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RESEARCH ARTICLE
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FORMULATION AND EVALUATION OF LIPOSOMAL GEL WITH DAPSONE

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Abstract:
The current investigation employed a method to assess the effectiveness of liposomes as a new type of lipid for delivering dapsone through the skin. To optimize the liposomes, adjustments were made to the concentrations of ethanol phospholipids and cholesterol. Ultimately, liposomes composed of soy lecithin, ethanol, and cholesterol were found to be the most optimal. The results revealed that the F7 formulation yielded spherical, unilamellar vesicles with a smooth surface, demonstrating superior outcomes. The topical administration of dapsone may offer an alternative method of acne valgeris as well as novel therapeutic uses for a known medication. Dapsone is challenging to include into standard formulations due to its physicochemical characteristics. The goal of the current study was to create a stable liposomal containing dapsone that could be modified for topical application.

Keywords: - Liposome, Dapsone, topical administration, Franz diffusion cell, and in-vitro release.

INTRODUCTION: -

A liposome is a spherical structure with an equal number of aqueous compartments surrounded by one or more phospholipid bilayers. Drugs that are hydrophilic or lipophilic can be enclosed in liposomes. Since the medicine must be released from the liposome before it can undergo metabolism and excretion, drugs enclosed in liposomes can maintain therapeutic levels for a long time. In recent times, there has been increasing interest in the administration of medications topically due to the localized accumulation of the highest drug concentration at the intended site of action. When he envisioned a medication delivery method that would specifically target the medicament to injured cells and gave them the name "magic bullets," Paul Ehrlich initiated the development of targeted delivery systems in 1906. [1,2]

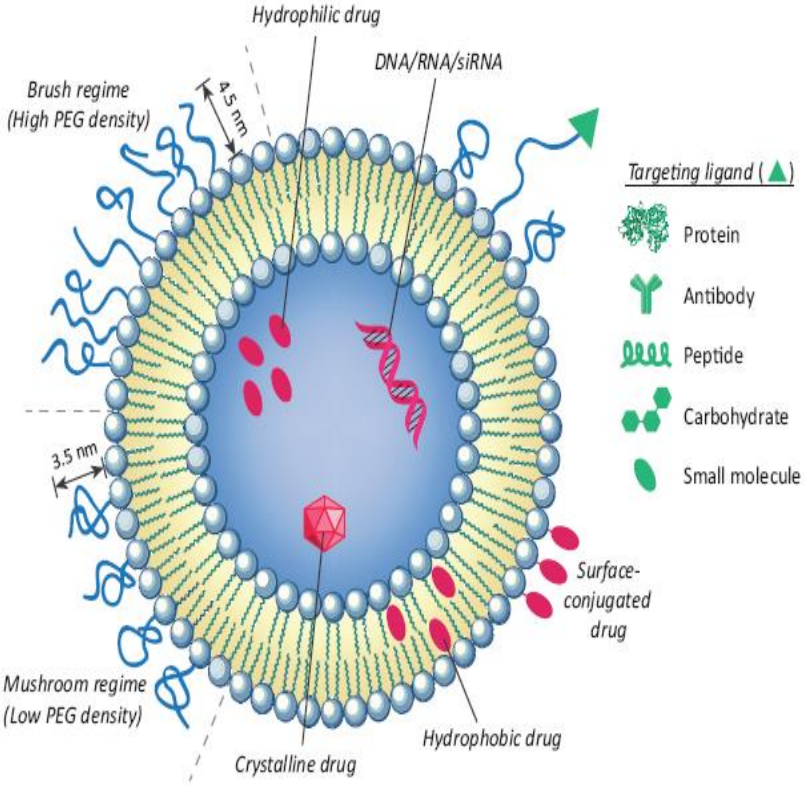


Fig1.1 Figure of liposomal structure

A benefit of liposomes is: [3,4]
It can transport both lipid- and water-soluble medications, and it can selectively target tumour tissues (liposomal doxorubicin).

Liposomes improved the therapeutic index and efficacy of the medication actinomycin-D.
It offers continuous release.

It can be given by a variety of routes.
It encourages the incorporation of both small and large molecules.
It also serves as a narcotics storage area.
Liposomes have the ability to control drug distribution.
It directly affects how the medicine interacts with cells.

Material and Method
Yarrow chem products generously offered dapsone. We bought soy lecithin from Central Drug House in New Delhi. SDFCL Fine-Chem Limited provided the cholesterol crystalline, Central Drug House provided the ethanol, Carbopol 934 provided the carbopol 934, and Central Drug House provided the Triethanolamine. [5]

Methods of preparation of Dapsone loaded liposomes
The approach of modified ethanol infusion was used to produce liposomes. After ethanol had been used to break down the cholesterol and phospholipids, the lipophilic medication was added. The mixture was then injected into the aqueous phase of the hydrophilic drug. Liposome formation occurred upon contact between the ethanol solution and the aqueous phase. The resulting liposome suspension was stirred and later subjected to ultra-centrifugation to extract the released medication. The extracted pellets were dispersed in a saline solution with phosphate. [6]

Ethanol injection technique for liposome preparation: -

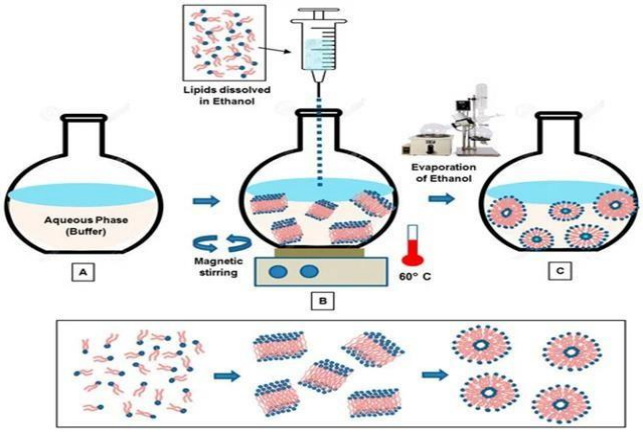


Fig 1.2 Ethanol injection technique

(F) Batch Code	(mg) Dapsone	(mg/ml) Phospholipids	(mg/ml) Cholesterol	(ml) Org/aq. Phase ratio
1	.1	20	4	10
2	.1	20	6	10
3	.1	20	8	10
4	.1	40	4	10
5	.1	40	6	10
6	.1	40	8	10
7	.1	60	4	10
8	.1	60	6	10
9	.1	60	.8	10

Table 1.1: Designing formulations

Liposomal Gel Preparation

Glycerol (10 ml) was added, and Carbopol 934 powder was dissolved in hot distilled water by swirling.

Also included were 0.01%-weighted amounts of methyl and propyl parabens, respectively.

Ensure that any lumps you produce are easily dispersed and give them 24 hours at room temperature to hydrate for swelling.

After that, the dispersion was neutralized using Triethanolamine (50 percent w/w).

A mechanical stirrer running at 25 rpm for two minutes was used to mix liposomes containing dapsone with carbopol gel.

Methodology

The appearance, color, and order of this substance are examined.

Calculating the melting point

USP technique was used to determine the melting point. A small amount of medication was added to a capillary tube that was sealed. The melting point device was used with the tube. The apparatus's temperature was gradually raised, and it was observed at what temperature the drug began to melt and at what temperature it finished melting. [7]

Drug pH measurement

Using a digital pH meter, the pH of Dapsone was determined.

Calculation of the partition co-efficient

In a separating funnel, a known amount of Dapsone was combined with 20 ml of phosphate buffer pH 7.4. and 20 ml of n-octanol. The mixture was then shaken periodically and allowed to equilibrate at 37 °C for two hours to attain two distinct phases. After any necessary dilution, the UV spectroscopic

approach was used to estimate the concentration of the medication in the aqueous phase and organic phase at max 290 nm. The apparent partition coefficient was obtained by utilizing the provided equation, which involves calculating the ratio between the drug concentration in each phase. [8]
K p = Organic /Aqueous
The organic phase refers to the concentration of the drug in the organic medium, while the aqueous phase indicates the concentration of the drug in the aqueous medium.

Studies on solubility
Semi-quantitative solubility measurements were made by introducing solvent to a glass tube with precisely measured solute. Any undissolved solute particles are then visually inspected after the system has been vigorously stirred. Solubility is expressed as a function of solute to solvent ratio. In order to conduct a solubility study on dapsone, the following solvents were used: methanol, distilled water acetone, ether hexane, ethanol, propylene glycol, chloroform, and phosphate buffer solution pH 7.4.[9]

Analysis using infrared spectroscopy
Moisture-free samples of soy lecithin and Dapsone were analyzed using an IR spectrophotometer to capture their Fourier infrared spectra. Infrared spectroscopy, By analyzing the different peaks in the FTIR spectrum, various groups in the structure of meloxicam were identified. Furthermore, FTIR spectroscopy allows for the investigation and prediction of physicochemical interactions between different components. With a resolution of 1 cm-1, the scanning range was between 4,000 and 400 cm-1.[10]

Vesicle Size and Shape:
An optical microscope was used to measure the size and shape of the vesicles produced by each formulation. The uniform distribution of 2 gm of each formulation onto a glass slide was checked for vesicular size and shape using an optical microscope. [11]

Drug Release from Formulated Liposomes
The dialysis membrane method was used in this study. A 100ml dissolution media was placed in a 50ml beaker and maintained at 37.5 °C on a magnetic stirrer. The liposome formulation was sealed in the dialysis membrane and suspended in the media. Five-milliliter aliquots of the media were collected at regular intervals and replaced with fresh media of the same volume. The drug concentration in each aliquot was determined using a spectrophotometer. [12]

Evaluation of Liposomal [13,14]
Physical evaluation of liposomal gel :-
Organoleptic qualities of the liposomal gel formulation were assessed.

Measuring the liposomal gel's pH
A homogenizer was used to combine 1 g of Dapsone liposomal gel with 100 ml of distilled water. After that, the electrode was submerged in the prepared gel solution while a digital pH metre took readings in triplicate and determined an average value.

Viscosity research
The Brookfield viscometer was used to measure viscosity by choosing the appropriate spindle number and rpm. During the lowering of the spindle groove, setting of the rpm, and measurement of the dial reading after three minutes, a 50 ml beaker holding 50g of the preparation was left there. Utilizing the reading, a factor was employed to calculate the viscosity. Three times the process was carried out, and the results were recorded as means.

Spreading capacity
It described how far the gel spreads when applied to the skin or the affected area. When a load is applied, it is measured in terms of the number of seconds it takes for two slides to separate from the gel that has been deposited in their interstices. Better spread ability is achieved by requiring less time to separate two slides.

The calculation is performed using the formula:

$$S = M.L/T$$

Where:
M represents the weight tied to the upper slide
L corresponds to the length of the glass slide
T indicates the time taken for the slide to spread.

Ratio of yield
Calculating the weight difference between a container containing the formulation and an empty container allowed for the realistic yield to be established.

Calculated by formula:

Percentage yield = Practical yield /Theoretical yield× 100

Grittiness and homogeneity
To assess the consistency of the formulation, a tiny amount of liposomal gel was rubbed on the skin of the back of the hand or pressed between the thumb and forefinger. The coarse particles will be visible if there are any.

In vitro release tests
This study's execution involved the dialysis membrane technique. A 50 ml beaker received 100 ml of dissolution media. The beaker was positioned on a magnetic stirrer at a temperature of 37.5°. In the sealed end of the dialysis membrane, the liposome formulation was filled and sealed. A dialysis membrane sample was suspended in a medium. At regular intervals, 5 ml aliquots from the medium were removed and immediately replaced with an equivalent volume of fresh medium. The aliquots' medication concentrations were measured with a spectrophotometer. [15]

Stability
Liposomal gel's stability research was calculated by putting the gel in the best-fitting glass vial at 40°c for a month, then placing the same vial at 25°c for a month, and finally at 40°c for another month. Liquid exudates separated after the liposomal gel was exposed to room temperature. The physical traits were evaluated three months afterwards [16].

Results and Discussions
Organoleptic characteristics and pre formulation studies:
The dapsone drug is of pastel yellow solid having unpleasant smell

Properties	Description
Appearance	Powdered
Color	Pastel yellow
Odor	Unpleasant aroma

Table 1.2 Properties

Solubility study: -

Solubility studies are performed to determine the the solubility of drug in different solvent.

S.no	(mg) Quantity of drug	Solvent	(ml) Quantity of solvent	Inference
1	50	Water	5	insoluble
2		ether	5	insoluble
3		Octane	5	Slightly soluble
4		Polyethylene glycol	5	Highly soluble
5		Ethanol	5	Very slightly soluble

Table 1.3 solvents

Melting point: -

It was established that dapsone had a melting point of 185°c. The average result of three measurements was noted.

Partition co-efficient:

The partition co-efficient was measured three times, with the mean value recorded. Dapsone was thus found to have an organic phase partition coefficient of 0.369 and an aqueous phase partition coefficient of 0.166.

Analysis Using FTIR

The analytical technique of infrared spectroscopy, commonly referred to as FTIR analysis or FTIR spectroscopy, is employed to distinguish and classify different types of materials, including organic compounds, polymers, and, in some cases, inorganic substances. where

DRUG (DAPSON)

DRUG+LECITHIN

DRUG+LECITHIN+CHOLESTROL

DRUG+LECITHIN+CHOLESTROL+DW

DRUG (DAPSON)

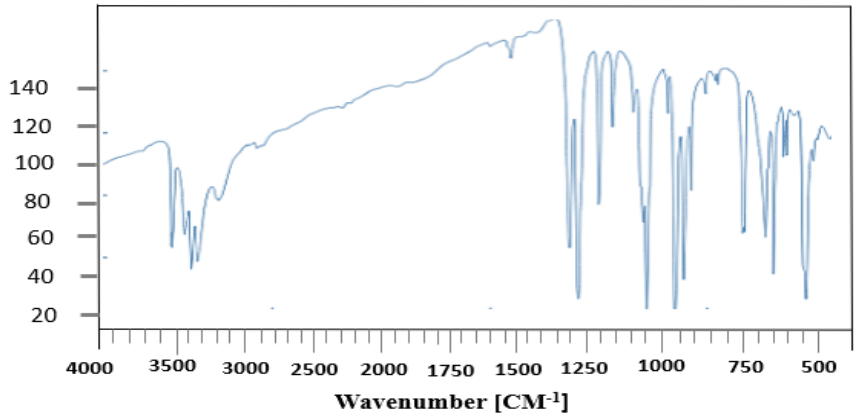


Fig 1.3 FTIR

Peak No.	Frequency (cm^{-1})
1	3814
2	3524
3	1280
4	1190
5	1186
6	1178
7	9980
8	766

Table 1.4 DRUG+LECITHIN

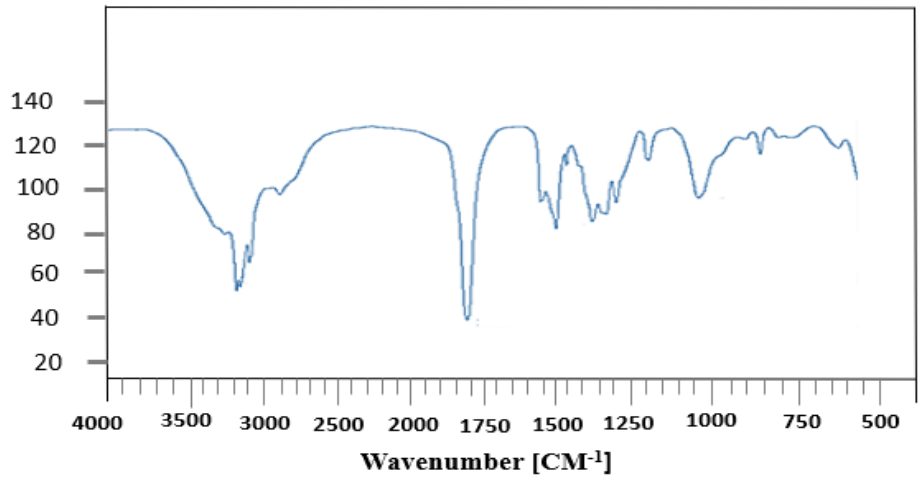


Fig 1.4

Peak No.	Frequency (cm^{-1})
1	3214
2	1624
3	1680
4	1190
5	1386
6	1179
7	1000
8	590

Table 1.5 DRUG+LECITHIN+CHOLESTROL

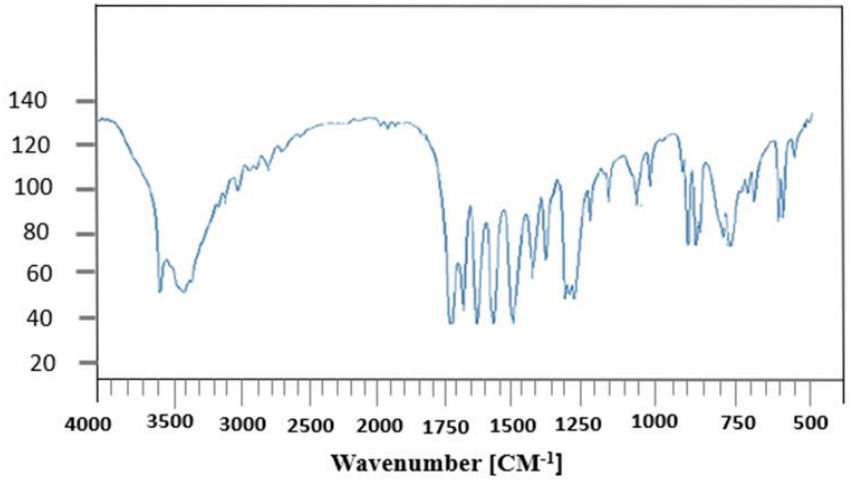


Fig 1.5

Peak No.	Frequency (cm^{-1})
1	3614
2	3524
3	1750
4	1720
5	1255
6	980
7	987
8	745

Table 1,6 1.8 DRUG+LECITHIN+CHOLESTROL+DW

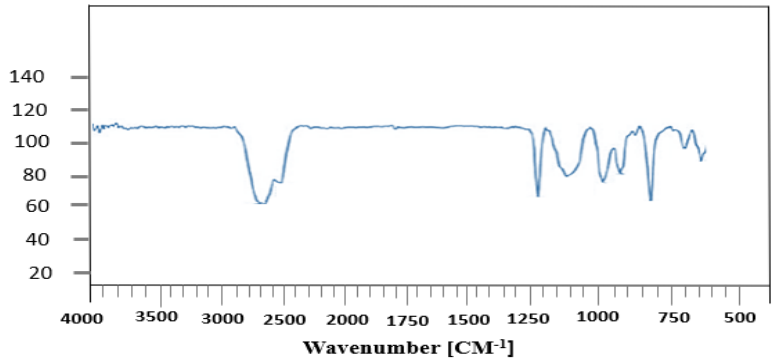


Fig 1.6

Peak No.	Frequency (cm^{-1})
1	2987
2	1140
3	987
4	876
5	1255
6	980

Table 1.7

Evaluation of Liposomes

Percentage of the medical product's intarpation

Five milliliters of the phospholipid formulation and 25 milliliters of the mobile phase were placed in a 100 milliliter volumetric flask, and they were sonicated in an ultrasonic bath for a short period of time. An additional 0.5 m membrane filter was used to filter the resultant combination. Through the use of the mobile phase, the filtrate was further diluted. The absorbance at 363 nm was measured with a Shimadzu 1700 UV-VIS spectrophotometer to determine the quantity of dapsone.

%EE = (Amount of drug entrapped in liposomes) / (Total amount of drug added).

Formulation (F)	Percentage of drug entrapment
1	35.29
2	28.58
3	38.57
4	46.71
5	41.43
6	56.14
7	61.29
8	63.86
9	72.87

Table 1.8: Percentage drug entrapment of Dapsone loaded liposomes

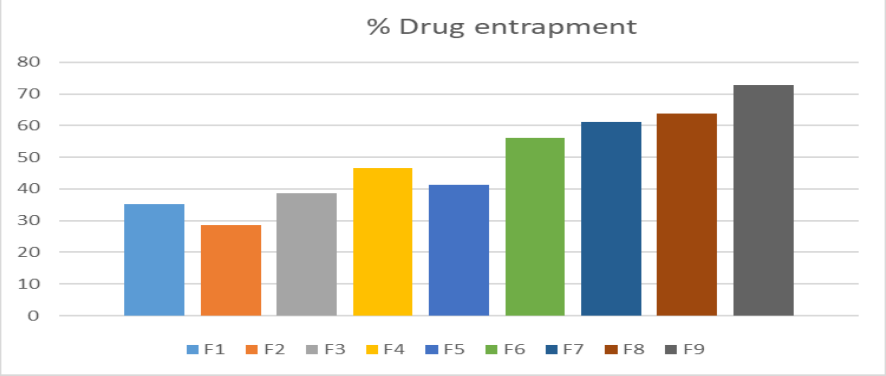


Fig 1.7: Drug Entrapment

SEM Evaluation

The substance is subjected to scanning with an electron beam, resulting in the generation of an enlarged image for detailed examination. This method, commonly referred to as SEM microscopy and SEM analysis, proves highly beneficial for the analysis of failures and microanalysis of solid inorganic materials.

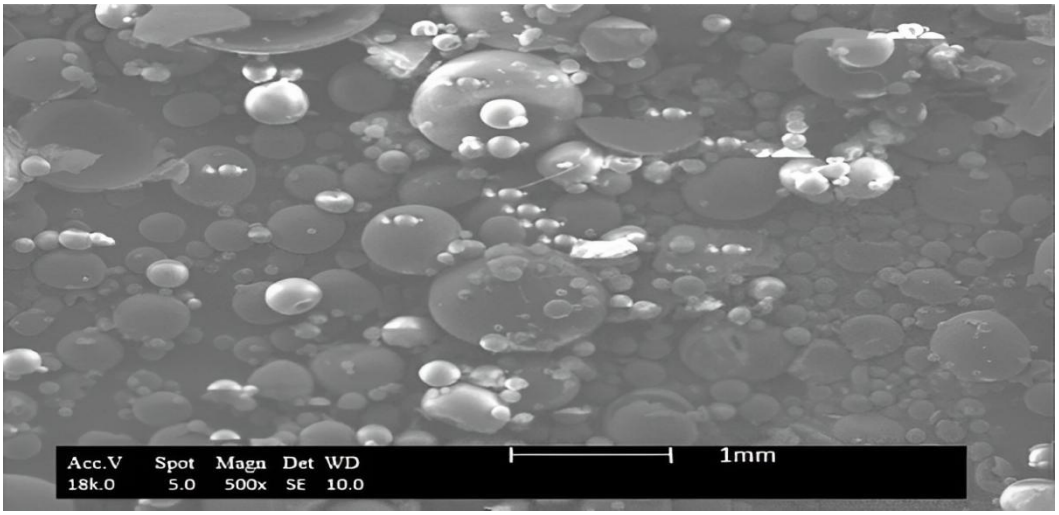


Fig 1.8: SEM

TEM Evaluation:

A beam of electrons is sent through a specimen during transmission electron microscopy in order to create a picture. Most frequently, the specimen is a suspension on a grid or an ultrathin slice that is less than 100 nm thick.

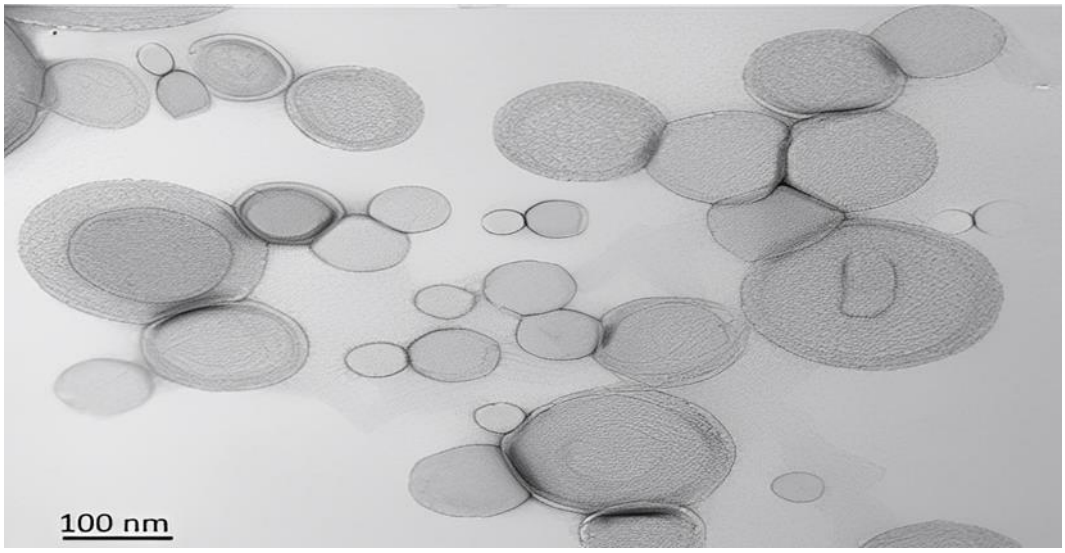


Fig 1.9 TEM

Drug Release IN VITRO Loaded Liposomes With Dapsone

The decay profile of details F1 to F6 at 2 hours (30.6-67.42%) was not very high due to medication discharge. All definitions show that the F9 drug discharge exhibits a perfect needs control discharge profile. During the first two hours of administration, 32.48% of the medicine was present, according to the F9 side effect. 61.1% of the medication was administered during the first 4 hours, while the remaining portion was administered during the next 4 hours. The least amount of a regulated drug dump is displayed by F7, nevertheless. The F7 tuft was therefore passed over for further analysis.

Time (H)	Cumulative percentage of drug release (F)								
	1	2	3	4	5	6	7	8	9
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	4.34	4.40	3.56	4.00	5.24	4.80	3.46	3.09	3.45
30	9.36	10.80	8.60	8.75	10.21	7.80	6.00	5.64	6.75
45	12.03	12.81	10.67	12.50	15.45	12.80	9.64	9.09	8.63
1	18.05	19.61	15.71	17.26	19.59	19.80	13.46	14.00	12.71
2	23.73	27.21	20.15	24.76	26.49	26.40	18.00	18.37	15.69
3	30.08	39.62	26.08	35.76	38.08	34.60	23.28	22.01	20.71
4	36.76	44.02	37.64	44.01	41.94	42.20	34.37	25.28	26.52
5	43.78	52.82	45.35	50.02	46.63	52.20	39.10	27.46	34.67
6	56.14	60.42	58.98	62.77	58.22	62.00	48.38	36.56	46.91
7	66.83	71.63	69.95	72.27	64.57	70.40	53.29	46.56	56.64

Table 1.9

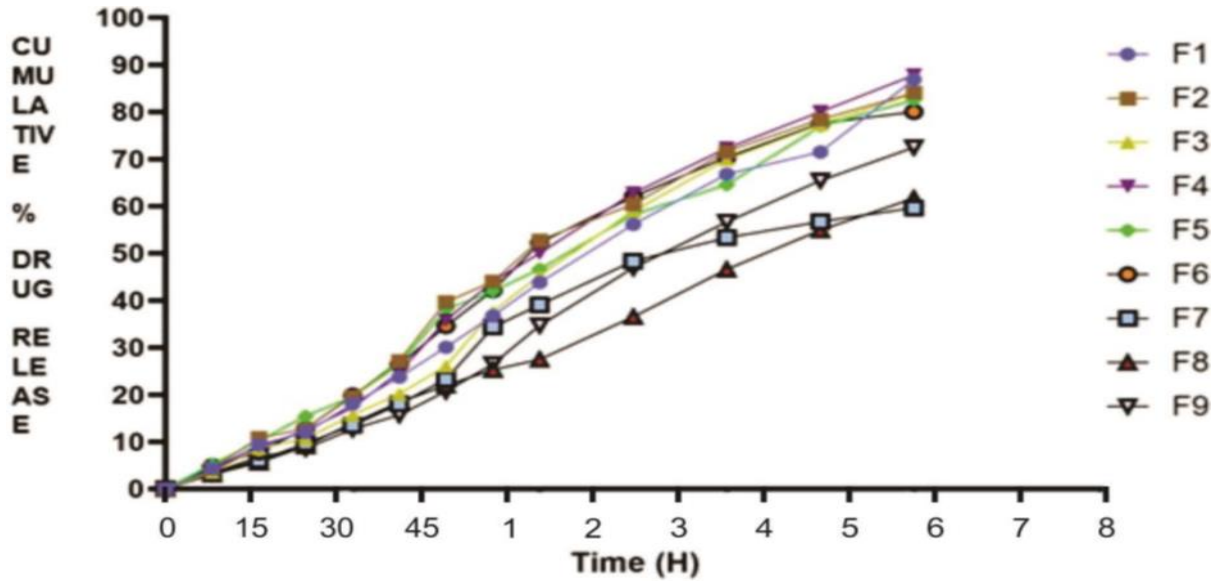


Fig. 2

Liposomal gel's physical analysis

Examining Dapsone's liposomal gel formulation

characteristics of organoleptic= light yellow to colourless

smell is a quality

appearance. = Smooth

Gel base and liposomal gel pH determination:

The average of three measurements of pH was recorded. It was determined that the Ph of Dapsone gel was 7.38.

Viscosity

Brookfield viscometer used to measure of the formulation. 478891.13 centipoise of viscosity was measured. In order to be stable and to apply, viscosity is crucial.

Spreadbility

Two 20 cm2 horizontal plates, the upper of which weighted 46.36 g and had a 200 g weight on top of it, were placed between gels that had been in contact with air for 24 hours. 5 mms into a 5 mm circle was used to measure the gel's diameter.

Homogeneity and grittiness

Liposomal gel was found to be homogenous and no grittiness was observed Smoothly gel.

physical appearance
It was found that optimised gel held for three months at 40°C, 25°C, and 40°C temperature settings showed no change in their physical appearance.

Phase separation
Phase separation was not present in the optimised liposomal gel.

Dapsone-loaded liposomal gel in-vitro drug release:
The drug release was assessed using a Franze diffusion cell and a cellophane membrane.

Studies on stability
Stability studies were performed over two months at 0 and 25 degrees Celsius. The drug retention behavior of the generated liposomal gel was assessed by monitoring vesicle size and drug concentration at these temperatures.

Time in Days	0°C	25°C	55°C
Percentage Drug leakage	Percentage Drug leakage	Percentage Drug leakage	
0	-	-	-
7	1.05	1.23	.89
15	1.33	2.53	1.49
30	2.79	3.26	2.09
45	2.88	3.26	2.64
60	3.45	7.67	3.01

Table 2: Stability parameters of all formulations

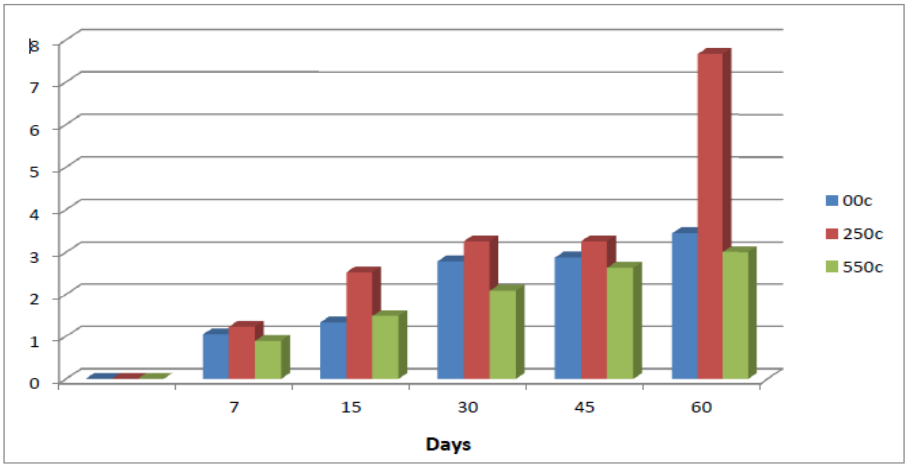
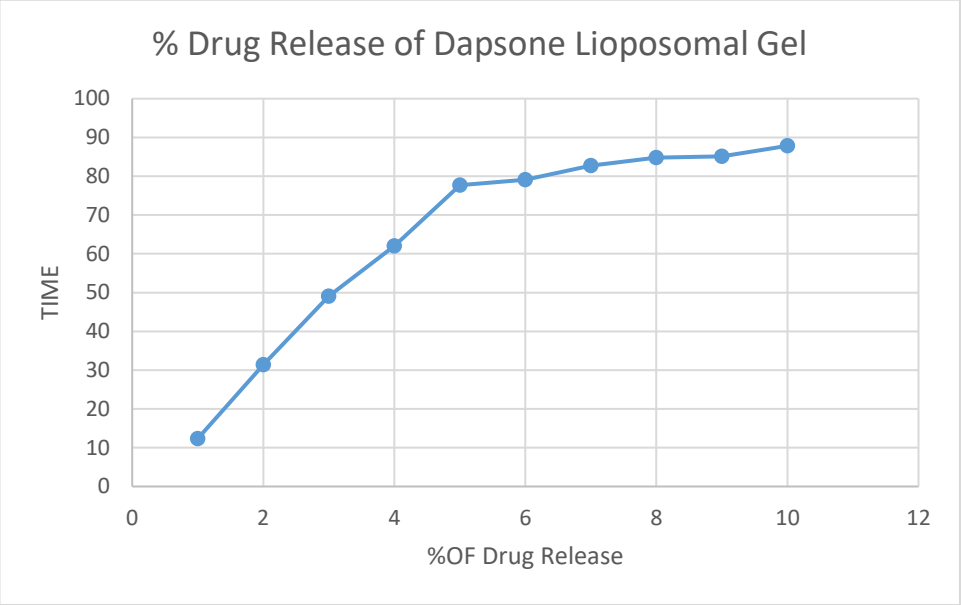


Fig 2.1 Graph: Stability parameters of all formulations

Dapsone-loaded liposomal gel in-vitro drug release:
The drug release was assessed using a Franze diffusion cell and a egg membrane membrane.

Time	% Cumulative drug release
0	0.00
15	12.34
30	31.36
45	49.03
1	62.05
2	77.73
3	79.08
4	82.76
5	84.78
6	85.14
7	87.83



Conclusion: -

Drugs were used in the development of the dapsone liposomes gel to enhance therapy, reduce toxicity, and boost therapeutic efficacy. In a number of fields, such as cancer therapy, immunology, ophthalmology, infectious illness, and immunology, liposomes are being employed more frequently. As a result of a variety of discoveries, targeting liposomes has led to medication accumulation at disease sites and decreased delivery to sensitive tissues. Stealth liposomes are a novel type of liposome that have been applied in a number of medical procedures. Due to their ability to circulate in the body for extended periods of time, liposomes are being used in medicine on a larger scale. If the aforementioned applications and hypotheses will be successful, only time will tell. But we may say that liposomes have a good track record.

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