FORMULATION, DEVELOPMENT AND CHARACTERIZATION OF LIPOSOMAL GEL OF RESVERATROL

Km Kavita Gupta, Dr. Sachin Kumar

NKBR College of Pharmacy and Research Centre, Meerut-Hapur Road, Phaphunda, Meerut-245206 Uttar Pradesh

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Abstract

The development and characterization of a liposomal drug delivery system for resveratrol was the main goal of this investigation. Resveratrol's incorporation into liposomes considerably enhances their qualities. In vitro studies of liposome-encapsulated Resveratrol have mostly focused on the assessment of a better way of Resveratrol delivery, despite the many benefits of employing liposomes as carriers to transport the medicine over the free form of the substance. High levels of drug entrapment, vesicle size, and drug release are seen in liposomes. The Resveratrol-loaded liposome was formulated in this work using the hand-shaking method. In order to get a satisfactory gel viscosity and accuracy, the pH for the liposomal gels was discovered to be 6.2.When using the hand shaking method, the average particle size, percent drug entrapment, and final drug release were determined to be 303-600, 70.15%, and 58.89%, respectively, at 5 hours. Swelling index of resveratrol gel was found to be in range of 42-50% respectively. Skin irritancy test was found that there was no redness, no irritation or dryness found on skin. As a result, as compared to pure drug, the hand shaking method was better for formulated Resveratrol liposomes due to stability and drug entrapment efficiency.

Keywords: liposomes, Skin, Resveratrol, entrapment efficiency

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INTRODUCTION

Drug delivery System act as a medium or service for management of medicine through numerous routes inside the body for the purpose of improving health or the system refers to transporting a pharmaceutical compound in the body as had to appropriately gain its favored healing effect called as drug delivery system **(Maiti, 2022).**

Liposomes are lipoidal vesicles with a lipid bilayer that act as drug carriers to enhance the absorption of pharmacological drugs. Oil-in-water (O/W) the emulsion with mean droplet dimensions between 50 and 1000 nm are known as nanoemulsions. A non-ionic, surfactant-based vesicle is called a niosome. Niosomes have a greater capacity for penetration. Drugs that are hydrophilic and lipophilic can be trapped by noisome, either in a layer of liquid or in a vesicle membrane made of lipid material.

In the beginning, an organic solvent is used to dissolve and sort the lipids. The mixture of methanol and chloroform (chloroform:methanol) will be used to carry out this procedure. The main goal is to provide a transparent lipid solution for complete lipid mixing. If the lipid is fully combined with an organic solvent, the solvent is released, resulting in the formation of a lipid film.

Small quantities of organic solvent (< 1 mL) can be evaporated in a fume hood utilising a dry nitrogen or the argon stream. A thin lipid layer should be produced on the edges of a round bottom flasks by rotating evaporation to remove the organic solvent from greater volumes **(Sackmann, 1982**). A lipid solution that has been dissolved in ether-methanol or diethyl ether is gradually added to an aquatic solution of the substance that has been enclosed at a temperature between 55 and 65 degrees Celsius or under condensed pressure. The creation of liposomes results from the elimination of ether when under vacuum. Detergents at critical a micelle concentration (CMC) is employed to solubilize the lipid. The micelles get progressively better at phospholipid as the detergents is separated, and they eventually combine to form LUVs. By using dialysis, the detergents were separated. The dialyses are carried out in dialysis bags. **(Kaes, 1991).**
Manufacture of nanoparticles, such as liposomes, nano-emulsions, nano-crystals etc.Take away dissolved gases from liquids (degassing) by sonicating the liquid while it is under a vaccum. very poor encapsulation effectiveness and interior volume, Potential degradation of the chemicals to be encapsulated and phospholipids big molecules are removed, the presence of MLV and SUV. (70 to 200 nm) The molecule is heterogeneous. The assertion that certain substances should be heated organic solvents. Even partially depleted detergent with extremely low CMC can be removed by detergent adsorbers. Colloidal carriers have drawn the most attention because they provide potential methods for delivering drugs through the skin. These systems can meet the requirements for crucial supplies such non toxicity, enough drug loading capacity, drug targeting potential, controlled release functionality, and physical and chemical storage durability. All colloidal systems share a submicron particle size as a common property. Strength of the carriers in terms of composition, materials, range of applications, and drug loading. Phospholipids are the main structural mechanism of the biological membranes in the human body, they exist in two forms i.e. sphingolipids and phosphodiglycerides, in combination with their corresponding hydrolysis products.

To encourage the drug transport across the skin barrier is termed as dispersion enhancers. Penetration enhancers caused the amount of water molecules that were free between the bilayer to rise, which enhanced the cross-section for the diffusion of polar drugs. **(Songkro, 2009**)
Through the transepidermal route, chemicals diffuse over the skin. Transepidermal penetration can occur intracellularly or intercellularly. Hydrophilic or polar solutes can be transferred intracellularly from side to side corneocytes, which are terminally differentiated keratinocytes. Lipophilic or non-polar substances can be dispersed through the continuous lipid matrix by transport via intercellular gaps. The transappendegeal pathway involves molecules travelling via hair follicles and sweat glands (**Kalia and Guy, 2001).** Various drug absorption routes are as follows.

Solvated polymer (lipophilic colloids) undergo gelation as a result of temperature changes. Hydrogen atoms are more soluble in hot water than in cold. Condensed temperatures result in condensed levels of hydration, which leads to gelation. For example: cellulose derivatives, gelatin, agar sodium and oleate, gummed, etc **.**

The gelation in this case is produced by adding precisely the right quantity of salt to achieve full precipitation. Make sure to combine quickly to prevent localised excessive precipitant concentration. For example: Ethyl cellulose, polystyrene, and benzene solutions may be quickly combined with the right amounts of a non-solvent and petroleum ether to form gels. It is not always

clear what happens when sodium are added to a hydrophobic solution in terms of coagulation and gelation. The gels created using the flocculation process behave in a thixotropic way. High attention of electrolytes can best have an effect on the hydrophilic colloids consisting of gelatin, proteins and acacia. By using the chemical relationship between the solvent and the solute, a gel is created in this method. For instance: The interaction of a aluminium salt with a sodium carbonate in an aqueous solution may be used to create aluminium hydroxide gel, and more attention to the reactants will result in the formation of a gel.

Method

Prior to calculating a partition coefficient, shaking on the experiment's temperature saturated the solvent system's levels. Highly pure analytical grade n-octanol & water were combined in a separating funnel at a 1:1 ratio in order to accomplish that. Then, the drug was added to one of the sections in a minimum amount (no more than 0.01 mol/liter), and the funnel was once more shaken for 30 minutes so as to allow for complete blending. After that, the funnel was allowed to stand for twenty-four hours to expand the phases that were saturated with one another, and after that, the amount of drug in every phase (n-octanol and water) was measured. The unionised compound concentration ratio between the two solutions is known as the partition coefficient. The pH of the water phase is adjusted so that the most common form of the chemical is un-ionized in order to evaluate the coefficient of partition of ionisable solutes. Log P is the logarithmic of the proportion of the unionised solute concentrations inside the solvents. **(Aulton, 2007).**

$$\log P_{oct\,/\,wat} = \log\left(\frac{[\text{solute}]\ \text{octanol}}{[\text{solute}]\text{un}-\text{ionized water}}\right)$$

Drug	Standard	Observed
Resveratrol	3.08	3.05

Table 1: Showing partition coefficient of Resveratrol

Determination of λ_{max} of Resveratrol:

A UV Spectrophotometric investigation will be conducted to determine the maximum amount of resveratrol in phosphate- buffer (pH 7.4). A common drug stock was created by dissolving 100 mg of the substance in a volumetric flask, and the volume was increased to 100 ml by adding a buffer of phosphate to get a resveratrol concentration of 1000 g/ml. 1 ml of the standard stock solution was pipette out into a 100 ml volumetric flask, and 100 ml of phosphate buffer were added to reach the desired concentration of 10 g/ml. 1 ml of this solution was pipette out into a 10 ml volumetric flask, and 10 ml of phosphate buffer was added to the mixture to reach an awareness of 1 g/ml. By using a UV-VIS spectrophotometer to scan the solution (1 g/ml) inside a wavelength range of 200 nm to 400 nm, the maximum wavelength (max) was obtained. Resveratrol's maximum absorbance was found to sit at 303 nm.

FTIR spectroscopy:

The structure of an intricate may be determined with sufficient accuracy using FTIR spectra, which serve as the master record. This approach produces a spectrum with a lot of absorption bands, which

disclose structural information, as opposed to the UV spectrum, which has relatively few peaks. FTIR spectrometer was used to get the pure drug's FTIR spectra.

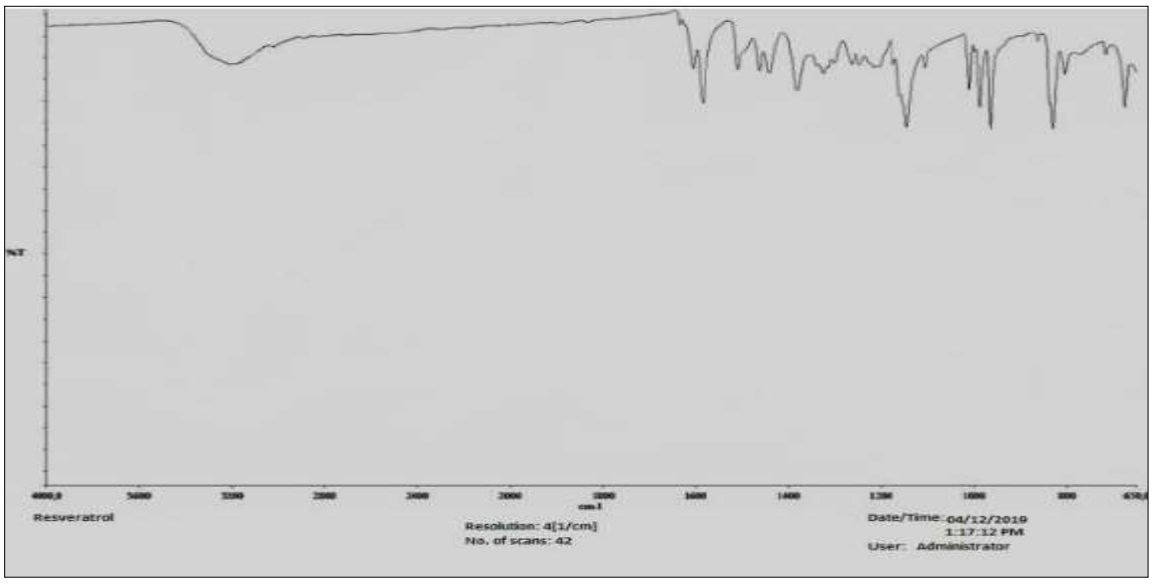


Figure 1: FTIR of Resveratrol (Standard)

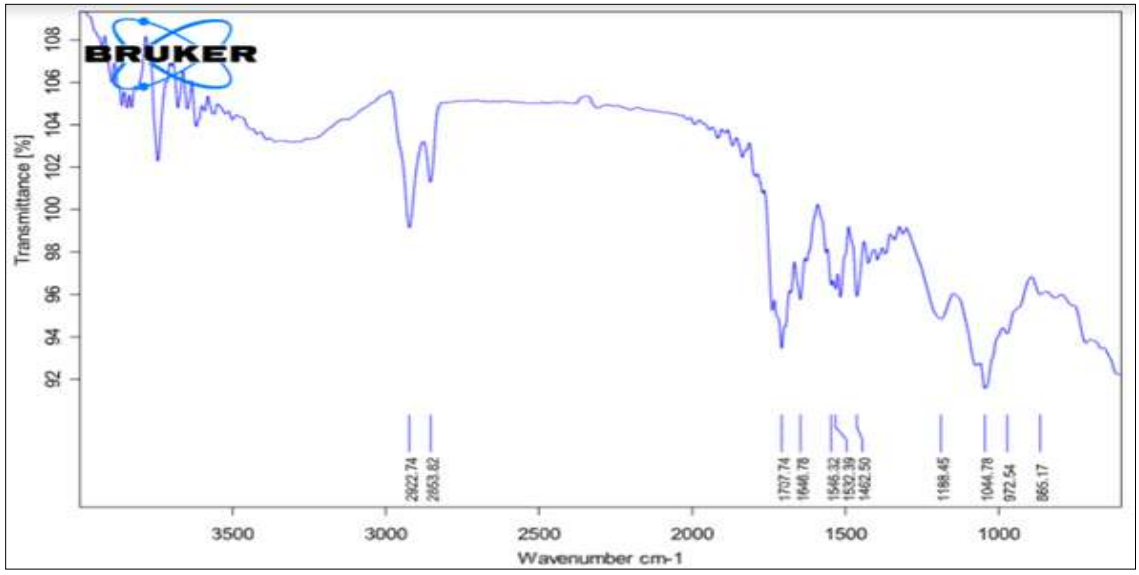
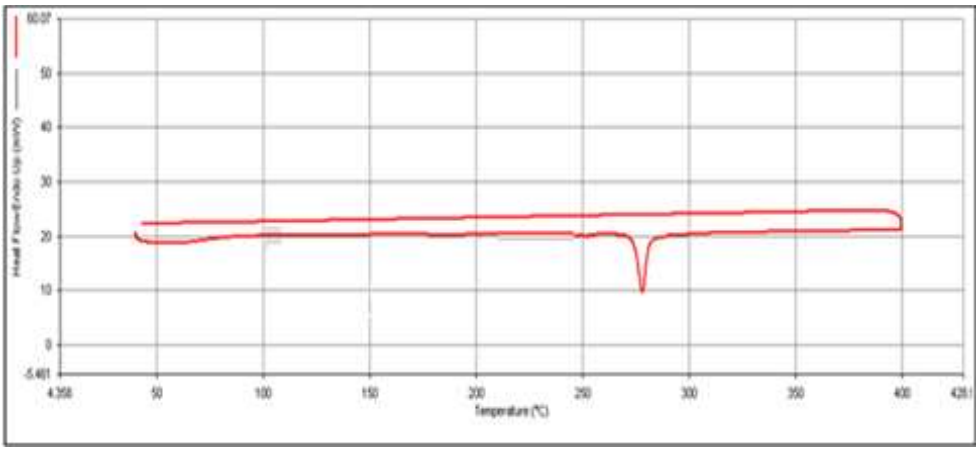
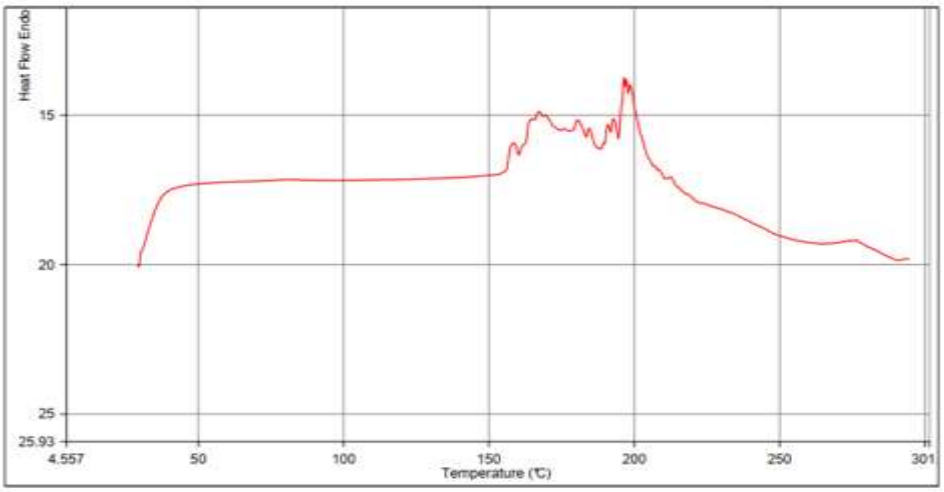


Figure 2: FTIR of Resveratrol (Sample)

Drug-Excipient Compatibility Study: - With the use of a differential scanning calorimeter, thermograms were captured. Weighed samples (5–10 mg) were placed in flat-bottomed aluminium pans and hermetically sealed. With alumina serving as the reference standard, these samples were subjected to heating between 50 and 400 ℃ at a steady rate of 100 ℃ per minute in an environment of nitrogen (200 ml/min).



(a)



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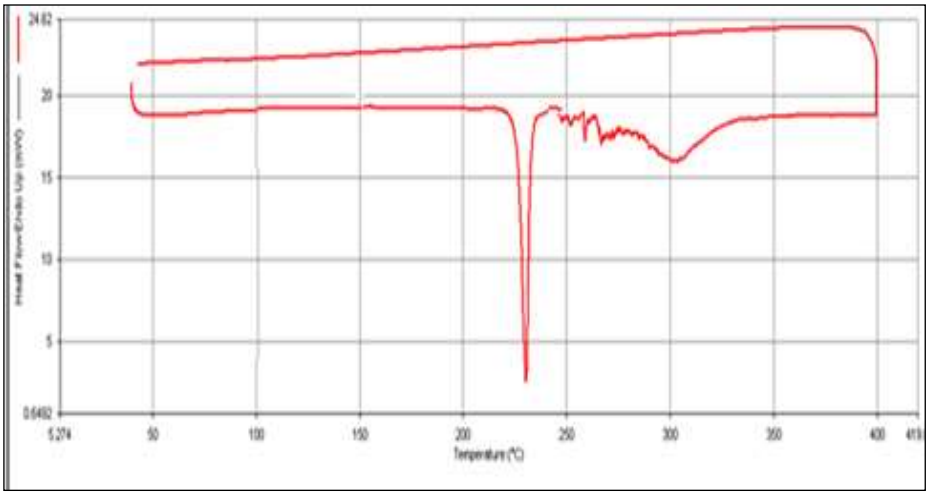

(c)

Figure 3: DSC thermogram of (a) pure drug, (b) PC, (c) drug + PC

Drug-Excipient compatibility study: Resveratrol's DSC thermogram showed a melting point of 2610 C. The medication and cholesterol combination that was exposed to a 30-day DSC study under

accelerated conditions of 400C and 75% RH. Resveratrol's typical melting point does not differ from 2610C, which indicates that there won't be any drug–PC interactions.

RESULT AND DISCUSSION

Preparation of standard curve: Preparation of PBS (pH 7.4):-

Prepare 1000ml of solution by dissolving 2.38g of disodium n hydrogen phosphate, 0.19g for dihydrogen potassium phosphate, and 8.0g of chloride of sodium in enough water.If required, change the Ph (IP, 2007).

Preparation of Resveratrol Standard Curve Using PBS :- In a 100 ml volume flask, 100 mg of merlot were dissolved in 100 ml of phosphate buffered saline pH 7.4. This principal stock solution had a 1000 g/ml concentration. From this initial stock solution, 10 ml was carefully pipetted out and placed in to a volumetric flask measuring 100 ml. The volume was then filled to 100 ml with 100 g/ml of phosphate buffer, pH 7.4, which was used to make up the difference. From the second stock solution, 1 ml of the mixture was removed and diluted up to 10 ml with phosphate buffered saline pH 7.4 in a succession of 1 ml to 5 ml volumetric flasks to create 5 to 30 g/ml concentration. Using a UV-visible double beam spectrophotometer, the absorption of these solutions was computed using the phosphate buffer 7.4 as a blank at a maximum wavelength of 303 nm. The concentration was then plotted on the Y-axis using a calibration curve, with absorbance measured in µg/ml.

S. No.	Concentration (µg/ ml)	Absorbance
1	5	0.081
2	10	0.153
3	15	0.216
4	20	0.289
5	25	0.374
6	30	0.451

Table 2: Absorbance of resveratrol with PBS (PH 7.4)

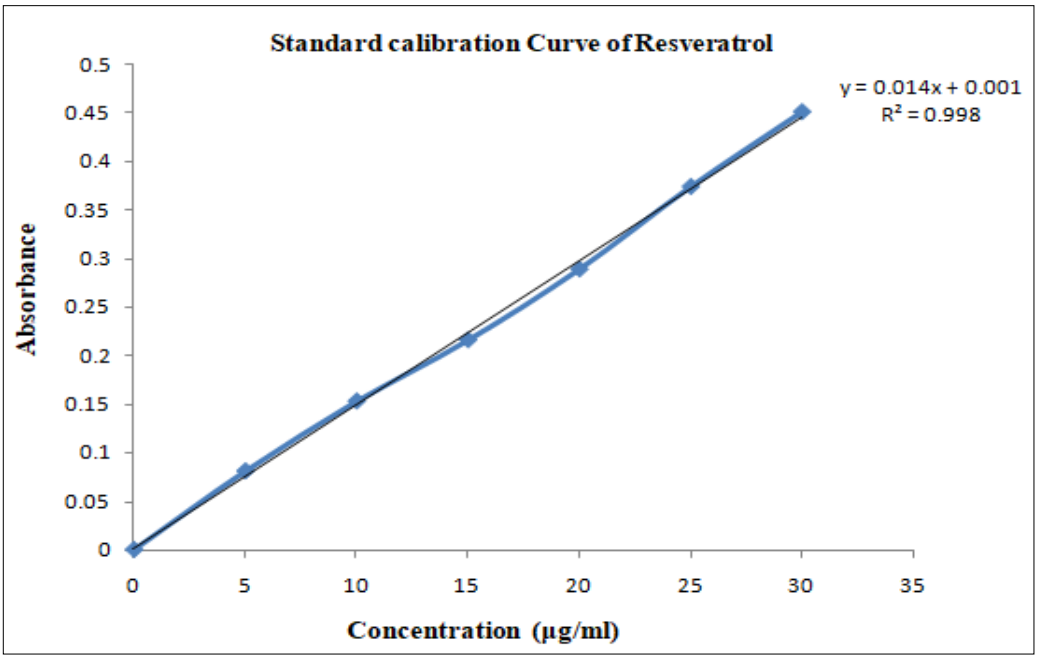

Figure 4 : Standard Calibration curve of Resveratrol with PBS (pH 7.4)

Preparation of liposome-

In an organic solvent, the (Chloroform: methanol)combination disintegrated and became muddled. The main objective is to create a clear lipid solutions for complete lipid mixing. If the lipid and organic solvent are well combined, the solvent is then removed, leaving behind a lipid film. Small amounts of natural solvent (less than 1 mL) can be evaporated in a fuming hood by using dried nitrogen or argon flow. Organic solvents must be completely removed from greater quantities by rotary evaporation, leaving a thin lipid coating around the flask's perimeter.

With the use of a vacuum pump, the film of lipids is thoroughly dried to eliminate any remaining organic solvent in a single day. The lipid(s) can also be dissolved in tertiary butanol or cyclohexane if using chloroform is unacceptable. Using either a dry ice block or a dry ice-acetone or alcoholic (ethanol or methanol) bath, the lipid solution gets transported to containers and frozen. When using the bath technique, care must be taken to ensure that the container being used can withstand unexpected temperature changes without splitting. The frozen lipid cake is placed on a vacuum pump after being completely frozen and is then lyophilized for one to three days, depending on the quantity. The lipid cake's thickness must not be more than the box's diameter, which is utilised for lyophilization.

Preliminary trial:

Firstly several batches of blank (without drug) liposomes were prepared using diverse polymer cholesterol, soya lecithin and also chloroform, methanol ratio. Keeping the ratio of chloroform and methanol altering but the method of preparation was similar for the formation of liposomes. The obtained batch in which liposomes created was then selected for incorporation of drug.

Formulation-

S. No.	Batch No.	Chloroform + Methanol (Ratio)	Result

1	A1	1:2	Liposomes were not formed
2	A2	1:3	Liposomes were not formed
3	**A3**	**2:1**	**Liposomes were formed**
4	A4	3:2	Liposomes were not formed
5	A5	3:9	Liposomes were not formed
6	A6	6:18	Liposomes were not formed

Table 3 : Formation of liposomes

Incorporation of drug in liposome:-

S. No.	**Batch no.**	**Chloroform + Methanol (Ratio)**	**Amount of drug Resveratrol (mg)**	**Result**
1	DL1	1:2	100	Liposomes were not formed
2	DL2	1:3	100	Liposomes were not formed
3	**DL3**	**2:1**	**100**	**Liposomes were formed**
4	DL4	3:2	100	Liposomes were not formed
5	DL5	3:9	100	Liposomes were not formed

Table 4: Drug loading into the liposome

Drug entrapment efficiency (DEE %):-

This is a quantity of drug loading that was estimated by the percentage of drug entrapment that was present in the formulation. . Formulation of each drugs were centrifuged and the supernatant was collected and after appropriate dilution with the solvent the sample was assayed for resveratrol and via UV spectrophotometry. Percentage of drug entrapped was calculated with the help of the formula given below **(PF Yue, 2009).**

$$\% \text{ Entrapment efficiency} = (\text{Entrapped drug}/\text{Total drug added}) \times 100$$

SEM (scanning electron microscope) -

Scanning electron microscopy was used to assess the surface morphology of the selected liposomal formulation.

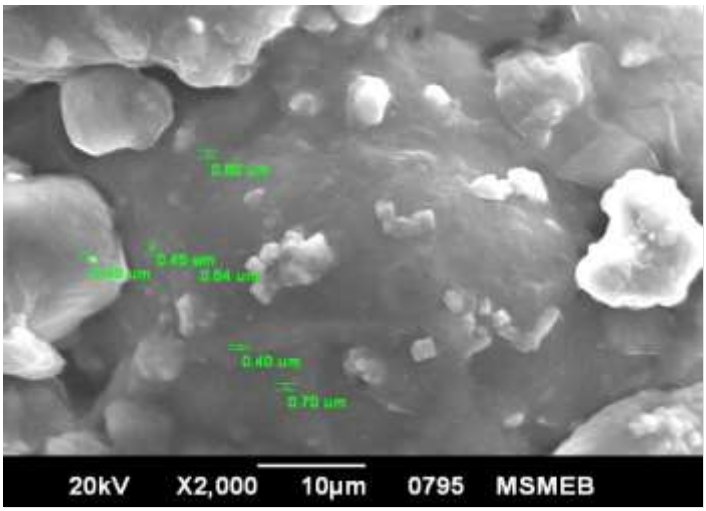

Figure 5: Scanning electron microscopy of drug loaded liposomal formulation

Drug release-

10gm of resveratrol liposomal formulation was kept in tube; last part of the tube was covered by membrane. The receptor compartment, which held 100 ml of PBS pH 7.4, came into touch with the whole membrane's surface. Using a magnetic stirrer, the receptor segment was continually stirred (100 rpm). 37 1°C was the sustained temperature. The research had a 5-hour approval period, with 10-minute, 20-minute, 30-minute, 1-hour, 2-hour, 3-hour, 4-hour, and 5-hour breaks. The sample was held for a set amount of time, and the same volume got replaced with brand-new 1 ml of phosphate buffer.

Time(Min.)	**% Drug release**
10	8.12
20	17.35
30	19.65
60	32.45
120	37.98
180	42.90
240	48.10
300	60.20

Table 5: Percentage drug release vs time of liposome of resveratrol

n=3 Average of three determinations

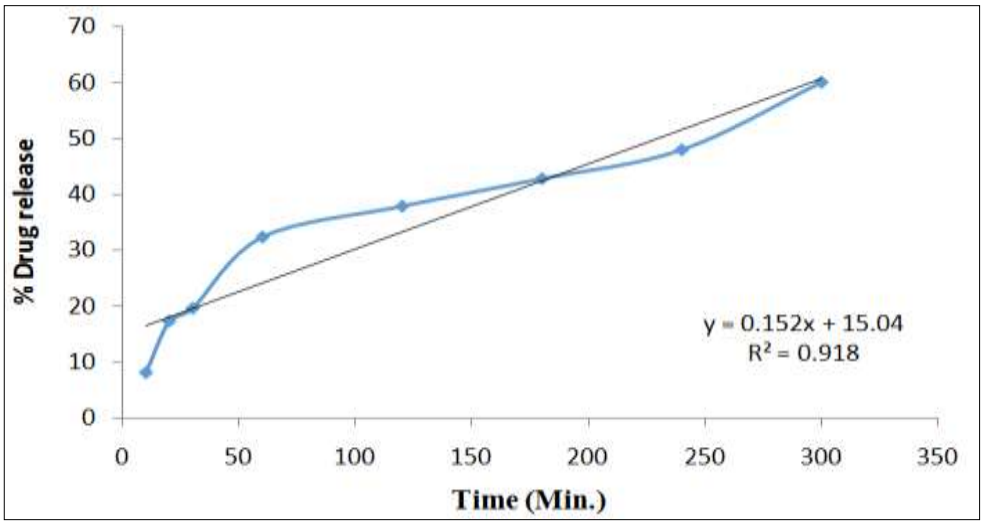

Figure 6:% drug release vs time of liposome

Formulation, developmental and evaluation of liposomal gel of resveratrol:-

S.No.	Ingredients	Quantity(gram)
1	Resveratrol	2.50
2	Carbopol-934	1.00
3	Carboxy methyl cellulose	1.00
4	Methyl paraben	0.02
5	Propyl paraben	0.02
6	Triethnolamine	0.20

Table 6: Preparation of liposomal gel of resveratrol

Development of gel

Accurately measured a one-gram of carbopol-934 was added to 60 ml of hot water while being repeatedly swirled to achieve a thick consistency. 30 cc of warm water, carboxy dimethyl cellulose, methyl paraben, and propyl paraben were added to a separate container and continuously agitated to create a firm gel. After 6 hour both of these were mixed and resveratrol was added and final volume was ready with water. Triethnolamine was added at the ending for pH adjustment.

Liposomal gel evaluation

pH

Using equipment called a digital pH metre, the pH of the formulation was determined.

Skin irritancy test

The test is carried out using a patch test. The test ingredients were placed to a 6 cm2 area of skin and coated with a gauze patch. For 4 hours, the gauze patch was loosely kept in place by a semi-occlusive dressing before being removed. An hour after the patch was removed, the observation was made. A good hydrogel base should not display any apparent response, such as erythema or strong erythema with edoema and vesicular erosion **(Das *et al.*, 2010).**

Spreadability-

There were two sets of slides that were made of glass and had the usual sizes. On one of the slides, the gel was situated. The gel was positioned between the two slides, separated by a space of 7.5 cm along the slides, with the second slide being positioned on top of the gel. Gel weighing 100 g was placed on the upper slides, where it was regularly pushed to produce a thin layer between the two slides. The 50 grammes weight was taken off, and the gel stuck to the slide was scraped off. The two slides in that place were fastened to a platform in such a way that only the top slides could be removed freely by forces of weights that was tied to it. A weight of 20g was applied to the higher slide, and it was known how long it took for the upper slides travel 7.5 cm and separate from the lower slide as a result of the weight. Three times the experiment was run, with the mean time being used for computation **(Jackson Roberto *et al.*, 2016).**

Utilising the following formula, spreadability was determined:

S= m × l where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in sec.

Swelling index-

The ready hydro gels' water absorption was assessed using the teabag technique. A teabag containing 0.05 gm of the sample being studied was placed in an appropriate volume of distilled water and kept at room temperature 25 ℃ for 30 minutes. The tea bag used to collect the sample is then removed from the water and left alone for about a minute to drain any extra water. The enlarged samples' weights were noted. **(Du Plessis J, *al.*, 1994).**

The change in mass before and after swelled was used to calculate the hydrogels' water absorption using the following equation:

$$\%\ \mathbf{Swellingindex} = \frac{\boldsymbol{W2-W1}}{\mathbf{W2}} \times \mathbf{100}$$

Where, W2- weight of the sample after absorption

W1- weight of the sample before absorption

Viscosity

The viscometer's spindle speed was set to 20 rpm and a viscometer made by Brookfield (S-62, model LVDVE) was used to measure the viscosity of the gel.

In-vitro Drug Diffusion Study:- The goal of the current investigation was to ascertain how the formulation's medication would release. Using a cellophane membrane holding 5ml of the selected formulation, an in-vitro drug release research was carried out. The receptor compartment, which contained 100 ml of buffered phosphate, was in touch with the whole membrane's surface. Using a magnetic stirrer, the receptor compartments was continuously stirred (100 rpm). 37± 1°C was the sustained temperature. The research was given for 5 hours at intervals of 10 minutes, 20 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours. At the scheduled time, the sample was removed, and an equal volume of brand-new 1 ml phosphate buffer was substituted. The Sample was spectrophotometrically examined at 303 nm for drug discharge. This allowed for the calculation of the data % drug release and the plotting of the medication release pattern.

S. No.	Parameter	Description
1	Formulation	Liposomal gel
2	Method	Diffusion cell method
3	Dissolution medium	100 ml of Phosphate buffer
4	pH of dissolution medium	7.4
5	Temperature	37˚C(±1)
6	Sampling time interval	10 min
7	Total duration period	5 hours
8	Sample amount	5 ml

Table 7: **various parameters used for the *in vitro* drug release**

TIME (Min.)	% DRUG RELEASE
10	7.39
20	16.85
30	18.89
60	30.25
120	36.56
180	40.25
240	47.32

300	58.89

TABLE 8: % drug release vs time of liposomal gel of resveratrol

n=3 Average of three determinations

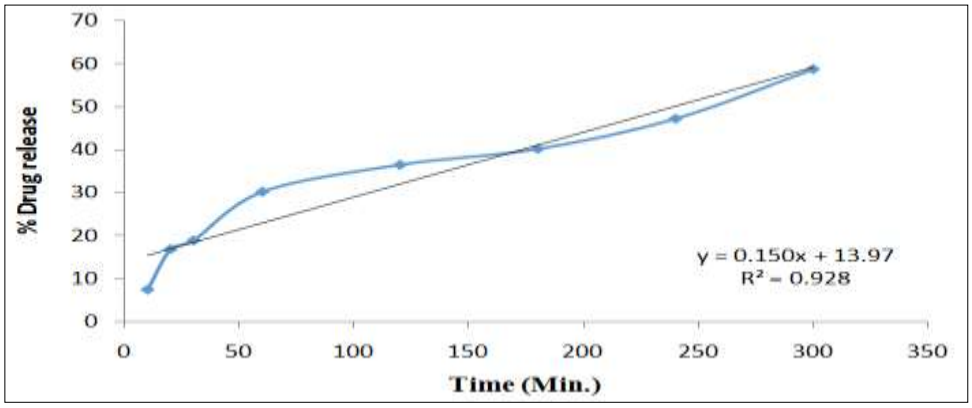

Figure 7: Time vs % drug release of resveratrol liposomal gel

Stability Studies

The liposomal gel was kept in temperate condition 4-80C (Refrigerator) to test the vesicles' capacity to preserve the medication resveratrol. Periodically, samples were taken out and examined for the effectiveness of the drug release and trapping.

DISCUSSION

Preformulation study-

Organoleptic properties:

The sample of Resveratrol was found to be a white to light white powder.

Solubility study-

The identification tests establish that resveratrol complies with the official standards. Solubility of the drug was determined in a variety of solvents at room temperature. Resveratrol was marginally soluble in water and highly soluble in chemical solvents such ethanol, DMSO, dimethyl formamide, and phosphate buffered saline (PBS) at pH 7.4.

Melting point of drug-

Resveratrol's melting point was discovered to be 262-263° C.

Determination of absorption maxima of resveratrol -

On scanning the standard solution of drug over the prescribed range (200-400nm), the absorption maxima of the drug exhibited at λmax 303 nm. All these observation confirmed the authenticity of resveratrol.

Resveratrol standard calibration curve in PBS (pH 7.4)-

The standard curve of resveratrol in PBS (phosphate buffered saline) at pH 7.4 was ready. The caliberation curve were plotted which exhibits good linearity. The medication complies with Beer-Lambert's law in the concentration range of 10–50 g/ml, as indicated by the R2 value of 0.998 in phosphate buffered saline (PBS) at pH 7.4. The approximation procedure was found to be reproducible and acceptably sensitive in the agreed concentration range.

Formulation Development

The preparation of delivery system was completed by hand shaking method.

Preliminary trials

Firstly several batches of blank (without drug) liposomes were ready using diverse polymer cholesterol, soya lecithin and also chloroform, methanol ratio. Maintenance the ratio of chloroform and methanol changing but the technique of preparation was similar for the formation of liposomes. The obtained batch, (A3) in which liposomes created was then selected for incorporation of drug. DL3 batch was incorporated with drug in which drug loaded liposomes were created.

Evaluation of liposomes

Efficacy of Liposome Drug Entrapment

Liposomal formulation's entrapment competence was discovered to be 70.15%.

Drug release-

In skin penetration studies, it was observed that in liposomal formulation highest amount of drug permeated via skin was 60.20% at 5 hours but in liposomal gel formulation maximum amount of drug permeated by skin was 58.89% at 5 hours.

Evaluation of liposomal gel

PH

In order to get a satisfactory gel viscosity and accuracy, the pH for the liposomal gels was discovered to be 6.2.

Appearance

Homogenous, transparent and colorless.

Swelling index

Swelling index of resveratrol gel was found to be in range of 42-50%.

Spreadability

The spreadability of resveratrol liposomal gel was high by having a low spread of time.

Skin irritancy

There was no redness, no irritation or dryness found on skin.

Viscosity

21402 was the viscosity of the gel as measured by a Brookfield viscometer (S-62, models LVDVE) at 250°C and 20 rpm spindle speed.

CONCLUSION

It was discovered that creating resveratrol-containing liposomes and dispersing them as carbopolgel was a sensible and effective way to produce stable liposomal formulation. The amount of phospholipid and stabiliser in a liposomal formulation, as well as other factors, have a significant impact on the size of the vesicle and the effectiveness of entrapment. The concentration of carbopol needed was clearly determined by rheological investigations of all liposomal gels made with 1% w/w carbopol. In comparison to controls, lipid dispersion and gels have been observed to enhance skin penetration and deposition. The conclusion drawn from the data is that resveratrol-containing liposomal gel may be used to treat skin conditions. In order to get a satisfactory gel viscosity and accuracy, the pH for the liposomal gels was discovered to be 6.2.When using the hand shaking method, the average particle size, percent drug entrapment, and final drug release were determined to

be 303-600, 70.15%, and 58.89%, respectively, at 5 hours. Swelling index of resveratrol gel was found to be in range of 42-50% respectively. Skin irritancy test was found that there was no redness, no irritation or dryness found on skin. As a result, as compared to pure drug, the hand shaking method was better for formulated Resveratrol liposomes due to stability and drug entrapment efficiency.

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