INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Formulation And Evaluation Of Benzocaine Transferosomes Containing Gel For Local Anesthetics Activity

Rutuja Patil[1*], Pranjal Chougule[1], Dr. Nilesh Chougule[2]

[1]Student of B. Pharm, Ashokrao Mane Institute of Pharmacy, Ambap, Dist. Kolhapur, Maharashtra India, 416112
[2]Assitant Professor, Ashokrao Mane Institute of Pharmacy, Ambap, Dist. Kolhapur, Maharashtra India, 416112
[3]Principal, Ashokrao Mane Institute of Pharmacy, Ambap, Dist. Kolhapur, Maharashtra India, 416112.

ARTICLE INFO

Received: 11 July 2024
Accepted: 28 July 2024
Published: 30 July 2024

Keywords:
Transferosomes, local Anesthetics, transferosomal gel

DOI:
10.5281/zenodo.13131925

ABSTRACT

This study presents the development and evaluation of benzocaine-loaded transferosomes incorporated into a gel matrix for enhanced local aneasthetic activity. Transferosomes, lipid-based vesicles known for their deformability and ability to encapsulate drugs, were prepared using the thin-film hydration method. Different concentrations of phospholipids were explored to optimize transferosome formulation. These transferosomes were then integrated into a gel base to create a stable topical formulation suitable for skin application. Physicochemical characterization revealed uniform particle size distribution and the deformable nature of the transferosomes, facilitating enhanced skin penetration. In vitro release studies demonstrated sustained release of benzocaine from the transferosomal gel formulation compared to conventional gels, promising prolonged analgesic effects. Permeation studies using excised rat skin demonstrated significantly enhanced benzocaine permeation with the transferosomal gel compared to conventional gels, attributed to the transferosomes' deformable nature. In vivo evaluation in rats corroborated these findings, showing prolonged analgesic effects with the transferosomal gel, indicating its potential for improved local anesthesia. This study underscores the promise of benzocaine-loaded transferosomal gels as a novel approach for enhancing local anesthetic activity, with implications for improving pain management in medical and surgical procedures. Further research is warranted to optimize formulation parameters and evaluate clinical efficacy and safety for broader applications.

INTRODUCTION

NOVEL DRUG DELIVERY SYSTEM

Novel Drug delivery System (NDDS) refers to the approaches, formulations, technologies, and

***Corresponding Author:** Rutuja Patil
Address: *Ashokrao Mane Institute of Pharmacy, Ambap, Dist. Kolhapur, Maharashtra India, 416112*
Email ✉: ruturajpatil0803@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. It may involve scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics; in any case, it is typically concerned with both quantity and duration of drug presence".

• The conventional dosage forms provide drug release immediately and it causes fluctuation of drug level in blood depending upon dosage form.

•NDDS is advanced drug delivery system which improves drug potency, control drug release to give a sustained therapeutic effect, provide greater safety, finally it is to target a drug specifically to a desired tissue.

•NDDS is a combination of advance technique and new dosage forms which are far better than conventional dosage forms

Advantages of NDDS

- Decreased dosing frequency.
- Reduced rate of rise of drug concentration in blood.
- Sustained and consistent blood level within the therapeutic window.
- Enhanced bioavailability.
- To achieve a targeted drug release
- Reduced side effects
- Improved patient compliance.

IMPORTANCE OF NOVEL DRUG DELIVERY SYSTEM

- Enhanced drug efficacy: Novel drug delivery systems can improve the therapeutic effect of drugs by delivering them to the target site in a controlled and sustained manner.
- Reduced side effects: By targeting drug delivery, these systems can reduce the exposure of healthy tissues to the drug, minimizing side effects.
- Improved patient compliance: Systems that reduce dosing frequency or simplify drug administration can improve patient adherence to treatment regimens.
- Enhanced drug stability: Some delivery systems can protect drugs from degradation, improving their stability and shelf life.

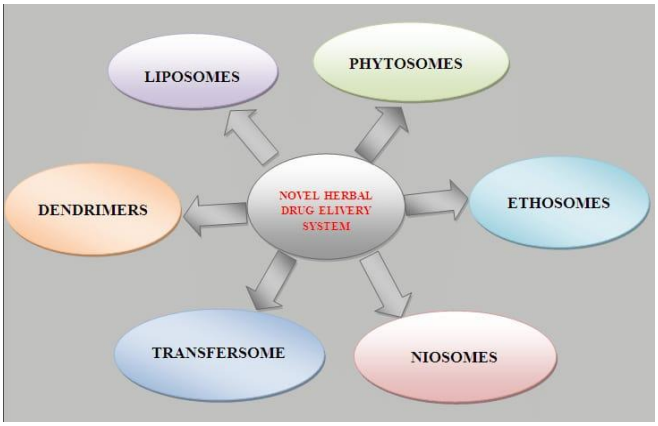


Figure 1: Classification of NDDS

TRANSFEROSOMES: Transferosomes are advanced drug delivery systems that aim to transport therapeutic agents across biological membranes, such as the skin. Here's a comprehensive overview of transferosomes:

Structure:

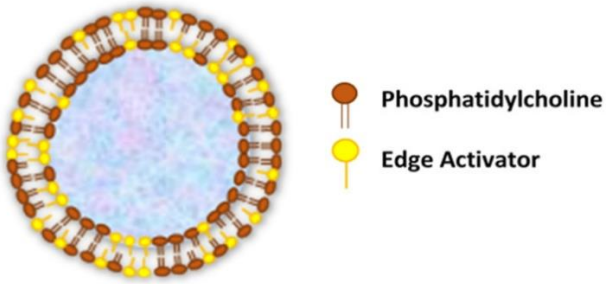


Figure 2: Structure of transferosomes

Transferosomes are ultra-deformable lipid vesicles, consisting mainly of phospholipids, surfactants, and water. They are characterized by their flexibility and ability to penetrate deep into the skin layers or even reach systemic circulation.

Components

1. Phospholipids: These form the lipid bilayer, similar to biological membranes, enhancing biocompatibility.

2. Surfactants: These provide the necessary flexibility to the vesicle, allowing it to squeeze through narrow pores without rupturing.

3. Water: Serves as the medium in which the lipids and surfactants are dispersed.

Composition of Transferosomes: Transferosomes are possessed majorly of phospholipids like phosphatidyl choline which assembles into lipid bilayer in an aqueous environment such that to form a vesicle upon closing. The second component in the composition of transferosomes is edge activator. It acts by increasing the lipid bilayer flexibility and permeability. An edge activator consists of single chain surfactant which destabilizes the lipid bilayer thus increasing its fluidity and elasticity.

By mixing suitable surfactants in the appropriate ratios, the flexibility of transferosomes membrane can be altered. The resulting transferosomes are flexibility and permeability optimized. Transferosomes can therefore acclimatize its shape to adjacent stress easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer. The flexibility is of significant importance because it minimizes the threat of complete vesicle rupture in the skin and allows them to track the natural water gradient across the epidermis, when applied under non occlusive condition.(1,2)

Mechanism of Penetration of Transferosomes

When the formulation (lipid suspension (transferosomes)) is applied on to the skin the water gets evaporated and there is a formation of "osmotic gradient", which is the major mechanism for transportation of transferosomes across the skin. Thus the transportation of these elastic vesicles is independent of concentration. When applied under suitable condition they can transfer 0.1mg of lipid per hour/ cm2 area across the intact skin. This value is considerably superior than which is typically driven by the transdermal concentration gradients8. Naturally occurring transdermal osmotic gradients i.e. "another much prominent gradient is available across the skin is the reason for this high flux rate(3).

Due to the stratum corneum, which acts as a skin penetration barrier this osmotic gradient is developed so that it prevents the water loss across the skin and maintains a water activity difference in the viable part of the epidermis (75% water content) & nearly completely dries the stratum corneum, near to the skin surface (15%water content) .The stability of osmotic gradient is very important because the ambient air acts as a perfect sink for the water molecules even when the transdermal water loss is un physiologically high(4). Due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water all polar lipids attract some water. Most lipid bilayers thus spontaneously resist an induced dehydration. As a result all lipid vesicles composed of polar lipid vesicles move from the rather dry location to the sites with sufficiently high water properties which is responsible for their greater deformability(5). Standard liposomes are confined to the skin surface, where they dehydrate completely and fuse, so they have less penetration power than transferosomes. Transfersomes attain maximum flexibility, so they can take full advantages of the transepidermal osmotic gradient (water concentration gradient) (5). Transfersomes vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer as shown in figure 2 in detail (6). So when lipid suspension is placed on the skin surface, that is partly dehydrated by the water evaporation loss and then the lipid vesicles feel this "osmotic gradient" and escape the complete drying by moving along this gradient. This can be achieved only when they are sufficiently deformable to pass through the narrow pores in the skin, because transferosomes composed of surfactants that have more suitable rheological anhydration.

Transferosomes work through a combination of osmotic gradient and elastic deformation:

1. Osmotic Gradient: Creates a driving force that pushes the transferosome into the deeper layers of the skin.
2. Elastic Deformation: The surfactants allow the vesicle to deform and pass through pores and skin barriers that are smaller than the vesicle itself.

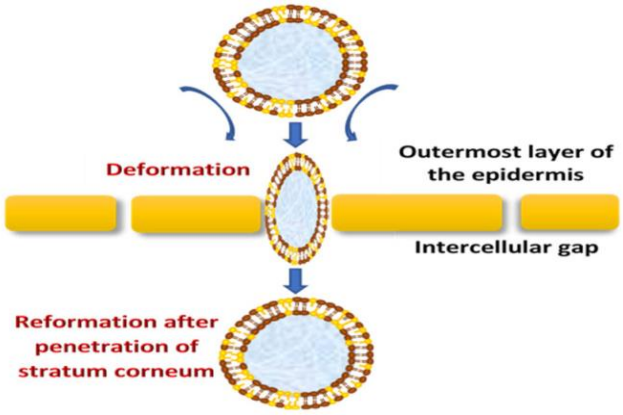


Figure 3: MOA of Transferosomes

Advantages

1. Enhanced Penetration: Capable of delivering drugs to deeper skin layers or into the bloodstream.
2. Reduced Dosage Frequency: Improved bioavailability can reduce the need for frequent dosing.
3. Targeted Delivery: Potentially offers more precise delivery to specific tissues or organs.
4. Non-Invasive: Provides an alternative to injections, improving patient compliance.
5. Self administration is also possible with this targeted drug delivery system.
6. Transferosomes also be used for drugs with a narrow therapeutic window.
7. Transferosomes can act as a carrier for high molecules as well as low molecular weight drugs example analgesic, Anesthetics, corticosteroids, sex hormone, insulin, anti-cancer and albumin. (7,8,9,10,1

Disadvantages

1.The formation of transferosomes is expensive.
2. They are chemically unstable because of their predisposition to oxidative degradation.
3. Purity of natural phospholipids is another criteria militating against adoption of transferosomes as drug delivery vehicles.(12,13,14)

Applications

1. Transdermal Drug Delivery: For drugs that are poorly absorbed orally or need to avoid first-pass metabolism.
2. Topical Treatments: Enhanced delivery of dermatological treatments for conditions like psoriasis or eczema.
3. Vaccines: Non-invasive delivery of vaccines through the skin.
4. Cancer Therapy: Targeted delivery of anticancer drugs to tumor sites.

MATERIALS

Benzocaine, soya lecithine, span 80, methanol, chloroform, toluene, cholesterol, distill water.

EXPERIMENTAL WORK

A] PREFORMULATION STUDY:

1. Melting point

The melting point is determined by using manual physical methods using Thieles tube and liquid paraffin. The melting point is typically around 91°C.

2. Solubility

The solubility is check in various solvents like water, ethanol, methanol, chloroform, dichloromethane

3. Calibration by Using UV Spectrophotometer

A UV spectrophotometric procedure for analyzing benzocaine in transferosomes involves preparing a benzocaine stock solution, preparing calibration standards, selecting a wavelength, constructing a calibration curve, and preparing a sample. The process involves weighing an accurate amount of benzocaine, dissolving it in a solvent, and preparing calibration standards. The maximum absorption wavelength (λmax) is typically around 285 nm. The sample is then prepared by centrifuging the benzocaine-loaded suspension, disrupting it with methanol, and diluting it to

within the calibration range. The absorbance of the sample solution is measured at the λmax, and the concentration of benzocaine is determined using the calibration equation. The method is validated by evaluating linearity, accuracy, precision, specificity, and LOD and LOQ based on the standard deviation of the response and the slope of the calibration curve.

4. IR Spectroscopy of Benzocaine

The IR is done by using the BRUCKER IR spectrometer is a crucial method for characterizing and confirming the presence of functional groups in chemical compounds and formulations, such as benzocaine transferosomes. To conduct IR spectroscopy on benzocaine transferosomes, a sample of benzocaine encapsulated in transferosomes is prepared, potassium bromide (KBr) is used for pellet preparation, an IR spectrometer is preferred for better resolution and sensitivity, a mortar and pestle for grinding the sample with KBr, a hydraulic press for making KBr pellets, and a sample holder or liquid cell depending on the sample's state.

The procedure involves preparing a small amount of benzocaine in a suitable solvent, placing the solution onto an IR-transparent material, setting up the spectrometer according to the manufacturer's instructions, and recording the spectrum over the desired wavelength range. The IR spectrometer is then used to analyze the recorded spectrum to identify characteristic absorption bands of benzocaine, compare it with reference spectra or literature data, and interpret the observed absorption bands in terms of structural features of benzocaine.

The results of the IR spectroscopy analysis are reported, including the identified absorption bands and their assignments to specific molecular vibrations of benzocaine. If necessary, differences or similarities between the experimental spectrum and reference data can be discussed.

B] EXPERIMENTAL DESIGN:

MATERIALS FOR TRANSFEROSOMES:

Transferosomes is a self adaptable and optimized mixed lipid aggregate and composed of phospholipids like phosphatidyl choline which self assembles into lipid bilayer in aqueous environment and closes to forma vesicle. A bilayer softening component (such as a biocompatible surfactant or an amphiphile drug) is added to increase lipid bi layer flexibility and permeability. This second component is called as edge activator. An edge activator consists usually of single chain surfactant that causes destabilization of the lipid bilayer thereby increasing its fluidity and elasticity. The newer elastic vesicles were introduced by Van den berg in 1998, consisting of non ionic surfactant as the edge activator 30. Flexibility of tranferosomes membrane can be altered by mixing suitable surface active agents in the proper ratios. The resulting, flexibility and permeability optimized, transferosome vesicle can therefore adapt its shape to surrounding stress easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bi layer. This flexibility also minimizes the risk of complete vesicle rupture in the skin and allows transferosomes to follow the natural water gradient across the epidermis, when applied under non occlusive condition. Vesicles composed of phospholipids as the main ingredient (soya phosphatidylcholine, egg phosphatidylcholine,), 10-25% surfactant for providing flexibility(methanol) and hydrating medium consisting of Toluene). (15,16,17,18)

Materials commonly used for the preparation of transfersomes are summarized in Table Below

Class	Example	Uses
Phospholipids	Soyalecithine	Vecicle forming component
Surfactant	Span 80	For providing flexibility

Alcohol	Methanol, Chloroform	As a solvent
Buffering agent	Toluene	As a hydrating medium
Combining agent	Cholesterol	Fluidizing agent

Table No.3 Materials for Transferosomes

FORMULATION OF TRANSFEROSOMES

Central Composite Design (CCD) is a statistical technique used for optimizing formulations by studying the effects of multiple factors and their interactions. When formulating transferosomes using soyalecithine and Span 80, CCD can help in determining the optimal concentrations of these components to achieve desirable characteristics such as particle size, encapsulation efficiency, and stability

Batch No.	Std	ID	Run	Build Type	Space Type	Factor A: Soya lecithine in mg	Factor B: Span 80
F1	12	9	1	NA	Central	150	3
F2	4	4	2	NA	Factorial	200	4
F3	3	3	3	NA	Factorial	100	4
F4	11	9	4	NA	Central	150	3
F5	6	6	5	NA	Axial	220.711	3
F6	5	5	6	NA	Axial	79.2893	3
F7	2	2	7	NA	Factorial	200	2
F8	8	8	8	NA	Axial	150	4.41421
F9	7	7	9	NA	Axial	150	1.58579
F10	13	9	10	NA	Central	150	3
F11	10	9	11	NA	Central	150	3
F12	1	1	12	NA	Factorial	100	2
F13	9	9	13	NA	Central	150	3

Table No.4: Formulation Of Transferosomes

METHOD OF PREPARATION FOR TRANSFEROSOMES

Thin film hydration technique is employed for the preparation of transfersomes which comprised of three steps

1. A thin film is prepared from the mixture of vesicles forming ingredients that is phospholipids and surfactant by dissolving in volatile organic solvent (chloroform methanol). Organic solvent is then evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50°C for Souyalecithine) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight.

2. A prepared thin film is hydrated with toluene by rotation at 60 rpm for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature.

3. To prepare small vesicles, resulting vesicles were sonicated at room temperature or 50°C for 30 min. using a bath sonicator or probe sonicated at 4°C for 30 min. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

C] CHARACTERIZATION:

1. PARTICLE SIZE:

The HORIBA HZ 100 particle size analyzer is a tool used to determine the particle size of benzocaine and other excipients. The process involves preparing the sample, selecting the appropriate dispersion medium, and dispersing the sample using an ultrasonic bath. The analyzer is set up, calibrated, and loaded into the sample cell. The sample is then measured using laser diffraction, and multiple measurements are recorded for accuracy and repeatability. The

HORIBA HZ 100 software provides a particle size distribution curve, with key parameters such as mean particle size, standard deviation, and other percentile values. The data analysis is done using the HORIBA HZ 100 software, which provides a summary of the particle size distribution. The sample cell is then cleaned thoroughly between different samples to avoid cross-contamination and using appropriate solvents to remove residues. The process ensures accurate and repeatable results. The 1 ml of solution is taken and diluted upto 10 ml water after that is it sonicate for 10 min using ultrasonicator for 10 min and analyse .

2. ENTRAPMENT EFICIENCY:

To determine the entrapment efficiency (EE) for benzocaine transferosome, follow these steps:

1. Prepare transferosomes by dissolving phospholipids and surfactants in an organic solvent. Add benzocaine to the mixture, evaporate the solvent, and hydrate the film with an aqueous buffer.
2. Separate the encapsulated and free drug using ultracentrifugation or dialysis. Measure the total benzocaine content using techniques like UV-Vis spectrophotometry or HPLC. After separation, measure the free benzocaine content.
3. Calculate the entrapment efficiency by substituting the measured values into a formula. If specific data is needed, consult a professional. Otherwise, follow the steps outlined in the experimental setup to determine the entrapment efficiency.

Batch No.	Std	ID	Run	Build Type	Space Type	Factor A: Soyalecithine in mg	Factor B: Span 80	Response 1:Particle size nm	Response 2:Entrapment efficiency %
F1	12	9	1	NA	Central	150	3	302	79.26
F2	4	4	2	NA	Factorial	200	4	297	92.83
F3	3	3	3	NA	Factorial	100	4	511	78.26
F4	11	9	4	NA	Central	150	3	368	79.26
F5	6	6	5	NA	Axial	220.711	3	241	95.33
F6	5	5	6	NA	Axial	79.2893	3	353	71.35
F7	2	2	7	NA	Factorial	200	2	242	94.27
F8	8	8	8	NA	Axial	150	4.41421	483	81.97
F9	7	7	9	NA	Axial	150	1.58579	480	64.22
F10	13	9	10	NA	Central	150	3	302	79.26
F11	10	9	11	NA	Central	150	3	302	79.26
F12	1	1	12	NA	Factorial	100	2	427	60.37
F13	9	9	13	NA	Central	150	3	302	79.26

Table No. 5 Method using CCD

SOLUTIONS

Number	Soyalecithine	Span	PS	Student err PS	EE	Student err EE	Desirability	
1	156.150	2.252	337.431	20.067	75.870	1.287	1.000	Selected
2	200.000	2.000	298.921	35.091	89.793	2.251	1.000	
3	200.000	4.000	320.231	35.091	90.300	2.251	1.000	
4	100.000	4.000	474.079	35.091	79.370	2.251	1.000	
5	100.000	2.000	423.769	35.091	59.532	2.251	1.000	
6	192.111	2.583	255.208	22.013	89.136	1.412	1.000	
7	192.864	2.189	287.072	27.165	87.865	1.743	1.000	
8	110.138	3.771	426.464	25.309	78.350	1.624	1.000	

Optimization of Batch

The batch no .1 is selected as a optimized batch.

a) 3D graph

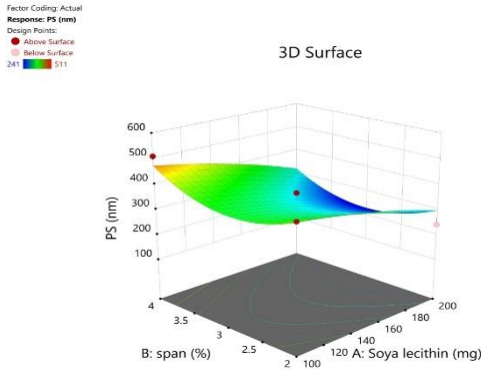


Figure 4: A

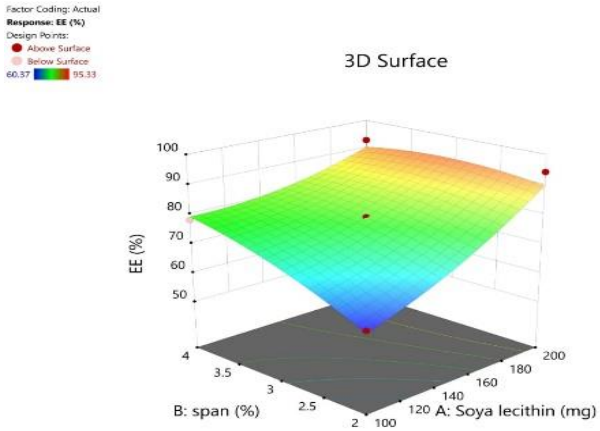


Figure 4: B

Figure 4: A The 3D surface morphology gives the rigid and abnormal graph. The particle size is mostly depend on the concentration of soyalecithine. The concentration of soyalecithine is increases the particle size will be decreases.

Figure 4: B The figure 8 B shows entrapment efficiency is dependable on the variables. The entrapment efficiency increases with the concentration of variables.

b) Counter Plot:

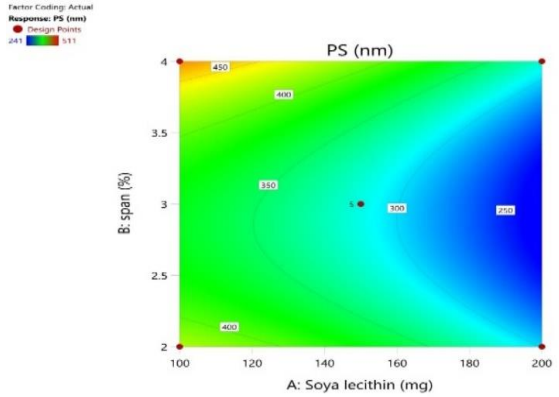


Figure 5: A

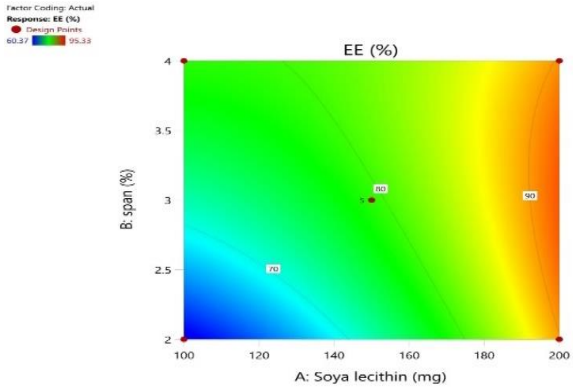


Figure 5: B

Figure 5: A Particle size is decreases with the increasing amount of variables. The variable partially affects by the amount of soyalecithine.

Figure 5: B The figure 9:B shows that entrapment efficiency is affected by the concentration of variable X that is soyalecithine are not affected by variable Y

c)Interaction:

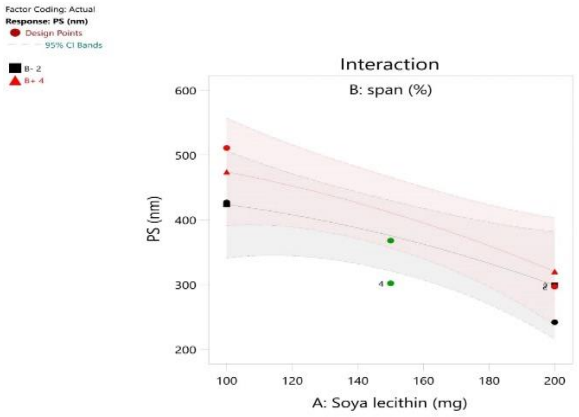


Figure 6: A

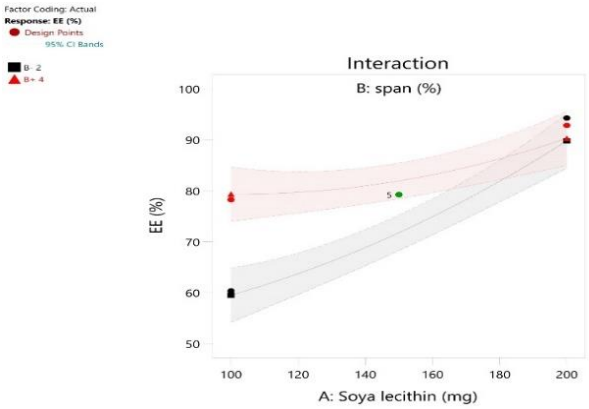


Figure 6: B

Figure 6: A The interaction graph and interaction points of soyalecithin and particle size are cross with each other and shows the interaction and dependency with each other.

Figure 6: B The line of both the variables shows 4 designs of points of interaction and shows the initial entrapment and dependency in lower concentration.

d) Predicted VS Actual:

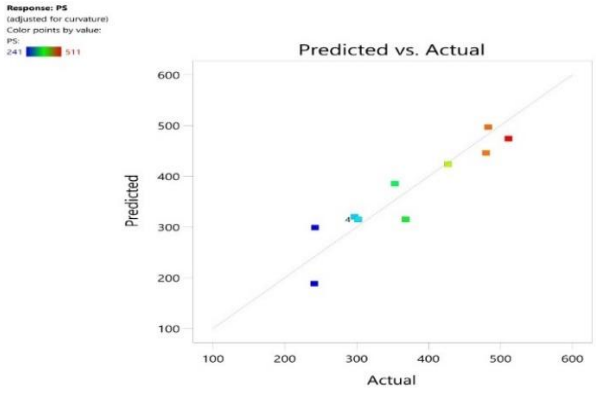


Figure 7: A

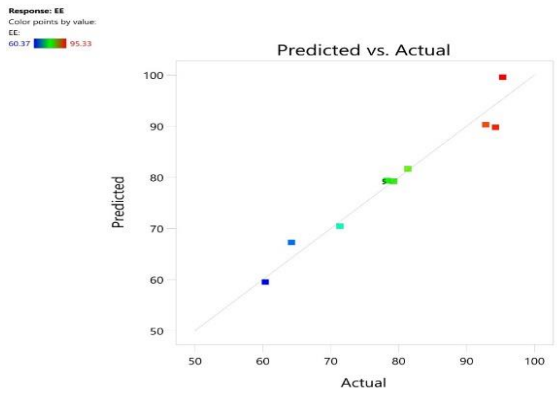


Figure 7: B

Figure 7: A The graph shows that total 9 points and all the points come near to diagonal line. So in case of particle size your actual values good performance with the predicted values.

Figure 7: B The figure 11 B shows 9 points and all of are into the diagonal line. So predicted values are match with actual values

e) ANOVA:

ANOVA for Quadratic model

Response 1: PS

Source	Sum of squares	df	Mean Square	F value	P value	
Model	88273.80	5	17654.76	8.96	0.0060	significant
A-Soyalecithine	38835.72	1	38835.72	19.71	0.0030	
B Span	2564.81	1	2564.81	1.30	0.2914	
AB	210.25	1	210.25	0.1067	0.7535	
A^2	1383.03	1	1383.03	0.7020	04298	
B^2	42486.42	1	42486.42	21.56	0.0024	

Residual	13791.27	7	1970.18			
Lack of Fit	10306.42	3	3435.49	3.94	0.1091	
Pure error	3484.80	4	871.20			
Cor Total	1.021E+05	12				

Table 7: A

Factor coding is Coded. Sum of squares is Type III - Partial The Model F-value of 8.96 implies the model is significant. There is only a 0.60% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

ANOVA for Quadratic model

Response 1:EE

Source	Sum of squares	df	Mean Square	F value	P value	
Model	1260.77	5	252.15	31.10	0.0001	significant
A-Soyalecithine	848.37	1	848.37	104.64	<0.0001	
B Span	206.96	1	206.96	25.53	0.0015	
AB	93.41	1	93.41	11.52	0.0115	
A^2	57.78	1	57.78	7.13	0.0320	
B^2	39.84	1	39.84	4.91	0.0622	
Residual	56.75	7	8.11			
Lack of Fit	56.75	3	18.92			
Pure error	0.0000	4	0.0000			
Cor Total	1317.53	12				

Table 8: B

Factor coding is Coded. Sum of squares is Type III - Partial The Model F-value of 31.10 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

3. IN VITRO DRUG RELEASE:

The transdiffusion procedure for benzocaine transferosome gel involves evaluating the drug's permeation through a biological membrane. The procedure requires the use of benzocaine transferosome gel, Franz diffusion cells, egg skin, phosphate buffer solution (PBS) 7.4 ph, magnetic stirrer, analytical balance, syringe, sampling vials, and a UV-Vis spectrophotometer for drug quantification. The membrane is prepared by removing any subcutaneous fat and hydrating it in PBS for at least an hour before the experiment. The Franz diffusion cell is assembled, consisting of a donor compartment and a receptor compartment, filled with PBS and maintained at a constant temperature. A magnetic stirrer is placed in the receptor compartment to ensure uniform drug distribution. The benzocaine transferosome gel is applied onto the membrane between the donor and receptor compartments. Samples are withdrawn at predetermined intervals and replaced with fresh PBS to maintain sink conditions. The concentration of benzocaine in the receptor compartment is determined using UV-Vis spectrophotometer, and the cumulative amount of

benzocaine permeated per unit area is plotted against time to obtain the permeation profile.

4. PREPARATION OF GEL:

Preparing benzocaine transferosomes gel involves several key steps: preparation of transferosomes, incorporation of benzocaine into these transferosomes, and formulation of the gel. Here's a step-by-step guide:

1.Preparation of Transferosomes

Transferosomes	Carbopol 934	Distilled water
250mg	3%	10
250mg	4%	10
250mg	5%	10

Table No. 3: Preparation of Transferosomes

Materials Needed:

1. Phosphatidylcholine (PC)
2. Edge activators (Span 80)
3. Benzocaine
4. Ethanol
5. Distilled water
6. Sonicator
7. Rotary evaporator (optional)

Steps:

1. Dissolve Lipids: Dissolve phosphatidylcholine and the edge activator in ethanol. The typical ratio might be PC: edge activator at 85:15 by weight.

2. Hydration: Hydrate the lipid mixture with distilled water. This can be done by slowly adding water to the lipid mixture while stirring.

3. Formation of Vesicles: Sonicate the mixture using a probe sonicator to form small vesicles. This step can also be done using a rotary evaporator to remove the solvent, leading to the formation of a thin lipid film which is then hydrated and sonicated.

4. Sizing: If needed, extrude the vesicles through a polycarbonate membrane to achieve the desired size.

2. Incorporation of Benzocaine

Steps:

1. Loading Benzocaine: Add benzocaine to the hydrated lipid mixture before sonication. Benzocaine can be dissolved in a small amount of ethanol if needed to aid in its incorporation.

2. Sonication: Continue sonication until benzocaine is uniformly distributed within the transferosomes.

3. Preparation of Benzocaine Transferosome Gel

Materials Needed:

- Carbopol 934 or another suitable gelling agent
- Distilled water
- Benzocaine-loaded transferosomes

Steps:

1. Prepare Gel Base: Disperse Carbopol 934 in distilled water (usually around 1-2% w/w). Allow it to hydrate completely, usually requiring a few hours with occasional stirring.

2. Neutralize: Slowly Carbopol to adjust the pH and form a clear gel. The pH should be around 6-7.

3. Incorporate Transferosomes: Add the benzocaine-loaded transferosomes to the gel base. Mix gently but thoroughly to ensure even distribution of the transferosomes within the gel.

4. Adjust Consistency: If needed, adjust the final consistency of the gel by adding more distilled water or additional Carbopol.

Storage: Store the gel in airtight containers at a cool temperature to maintain stability.

EVALUATION TESTS FOR GEL

Evaluating benzocaine transferosomes-containing gel involves various analytical techniques to ensure its quality, efficacy, and safety. Below are detailed descriptions of the essential tests:

1. Physical Appearance:

- Objective: To assess the visual and tactile properties of the gel.
- Procedure: Observe and document the gel's color, homogeneity, presence of any aggregates, phase separation, and any other visible inconsistencies.

2. pH Measurement:

- Objective: To ensure the gel's pH is suitable for topical application.
- Procedure:
- Prepare a 1% gel solution in distilled water.
- Measure the pH using a calibrated pH meter.

3. Viscosity:

- Objective: To determine the gel's spreadability and consistency.
- Procedure:
- Use a viscometer.
- Measure the viscosity at different shear rates.

4. Drug Content Uniformity:

- Objective: To ensure even distribution of benzocaine within the gel.
- Procedure:
- Accurately weigh several samples of the gel.
- Dissolve each sample in a suitable solvent.
- Analyze benzocaine concentration using UV spectroscopy.
- Calculate the drug content uniformity across samples.

5. In Vitro Release Studies

- Objective: To study the release profile of benzocaine from the gel.
- Procedure:
- Use Franz diffusion cells with synthetic membranes.
- Fill the receptor compartment with phosphate buffer (pH 7.4).
- Place a known amount of gel in the donor compartment.
- Maintain the system at 37°C.
- Collect samples from the receptor compartment at predetermined intervals.
- Analyze the samples for benzocaine content using UV spectroscopy.

6.Skin Irritation Studies

-Objective: Ensures safety for human skin.

- Procedure:

Apply the gel to human skin and observe for any signs of irritation.

RESULT AND DISCUSSION

A] PREFORMULATION STUDY

1. Organoleptic Properties:

Criteria	Observations
Colour	White
Odour	Odourless
Nature	Crystalline powder

Table No :4 Organoleptic Properties

2.Solubility of Benzocaine:

Benzocaine is having low solubility in water, but it is moderately soluble in organic solvents like ethanol, methanol.

Solvent	Solubility
Water	Low solubility
Ethanol	Soluble
Methanol	Soluble

Table No :5 Solubility of Benzocaine

3. Melting Point:

For Benzocaine the experimentally determined melting point typically falls within a specific range. The melting point of Benzocaine is generally reported to be 88-92°C.

4. Characterization by UV Spectrophotometer:

The λmax (wavelength of maximum absorbance) for benzocaine typically falls around 254 nm in UV-Vis spectroscopy. However, it's essential to verify this value in your specific experimental conditions, as factors like solvent, concentration, and pH can influence it. Conducting a calibration curve with known concentrations of benzocaine can help determine the exact λmax for your setup.

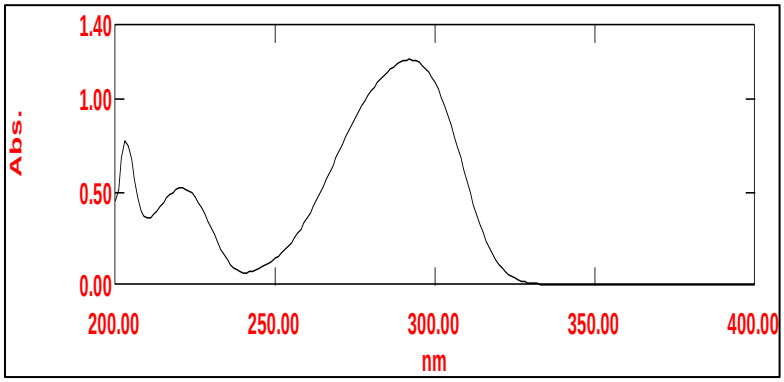


Figure:8 Calibration of Benzocaine ƛ max=292 nm

Calibration curve of benzocaine in Methanol:

Concentration	Absorbance
0	0
1	0.2518
2	0.4361
3	0.6616
4	0.8317
5	0.9894

Table.6: Calibration curve of benzocaine in Methanol

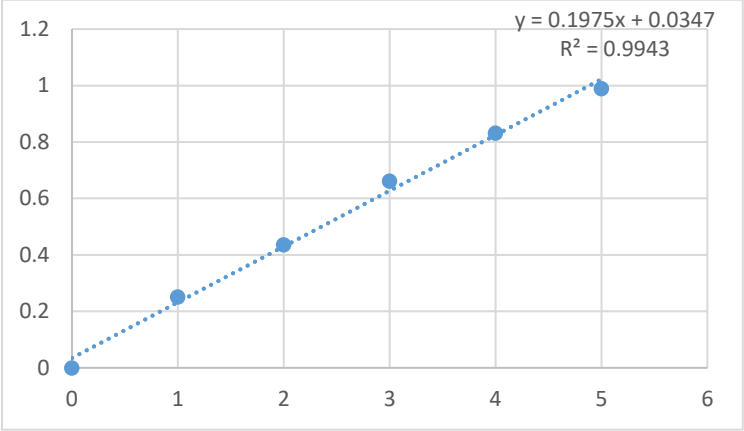


Figure 9: Calibration curve

5. Characterization By IR

a. Drug FTIR

The sample is prepared by grinding benzocaine with potassium bromide powder and pressing into a pellet, or by placing a thin film of the liquid on a KBr or NaCl window. The FTIR spectrometer is set up, calibrated, and the sample is placed in the sample holder. The spectrometer is then run to identify peaks in the spectrum, such as the ester C=O stretch (1735-1750 cm^{-1}), aromatic C-H stretch (3050-3100 cm^{-1}), aromatic ring vibrations (1400-1600 cm^{-1}), and ester C-O stretch (1050-1300 cm^{-1}). The data is then compared with reference spectra for confirmation.

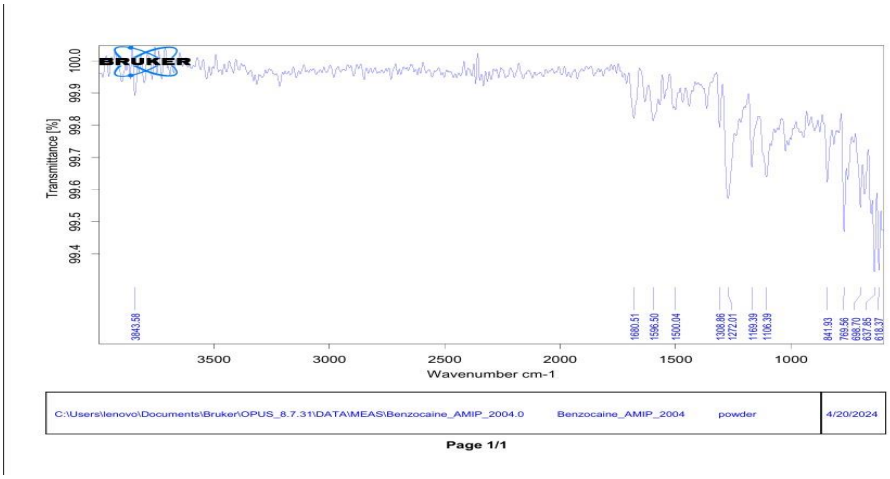


Figure 10: Drug FTIR

Functional group	Standard	Observation (cm-1)
NH2	3500-3100	3450-3200
Aromatic C-H	3100-3000	2983
Carbonyl	1900-1600	1679
Ketone	1300-1230	1272
C=O	692	770-700

Table No. 7: Functional groups present in drug FTIR

b. Drug And Polymer FTIR

The FTIR procedure for benzocaine with soy lecithin and Span 80 involves combining benzocaine, soy lecithin, and Span 80 in appropriate proportions. The mixture is ground with potassium bromide powder and pressed into a transparent KBr pellet. If the mixture is liquid or needs to be dissolved, a thin film is placed on a KBr or NaCl window. The FTIR spectrometer is set up, and a background scan is run with nosample or a blank KBr pellet for calibration. The sample is placed in the sample holder and scanned over the typical wavenumber range (4000 to 400 cm^{-1}). Data collection involves recording the absorption spectrum produced by the spectrometer. The absorption peaks identified include benzocaine, soy lecithin, and Span 80, with benzocaine having the most significant absorption peaks.

Functional group	Standard (cm-1)	Observation (cm-1)
NH2	3500-3100	3450-3200
Aromatic C-H	3100-3000	2983
Carbonyl	1900-1600	1679
Ketone	1300-1230	1272
C=O	692	770-700

Table No.8: Functional groups present in drug and polymer FTIR

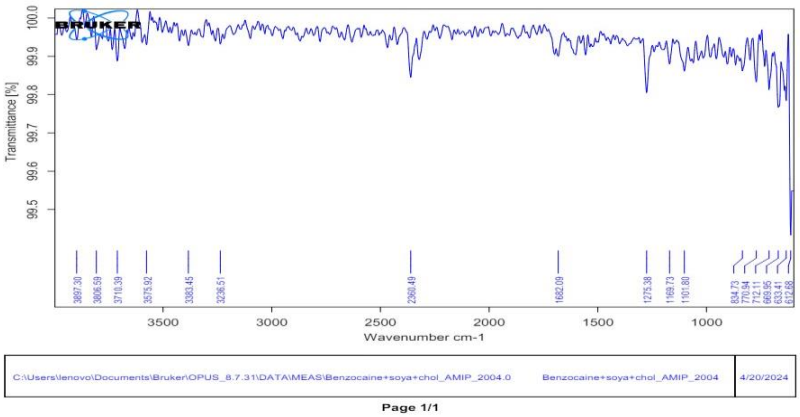


Figure 11: Drug And Polymer FTIR

B] Method using Central Composite Design:

Batch No.	Std	ID	Run	Build Type	Space Type	Factor A: Soyalecithine in mg	Factor B: Span 80	Response 1: Particle size	Response 2: Entrapment efficiency
F1	12	9	1	NA	Central	150	3	302	79.26
F2	4	4	2	NA	Factorial	200	4	297	92.83
F3	3	3	3	NA	Factorial	100	4	511	78.26
F4	11	9	4	NA	Central	150	3	368	79.26
F5	6	6	5	NA	Axial	220.711	3	241	95.33
F6	5	5	6	NA	Axial	79.2893	3	353	71.35
F7	2	2	7	NA	Factorial	200	2	242	94.27
F8	8	8	8	NA	Axial	150	4.41421	483	81.97
F9	7	7	9	NA	Axial	150	1.58579	480	64.22
F10	13	9	10	NA	Central	150	3	302	79.26
F11	10	9	11	NA	Central	150	3	302	79.26
F12	1	1	12	NA	Factorial	100	2	427	60.37
F13	9	9	13	NA	Central	150	3	302	79.26

Table No.9: Method using Central Composite Design

C] Physicochemical evaluation of Gel

1] Physical Appearance

Physical appearance of gel

Sr. no.	Batch	Color	Appearance
1	F1	Yellow	Light Yellow
2	F2	LightYellow	Light Yellow
3	F3	Dark Yellow	Light Yellow

Table No.10: Physical Appearance

All formulation batches were found to be homogeneous light yellow gel preparations

2] Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container.

Homogeneity of formulation

Sr. No.	Batch	Homogeneity
1	F1	Homogeneous
2	F2	Homogeneous
3	F3	Homogeneous

Table No.11: Homogeneity

3] Measurement of pH

The pH values of all prepared formulation ranged from 6-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5

4] Spreadability

The time in seconds require to separate the two slides was taken as measure of spreadability.

Sr. No.	Batch	pH	Spreadability (gm.sm/sec)
1	F1	6.8 /±0.03	16.15/±0.005
2	F2	7.0 /± 0.03	15.40/±0.005
3	F3	7.1 /± 0.03	15.39/±0.005

Table No.12: pH and Spreadability of gel formulation

5] Viscosity

Viscosity of gel was determined by using Brookfield rotational viscometer at 5, 10, 20, rpm. Each reading was taken after equilibrium of the sample at the end of two minutes. The samples were repeated three times.

Sr. No.	% of Carbopol	Viscosity (Cps)
F1	3	2112
F2	5	2330
F3	12	3680

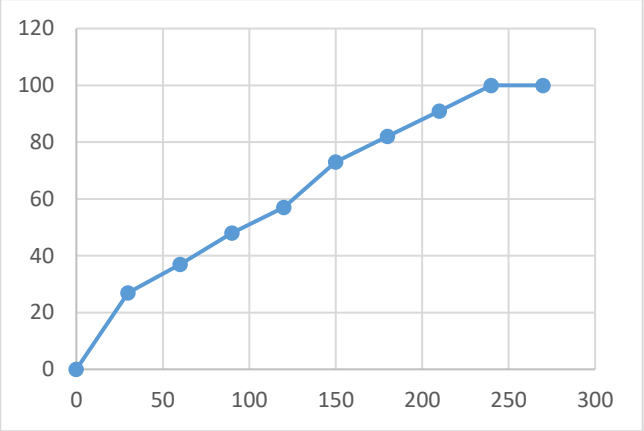
Table No.13: Viscosity

6] In vitro drug release

The transdiffusion procedure for benzocaine transferosome gel involves evaluating drug permeation through a biological membrane using various tools. The membrane is prepared, Franz diffusion cells are assembled and a magnetic stirrer is placed in the receptor compartment. The benzocaine transferosome gel is applied and the concentration of benzocaine in the receptor compartment is determined using a UV-Vis spectrophotometer.

Time interval	Drug release (%)
0	0
30	27
60	37
90	48
120	57
150	73
180	82
210	91
240	100
270	100

Table No. 14: In vitro drug release



Optimization of Batches

The batches were optimized by studying the evaluation parameters off all batches, batch F2 From spreadability and viscosity gel formulation were be optimized

CONCLUSION

The study on benzocaine-loaded transferosomes for local Anesthetics activity showed promising results. The ultra-flexible nature of transferosomes improved permeability, allowing deeper penetration through the skin barrier. The formulation provided a controlled and sustained release of benzocaine, potentially prolonging the Anesthetics effect and reducing the frequency of application. In vivo studies showed markedly enhanced Anesthetics efficacy with transferosome delivery, with a more rapid onset and extended duration of Anesthetics action in animal models. The biocompatibility of the transferosomal formulation was confirmed, indicating its safety and effectiveness. The study identified optimal formulation parameters, including the concentration of phospholipids and surfactants, for maximum drug encapsulation efficiency and stability. The enhanced performance in preclinical tests suggests strong potential for clinical application, improving patient outcomes in managing localized pain. Future studies should focus on clinical trials to validate these findings and explore commercialization potential.

REFERENCE

1. V. Dubey, D.Mishra, A.Asthana, NK Jain. Transdermal Delivery of a pineal Hormone: Melatonin via elastic Liposomes. Biomaterials. 27; 2006: 3491-3496.
2. Panchagnula R. Transdermal delivery of drugs. Indian Journal Pharmacology. 29; 1997:140-156.
3. G. Cevc. Isothermal lipid phase- Transitions Chemistry and Physics of Lipids. 57; 1991: 293- 299.
4. Boinpally R.R., Zhou S.L., Poondru S., Devraj G. and Jasti B.R. Lecithin vesicles for topical delivery of diclofenac. European journal of Pharmaceutics and Biopharmaceutics. 56(3); 2003:389-92.
5. Cevc G, Blume G, Sehatzlein A, Gebauer D and Paul A. The skin a Pathway for Systemic Treatment with Patches and Lipid-Based Agent Carriers. Advance Drug Delivery Reviews. 18; 1996: 349- 378.
6. J.R .Walve, S.R Bakliwal, B.R Rane, S.P Pawar. Transferosomes: A surrogated carrier for transdermal drug delivery System. International journal of applied biology and pharmaceutical technology. 2(1); 2011: 2014-213
7. Cevc, G.; Schatzlein, A.G.; Richardsen, H. Ultradeformable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements. Biochim. et Biophys. Acta (BBA)-Biomembr. 2002, 1564, 21–30.
8. Rajan, R.; Jose, S.; Mukund, V.P.B.; Vasudevan, D.T. Transferosomes—A vesicular transdermal delivery system for enhanced drug permeation. J. Adv. Pharm. Technol. Res. 2011, 2, 138–143.
9. Cevc, G. Lipid vesicles and other colloids as drug carriers on the skin. Adv. Drug Deliv. Rev. 2004, 56, 675–711.
10. Walve, J.R.; Bakliwal, S.R.; Rane, B.R.; Pawar, S.P. Transfersomes: A surrogated carrier for transdermal drug delivery system. Int. J. Appl. Biol. Pharm. Technol. 2011, 2, 204–213.
11. Sivannarayana, P.; Rani, A.P.; Saikishore, V.; VenuBabu, C.; SriRekha, V. Transfersomes: Ultra deformable vesicular carrier systems in transdermal drug delivery system. Res. J. Pharm. Dos. Forms Technol. 2012, 4, 243–255. Journal of Pharmaceutical Negative Results ¦ Volume 13 ¦Special Issue 8 ¦ 2022 4321
12. Sachan, R.; Parashar, T.; Soniya, S.V.; Singh, G.; Tyagi, S.; Patel, C.; Gupta, A. Drug carrier transfersomes: A novel tool for transdermal drug delivery system. Int. J. Res. Dev. Pharm. Life Sci. 2013, 2, 309–316
13. Li, J.; Wang, X.; Zhang, T.; Wang, C.; Huang, Z.; Luo, X.; Deng, Y. A review on phospholipids and their main applications in drug delivery systems. Asian J. Pharm. Sci. 2015, 10, 81–98.
14. Bhasin, B.; Londhe, V.Y. An overview of transfersomal drug delivery. Int. J. Pharm. Sci. Res. 2018, 9, 2175–2184.
15. Gupta A., Aggarawal G., Singla S., Rora R., A, "Transfersomes: A Novel Vesicular Carrier for Enhanced Transdermal Delivery of Sertraline: Development, Characterization and performance Evaluation," Sci Pharm, 2012; 80: 1061-1080.
16. Wavle J.R BakliwalS.R, Rane B.R, Pawer S.P, "Transfersomes: A surrogated Carrier For Transdermal Drug Delivery System" International Journal of Applied Biology and Pharmaceutical Technology, Jan-Mar-2011; 2(1).
17. Kavitha K, Bharath N, Mani T T., "Physical Permeation Enhancers for Transdermal Drug Delivery "Research of Journal of

pharmaceutical, Biological and Chemical sciences, October- December 2011; 2(4): 66.

18. Dave V., Kumar D., Lewis S., Paliwal S., "Ethosome for Enhanced Transdermal Drug Delivery of Aceclofenac" International Journal of Drug Delivery, 2010; 2: 81-9.

HOW TO CITE: Rutuja Patil, Pranjal Chougule, Dr. Nilesh Chougule, Formulation And Evaluation Of Benzocaine Transferosomes Containing Gel For Local Anesthetics Activity, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 7, 2188-2205. https://doi.org/10.5281/zenodo.13131925