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Formulation and Evaluation of Noisome By Using Tretinoin

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ABSTRACT
Acne is the most frequent multifactorial cutaneous condition in teenagers, with a frequency of 70–85 per cent. The majority of acne sufferers have mild to moderate acne at first, which can escalate to severe acne in certain circumstances. In minor acne, topical treatment is the early line of action, but in moderate and severe acne, systemic therapy is necessary in addition to topical therapy. Several topical treatments are already available that target at least one of the primary pathogenetic elements that cause acne to develop. Although topical therapy plays a significant part in acne treatment, the adverse issues connected with many topical antiacne agents and the unfavourable physicochemical features of several key agents like tretinoin and benzoyl peroxide limit their utility and patient compliance.
Keywords: Formulation of noisome using tretinoin and Evaluation and stability of noisome.

I. INTRODUCTION

NDDS - Novel drug delivery system

In recent decades, the development of novel NDDS has attracted a lot of interest. In theory, the NDDS should fulfil two requirements. The medication would initially be administered over the course of treatment at a pace determined by the body's needs. It must also direct the active entity to the area of impact. Conventional dose forms, even those with prolonged release, cannot meet any of these requirements. No drug delivery system now in use acts optimally, despite sincere efforts to do so by employing a range of cutting-edge drug delivery approaches**.**

NDDS Merits

- Minimises both the systemic and local side effects by reducing the total quantity of medication supplied over the course of pharmacological treatment.
- The toxicity to healthy tissues is decreased when the medication molecule is targeted towards the organ or tissue.
- The medicine is released by pulsatile and pH-dependent mechanisms as needed by the organism.
- Increased patient compliance as a result of the decrease in dosage frequency needed to sustain the appropriate therapeutic response.
- First-pass metabolism and gastrointestinal degradation are not present.

Restrictions of NDDS

- To manage when and how much medication is delivered to the pharmacological receptor.
- Targeted delivery of medication exclusively to affected cells or specific areas of the body.
- For instance, intracellular locations, bacteria, viruses, parasites, etcTo make sure that medicine stays ouinde the body until it reaches its target organ.
- Reduction in medication dosage and adverse effects.

Targeted delivery system of drug

Drug targeting is the process of precisely directing a therapeutic material to the desired location of action while minimising contact with unintended tissues. intended medicine distribution involves restricting access to neighbouring healthy cells in order to reduce the therapeutic index and achieve a therapeutic concentration of the active ingredient at the intended target location. By actively delivering the medicine to a select group of precisely chosen and extraordinarily exact compartments, site-specific targeted drug delivery raises the medication's effectiveness by reducing its concentration in healthy cells.Different scientific approaches that have been highly successful in targeting the medicine to intracellular places, viral cells, bacterial cells, and parasites. The advantages of targeted medication delivery are undoubtedly maximized by little parent drug distribution to non-target locations and increased and efficient concentration at the targeted site.

Ideal targeted medication delivery characteristics

- Developing a medication delivery system

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should be uncomplicated, replicable, and cost-effective.

- The carriers should have physicochemical stability and be non-toxic both in vivo and in vitro.
- A crucial component of the drug should be the capacity to regulate and forecast the rate of release.
- The carriers should naturally degrade and be easily eliminated from the body.
- The carriers utilized should be inert or devoid of any therapeutic effects.
- The release of the drug should not affect its distribution.

1.4 In the following circumstances, targeted medication delivery systems are preferred:

- The drug's stability is weak.
- Insoluble substances.
- Drugs that are poorly absorbed, have low therapeutic indices, and have low specificities.
- Medicines have short biological half-lives.
- Substances with extensive dispersion.

Types of targeting the drug

- **Passive Targeting:** In this type of targeting the particle system is captured by physiological mechanism such as filtration or macrophage (Reticulo endothelial system) sequestration. (i.e) drug targeting occurs because of the body's natural response to physicochemical characteristics of the drug or drug carrier system. It is concentration dependent, so external energy is not necessary.
- **Inverse Targeting**: To achieve inverse targeting, RES normal function is suppressed by preinjecting large amount of blank colloidal carriers or macromolecules like dextrin sulphate. This approach leads to saturation of RES and suppression of defense mechanism. This type of targeting is a effective approach to target drug(s) to non-RES organs.
- **Active targeting:** Using a surface modification method, active targeting is performed. The active ingredient is coated onto the carrier system, which can be glucose and galactose-containing carbohydrates or monoclonal antibodies. By adding alterations to its surface, the drug travels to a specific location rather than being naturally absorbed by RES. There are three types for active targeting.

a) 1st order targeting: A drug carrier system must be delivered to the target site's or organ's capillary bed. Brain ventricles, and the lymphatic system are a few examples.

b) 2nd order targeting: It entails giving medication to specific cells in living things, such kupffer cells or cancer cells**.**

c) 3rd order targeting: So as to transfer foreign DNA and genes to the nucleus, third order targeting is necessary. Targeting is based on a cell's internal structure. the more precise active targeting for liver's kupffer cells and parenchymal cells like hepatocytes.

Dual Targeting:

Due to the therapeutic activity of the carrier molecule utilised in this targeting technique, the drug's therapeutic effectiveness is increased. For instance, it is possible to load a medication onto a carrier molecule that is already active against viruses, and it has been demonstrated that this results in a net synergistic effect of the pharmacological combination.

Double Targeting

Double targeting refers to the use of both temporal and spatial techniques to target a carrier system. Drugs are targeted to certain organs by spatial placement.

Combination targeting

Combination targeting is used to deliver proteins and peptides to particular sites. Carrier and polymer are included in the targeting systems. This approach is better tailored to gene therapy.

Drug delivery carriers

Employing carriers allows for targeted medicine delivery. It is essential for the effective distribution of loaded medications to the intended sites that a special molecule or system known as a carrier be used. Using encapsulation or a spacer moiety, they are developed vectors that carry or distribute the medication to the target cell's location while holding the drug within or onto them.

Several carrier-based drug delivery methods include:

Vesicular system – carrier for drug delivery

In multiple fields, including genetic engineering, membrane biology, diagnostics, and immunology, lipid vesicles have become the preferred method for drug administration. These vesicles offer important benefits in terms of conveying and concentrating active ingredients, as

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well as simulating biological membranes. Vesicular medication distribution has a number of benefits:

- Like other targeted delivery techniques of drug, direct medication delivery to the location of infection decreases toxicity and lengthens the drug's stay in the systemic circulation.
- Vesicles operate as a prolonged release mechanism by delaying the clearance of quickly metabolizable pharmaceuticals. Compounds that are hydrophilic or lipophilic can both enter vesicles.

Several significant vesicular drug delivery methods include,

i) Liposomes

Liposomes are tiny vesicles with a membrane made completely of lipid molecules around an aqueous volume. They have lipid bilayer architectures. Liposomes are made mostly of two substances: phospholipid and cholesterol Sphingolipids, phosphoglycerides, and their hydrolytic byproducts are examples of the phospholipid class of substances.

ii) Sphinosomes

Numerous stability problems affect liposomes. Among other chemical reactions, liposomal phospholipids can be hydrolyzed and oxidized to deteriorate. Sphingolipid is utilised to make stable liposomes called sphingosomes.One definition of sphingosomes states that they are "concentric, bilayered vesicles in which an aqueous volume is totally surrounded by a membranous lipid bilayer composed primarily of natural or synthetic sphingolipid."

iii) Transferosomes

Many low and high molecular weight medications can now be administered trans dermally with the help of transferosomes.The lipid droplet known as a transferosome serves as a transfer agent and is extremely flexible, allowing it to easily pass through openings that are considerably smaller than the droplet size. They prevent metabolic breakdown of the medication that is encapsulated. As a result, the quasi-metastable state frequently aggregates, which encourages the formation of extremely curved bilayers.

iv) Niosomes

Niosomes are small lamellar structures formed by combining non-ionic surfactants and cholesterol in an aqueous environment. They resemble liposomes and have been studied as a drug delivery method. Niosomes show potential in releasing pharmaceutical substances and effectively transporting them to targeted tissues. They offer a promising approach for addressing various diseases.

v) Pharmacosomes

According to Goymann and Hamann (1991), the word "pharmacosome" refers to two primary components: Pharmacon (active principle) and some carriers. According to Vaizogle and Speiser's (1986) theory, pharmacosomes are self-assembling vesicles made of amphipathic drugs. A colloidal dispersion of a drug that has been covalently linked to lipid may contain pharmacosomes, which are hexagonal aggregates or ultrafine micelles.

Outstanding topographies of niosomes

- Niosomal surfactants are immune-suppressive, biodegradable, and biocompatible.
- Drugs contained within niosomes have improved stability.
- Niosomes provide an effective substitute for liposomes, resolving any potential drawbacks of liposomal distribution.
- Niosomes enable topical, parenteral, or oral administration to the targeted location of action.
- Niosomal surfactants don't need to be used under some circumstances.
- Niosomes show osmotic action and show stability.
- By extending the period that medication molecules are in circulation, niosomes help improve therapeutic efficacy.
- The adaptability of niosomes, including their composition, fluidity, and size, enables customization to meet unique requirements in a variety of settings.

Structure of niosomes

Niosomes mostly consist of cholesterol and nonionic surfactants. Cholesterol aids in the stiffening and correct morphology of niosomes. Surfactants have a substantial impact on niosome formation. Non-ionic surfactants such as brijs (brij 30,35,52,58,72,76), spans (span60,40,20,85,80) and tweens (tween 20,40,60,80)are frequently used in

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the production of noisome. The hydrophilic head and hydrophobic tail of the non-ionic surfactants is opposites.

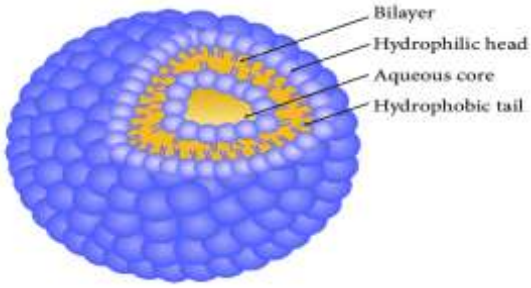

Figure .1: Interior structure of noisome

II. METHOD AND MATERIAL

Table no 1. Method Of Material

Utilization of the Material	**Origin**
Tretinoin	Bafnapharmaceuticals,Mumbai
Sodium chloride	S.D. Fine Chem Ltd, Boisar
Potassium di hydrogen phosphate	S.D. Fine Chem Ltd, Boisar
Polysorbate 20 (tween 20)	S.D. Fine Chem Ltd, Boisar
Triton X – 100	S.D. Fine Chem Ltd, Boisar
Ethenol	QualigensChem Ltd, Boisar
Disodium hydrogen phosphate	QualigensChem Ltd, Boisar
Sorbitan mono laurate (Span 60)	QualigensChem Ltd, Boisar
Dicetyl phosphate	QualigensChem Ltd, Boisar
Chloroform	QualigensChem Ltd, Boisar

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Cholesterol	QualigensChem Ltd, Boisar

Description of the drug:
The drug's organoleptic properties were evaluated and documented utilizing illustrative terminology **[49].**

Melting point:
A volumetric flask with one end closed is filled with dry powder to make a 0.25cm to 0.35cm column at the end of the opening, which is compressed as firmly as possible by moderate pounding on a solid surface. The equipment is set up and operated according to industry standards. The block is heated to around 30 degrees Celsius below the estimated melting point. The capillary is placed on the hot plate, and the temperature is gradually increased at a rate of 1 to 2 degrees Celsius per minute until the melting is complete.The temperature at which the sensor signal departs its starting value is defined as the commencement of melt, and the temperature at which the sensor signal achieves its ultimate value is characterised as the melting point. These two temperatures are both within the melting range **[50].**

Solubility Studies
The solubility of the drug sample was evaluated and studied in the number of solvents for example, distilled water, Chloroform,Ethanol, (Isopropyl alcohol), and Octaneformamide.

PREFORMULATION STUDIES:
Partition coefficient studies
In a separating funnel, a fixed amount of tretinoin was combined with 20 ml of ethanol and phosphate buffer (pH 7.4), respectively. After that, the mixture was periodically shaken while being allowed to attain equilibrium for two hours at 37°C. The mixture of the drug in both the aqueous and organic phases was then ascertained using UV spectroscopy at a wavelength of 340 nm after the aqueous and organic phases had been properly diluted.

The ratio of drug concentration in each phase was evaluated using the subsequent equation to estimate the apparent partition coefficient:

Kp = C organic / C aqueous

Determination of Absorbance maximum (λ_{max}).
Tretinoin was effectively dissolved in phosphate buffer saline at a pH of 7.4. To create a solution with a mixture of 20 µg/ml, appropriate dilution techniques were employed.

A UV spectrophotometer was employed to examine the Tretinoin medication in solution by measuring its absorption within the wavelength range of 200 to 400 nm. A blank solution consisting of phosphate-buffered saline with a pH of 7.4 was used. The highest absorption peak was observed at 340 nm. Subsequently, the drug dosage was determined by calculating the absorbance specifically at 340 nm in phosphate-buffered saline with a pH of 7.4.

Standard curve for Tretinoin (by UV method)
A 100 mg dose of tretinoin was dissolved in a 100 ml volumetric flask containing pH 7.4 phosphate buffer saline. After agitation, the volume was adjusted to the mark using pH 7.4 phosphate buffer saline, resulting in a solution concentration of 1000 µg/ml.
To create the stock solution, 2 ml of the primary stock solution was transferred to a 100 ml volumetric flask. The volume was then adjusted with pH 7.4 phosphate buffer saline, yielding a stock solution concentration of 20 µg/ml.
The standard Tretinoin secondary stock solution was divided into 1 to 10 ml portions and transferred to 10 ml volumetric flasks. These volumes were adjusted using pH 7.4 phosphate buffer saline to achieve concentrations of 2, 4, 6, 8, 10, 12, 14, 16, and 20 g/ml. The absorbance of each solution was measured at 340 nm, with pH 7.4 phosphate buffer saline as the blank. A concentration-absorbance graph was plotted to illustrate the relationship.

FTIR study of the compounds
To study the presence and absence of the functional group, FTIR spectrum analysis was carried out.

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Optimization process for niosome preparation

Niosomes containing retinol were produced by means of the thin film hydration method. Lipid and surfactant were dissolved in a 2:1 volume ratio solution of methanol and chloroform in a volumetric flask of 100 ml. The medicine and the precisely weighed diacetyl phosphate were then added to the solvent combination. The liquid phase of the solvent combination was eliminated with a rotary evaporator at 150 rpm and 60 °C, leaving a thin film on the flask wall. The use of a Hoover guaranteed that all solvents were completely removed. The dried lipid film was then hydrated at 60°C with 5 ml of pH 7.4 phosphate buffer saline. All batches underwent a 2-minute sonication process using a probe sonicator. Table 2 lists the formulation ratios of the surfactant and cholesterol used.

Table no .2 .Formulation of niosome.

Formulation code (F)	Tretinoin (mg)	Surfactant	Surfactant: Cholesterol (µM)
1	10	Span 60	100:100
2	10	Span 60	200:100
3	10	Span 60	300:100
4	10	Span 60	100:200
5	10	Tween 20	100:100
6	10	Tween 20	200:100
7	10	Tween 20	300:100
8	10	Tween 20	100:200
9	10	Tween 20	100:300

Time for hydration: 2 hours
Solvent: A combination of methanol and chloroform in a volumetric ratio of 2:1
Concentration of diacetyl phosphate: 15µM
Hydration medium: Buffer of Phosphate saline consisting of 5 ml with a pH of 7.4

Characterization of the noisome:

Using scanning electron microscopy and a drug entrapment percentage calculator, the niosomes were characterized.

Scanning Electron Microscopy:

In the process of SEM analysis, a small amount of niosome suspension was applied onto a carbon-coated copper grid to create a thin liquid layer. Subsequently, the liquid film was negatively stained for a duration of one minute using a 2 percent solution of phosphotungstic acid. The excess phosphotungstic acid solution was removed by filtration using filter paper. The stained sample was then characterized using an accelerating voltage of 80 Kv. Percent Entrapment:

Percent Entrapment:

In order to calculate the percentage of entrapment, researchers followed a procedure that involved transferring 5 mL of niosome into a 100 mL volumetric flask containing 25 mL of mobile phase. Subsequently, they performed a short-duration sonication in an ultrasonic bath and filtered the mixture through a 0.45 m membrane filter. Utilising the mobile phase, the filtrate was diluted. The Shimadzu 1800 UV-VIS spectrophotometer provided the correct absorbance at 340 nm. Additionally, the amount of tretinoin was calculated. The algorithm below was used to calculate the proportion of medication that was entrapped. %EE = amount of drug added in niosome/total drug added.

vitro release study for niosomal preparation

The niosomal formulation was delivered by suspending a 5 cm dialysis membrane in a

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beaker with 100 ml of pH 7.4 phosphate-buffered saline as the diffusion medium. The medium was maintained at 37 ± 0.5 °C with constant stirring. To maintain a constant volume, 1 ml of sample was replaced with fresh buffer every hour. Withdrawn samples (up to 10 ml) were prepared using pH 7.4 phosphate buffer saline and measured at 267 nm using spectrophotometry.

Stability study of Tretinoin niosomes

The formulations were subjected to storage for three months at three distinct temperatures: 4 °C, ambient temperature, and 45 °C with a relative humidity of 75%. Each formulation was placed in a 20 ml glass container and tightly sealed. Samples from each batch were evaluated after a one-month break to assess the efficiency of drug trapping and release in an in vitro environment.

Evaluation of the tretinoin niosomes

pH study

The formulation process involved adding a precisely weighed quantity (1 g) of gel to 100 mL of filtered water. The pH of the resulting mixture was measured using a calibrated digital pH meter and standard buffer solutions at pH levels of 4.0, 7.0, and 9.0. The pH of the final product underwent three separate tests, and the average readings were recorded.

Spreadability analysis

To evaluate the spreadability of a Tretinoin niosomes gel, a 0.5 g sample of the formulation was applied on a 20X20 cm surface area. Another glass plate was positioned on top, and a weight of 0.5 kg was placed on the upper glass slide for a duration of 5 minutes. This process resulted in the expansion of the premarked zone, leading to a gel spread with a diameter of 10 mm. The spreadability of the gel was assessed three times to ensure consistent results.

Viscosity Measurement

The Brookfield Engineering Laboratories, Inc., located in Middleboro, Massachusetts, USA, utilized their R/S CPS Plus Rheometer and RHEO3000 software to calculate the viscosity of the gel formulation in its original, undiluted state.

Grittiness & Homogeneity

To determine the uniformity of the product's composition, a small amount of unpleasant-smelling gel was applied either between the thumb and forefinger or rubbed onto the skin on the back of the hand. The presence of any rough particles will become apparent.

III. RESULTS AND DISCUSSION:

Drug Tretinoin Properties

The drug's appearance, colour, odour, melting point, and solubilities were all examined. While the table below includes information on solubility studies.

a. Appearance: Powder
b. Colour: Yellowish
c. Odor: Floral
d. Melting point: 180℃
e. Solubility:

Table 3: Studies on the solubilities of the drug.

S. no.	Solvent	Inference
1.	DMSO	Soluble
2	Ethanol	Soluble
3.	Isopropyl alcohol	Easily soluble
4.	Octane	Soluble
5.	Water	Insoluble
6.	Chloroform	Soluble

Partition coefficient:

The partition coefficient, which reflects a drug's capacity to permeate the cell membrane, is a measure of lipophilicity. It is described as the proportion of un-ionized medicine spread between the organic and aqueous layers at equilibrium.

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Table 4: Partition coefficient determination

Concentration	Drug amount in aqueous phase	Drug amount in oil phase	Partition coefficient
Octanol : Water	1.8	3.2	1.7
Octanol : PBS	0.9	2.0	2.8

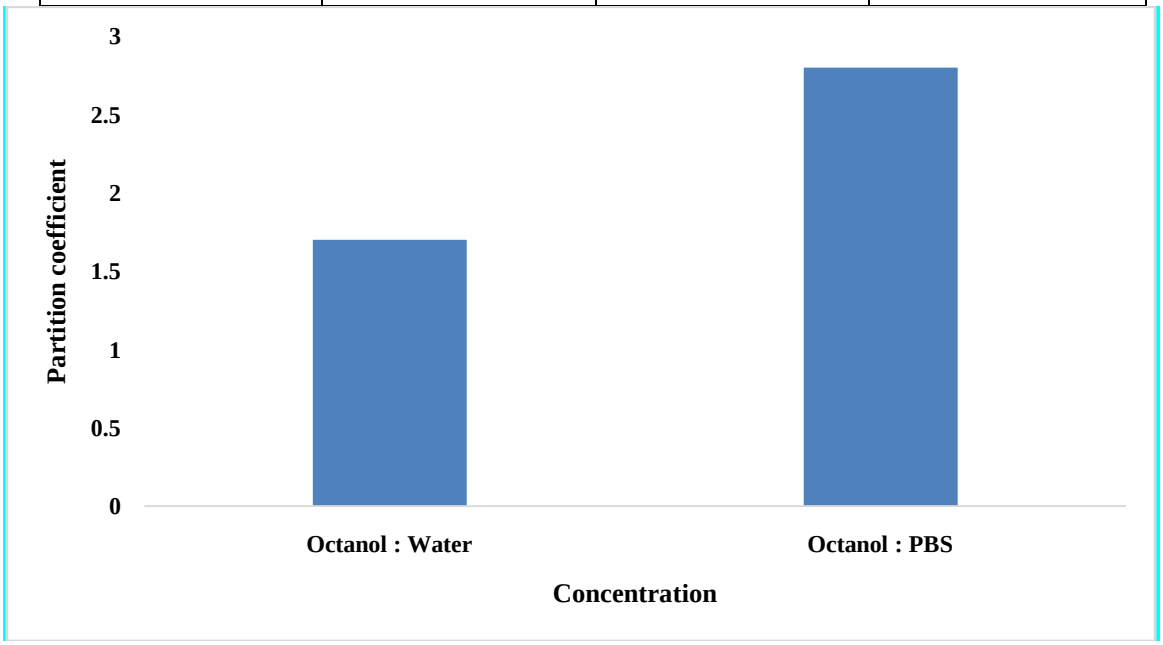

Figure 2. Determination of the partition coefficient

Standard curve analysis of Tretinoin

The analysis of the standard curve of Tretinoin was carried out by UV method the results were explained in the following table and graph.

Table 5: Standard curve data for Tretinoin

Concentration(µg/ml)	Absorbance at 349 nm
2	0.124
4	0.195
6	0.290
8	0.385
10	0.483
12	0.576
14	0.671
16	0.769
18	0.864
20	0.939

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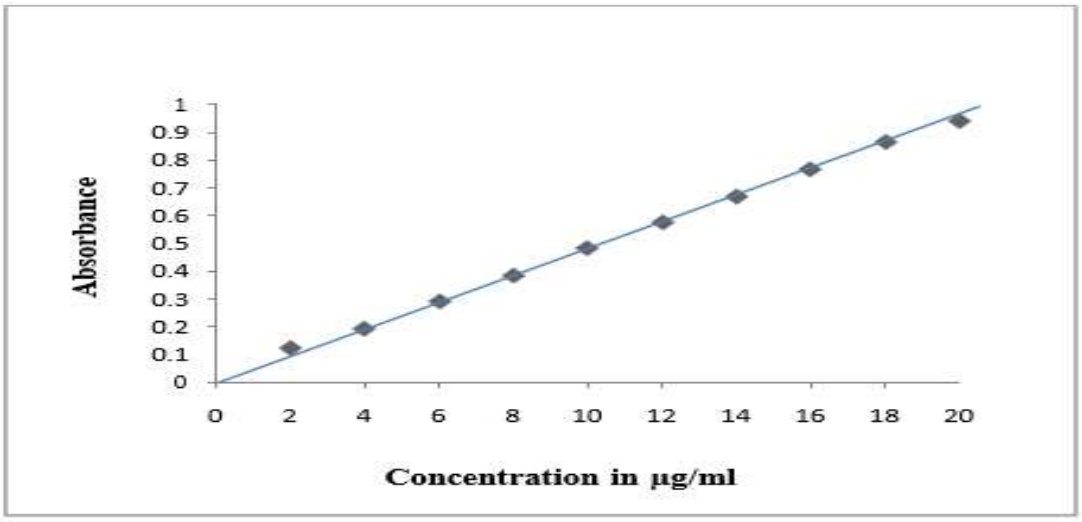

Figure3 : Standard curve of Tretinoin

FTIR ANALYSIS:
DRUG:

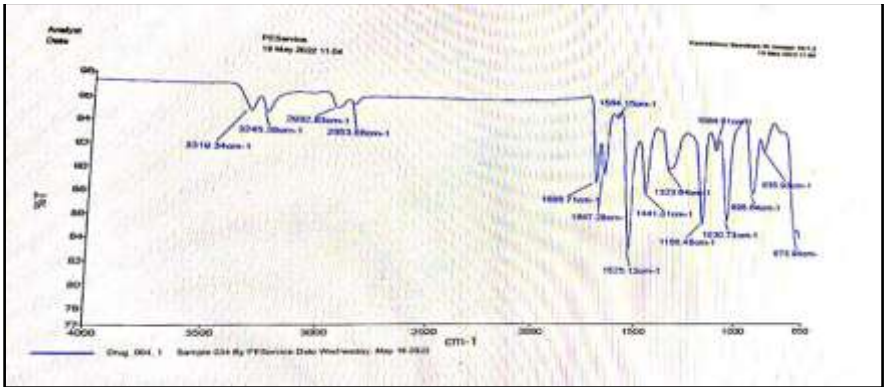

Fig no 4. 1 drug.

SPAN 60

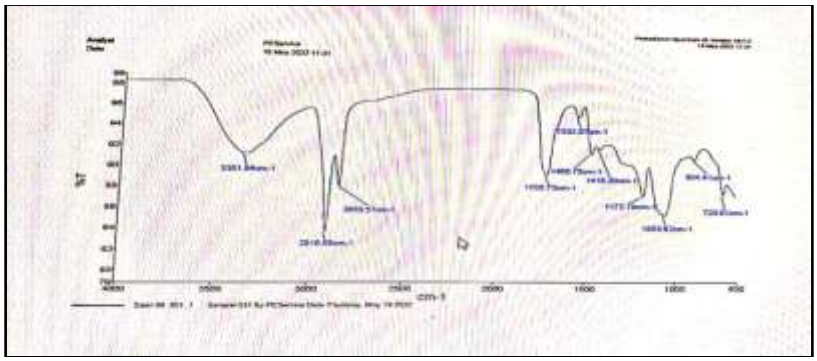

Fig no5 .Span 60

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CHOLESTROL

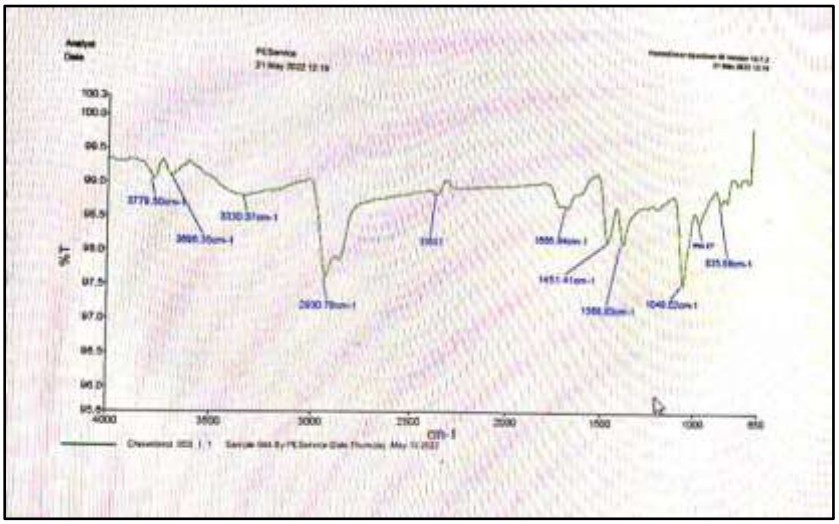

Fig no.6.Cholesterol

DRUG + SPAN 60

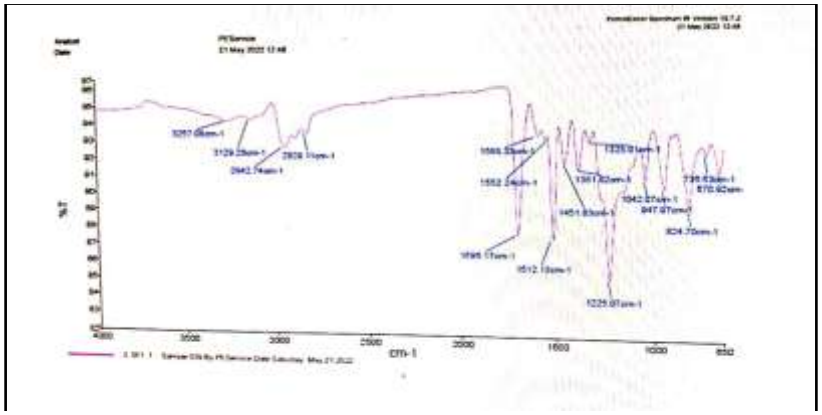

Fig no7.Drug + span 60

SPAN 60+ CHOLESTROL

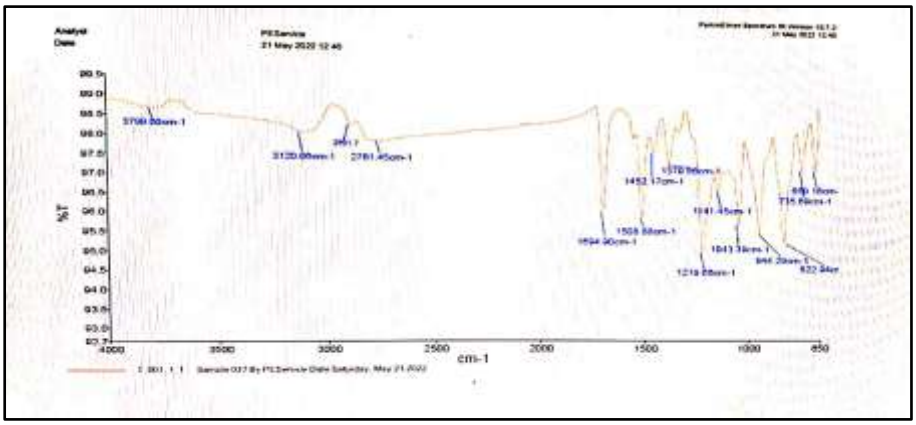

Fig no .8. Span 60+ cholestrol

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DRUG + SPAN 60+ CHOLESTROL

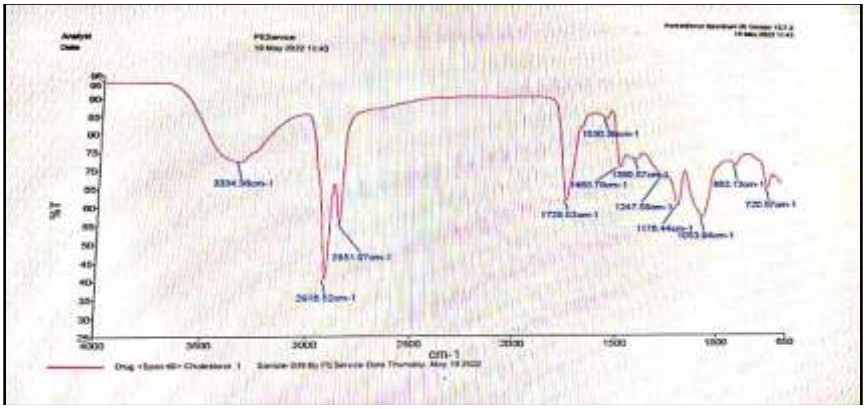

Fig no.9.Drug + span 60+ cholestrol

The FTIR spectrometer employed the pressed pellet technique to handle the material. This approach included the addition of an appropriate sample quantity and its thorough integration with potassium bromide. Subsequently, the blend was compressed using specialized discs under significant pressure to form a transparent pellet. This pellet was subsequently inserted into a designated container within the IR spectrometer.
Peaks were detected in both spectra after the pellets were scanned in an FTIR spectrophotometer from 4000 to 400 cm-1. Peaks showing functional groups and the wave number at which they first emerged are shown in the table.

The IR spectra of the medication, surfactant, and cholesterol in their physical mixtures as well as the drug itself were measured. Contrasting pharmacological spectrums and physical combinations were used.Both the physical mixture and the pure medication exhibit the same bands. As a result, there was little to no interaction between the excipients and the medication.

Tretinoinniosomes Development

This study involved the creation of retinol-loaded niosomes utilizing non-ionic surfactants and cholesterol such as Tween 20 and Span 60. The solvent employed was a combination of methanol and chloroform (2:1 v/v).Thin film was created once the formulation's solvent evaporated. By using buffered phosphate saline pH 7.4, the thin film was hydrated and eliminated. By ultrasonically processing the formulation in the Probe sonicator, the size of the vesicles was decreased.

Different surfactant and cholesterol ratios were prepared in the formulations.vesicle size estimation, Niosome morphology and release of drug profile stood among the physicochemical properties studied. Additionally, the optimised formulation's temperature stability was assessed.The formulation additionally contains the charge-inducing chemical dicetyl phosphate (DCP). Charge-inducing agent (DCP) insertion hindered vesicle fusion and aggregation. Dicestyl phosphate also keeps things consistent and in order.An effective niosomal drug delivery system must have high drug loading and durable encapsulation. It should also have excellent physical and chemical stability while stored.

Process related variables Optimization

A number of variables, including rotational speed, hydration volume, hydration medium, and vacuum, had an impact on the prepared niosomal vesicles. These elements ought to be optimised using an empty vesicle before adding the medication.

350 mmHg of vacuum was utilized to dry the thin film. When niosomes were hydrated, vacuum pressures below 350 mmHg were insufficient to completely remove the solvent from the formulation. A vacuum above 350 mmHg caused the solvent to evaporate quickly, trapping air bubbles on the film's surface. This led to inadequate drug entrapment in niosomes, and the lipid film generated by the 350-mmHg vacuum had noticeable drug entrapment. Therefore, the ideal range of hoover was thought to be 350 mmHg.The hydration of the lipid film took place over a period of 60 to 120 minutes. Niosomes were generated with the specified size range and spherical shape after 120 minutes of hydration. Therefore, the ideal hydration parameters were determined to be 120 minutes and 5 ml.

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The speed at which the round bottom flask rotated had an impact on the thickness and homogeneity of the thin layer. 150 rpm was chosen as the ideal speed. At this rate of rotation, a uniform thin layer was created. A rotating evaporator's optimal bath temperature was maintained at 60°C.

Process-related variables Optimization:

Table 6: Tabular representation of the related variables.

Hydration Time (min)	Hydration volume	Surfactant: Cholesterol	Speed of Rotation (rpm)	Chloroform: methanol	Vesicle Size (µM)
30	5 ml	100:100	75	2:1	10.29±1.48
60	5 ml	100:100	100	2:1	9.41±1.09
120	5 ml	100:100	125	2:1	9.11±1.88
120	5 ml	100:100	150	2:1	8.69±1.88

Table 7: Percentage entrapment, hydration time and volume of medium

Percentage entrapment (%)	Hydration time (min)	Volume of hydration medium (ml)
60.74 ± 0.98	60	3
68.84 ± 0.76	60	4
73.38 ± 0.58	60	4
89.45 ± 0.88	120	5

Scanning electron microscopy

SEM was used to examine the surface properties of the Tretinoin niosomal formulation. SEM image of the niosome formulation demonstrates the presence of a surfactant and cholesterol coating on the medication particles. Due to the handling and processing involved, some of the particles in the images appear to be fragmented.. The majority of the vesicles have sharp, distinct edges and spherical shapes. Niosome vesicles look smooth in scanning electron micrographs, indicating a thin and even coating over the medication. There is no discernible change in particle size at the microscope scale. The observation demonstrates unequivocally that the surfactant layer prevents any particle agglomeration.

Figure10 : SEM image

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Percent drug entrapment:

Table no.8. Percent drug entrapment

S. No.	F. code	Percentage entrapment Efficiency (%)
1	F_1	63
2	F_2	74
3	F_3	68
4	F_4	62
5	F_5	72
6	F_6	84
7	F_7	88
8	F_8	79
9	F_9	45

The effectiveness of the niosomes' trapping depends on the formulation's ability to maintain the drug molecule in the vesicles' bilayer membrane or aqueous core. The entrapment of each formulation was assessed after the substance that wasn't trapped was taken out. Different surfactant and cholesterol ratios altered the effectiveness of entrapment. The presence of cholesterol, the ratio of medicine to lipids, and the concentration of lipids all affect how successful trapping is.

It was discovered that Formulation F1 had an entrapment efficacy of 63%. Entrapment efficiency was raised to 74% in formulation F2 by adding more surfactants. Increasing the surfactant level in F3 resulted in a 68% improvement in entrapment efficiency, attributed to the absence of cholesterol.To increase entrapment effectiveness, the content of cholesterol in formulations F4 and F5 was increased to 200 M.

Because increasing cholesterol levels will increase the effectiveness of trapping. Drug entrapment did not significantly improve. This is because Span 60, a surfactant, was utilised in those formulations. Due to the greater hydrophobicity of Span 60, the hydrophilic medication is solely contained in the watery core.The increase in entrapment efficiency is assumed to be caused by cholesterol and surfactant's capacity to seal the bilayer membrane's leaky region.This allows for increased drug levels in niosomes. The improved entrapment efficiency was attained in tween 20 compared to Span 60. The hydrophilic drug tretinoin divides into vesicle membranes and becomes encapsulated in tweens because they are more water soluble than adults.

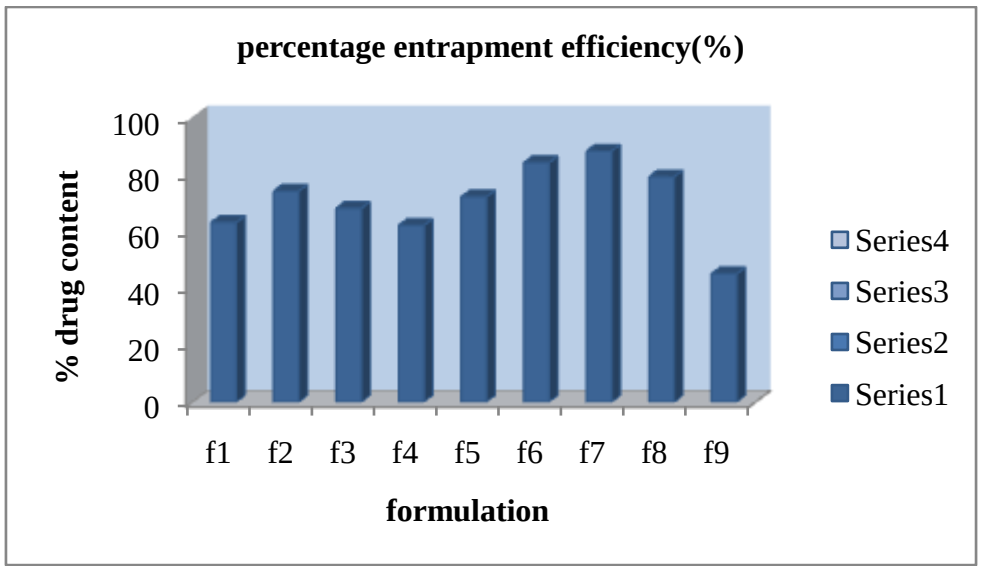

Fig. 11 : Entrapment efficiency of drugPercentage

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Analysis of in vitro release

Using a technique called membrane diffusion, the release of tretinoin from niosomes was assessed. The 24-hour release study's results are displayed in the following graphs. Phosphate buffer was used as the diffusion medium in the in vitro drug release experiment, and a biphasic pattern was seen. This suggests that the dialysis membrane and the lipid bilayer both had an impact on the release. Cholesterol was added, and this had an effect on how quickly the medication was released from the capsules. Formulations comprising two various surfactants were contrasted in terms of their in vitro drug release characteristics. Retinoinniosomes were used to study two different amounts of cholesterol and surfactants.It was determined that 61.58%, 72.69%, and 67.64% of the medication was released from formulations F1, F2, and F3 in 16 hours, 17 hours, and 16 hours, respectively. The release was not sustained up to 24 hours due to the low cholesterol level in such formulations.

The formulas F4, F5, F6, and F7 now include 200 M cholesterol instead of 100 M. The respective 24-hour, 72-hour, 24-hour, and 24-hour release rates for F8, F9 were 59.57%, 71.65%, 81.97%, and 70.63%.Even though longer release was achieved, the formulations' drug release percentage fell short of expectations. It was found that the release was 81.83% higher. This is due to Span 60's inability to dissolve in water.

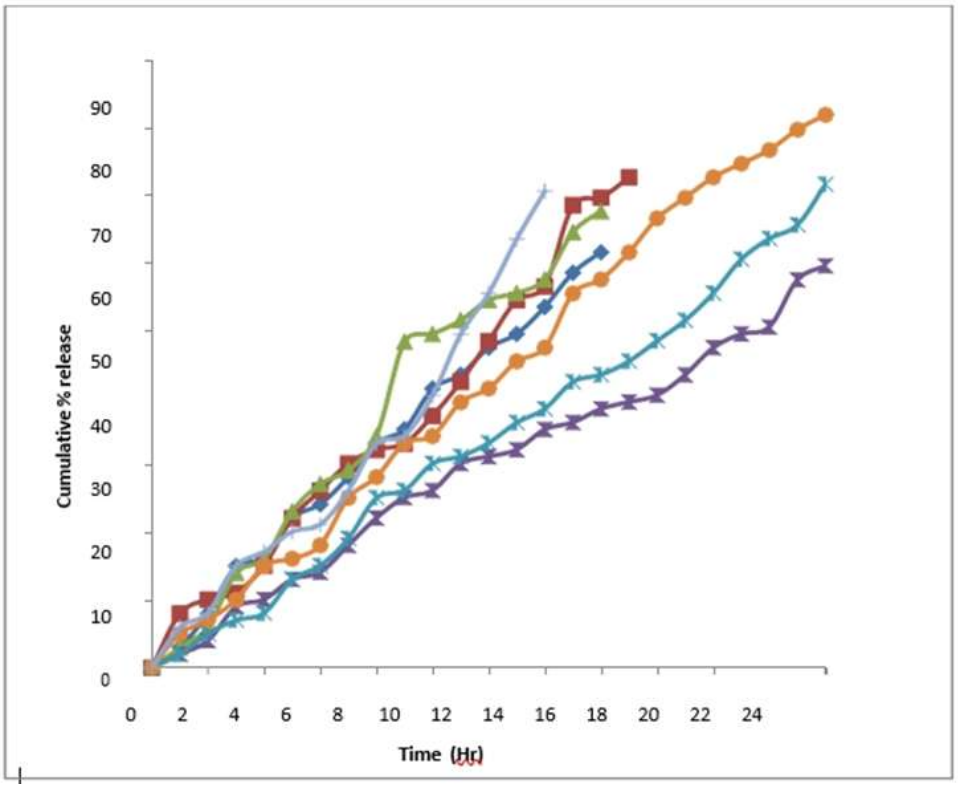

Fig 12: Relative in vitro release study of formulations of Tretinoin niosomal

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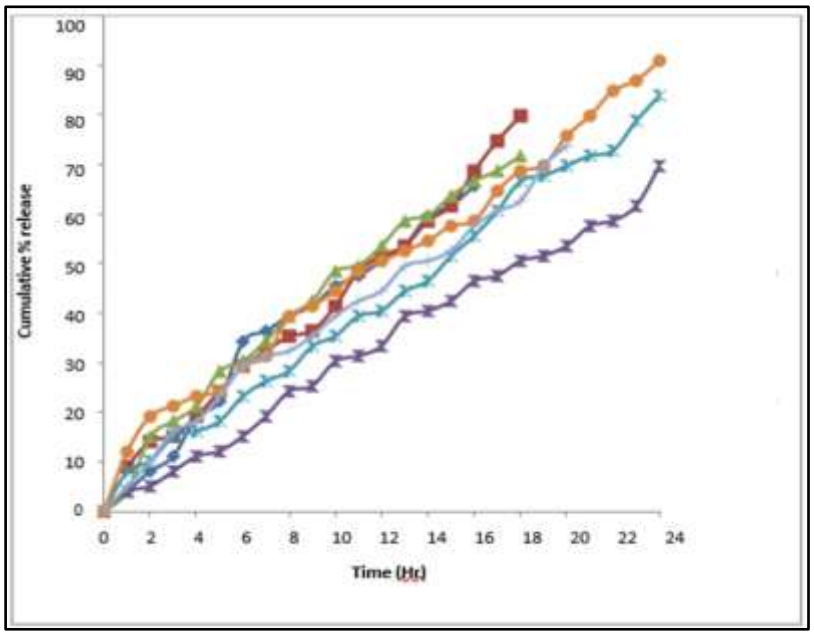

Fig 13: Comparative in vitro release study of formulations of Tretinoin niosomal.

Stability studies

Three months were spent studying the stability of the improved Tretinoin niosomal formulation (F13) at 450C/75%RH and 40C, room temperature. Niosomes were assessed for in vitro release and trapping effectiveness after a one-month delay. In contrast to room temperature, niosomal formulations show improved stability when stored at 4°C (in a refrigerator) and at 45°C/75%RH.

Table.9: Stability studies

Temperature	**Rate of entrapment within a one-month period (%).**			**Rate of entrapment within a two-month period (%).**			**Rate of entrapment within a three-month period (%).**		
	F3	F6	F9	F3	F6	F9	F3	F6	F9
4°C	90	91.5	92.8	89	89.8	90.5	85	86	87
25°C	87	88.9	89.5	88.6	87.8	88.4	80	82.1	81
45°C	84	84.5	83.9	81	82.4	82.9	74	76.8	78.4

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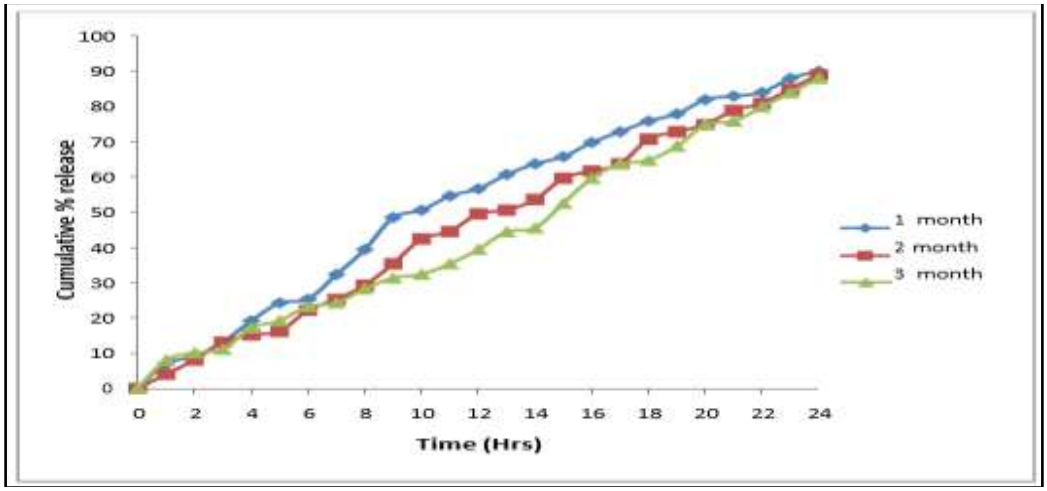

Fig14:Release of formulation stability study findings after three months at 4°C

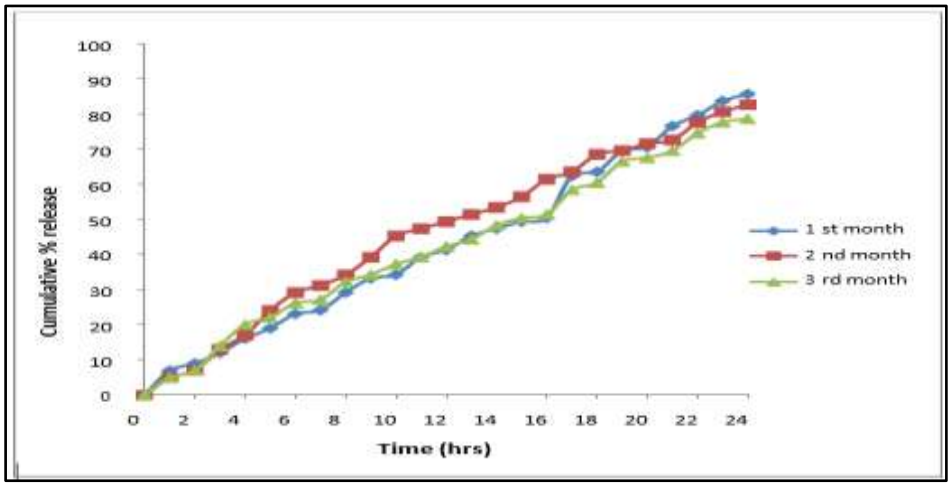

Figure15 :Formulations stability study at 25

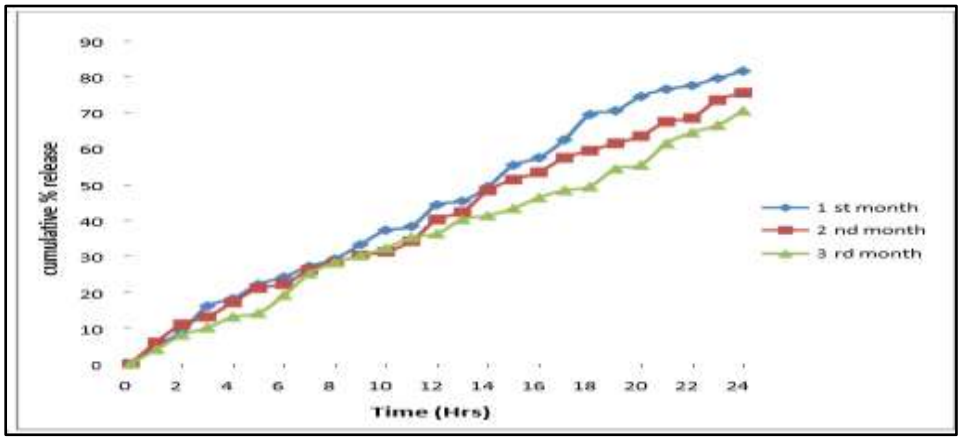

Fig 16: Data from formulations stability studies that were released after three

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IV. CONCLUSION:

In this research, a niosomal delivery of drugs system was created employing a non-ionic surfactant and a thin film hydration approach to incorporate Tretinoin.Theniosomal vesicles that had been created were quite robust. SEM, entrapment effectiveness, in vitro release, and Zeta potential experiments were performed on the formulations. Based on the findings of an experimental investigation, we came to the conclusion that formulations F1 containing drugs had larger percentage entrapment with Tretinoin's predicted prolonged release when the ratio of surfactant to cholesterol was 300:200 mol. As a result, Formulation F1 was selected as the best. In vitro drug release study from an improved Tretinoin niosomal formulation (F1) revealed a 24-h release. The vesicles are generally spherical and consistent in size, according to the SEM image. There really is no agglomeration between the particles, as shown by a scanning electron micrograph. In zeta potential analysis, a negative zeta prospective value was discovered. The existence of a negatively charged generating ingredient in the formula was established. The sterility of the composition was tested according to the I.P. criteria. The sterility test is passed by the adjusted formulation. A three-month durability study was conducted under a variety of storage settings. The formulation remained stable at 4°C, according to the data. The linearity of the graph of Time vs. accumulated percentage medication release demonstrated that the improved formulation followed a zero-order distribution pattern. We discovered that the niosome promoted the drug's release through a diffusion-regulated mechanism with zero-order kinetics based on our analysis of the drug release kinetics.

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CONFLICT OF INTEREST

The authors confirm that they have no known financial or personal conflicts of interest that would affect the research presented in this study.

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