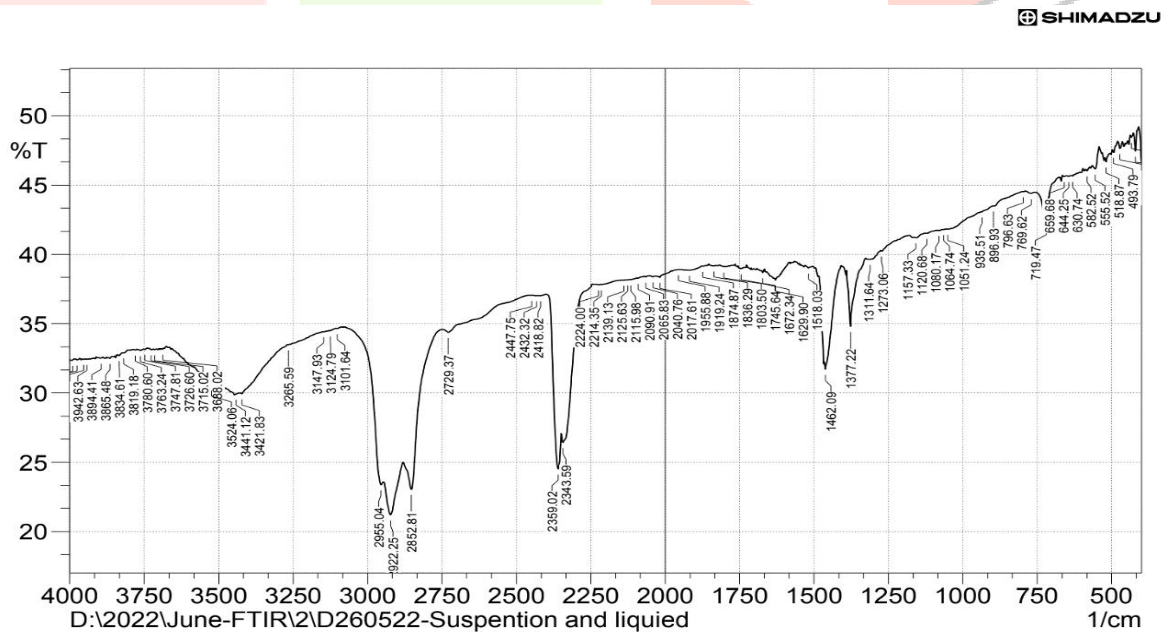
Graph showing FTIR spectra of Geraniol



The FTIR spectra of geraniol was found to be similar to that of standard spectra as stated in literature survey.

D) Compatibility of Oil and Excipients study

Graph showing FTIR spectra of oil with soy lecithin and cholesterol



Evaluation of Liposomes and Liposomal Cream:

The liposomes were formulated and evaluated by as follows:-

Microscopic Evaluation of Liposomes: Liposomes were analyzed by simple microscope under 10x aperture.

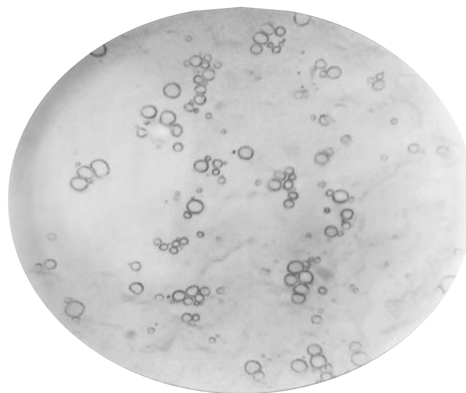


Fig. Microscopic images of Liposomes

Determination of Particle Size: Particle size measurements were carried at 25 ºC by scattering light technique scattering angle as kept at 90 ^{0} using instrument Malvern Zetasizer Nano Z®. A dynamic light scattering instrument (Malvern Instruments Inc., Malvern, UK) was used to measure zeta potential of the liposomal formulation. The sample was analyzed at 25 °C.

The Polydispersity index (PI) was found to be 0.838 which indicates highly poly disperse sample with multiple particle size populations. The Total particle sizes were in range from 0.34nm (lowest) to 8510.56 nm (highest). Hence, the Z average concluded was 386.3 nm diameter of particle size indicating saturation of particles size in liposomes.

The zeta potential mean at 25 ^{o}C calculated was 10.3 mV and viscosity of dispersion was 0.894 mPa^{-s}

Scanning Electron Microscopy (SEM)

The determination of shape and surface morphology was done by scanning electron microscopy on Hitachi S-3700N. The liposomes photographic images were taken at appropriate magnification of 10.0 kV and acceleration voltage) was used to analyze liposomes.

SEM analysis revealed that liposomes were spherical in shape with varying sizes, as shown in photographic images the sizes of liposomes were at 1.1 mm 114 um (smallest) and in between the varying sizes liposomes was observed as, 128 um, 137 um, 139 um, 161 um, 171 um, 203 um, 206 um, 211 um, 218 um, 235 um and 263 um (largest) respectively. From the observation it was concluded that as due to heterogeneous sizes and with multiple particle sizes liposomes were large size termed as giant liposomes.

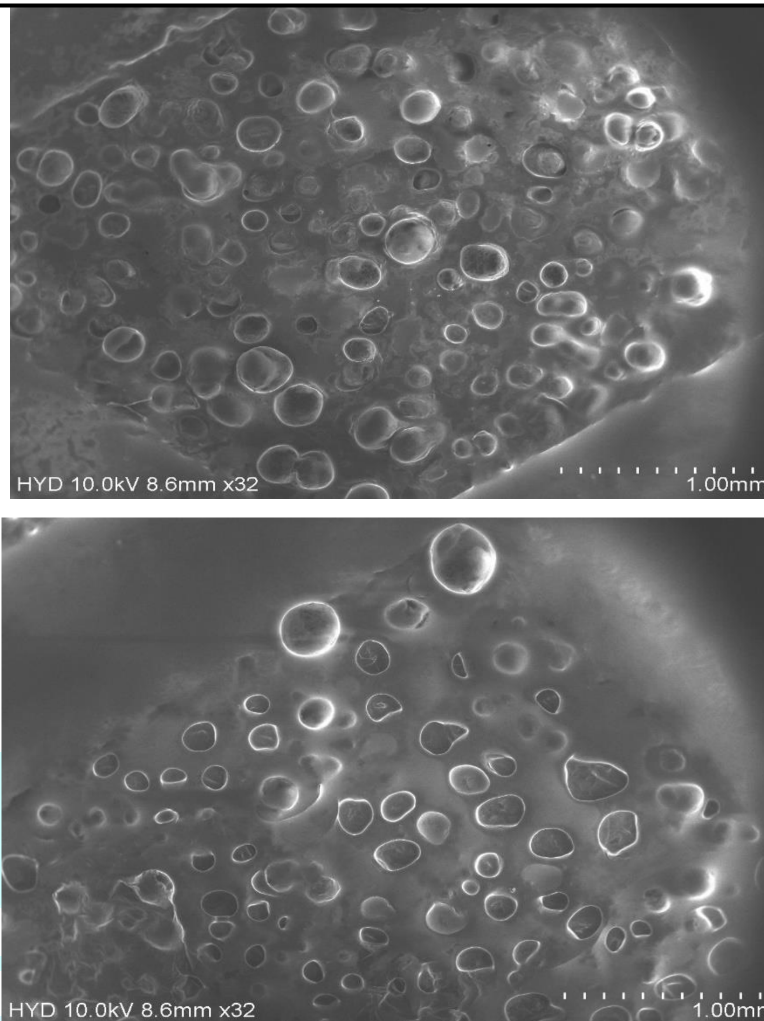


Fig. Showing photographic Images of Liposomes under SEM analysis

Evaluation of Liposomal Cream: The cream batches prepared was evaluated and following observations were made.

Organoleptic Evaluation: The cream was observed for the color, odor and appearance.

Color: Color for each formulation was observed and noted as-

F1=White, F2=White, F3=White, F4=White

Due to presence of white bees wax and no other color were added into preparation so.

Odor: Odor for each formulation was determined by sensory evaluation and for each formulation it was observed and noted as-

F1=Pleasant aromatic, F2= Pleasant aromatic, F3= Pleasant aromatic, F4= Pleasant aromatic

As addition of rose oil was done so pleasant aromatic odor was smelled.

Homogeneity: The homogeneity of the formulated cream was judged by the visual appearance and touch.

F1 Batch: poor Homogeneity – due to some particles of white bees-wax which does not evenly mixed into formulation.

F2 Batch: Poor Homogeneity- When touched and rubbed between fingers, the particles of borax which were not mixed homogenously with water were felt.

F3 Batch: Poor Homogeneity- Liquid paraffin was in excess amount and water added was in less quantity hence, phase separation of both was observed.

F4 Batch Good Homogeneity- When rubbed between fingers, no particles were felt and also no phase separation was seen in cream.

Spread ability: The cream was applied onto skin and the extent to which the cream easily spread was determined. All the four batches were determined F1 formulation was irritable due to presence of particle and hence it was not evenly spread. For F2 formulation when spread, it was again not homogenous as observations were made, and Formulation F3 was too much greasy and oily and for F4 formulation when applied it was evenly spread as force were applied when spreading and was evenly mixed onto skin surface.

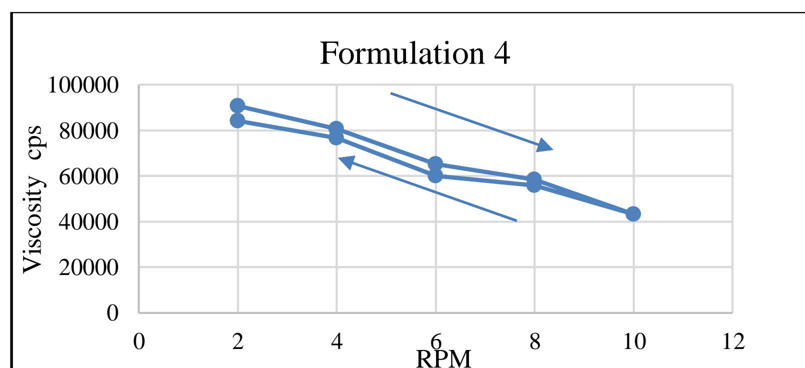
Rheology Study: Determination of viscosity and flow of formulation and results was obtained as:-

Table Showing Viscosity of Different formulations of cream

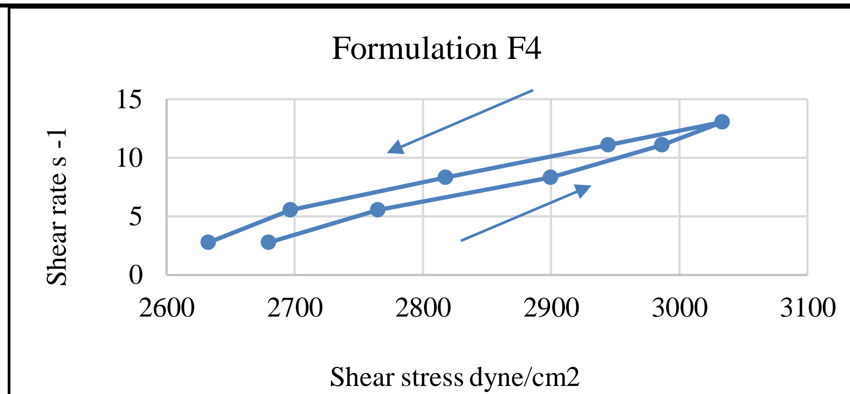
RPM	F1	F2	F3	F4
2	290342	316634	328131	90718
4	195216	161342	178917	80644
6	167832	107636	98812	65240
8	96312	85820	49019	58431
10	66045	64782	35735	43285
8	82494	76284	42345	55849
6	109988	95602	60000	60045
4	165000	111284	107766	76770
2	265142	208602	253960	84291

Table showing Rheological Study of different formulation of Cream

	Shear Stress (dyne/cm2)			
Shear Rates s^{-1}	F1	F2	F3	F4
2.78	3391	3909	2797	2680
5.54	3625	4168	2988	2765
8.32	3836	4216	3231	2900
11.09	3983	4316	3566	2987
13.06	4285	4421	3632	3034
11.09	3585	4244	3386	2945
8.32	3244	3969	3074	2818
5.54	2929	3625	2782	2697
2.78	2756	3243	2315	2633



Graph showing Viscosity of Formulation 4



Graph Showing Rheological behaviour of formulation F4

The viscosity of formulations were studied using Brookfield viscometer. The RPM vs Viscosity graph of formulations were plotted. From RPM 2 to RPM 10 and again from RPM 10 to RPM 2 readings were noted down and accordingly, graphs were plotted. From the graph we can conclude, that at RPM 2 the viscosity is high and at 4, 6, 8 and 10 it decreases. And from 10 to 2 RPM the viscosity goes from low viscous to high viscous.

The flow of cream was determined using graphs of shear rate vs shear stress, shear stress is the amount of force required to evenly spread the cream on to skin surface hence higher the shear stress higher is the viscosity and more will be the force required. Hence, the shear rate vs shear stress graphs of formulation indicated a non-linear relationship which confirms about Non-Newtonian system of formulation.

The flow of the cream was found to be pseudo plastic and Thixotropic behavior stated that apparent viscosity decreases with increase in shear stress, indicates that the cream is easily spreadable by small amount of shear confirming shear thinning system. From the above observations and result we can interpret that the F1 formulation required more shear rate and subsequently for F2 and F3 was observed. F4 formulation batch was selected for Liposomal Cream. The F4 batch was selected and again 3 Batches (F1, F2 and F3) of liposomal cream from the formulation itself was prepared and accelerated stability testing of formulation containing varying amount of liposomal essential oil was added to it. While simultaneously comparison of perfume stability of prepared liposomal cream was done with the conventional formulation cream.

Liposomal Cream and its Mechanism of action of preserving perfume:

The formulation F1, F2 and F3 were prepared from selecting the F4 batch of optimized cream. The liposomal essential oil was then added to it making the cream liposomal essential oil cream.

Here, the essential oil is encapsulated into liposomes which is responsible for the perfume of cream. Here, the perfume of rose oil is not directly added to cream whereas, they are encapsulated into liposomes and the suspension is further added to cream. So due to this when the cap of container is kept open even for a longer period the perfume is preserved. When cream is applied onto skin the liposomes having perfume inside it gets ruptured and perfume is released into atmosphere and even though the smell can be sensed by organoleptically too.

Fig showing Formulated Liposomal Cream

Table showing Stability Studies of Formulation

Batch No	Days	Oil in (ml)	Physical Evaluation	Refrigerated temperature	Room Temp	45 ^{0}C
F1	10	1	White Colored	Stable	Stable	Stable
	20		White colored	Stable	Stable	Stable
	30		White colored	Stable	Stable	Stable
F2	10	1.5	White Colored	Stable	Stable	Stable
	20		White colored	Stable	Stable	Stable
	30		White colored	Stable	Stable	Stable
F3	10	2.5	White Colored	Stable	Stable	Stable
	20		White colored	Stable	Stable	Stable
	30		White colored	Stable	Stable	Stable

Table showing comparison between marketed and liposomal cream

Batch No	Days	Oil in (ml)	Perfume stability of marketed cream containing rose oil	Perfume stability of rose essential oil liposomal cream
F1	10	1	Satisfactory	Better than marketed Formulation
	20		Satisfactory	Better than marketed Formulation
	30		Satisfactory	Better than marketed Formulation
F2	10	1.5	Satisfactory	Better than marketed Formulation
	20		Satisfactory	Better than marketed Formulation
	30		Satisfactory	Better than marketed Formulation
F3	10	2.5	Satisfactory	Better than marketed Formulation
	20		Satisfactory	Better than marketed Formulation
	30		Satisfactory	Better than marketed Formulation

Stability testing of liposomal cream

Accelerated Stability Testing of Liposomal Cream: It was checked by Accelerated stability study, the cream was subjected to different temperature at Room temperature, Refrigeration temperature and 45^{0}C for 10, 20 and 30 days respectively, and any physical changes in the cream at the end of interval period was observed observations were made, after 10 days each formulation was checked thoroughly for any kind of changes or phase inversion. These observations made was, all three formulation of F1 , F2 and F3 subjected to different temperature as mentioned above was as follows- F1 Formulation, was stable at these temperature and no phase inversion or any kind on changes were observed in the cream. F2 formulation, it was also stable at the mentioned temperature and no physical or any kind of changed were noticed in this formulation also. F3 formulation was also stable and no changes were observed in this too. Hence it can be concluded that the different temperature conditions have a no little effect on the prepared formulations. No physical changes in the cream were observed nor, any signs of phase inversion were there in the formulations.

Perfumery Stability Testing of Liposomal Cream: For enhancement of perfume the prepared cream was compared with marketed formulation of cream and perfume stability of both were observed during the process. Total 30 days of testing was done 10, 20 and 30 and during interval of 10 days, each batch was observed for the perfume stability. Formulation F1 was better than the conventional marketed formulation, F2 was also better in case with conventional one and F3 also shown same result. It was due to the liposomes which encapsulated the perfume of rose oil and due to the encapsulation of oil into liposomes it was able to preserve the perfume and give a long lasting effect with regard to the fragrance. The liposomes are basically vesicle which has oil entrapped into it. And this, liposomal essential oil as a whole, were added as a suspension to prepared cream formulation. When the cream was applied onto skin the liposomes get ruptured and the oil which was encapsulated get free from them and hence we got the long lasting action of perfume. The stability

of liposomal essential oil and perfume stability was both determined and no significant changes in the formulations were observed.

SUMMARY: Before beginning of the main formulation the preformulation of oil and excipients was done for oil it assessed at different parameters by visual appearance, RF, pH etc. Then FTIR study of soy lecithin, cholesterol, oil and mixture was done to ensure the compatibility which was compatible with the excipients and hence, the formulation can be successfully formed. The liposomes were formulated using the rotary vacuum evaporator method in this study. Soy lecithin and cholesterol were optimized by optimization. After optimization of them, incorporation of essential oil was done in liposome and on the basis of encapsulation efficiency of liposomes the selected batches were used for preparation of liposomal cream. Every batch was closely observed conclusion were made accordingly. The liposomal cream was prepared by addition of sufficient amount liposomal essential oil into cream and stability testing resulted into that the liposomes can be a potential carrier of essential oil for perfume enhancement thus preserving the natural and authentic perfumes of essential oil.

CONCLUSION: The present study had been a satisfactory attempt to formulate and evaluate liposome of essential oil of Rose (Rosa damasceana) and taking geraniol its components which is responsible for fragrance of rose oil. Pre formulation studies ensured the compatibility of our oil with the excipients. Formulation of liposomes were done by optimization of soy lecithin and cholesterol, then the optimized quantity was use for preparation of liposomal essential oil. Encapsulation efficiency ensured us that the liposomes were encapsulated at a satisfactory % into liposomes which later accounts for preparation of liposomal cream which was main formulation of carrier of liposomal essential oil for perfume enhancement. Stability studies reveal that the formulation was stable when it was exposed to different temperature conditions. Perfume stability was carried out by sensory smelling of perfume in liposomal cream which was compared with the marketed rose oil containing cream, and liposomal cream showed the better result than the conventional one. Which happened because the perfume was encapsulated into liposomes vesicle and when it was use for application on to skin the liposomes which was vesicles they ruptured when applied to skin perfume was released which gives a long lasting effect of perfume, pleasant aromatic feel was felt. Hence, study concluded that Essential oil can satisfactorily encapsulated into liposomes and it can be loaded as a suspension into cream.

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