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RESEARCH ARTICLE

In Vitro and *Ex Vivo* Characterisation of Capsaicin-loaded Liposomal Film for Transdermal Delivery

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Abstract

Background: Capsaicin has anti-inflammatory and analgesic effects. It undergoes extensive first-pass metabolism and is limited to clinical use due to its pungency. **Aim:** To overcome the associated drawbacks, the study aimed to develop a CAP-liposomal film for dermal delivery. **Methods:** The CAP-liposomes were developed by the ethanol injection technique and evaluated for particle size distribution, zeta potential, % Entrapment Efficiency (EE) and *in vitro* drug release studies, respectively. The films were developed using sodium carboxymethylcellulose as a film base by the solvent casting method and evaluated for particle size distribution, zeta potential, folding endurance, % drug content, *in vitro* drug release, and *ex vivo* permeation studies, respectively. **Results:** CAP-liposomes were optimised based on the results of particle size distribution, zeta potential, % EE, and in vitro drug release, which were found to be in the ranges of 234 to 380 nm, -24.1 to -41.4 mV, 40.67 to 76.52%, and 61 to 94%, respectively. For *in vitro* drug release of CAP-lipofilm and SALONPAS®, it showed 87.6% at 12h and 82.9% at 6 h, whereas for *ex vivo* permeation studies, it showed 75.8% at 12h and 71.6% at 6h, respectively. **Conclusion:** The CAP-lipofilm showed superior results as compared to other test films in all characterisation parameters. Based on the key findings of the current study, the lipofilm showed prolonged permeation of CAP rather than SALONPAS®. Therefore, CAP-lipofilm was thought to offer maximum penetration and controlled release for anti-inflammatory treatment.

Major Findings: The CAP-lipofilm was successfully designed with an adopted formulation composition, and it showed better results than SALONPAS®. These findings may be helpful for *in vivo* activity in future research.

Keywords: Liposomes, Lipofilm, Permeation, Phospholipid, Stability

Abbreviations: CAP: Capsaicin; BCS: Biopharmaceutical classification system; Lipofilm: liposomal film; EE: entrapment efficiency; FTIR: Fourier transform infrared spectroscopy; PEO: polyethylene oxide; PDI: Polydispersity index.

1. Introduction

Capsaicin (CAP) is chemically ((E)-N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methylnon-6-enamide), the primary pungent ingredient found in hot chillies. It has been extensively applied for both pharmacological and physiological properties on the cardiovascular, respiratory and nervous systems. CAP has a low biological half-life of 1.6 hrs, poor aqueous solubility (belonging to BCS-II) and extensive first-pass

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metabolism via the oral route[1,2]. To overcome the associated disadvantages of CAP, the study proposes to develop a CAP-liposomal film for dermal delivery to enhance skin retention and reduce skin irritation[3,4]. Colloidal dosage forms such as liposomes, solid lipid nanoparticles, micro microemulsions have been extensively investigated against dermal delivery. Among these formulations, liposomes have a prospective role in dermal drug delivery. Liposomes offer properties like nontoxic, biocompatible, biodegradable and bioabsorbable, etc. Liposomes can improve the drug solubility by encapsulating into their suitable compartment and modify their biophysical, pharmacokinetic and pharmacodynamic properties with lower dose and reduced toxicity[5-7].

Many researchers have reported the role of liposomes for dermal drug delivery. The current study describes a few research works that were demonstrated by Sudhakar *et al.*, who developed terbinafine liposomes by the ethanol injection method using Phospholipon 90H and Dimethyl Phosphatidylcholine as components. In this study, the encapsulation efficiency of liposomes was shown 70% and the *in vitro* permeation was shown to be 72.62±0.22% up to 12 hrs. At the end of their study, they concluded that liposomal *in vitro* and *ex vivo* behaviour was influenced by the concentration of phospholipids and cholesterol[8]. Another research work on liposomes was reported by Manconia M *et al.* They developed phycocyanin liposomes using the film hydration technique. They reported that phycocyanin (C-PC) loaded liposomes could be accumulated onto the stratum corneum and are unable to penetrate deep into the skin layers[9].

Other research work of Schleining JA *et al.*, developed diclofenac-loaded liposomes for the treatment of inflammation in an acute synovitis model horse. The inflammation and lameness associated with osteoarthritis can be effectively treated by diclofenac liposomes[10]. Gupta V *et al.*, studied aceclofenac pro-liposomes for topical delivery. The pro-liposome was developed by the film hydration technique and reported that pro-liposomes deliver the drug in a sustained manner and exhibited superior stability[11]. Canto GS *et al.* developed piroxicam liposomes by film hydration technique followed by extrusion process and reported that piroxicam liposomes increased the topical anti-inflammatory effect[12]. Du Plessis *et al.* developed cyclosporine-A liposomes for transdermal delivery. They reported that phospholipids mix with intercellular skin lipids and lead to form the intracutaneous depot, and liposomes enhance skin permeation[13]. Varde NM *et al.*, formulated prednisolone liposomes for topical delivery. They reported that liposomal dispersion and their gels were found to improve skin permeation and deposition compared to control formulations[14]. Recently, a simvastatin niosome buccal film was developed by the authors' laboratories. The simvastatin permeation (96.12% up to 24hrs) was improved against the porcine buccal cavity[15]. Based on the above literature, the current study is projected to develop CAP liposomes in a controlled manner for dermal delivery.

2. Materials and Methods

The drug and excipients procurement and adopted methodology for the current research work was mentioned as follows

2.1 Materials

CAP was procured from Praveena Life Sciences, Hyderabad, and phospholipon 90H was procured from Lipoid Pvt Ltd, Germany. Cholesterol was purchased from Merck India Pvt Ltd. Sodium carboxymethyl cellulose and Glycerin were purchased from Sigma-Aldrich, India. All chemicals used were of analytical grade.

2.2 Methods

The estimation of CAP by analytical assessment and preparation method of liposomes are clearly described under this section.

2.2.1 Analytical Method for Estimation of CAP

The CAP was measured at a λmax of 280nm using a UV spectrophotometric technique with phosphate buffer pH 6.8. From the stock solution of 100 µg/mL, 2, 4, 6, 8 and 10mL of solutions were withdrawn and further made up to 10ml using pH 6.8 phosphate buffer to get 20, 40, 60, 80, 100 µg/mL standard solutions, respectively[15].

2.2.2 Preparation of Liposomes

Liposomes were developed by the ethanol injection method[16,18]. Phospholipon 90H, cholesterol, stearic

acid, and precisely weighed amounts of CAP were added in a beaker with a weighed quantity of ethanol and dissolved at a temperature between 50˚ and 60˚C. Ethanol was injected slowly through a 14-gauge needle at the rate of 0.25ml/min into a beaker containing measured amounts of phosphate buffer pH 6.8, which was kept at a temperature of 60˚C and stirred at 500 rpm using a Remi magnetic stirrer. The system was left to evaporate for 45 min in order to evaporate ethanol, and the vesicles were produced as a suspension. The phosphate buffer, pH 6.8, was added to make the required volume of vesicular solution. The obtained dispersion system was filtered using a 0.2µm filter (Ultipor GF Plus®, Pall Corporation, Pall India Pvt Ltd, India). The formulated liposomal dispersion batches were labelled from F1 to F9. The labelled formulations were stored at 2 to 8°C, and the composition of various CAP-liposomes is shown in Table 1.

2.3 Characterisation of Liposomes

The produced CAP-liposomes were characterised for % drug content, pH, particle size, polydispersity Index (PDI), zeta potential, percentage entrapment efficiency (% EE), and *in vitro* cumulative percentage drug release studies, respectively.

2.3.1 Drug Content (%)

One millilitre of liposomes was lysed in methanol. Using a phosphate buffer with a pH of 6.8, it was further diluted. At 280nm, the diluted samples were examined using a UV-visible spectrophotometer[17].

Table 1. Composition of CAP-liposomes

Batch Code	Phospholipon 90H (mM)	Cholesterol (mM)	CAP (mg)	Stearic Acid (mg)	Phosphate Buffer pH 6.8 (ml)
F1	0.1	0.1	50	20	20
F2	0.1	0.2			
F3	0.1	0.3			
F4	0.2	0.1			
F5	0.2	0.2			
F6	0.2	0.3			
F7	0.3	0.1			
F8	0.3	0.2			
F9	0.3	0.3			

2.3.2 Determination of pH

A Thermo Scientific pH meter was used to measure the pH of the liposomal suspension at a temperature of 25±5˚C[8].

2.3.3 Determination of Particle Size, Polydispersity Index (PDI) and Zeta Potential (ζ)

Zeta potential and particle size were measured using the disposable capillary cell. A 0.1M sodium chloride solution was used to dilute the test liposomes (1:10). To measure zeta potential, the diluted liposomes were stored in single-use capillary cells and placed within the sample holder of the Malvern Nano ZS90 device (Malvern, UK). Particle size and zeta potential studies were made at 25°C and a light scattering angle of 90° [15,17].

2.3.4 Determination of % Entrapment Efficiency (EE)

The ultracentrifugation method was used to calculate the liposomes' %EE. A Remi cooling centrifuge was used to centrifuge two ml of liposome suspension for two hours at 25,000rpm and 4°C. The drug-containing supernatant was extracted and measured at 280nm using a UV-visible spectrophotometer[8,18].

2.3.5 In vitro Drug Release Studies by Franz Diffusion Cell

For the *in vitro* drug release studies, a vertical Franz diffusion cell with a 28ml capacity and an effective diffusional area of 4.52cm^2 was employed. A liposomal suspension of 5mg was added to the donor compartment. The required quantity of pH 6.8 phosphate buffer was added to the receptor media, and the temperature of the diffusion cell was kept at 32±0.5°C throughout the experiment by using a magnetic stirrer that rotated at 100rpm. The donor and receptor compartments were separated using a cellulose dialysis membrane (Membra–Cel MD 34-14, cutoff 14KD) after an overnight soak in the receptor medium. At predetermined intervals of 1, 2, 4, 6, 8, 10, and 12 hrs, one ml aliquot was removed from the sampling port and replaced with an equivalent volume of fresh buffer medium to maintain a constant volume. The gathered samples were analysed with a UV-visible spectrophotometer set to 280 nm[19-21].

2.4 Preparation of Plain and Liposomal Films

The optimised liposome suspension was used to prepare a lipofilm. Based on literature and method reproducibility, the solvent casting method was used to prepare the lipofilm and plain by utilizing sodium carboxymethylcellulose (Na-CMC). While 50mg/ 20ml of pure CAP solution was used to produce the plain film and kept for stirring, CAP-liposomes were combined with precisely weighed amounts of polymer, plasticizer, and glycerin to prepare lipofilm. This solution was put into a large petri dish and heated to 40˚C in a hot air oven to dry it out. Each 2x2cm^2 film containing 5mg of drug was optimised with all formulations, and 50mg of drug was included in a film in accordance with the petri plate's (40 sq. cm) requirements[21-23]. The images of prepared films are shown in Figure 1.

2.5 Characterisation of Plain and Liposomal Films

Size, zeta potential, percentage EE, surface pH, weight variation, thickness, folding endurance, percentage drug content, *in vitro* drug release, *ex vivo* permeation studies, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and stability studies were among the physicochemical characteristics of the films that were assessed.

2.5.1 Particle Size, PDI and Zeta Potential

Zeta nanosizer (ZS Nanosizer) was used to dissolve the liposomal film using phosphate buffer pH 6.8, dilute it (1:100) with sodium chloride solution (0.1M), and then reapply the mixture. The analysis was performed with the angle of detection set at 90° and at 25°C. The unit of measurement for the mean vesicle particle size was d (0.9) nm[23,25].

2.5.2 Percent of Drug Content (%)

After cutting and dissolving the 2x2cm^2 plain and liposomal films with 5ml of methanol, 10ml of phosphate buffer with a pH of 6.8 was added. UV spectroscopy was used to estimate the drug content[23, 25].

2.5.3 Percent EE (%)

To ascertain the percentage EE, the disintegrated liposomal film (2ml) was centrifuged using ultra-centrifugation at 20000 rpm for two hours at 4°C. Using UV spectroscopy, the CAP concentrations in the supernatant and pallets were calculated at a wavelength of 280nm[23, 25].

2.5.4 Surface pH

By letting the film expand in contact with 1.0ml of phosphate buffer pH 6.8 for one hour on a watch glass, the surface pH of plain and lipofilm was ascertained[25]. pH paper was used to record the surface pH.

2.5.5 In Vitro Disintegration Study

Using distilled water and a disintegration tester (Pharmatest, Germany) at 32±0.5°C, the *in vitro* disintegration time of plain and lipofilm (2x2 cm^2) was measured[23,25].

2.5.6 Thickness

In order to verify the thickness of the produced films, the thickness of both plain and lipofilm was measured

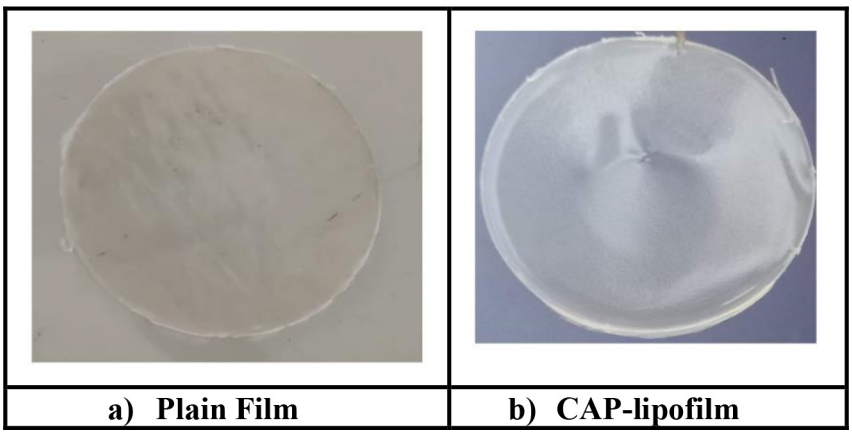


Figure 1. Images of plain and lipofilm.

using a vernier calliper at various positions along the film. The average thickness and standard deviation for the same were then calculated[25].

2.5.7 Folding Endurance

The plain and lipofilm were folded 300 times without breaking, which is regarded as the folding endurance value, or repeatedly at a 180° angle of the plane at the same plane until it broke. The mean of three determinations was calculated[25].

2.5.8 Percentage of Moisture Content (%)

The produced films must be weighed separately and stored at room temperature in a desiccator that contains fused calcium chloride [15,23]. The films must be reweighed after 24 hours to calculate the moisture content percentage using the formula below:

$$Percentage\ moisture\ content = \frac{W2 - W1}{W1} \times 100$$

Where, W_1= initial weight; W_2= final weight

2.5.9 In Vitro and Ex vivo Drug Release Studies

Franz diffusion cells were used for the *in vitro* drug release examinations from test formulations, whereas rat abdominal skin was used for the *ex vivo* drug release investigations. CAP at a dose comparable to 5mg was used in all test formulations. For *ex vivo* studies, rat abdominal skin was used and the experimental protocol, Andhra University Institutional Animal Ethics Committee authorised the experimental protocol (Reg. No. 528/PO/c/01/IAEC/12). The experimental protocol followed that of the *in vitro* research. The dorsal side of the rat abdomen skin was in contact with the receptor solution when it was placed on the Franz diffusion cell. The previously described approach is used to carry out the diffusion process[26,27].

2.5.10 Drug-Excipient Compatibility Studies by FTIR

Drug release from the formulation is significantly influenced by drug-excipient interactions. Because of their close contact, drug and polymer interactions are possible. Thermo Scientific's NicoLET 6700 model Bruker FTIR spectrophotometer, which was outfitted with Opus software, was utilised to capture the infrared spectra of the samples. Test samples' infrared spectra were collected between 400 and 4000cm^{-1} [24].

2.5.11 Surface Morphological Studies by SEM

Using a HITACHI S3400 SEM, the surface morphology of the broken lipofilm was examined. The sample was examined between 20 and 20,000 times magnification[27,28].

2.5.12 Stability Studies of Test Products

The chosen test formulations underwent stability testing in accordance with the International Congress of Harmonisation (ICH) guidelines[27,28].

3. Result and Discussion

3.1 Characterisation of Liposomal Dispersion

CAP, preferably used in the treatment of various diseases such as arthritis, osteoarthritis pain, minor pain, muscle pain and backache. Dermal drug delivery is a choice of drug treatment through the skin by providing prolonged treatment with minimal side effects. Hence, the present work was designed to promote controlled drug delivery by developing the CAP-lipofilm through the dermal route. Moreover, several techniques are available to prepare liposomes; based on the feasibility of our laboratory conditions, the ethanol injection method was adopted, which offers unilamellar vesicles. The phospholipon 90H was used as a lipid base in the current investigation because of its nontoxicity, biocompatibility, and affordability[3]. It is well known that sterols, such as cholesterol, enhance liposome stability and drug entrapment[28]. The vesicle surface charge was imparted by the addition of stearic acid as a charge inducer, which reduced or eliminated clumping and increased the liposomes' physical stability[15].

The % drug content of prepared liposomal formulations was found in the range of 94.93% to 101.34%, and values are shown in Table 2. The result showed a consistent distribution of the drug in each liposomal formulation based on experimental results of generated liposomal dispersions. All liposomal formulations were found to have a pH of approximately 6.8, and the results showed that all formulations had the same dispersion medium pH. It was observed that the particle size distribution ranged from 234nm to 380nm, respectively. The observation resulted that phospholipon 90H and cholesterol played a

Table 2. Physicochemical characterisation of CAP-liposomes

Batch code	Drug Content (%)	Vesicle Size (nm)	PDI	Zeta Potential (mV)	EE (%)
F1	98.7±0.8	380±0.2	0.318±0.3	-19.8±0.1	40.67±0.2
F2	97.2±0.1	341±0.1	0.412±0.7	-24.2±0.2	56.32±0.6
F3	93.4±0.6	318±0.2	0.291±0.8	-28.2±0.6	49.34±0.1
F4	95.5±0.9	295±0.1	0.321±0.2	-36.1±0.1	57.41±0.8
F5	100.4±0.6	234±0.3	0.256±0.5	-41.4±0.2	76.52±0.2
F6	97.8±0.3	268±0.4	0.289±0.3	-31.4±0.1	60.20±0.4
F7	101.3±0.2	331±0.1	0.421±0.2	-28.7±0.8	54.92±0.1
F8	96.8±0.3	303±0.3	0.512±0.4	-33.9±0.4	62.86±0.1
F9	94.9±0.7	312±0.2	0.441±0.6	-31.1±0.6	49.93±0.2

Each value represents mean±s.d. (n=3)

rate-limiting role in the particle size distribution[28]. In view of phospholipid, the particle size was found at the lower level (0.1 molar level) of 318nm to 380 nm, at the middle level (0.2 molar level) of 234nm to 295nm, and at the higher level (0.3 molar level) of 303nm to 331nm, respectively. The observed result indicated that the middle level showed optimum particle size compared to the lower and higher levels. It seems that the middle level of phospholipon 90H is able to solubilise and encapsulate the selected amount of CAP, which leads to optimum particle size. The above result is well correlated with the research work of Catherine Charcosset *et al*., who stated that the saturated state of phospholipon 90H promotes maximum drug release and is compatible with other excipients[29].

Cholesterol concentration also plays a significant role in particle size distribution. It has multiple roles in the formation and stability of liposomes. During development trials, heterogeneous particle size distributions were observed in the absence of cholesterol. Cholesterol is capable of controlling the size, vesicle rigidity, encapsulation, drug release, stability, etc[28]. During the formation of liposomes, cholesterol fills the gaps between bilayered fatty acids and controls the influx and expulsion of internal aqueous core volume into the external dispersion medium. The obtained particle size distribution indicated that cholesterol at the lower level (0.1 molar level) allowed the internal aqueous core to obtain a larger particle size than at the middle and higher levels. At the middle level (0.2 molar level), liposomes are obtained with optimum particle size, whereas at the higher level (0.3 molar level), liposomes have a lower particle size than at the middle and lower levels. It indicated that cholesterol has a stronger affinity than CAP for phospholipon 90H. Hence, a lower particle size was observed. The obtained PDI results indicated that liposomes were found in a homogeneous suspension.

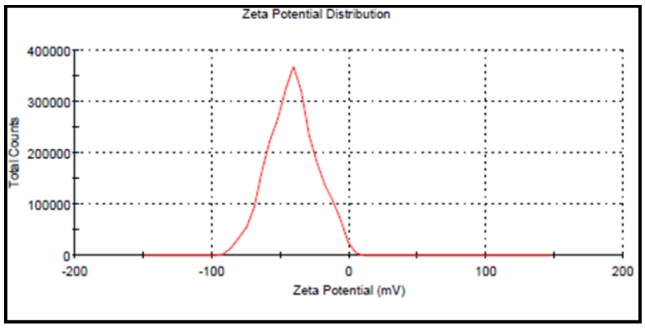


Figure 2. Zetapotential of optimised liposomes formulation (F5).

Zeta potential is one of the important factors that influence the *in vitro* stability of liposomes. The obtained results were found in the range of -19.8±0.1 mV to -41.4±0.2 mV, respectively. It indicated that optimised liposomes have sufficient surface charge to avoid aggregation and fusion during their stability. Stearic acid has a greater effect on zeta potential than other components of liposomes[28]. The PDI of liposomes was found in the range of 0.256 to 0.512. The graph representing the size distribution of the optimised liposomal formulation (F5) is shown in Figure 2. The values of the zeta potential of the liposomal formulations are found in the range of -19.8 to -41.4 mV, respectively.

The entrapment efficiency of liposomes was determined after separating the entrapped and unentrapped drugs by ultracentrifugation. The % EE was varied for all the formulations was found to be 40.67% to 76.52% respectively. Among all formulations, the F5 formulation showed the maximum % drug release of 94.1% at the end of 12h. In the literature of Moon Kyu Kwak *et al*. reported that cholesterol was used for the rigidity of liposomes, and it also acts as a thickener for the membrane. Finally, it leads to promoting controlled drug release due to the slow diffusion of particles into deep layers of the skin[30]. The present work obtained the same findings as the existing literature. The middle level

of phospholipon 90H and cholesterol (0.2Mm: 0.2mM) showed maximum % EE of 76.52. It was observed that the obtained % EE of liposomes was correlated with the existing particle size distribution, and it was inversely proportional to the existing % EE of liposomes. Where the particle size is larger, there might be a low % EE observed. It indicated that the poorly water-soluble CAP is unable to encapsulate into the internal aqueous core; it might be encapsulated into the lipophilic bilayered vesicle.

It was discovered that liposome drug release *in vitro* ranged from 49.3% to 94.1%, respectively. F5 batch liposomes demonstrated the greatest drug release of all the test formulations, reaching 94.1% at the 12-hour mark. Up to a certain point, the percentage drug release rose when lipid and cholesterol concentrations rose, and it reduced as lipid and cholesterol concentrations increased. The increased stiffness of the liposome bilayer phospholipids may be the cause of this effect[27]. The F5 formulation was chosen to load into film and assessed against SALONPAS® based on the obtained results. All formulations followed zero-order drug release, according to the findings of fitting the collected data to first- and zero-order kinetics. However, the F5 formulation had the greatest "r" value (0.921), which led to its optimisation. Diffusion was the mechanism that followed drug release, as evidenced by the larger "r" value (0.986) of the Higuchi plot and the non-Fickian "n" value (0.356) of the Peppas plot, respectively.

3.2 Characterisation of CAP-Plain and Lipofilm

Sodium carboxymethyl cellulose was used as the film foundation in the solvent casting procedure to create the plain and lipofilm. The physicochemical properties of both films were assessed. The percentage drug content of plain and lipofilm was found to be in the range of 95.2±0.4% to 97.5±0.6%, respectively. The particle size distribution of CAP-lipofilm, PDI, zeta potential, and % EE was found to be 298.00±0.9nm, 0.986±1.2, 27.00±1.10mV, and 68.00±0.56%, respectively. The particle size distribution of CAP-lipofilm increased from 234±0.3nm to 298.00±0.9nm, the zeta potential decreased from -41.4±0.2mV to -27.00±1.1mV, and the % EE also decreased from 76.52±0.2% to 68.00±0.56%, respectively.

The percentage drug content of the plain and lipo films also confirmed that the drug was dispersed uniformly throughout the polymeric film. The percentage drug content was found to be near 100%; no drug was found in either film, respectively. In the evaluation of CAP-lipofilm, all the parameters were altered from the suspension form. It was observed that the surface net charge was changed in dried film, which might decline in zeta potential from -41mV to -27mV, and increased particle size from 234±0.3 nm to 298.00±0.9 nm, respectively.

Alireza Fazeli *et al.* declared that zeta potential is an indicator to stabilise colloidal dispersion systems, and the net surface charge is used to determine the particle aggregation tendency. Moreover, it also interferes with biological interfaces due to its nano-range. In our current research, it was also proven that in dry film, the dispersion system stability is higher, which may be due to the standardisation of the dispersion system in the film, and the surface net charge is lower. These consequences lead to low zeta potential[31].

The pH of the test films was found within the limits. The plain and lipofilm have a smooth surface and elegant texture with a white colour. The film is flexible without any sign of cracking. The percentage elongation break test indicates that both films (plain and lipofilms) are flexible and show the elastic nature of the polymer. The plain film and lipofilm have shown uniform thickness for the entire film and were found to be 0.48mm and 0.51mm, respectively. The weights of plain (2x2cm^2) and lipofilm were found to be 46.6mg and 49.3mg, respectively, and the films were found to be uniform in weight. The folding endurance for plain and Lipofilm was measured, and films were folded repeatedly until they were broken. Folding endurance was found to be >300 for both formulations. The result indicates that both films were flexible. The percentage moisture content of plain and lipofilm was found to be 7.4% and 3.1% respectively. The percentage elongation break tests for plain and lipofilm were found to be 11.2% and 13.3%, respectively, and the results are illustrated in Table 3.

3.3 *In Vitro* Comparative Drug Release Studies with Marketed Formulation

Franz diffusion cells utilising dialysis membranes were used to accomplish the *in vitro* drug release

Table 3. Physiochemical properties of plain and lipofilms

Parameter	Plain Film	Lipo Film
Particle size distribution (nm)	NA	298.00±0.9
PDI	NA	0.986**±1.2**
%EE	NA	68.00±0.5
Zeta potential (mV)	NA	-27.00±1.1
Thickness (mm)	0.48**±0.03**	0.51±0.03
Weight variation	46.6±0.2	49.3±0.7
Folding endurance	>300	>300
Drug content (%)	97.5±0.6	95.2±0.4
% Moisture content	3.1**±0.7**	7.4**±0.8**
% Elongation beak test	13.3**±0.2**	11.2**±0.5**

Each value represents mean±s.d. (n=3)

of SALONPAS® (capsicum extract 0.025%), plain film, lipofilm, and CAP-liposomal formulation (F5). Figure 3 displays the relative % drug release for each test formulation. The results for the plain film and lipofilm were 56.4% and 87.6%, respectively, after 12 hrs, whereas SALONPAS® demonstrated 82.9% after 6 hrs. The outcome suggests that SALONPAS® cannot release the medication for a prolonged period of time in a controlled manner. The lipofilm demonstrated regulated drug release for up to 12 hrs longer than SALONPAS® and plain film, respectively. The hydrophilic characteristic of sodium CMC facilitates solvent molecule entry into the film's polymer matrix, thereby enhancing penetration into the skin[32].

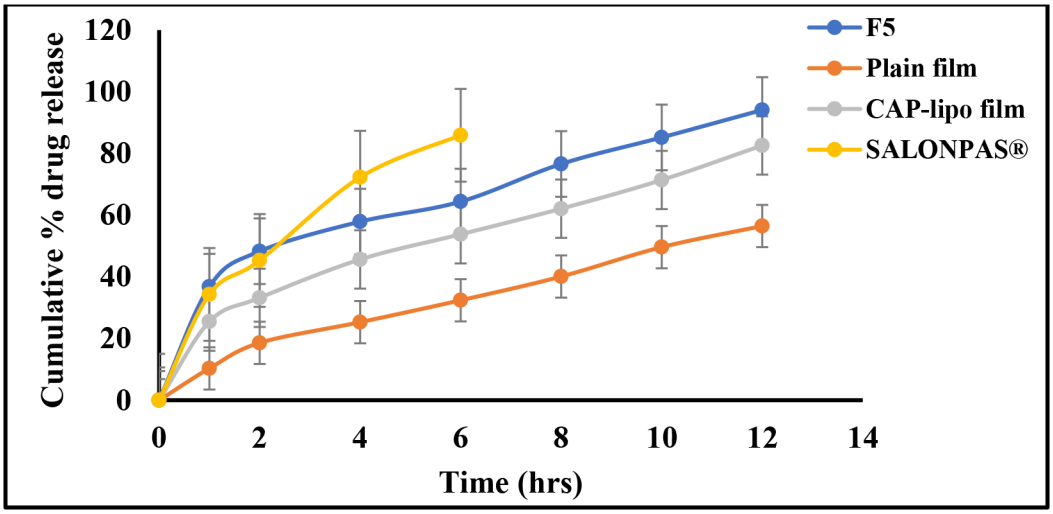


Figure 3. Comparative *in vitro* drug release profiles of test formulations.

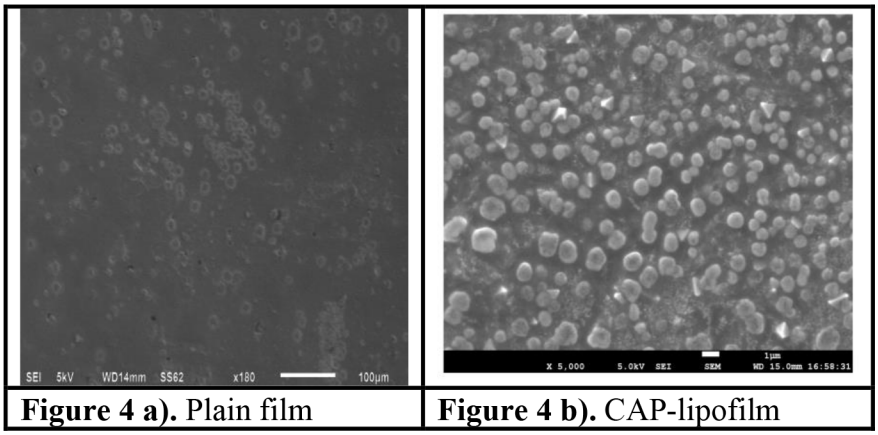


Figure 4 a). Plain film | **Figure 4 b).** CAP-lipofilm

Figure 4. SEM photographs of films at a scale of 100µm.

3.4 Scanning Electron Microscopy (SEM)

The morphology of plain and lipofilm was studied using the SEM by the HITACHI Benchtop SEM T3030 instrument. The SEM images of films are shown in Figure 4.

3.5 *Ex Vivo* Diffusion Studies Using Rat Abdominal Skin

Using Franz diffusion cells and rat abdomen skin, the *ex vivo* drug release of test formulations F5, plain film, lipofilm, and SALONPAS® was carried out. Figure 5 compares the *ex vivo* percentage drug release of each test formulation. The F5 formulation, CAP-lipofilm, and plain film all showed *ex vivo* drug release rates of 82.7%, 75.8%, and 42.4% at the conclusion of 12 hrs, while SALONPAS® demonstrated 71.6% within 6 hrs. When compared to SALONPAS®, the results showed that liposomes and their film formulation demonstrated regulated drug release for up to 12 hrs.

In the *ex vivo* release studies, the percentage of drug release decreased compared to the *in vitro* release. It was observed that the test formulation's drug release tendency was similar to that of *in vitro* drug release, respectively. As a result, lipofilm produces the drug release over an extended period of time, up to 12 hrs, and does so after the drug has been released from the liposomal vesicle. Similar effects were also reported by Asadullah Madni *et al.* The result of literature stated that *in vitro* drug release is in 16 hrs and obtained >90% drug release, whereas *ex vivo drug release was* conducted for 12 hrs and achieved a maximum of 80%. The current research parameter was well correlated with the existing literature[33, 34].

3.6 Drug and Excipient Compatibility Study by FTIR

The purpose of the FTIR investigations was to find any potential interactions between the medicine and the excipients. Figure 6 displays the FTIR spectra of lipofilm, cholesterol, phospholipon 90H, CAP, and stearic acid. The principal peaks were found to appear at 3315 cm^{-1} for N-H stretching, 1597cm^{-1} for aromatic C-H stretching, 2856cm^{-1} for out-of-plane C-H stretching, 1650cm^{-1} for olefin C=C stretching, 967cm^{-1} for out-of-plane C-H bending, 1515cm^{-1} for N-H bending and C-H stretching, 1515cm^{-1} for asymmetric C-O-C stretching, and 1202cm^{-1} for C-O stretching, according to the IR spectra of CAP.

The phospholipon 90H FTIR spectra revealed aliphatic C–H bands between 2917.23 and 2649.09, C=O stretching bands between 1702.73 and 1432.59cm^{-1}, and C=C stretching bands between 1434.45 and 1432.59cm^{-1}. The O-H stretching vibration at 3398.38 cm^{-1}, the C-O alcoholic stretching vibration at 1022.58 cm^{-1}, the aliphatic C-H stretching vibration at 2933.42 to 2849.37 cm^{-1}, and the C=C stretching vibration at 1467.47 and 1375.42 cm^{-1} were all visible in the cholesterol FTIR spectrum.

Stearic acid FTIR spectra revealed C=O (acid) stretching vibration at 1703.34cm^{-1} and C-H aliphatic stretching vibration at 2954.60cm^{-1}. Peaks in the lipofilm FTIR spectrum were seen at 3315 cm^{-1} for

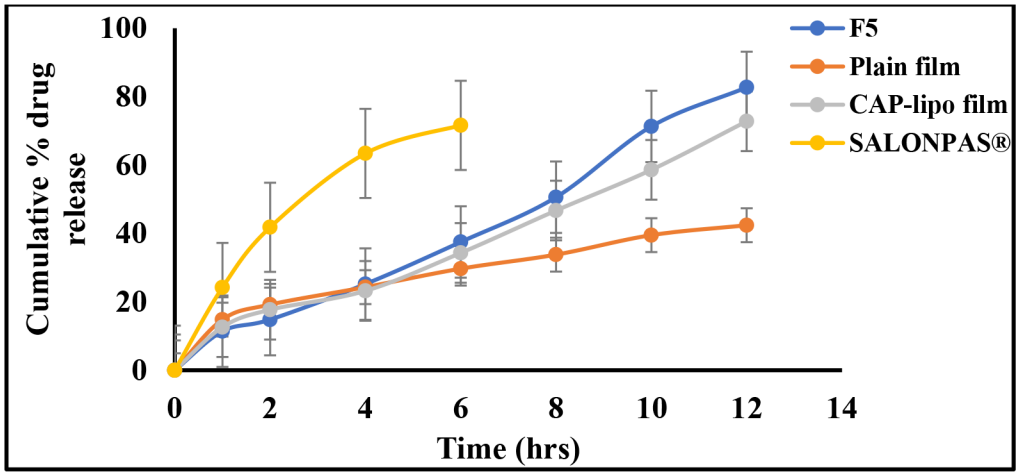


Figure 5. Comparative *ex vivo* drug release profiles of test formulations.

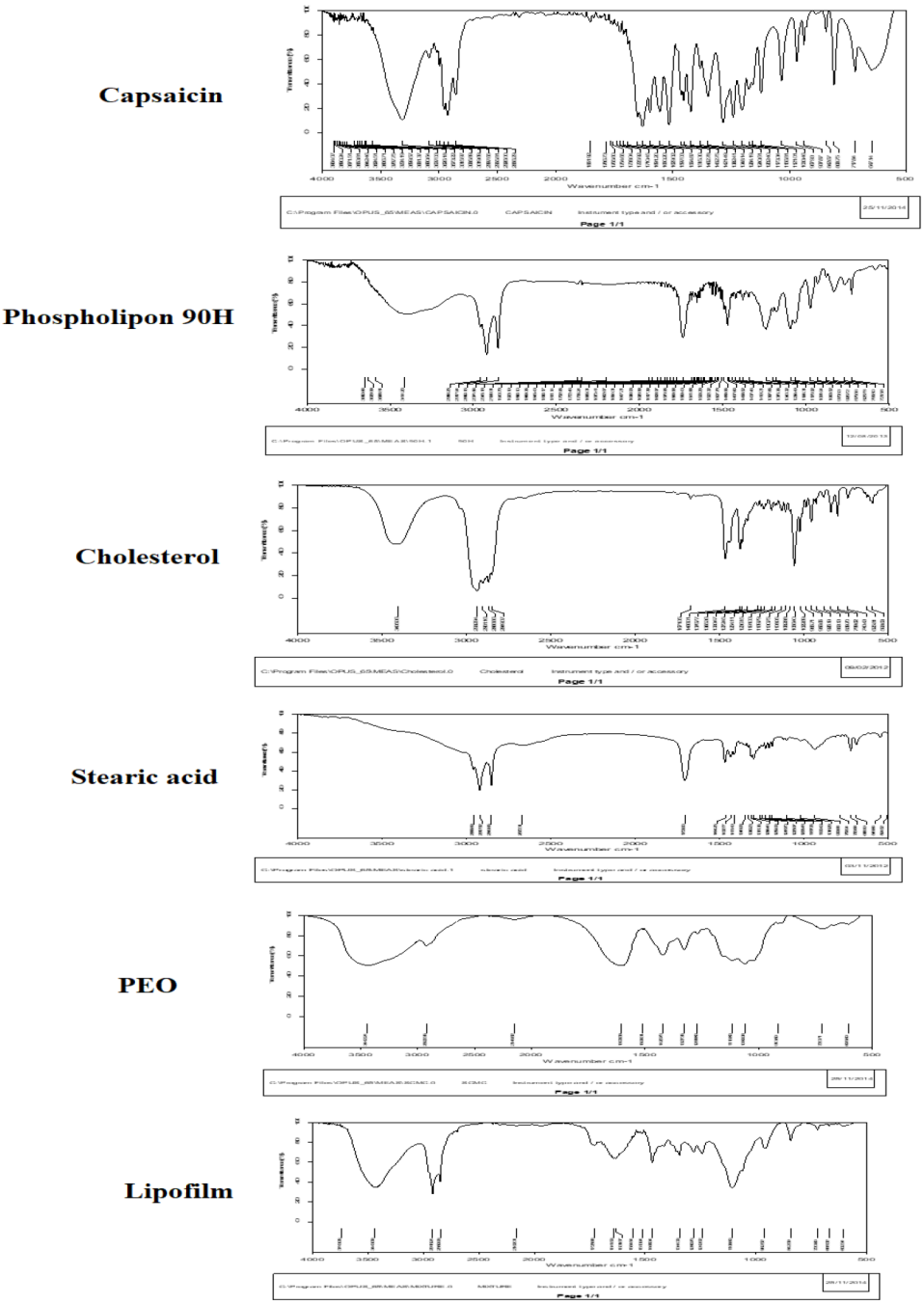


Figure 6. FTIR spectra of CAP, Phospholipon 90H, cholesterol, stearic acid, PEO and lipofilm.

N-H stretching, 1597 cm^{-1} for aromatic C-H stretching, 2856 cm^{-1} for out-of-plane C-H stretching, 1650 cm^{-1} for olefin C=C stretching, 967 cm^{-1} for out-of-plane C-H bending, and 1515 cm^{-1} for N-H stretching.

The principal peaks of CAP and excipients showed a strong correlation with the theoretical presence of functional groups. Additionally, it showed that the medicine and excipients were confirmed to be compatible. The observed distinctive peaks of pure medicines, excipients, and test formulations from the FTIR tests were in good agreement with the functional group theoretical prediction. No significant changes were observed in the principal peaks of CAP when phospholipon 90H, cholesterol, stearic acid, or sodium CMC were present. The result indicates that there is no incompatibility among the drugs and excipients used in the study.

Table 4. Stability study protocol of test products

S. No	Test Product	Stability Condition		Time Interval
1	F5 and CAP-Lipo Film	2-8°C	Long term	Stable up to 3 months in all the conditions
2	F5 and CAP-Lipo Film	25°C ± 2°C/60 % RH ± 5% RH	Accelerated stability	
3	Plain Film	25°C ± 2°C/60 % RH ± 5% RH	Long-term stability	
		40°C ± 2°C/75% RH ± 5% RH	Accelerated stability	

3.7 Stability Studies of Selected Test Products

The F5 formulation and CAP-lipo films are more stable at 2–8°C for three months, according to the stability studies. The F5 and CAP-lipo film physicochemical properties are much different from the first findings. While the remaining attributes were changed, the percentage of drug content was determined to be between 90% and 110%, which is within the limit. The physicochemical characteristics of the CAP-lipo films and the F5 formulation were considerably changed under accelerated conditions. The simple film stability indicates that the accelerated condition of 40°C±2°C/75%RH±5%RH is less suited than the 25°C±2°C/60%RH±5%RH condition (Table 4). The stability was affected by several instability pathways, including the oxidation process, temperature, and hydrolysis. Many factors, including the phospholipon 90H phase transition, intermolecular forces, cholesterol concentration, gravitational force, medium pH, etc., all had an impact on the stability of CAP-liposomes (F5) in the form of suspension. A temperature condition between 2 and 8°C was more appropriate following three months of stability, according to the data obtained for physicochemical parameters.

4. Conclusion

CAP-liposomes were well developed by the ethanol injection technique and evaluated by various parameters. Prepared liposomes were examined thoroughly and reported as positive in all parameters. In 12 hrs of drug release, the optimal concentration of phospholipon 90 H and cholesterol-containing F5 formulation showed the maximum cumulative percentage of drug. Further, it developed into a suitable film with sodium CMC as a film base using the solvent casting method. The CAP-lipofilm was evaluated against a marketed film (SALONPAS®). Based on *in vitro* and *ex vivo* studies, *SALONPAS® is* unable to release the drug in a controlled manner, based on the research evaluation, and hence it is not suitable for prolonged treatment. We are planning to conduct animal studies to prove the statement with much more clarity by maintaining therapeutic drug concentration levels in the future. Finally, the FTIR study confirmed the compatibility among the existing excipients and proved that CAP-lipofilm improved the physicochemical properties of CAP by incorporating it into a stabilised form. Hence, lipofilm was considered an alternative to improve dosage potential through dermal delivery.

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6. Author Contribution

All five authors equally contributed to all aspects. All authors have read and agreed to the published version of the manuscript.

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