Development and Characterization of Resveratrol Niosomal Drug Delivery System

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ABSTRACT
The present study was focused on formulating and evaluating Resveratrol containing niosomes formulation for in vitro studies. Niosomal formulations were prepared by using different ratio of surfactant (Tween 40 and Tween 80) and cholesterol by thin film hydration method and were evaluated for in vitro characteristics, stability studies. Tween 80 containing niosomal formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that niosomal formulation was spherical in shape. Niosomes containing tween 80 displayed higher percentage of drug release after 8h as compared to other formulations.F6 formulation was found to be stable at the end of the study on storage condition. The present study suggested that niosomal formulations provide sustained and prolonged delivery of drug with enhance bioavailability.
Keywords: Niosomes, Resveratrol, bioavailability, thin film hydration technique, in vitro drug release studies.

I.INTRODUCTION

Nano carriers are an effective material for encapsulating phenolic compounds and increasing their bioavailability. [1] Niosomes are one of the best among these carriers. The self-assembly of non-ionic surfactants into vesicles. [2] Niosomes (non-ionic surfactant vesicles) obtained on hydration are microscopic lamellar structures formed upon combining non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class with cholesterol. [3] Resveratrol (3, 5, 4′-Trihydroxystilbene) is found in grapes and wine. This stilbene-structured polyphenol has anti-inflammatory, antioxidant, anticancer, and immunomodulatory properties.[4] Resveratrol has lipophilic properties, resulting in high absorption in humans but low bioavailability when taken orally.[5] The purpose of this research was to determine the ability of niosomes to increase the bioavailability of resveratrol through in vitro method. Further in this study the particle size, and shape, entrapment efficiency, of the niosomes were studied.

II. MATERIALS

Resveratrolwas obtained from Hetero Lab, HYD. Cholesterol and Tween 40, 80were procured fromSynpharma Research Labs, Hyderabad, and other chemicals, and the reagents used were of analytical grade.

METHODOLOGY

Fourier Transform Infrared Spectroscopy (FTIR) study

FTIR is a useful technique to check and confirm any interaction that may occur between excipients and drug. The FTIR spectra of drug, excipients, briefly, solid sample (1 mg) along with 100 mg dried potassium bromide was compressed into a disc. For liquid sample, few drops of the sample were dripped onto NaCl or KBr aperture plate and sandwiched it under another aperture plate, such that no gas bubbles were trapped. The sample allowed formation of a thin liquid membrane between the two aperture plates. Thereafter, sample was scanned for absorbance over the range from 4000 to 400 (cm-1) wave numbers. The obtained spectrum was then compared with standard group frequencies of Resveratrol.[6]

Preparation of Niosomes

Niosome preparation: The Nano sized vesicles were prepared using Thin Film Hydration Method. The specified quantities of cholesterol, non-ionic surfactant were completely dissolved in 10 ml chloroform contained in a clean and dry Round Bottom Flask. The transparent solution was reduced to a thin dry film using Rotary Vacuum Evaporator (Perfit, India) at 50±2.00°C. Drug was dissolved in phosphate buffer pH 7.4 and the thin dry film was hydrated using this buffered drug solution. The film is allowed to hydrate for about 1 hour for the formation of niosomes. Milky dispersion is prepared which is kept at 4°C for 24 hours for maturation of the formed vesicles.[7]

Table-1: Composition of Niosomal Resveratrol (F1 to F8)

S.No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8

1	Resveratrol(mg)	250	250	250	250	250	250	250	250
2	Cholesterol(mg)	250	250	250	250	250	250	250	250
3	Tween 40(mg)	50	75	100	-	-	-	50	75
4	Tween 80(mg)	-	-		50	75	100	50	75
5	Chloroform (ml)	5	5	5	5	5	5	5	5

Evaluation of Niosomes

Zeta-potential:

The ZP of niosomal dispersions corresponds to the sum of surface charges, which is dependent on the components used in the formulation. Analysis of the surface charge of the niosomes is vital to estimate the stability of the technology long-term Niosomes with a large negative or positive charge exhibit negligible aggregation in a dispersion compared to neutral and slightly charged niosomes. This is attributed to the lack of electrostatic repulsive forces in neutral dispersions. In the measurement of ZP is used to estimate the changes in intensity of the scattered light due to the mobility of niosomes as a result of the impact of the electric field applied on particle charges. The charge on the surface of niosomes governs mobility, which changes the intensity of the scattered light.[8]

Size and size distribution:

Size and size distribution studies were done for niosomes prepared from Niosomes hydration. The Niosomes (100 mg) was hydrated in a small glass test tube using 10 ml of pH 7.4 phosphate buffer solution. The dispersion was observed under optical microscope at 40X magnification. Size and size distribution of 200–300 niosomes were noted using calibrated stage and ocular micrometers (Elico Instruments, Hyderabad). Similarly, size was noted for niosomes formed spontaneously from Niosomes after hydration without agitation in a cavity slide.[9]

Entrapment efficiency

The unentrapped drug in niosome formulation can be separated by using centrifugation method. In the centrifugation method, a specific amount of niosomal dispersion is subjected to cold centrifuge (4 °C) at a specific speed (rpm) for a particular time. Then remove the supernatant liquid from Eppendorf tubes. Suspend the settled product and again subjected to centrifuge, then remove the unentrapped drug with no void volume by washing it twice, and an assay of free drug determined by measuring the absorbance at a specific wavelength by using UV-spectroscopy.[10] Calculate the entrapment efficiency and drug loading from the following equations:

$$\mathbf{EP = [(C_t - C_r)/ C_t] * 100}$$

Where,

Ct, concentration of total Resveratrol, Cr, concentration of free Resveratrol.

SEM analysis

The surface morphology of the formulation was studied by using SEM. The prepared niosomal formulation was deep frozen and lyophilized prior to SEM. A double-sided conducting tape was taken, and the lyophilized sample was spread over it. Then, the coating was done with gold by using gold sputter under vacuum condition but in the presence of argon gas at 50 mA for 100 s. Finally, the sample was analysed under the microscope.[11]

***In vitro drug* release Study**

It is characterized by the Franz diffusion cell. It consists of a donor and receptor compartment. Niosomal dispersion is placed over the donor, and the receptor filled with buffer. In between these compartments, a cellophane membrane is placed, and this whole assembly is kept on a magnetic stirrer with a certain speed (rpm) at 37 °C. Aliquots of drug samples were withdrawn at certain time intervals and replaced the same volume of fresh medium to maintain the sink conditions. Check the absorbance at a specific wavelength by using UV-Spectroscopy.[12]

Drug release kinetics and drug analysis[13]

In vitro, the drug release date is fitted into various kinetic equations to understand the mechanism of drug release by determining the correlation coefficient and "n" value.

a.Zero-order, as cumulative % drug release vs. time

b. First- order, as log cumulative % drug retained vs. time

c. Higuchi's model, as cumulative % drug release vs. square root of time

d. Peppa's model, as log cumulative % drug release vs. log time and determine the "n" value from slope

Stability Studies

Stability studies of the different formulations were carried out under different temperature conditions so as to check the effect on: physical appearance, entrapment efficiency and drug content. The niosomal formulations were stored at 2- 8°C and at

room temperature (30±2°C) in air tight containers for 90 days and 2 ml samples were withdrawn every 30 days and at the end of 90 days. The analysis of the samples was then done spectrophotometrically at 286 nm after lyses of the niosomes and further preparing their suitable dilutions.[14]

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

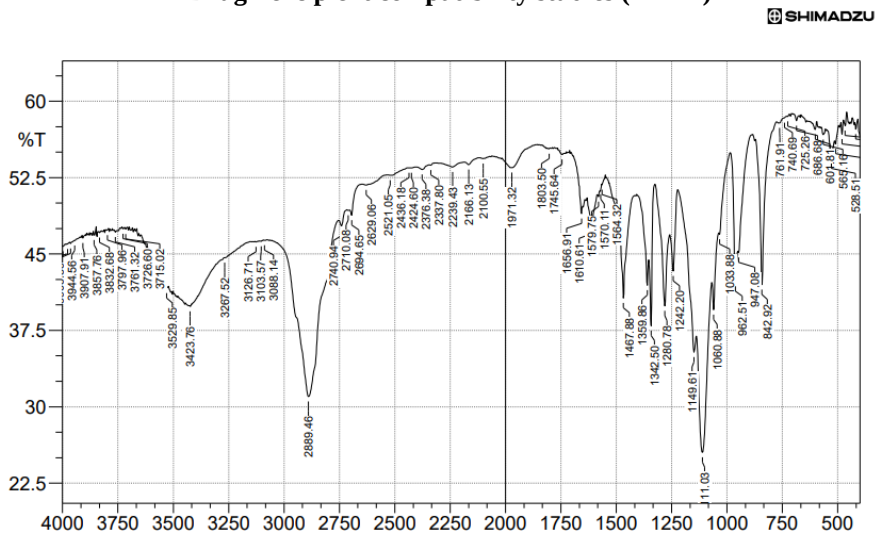

Fig-1: FT-IR Sample for Resveratrol

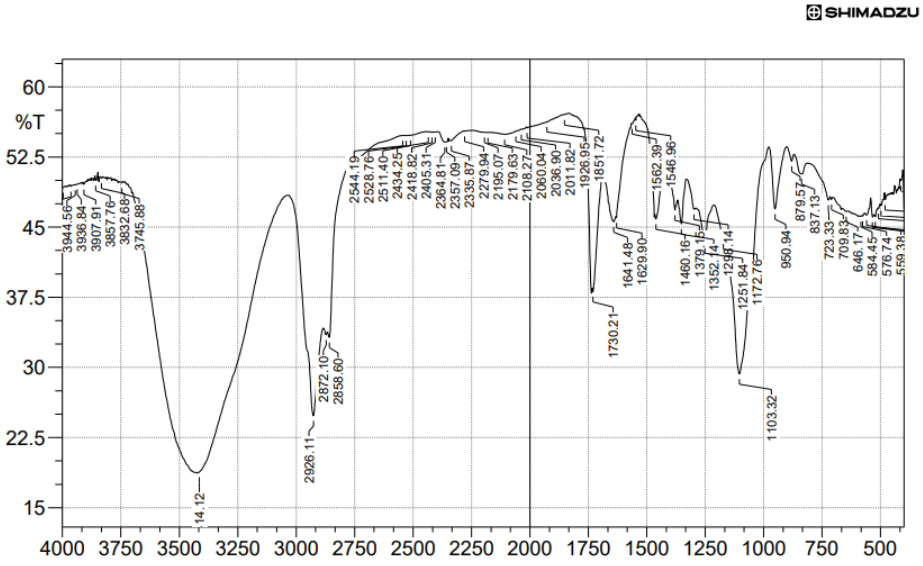

Fig-2: FTIR Spectra of physical mixture of drug and excipients

The IR spectrum of Resveratrol and Drug Excipients mixture was shown in respectively. In the present study, it has been observed that there is no chemical interaction between drug and the polymers used. From the figures it was observed that there were no changes in these main peaks in IR spectra of mixture of drug and polymers, which show there were no physical interactions because of some bond formation between drug and polymers. This further confirms the integrity of pure drug and compatibility of them with excipients.

EVALUATION PARAMETERS:

Entrapment Efficiency:

The unentrapped drug was separated from the niosomes by ultracentrifugation. Briefly, 1.5 mL of each niosome suspension was ultracentrifuge for 1 h at 16,000 rpm at 4 °C. The supernatant (unentrapped drug) was removed, and the niosomes were washed three times with PBS and ultracentrifuged under the same conditions. The content of the entrapped drug was determined after dissolving 0.1 mL of the niosomes in 1 mL of isopropanol until they were clear and then diluted up to 10 mL with PBS; after that, the samples were sonicated for 5 min at room temperature (RT). The amount of entrapped Resveratrol was determined by UV. The EE % was calculated using the following eq 1:

EE (%) = Amount of drug entrapped Total resveratrol amount×100%

Table-2: Drug entrapment efficiency of all formulation

F.no	Drug entrapment efficiency

F1	73.69±1.34
F2	72.50±1.26
F3	75.89±1.30
F4	76.98±1.19
F5	73.69±1.16
F6	79.88±1.25
F7	76.24±1.27
F8	71.88±1.16

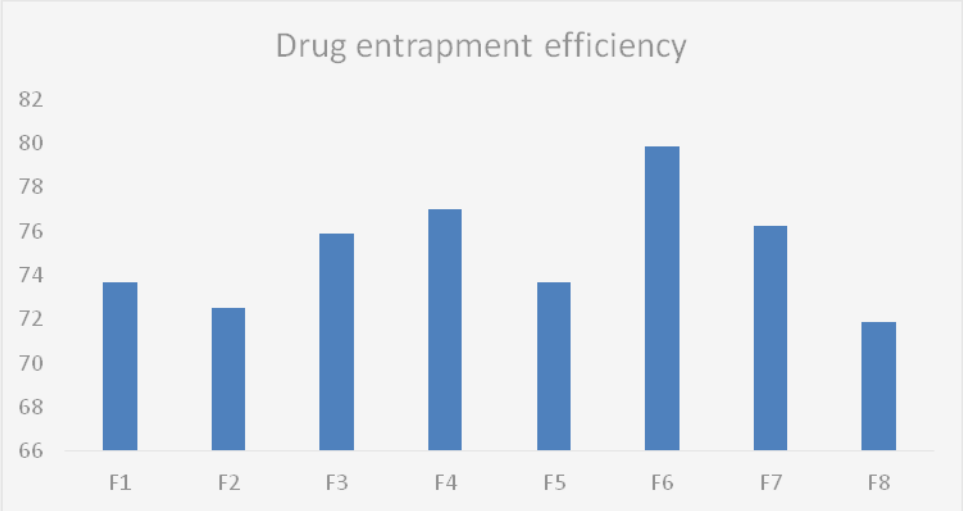

Fig-3: Drug entrapment efficiency of all formulation

Determination of Vesicle morphology and Size

The morphological characteristics of formulated niosomes were carried by using Scanning electron microscopy (SEM). A small drop of niosomal suspension was placed between two rivets fixed on a gold plated copper sample holder. The whole system was slushed under vacuum in liquid nitrogen. The sample was heated to -85^{0}C for 30 min to sublime the surface moisture. Finally the sample was coated with gold and allowed the SEM to capture the images at a temperature of -120^{0}c and voltage of 5kV.

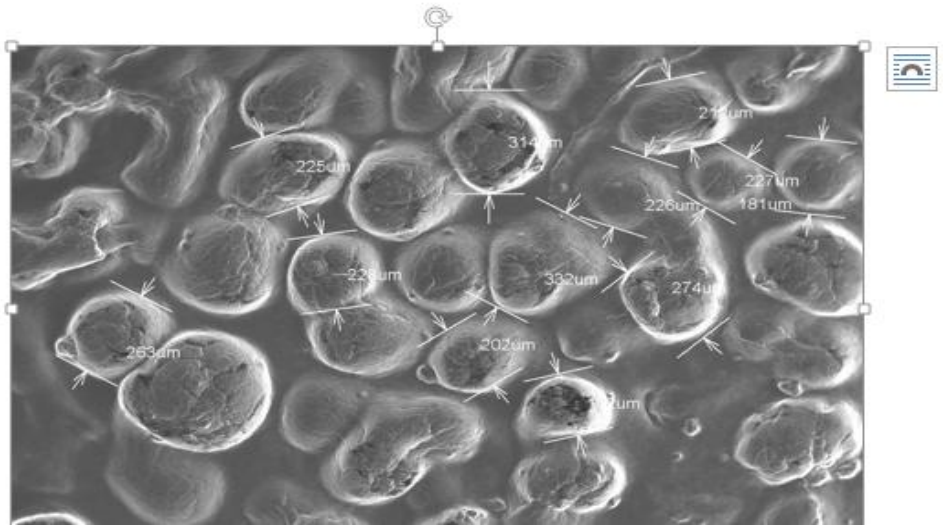

Fig-4: SEM analysis of Optimized niosomes

ZETA POTENTIAL

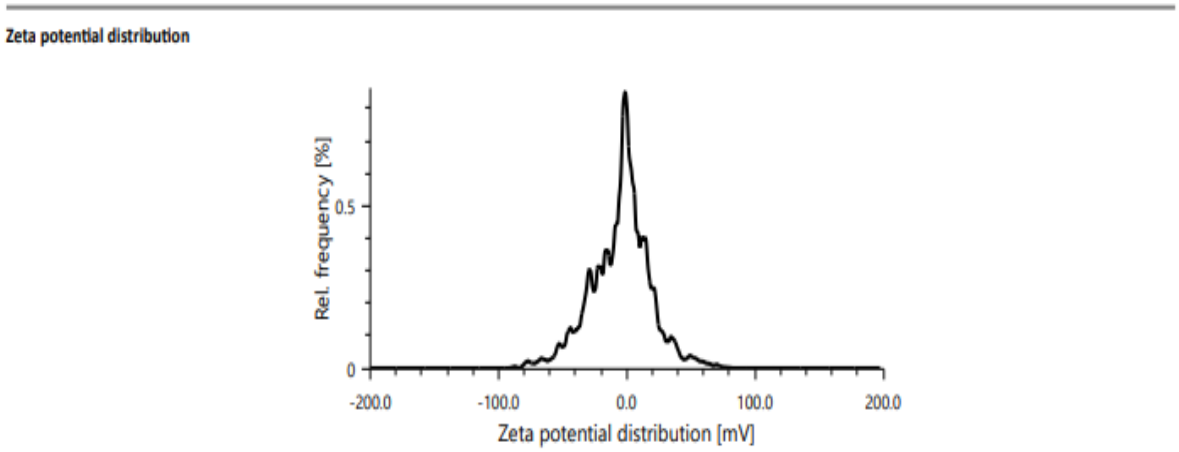

Fig-5: Zeta potential analysis of Optimized niosomes

TABLE-3: EVALUATION STUDIES OF PARTICLE SIZE AND ZETA POTENTIAL NIOSOMES

F. No	Particle size (nm)	ZETA POTENTIAL
F1	301.30	-25
F2	298.21	-32
F3	320.17	-27
F4	325.22	-34
F5	340.20	-31
F6	289.56	-29
F7	278.15	-30
F8	289.55	-35

***In vitro* drug release studies:**

The release of Resveratrol from niosomal formulations were determined using membrane diffusion technique. The niosomes left after removal of un-entrapped drug were dialyzed into a beaker containing 100 ml of PBS pH 7.4 containing 10% v/v methanol (to maintain sink condition), which acted as receptor compartment. The temperature of the receptor medium was maintained at 37 ± 0.5°C and agitated using a magnetic stirrer. Aliquots of 5 ml sample were withdrawn periodically and after each withdrawal, same volume of the medium was replaced. The collected samples were analysed using a UV spectrophotometer at 286 nm. The tests were carried out in triplicate.

Table-4: *In vitro* drug release profiles of Resveratrol niosomes (F1-F8)

Time (hrs)	% Cumulative drug released							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	25.86±1.32	24.78±2.35	25.10±1.32	23.65±1.32	26.98±1.41	24.20±1.74	25.10±1.16	21.46±1.25
2	36.98±1.15	35.66±2.35	34.80±1.25	33.69±1.32	35.87±1.25	34.82±1.18	36.89±1.25	35.28±1.31
3	47.80±2.13	45.28±2.09	44.96±1.30	45.12±1.32	46.98±1.16	47.14±1.23	45.68±1.26	43.51±1.21
4	53.61±2.50	52.10±1.61	50.12±1.32	48.25±1.39	51.24±1.30	53.69±1.26	55.58±1.16	52.27±1.18
5	67.89±1.96	65.32±2.20	68.84±1.32	56.85±1.30	58.96±1.21	61.20±1.16	62.14±1.13	68.12±1.18

6	73.25±1.36	72.15±1.36	75.80±1.32	78.96±1.47	79.86±1.26	80.10±1.26	83.69±1.24	81.23±1.42
7	82.52±1.65	83.95±1.32	81.16±1.32	83.69±1.25	85.84±1.29	86.98±1.20	85.13±1.10	87.85±1.43
8	93.56±2.20	94.68±1.24	95.12±1.32	96.20±1.36	97.12±1.30	98.52±1.26	97.85±1.26	95.89±1.43

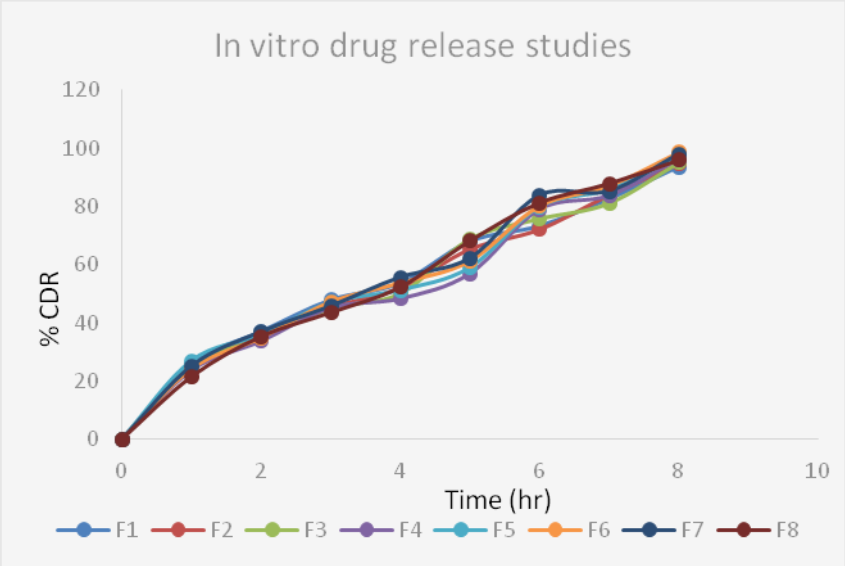

Fig-6: Drug release formulations

Release order kinetics

Zero order kinetics

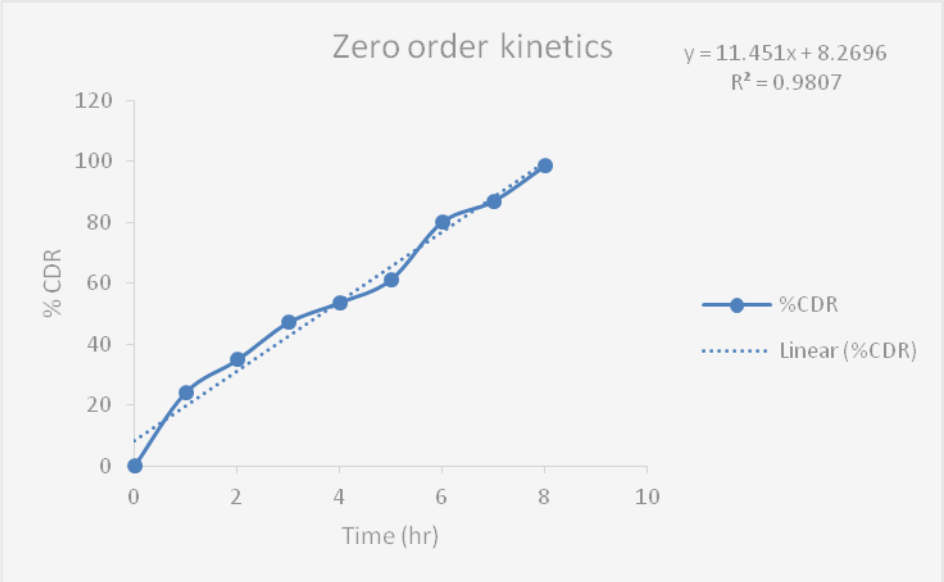

Fig-7: Zero order kinetics of optimized formulation

First order kinetics

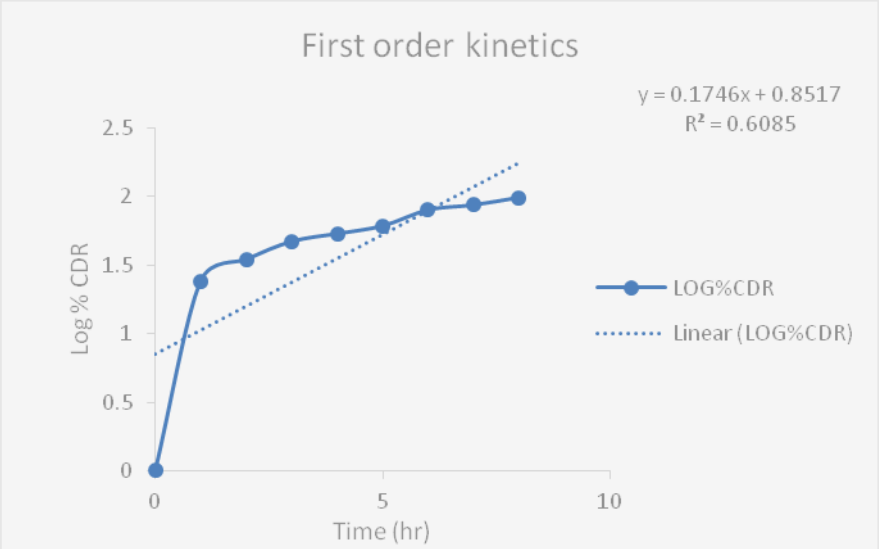

Fig-8: First order kinetics of optimized formulation

Higuchi model

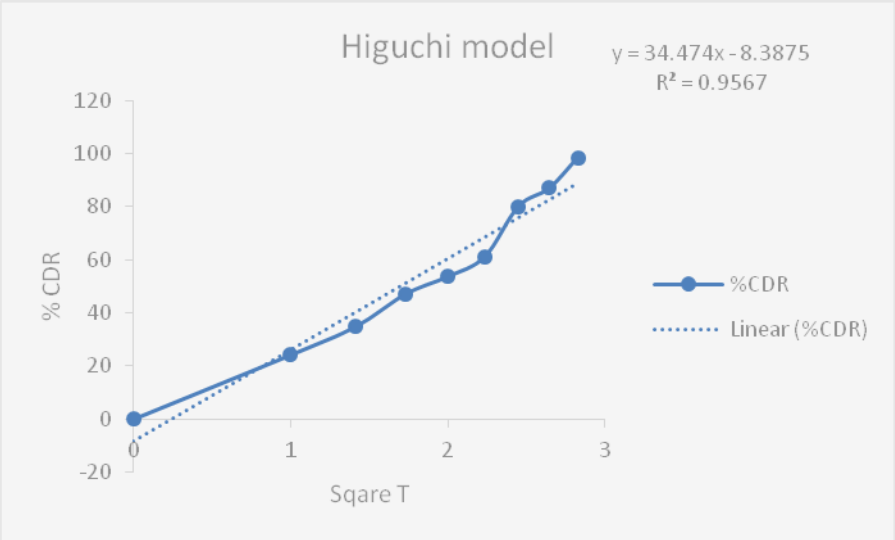

Fig-9: Higuchi model of optimized formulation

Korsmeyer peppas

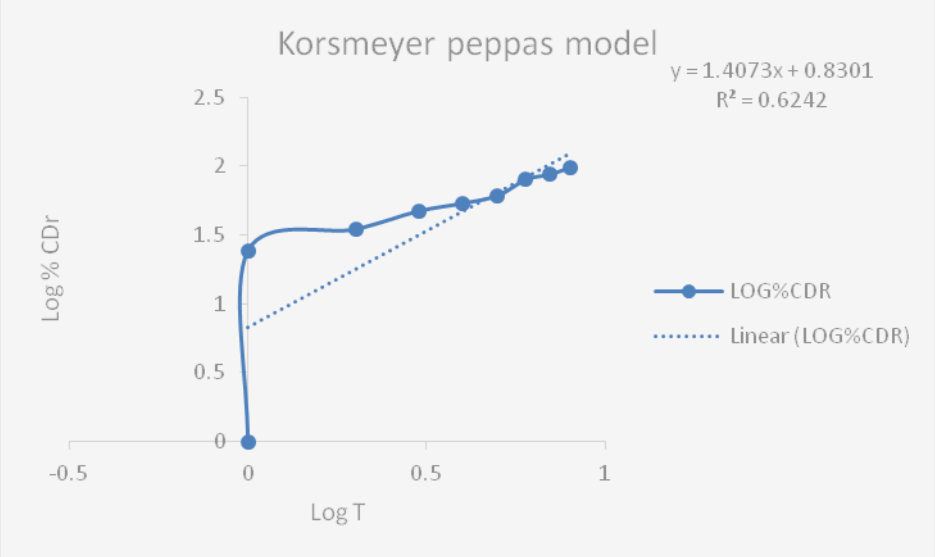

Fig-10: Korsmeyer peppas of optimized formulation

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Pappas were respective.**Stability studies:**

Optimized formulations F6 was selected for accelerated stability studies as per ICH guidelines. The patches were observed for color, appearance and flexibility for a period of three months. The folding endurance, weight, drug content, % cumulative drug release of the formulation was found to be decreasing. This decrease may be attributed to the harsh environment (40^0C) maintained during the studies.

Table-5: Stability studies of optimized formulations at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

Formulation Code	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-6	98.52±1.26	97.89±1.30	96.24±1.58	95.34±1.48	**Not less than 85 %**
F-6	98.52±1.26	97.58±1.43	96.20±1.36	95.15±1.69	**Not less than 85 %**
F-6	98.52±1.26	97.42±1.35	96.17±1.35	95.05±1.53	**Not less than 85 %**

CONCLUSION

The goal of the current work was to develop and characterize of Resveratrol niosomal drug delivery system in vitro. By adjusting the ratios of Tween 40, Tween 80, cholesterol, the niosomal solution was made using the thin film technique, and the prepared 8 formulations were tested for various parameters.Niosomes containing Resveratrol were created utilising the lipid thin film process and non-ionic surfactants Tween 40, Tween 80 and cholesterol in various ratios. Niosome preparation was optimised for maximum drug entrapment.

The goal of the current formulation study on Resveratrol is to create a niosomal drug delivery system and assess how well it functions in vitro. Different proportions of cholesterol and surfactant were used to create the formulations. High entrapment efficiency is regarded as the ideal or best niosome formulation. This study discovered that the ratio of cholesterol to surfactant affected entrapment efficiency. Formulations were discovered to guarantee the drug's good oral bioavailability. The niosomes were seen to be smooth-surfaced, spherical vesicles. The highest entrapment efficiency was demonstrated by Formulation F6. These facts lead to the conclusion that niosomes may be a promising method for increasing Resveratrol bioavailability.

REFERENCES

1. B. Yang, Y. Dong, F. Wang, Y. Zhang. Nano formulations to Enhance the Bioavailability and Physiological Functions of Polyphenols. Molecules 2020, 25 (20), 4613.
2. Allen TM. Liposomal drug formulations: Rationale for development and what we can expect for the future. Drugs. 1998; 56:747–56.
3. Malhotra M, Jain NK. Niosomes as drug carriers. Indian Drugs. 1994; 31:81–6.
4. B.S. Chhikara. Current trends in nanomedicine and Nano biotechnology research. J. Mater. Nanosci. 2017, 4 (1), 19–24.
5. B.S. Chhikara, R. Kumar, B. Rathi, S. Krishnamoorthy, A. Kumar. Prospects of Applied Nanomedicine: potential clinical and (bio) medical interventions via nanoscale research advances. J. Mater. Nanosci. 2016, 3 (2), 50–56.
6. Sharma S. Span-60 Niosomal Oral Suspension of Fluconazole: Formulation and invitro evaluation. Asian journal of pharmaceutical research and health care. 2009; 1: 142-156.
7. Suzuki K, Sokan K. The Application of Liposomes to Cosmetics. Cosmetic and Toiletries.1990; 105: 65-78.
8. Tabbakhian M,TavakoliN, JaafariM, Daneshamouz S. Enhancement of follicular delivery of finasteride by liposomes and niosomes. Int. J. Pharm. 2006; 323: 1-10.
9. NamdeoA,JainN.Niosomesas drugcarriers. Indian Journal of Pharm.Sci.1996; 58:41-46.
10. Rai A. Niosomes: An approach to current drug delivery-A Review. International Journal of Advances in Pharmaceutics. 2017; 06: 41-48.
11. SrivastavA,DasP.To Study the Formulationof Niosome of Ofloxacin and Its Evaluation for Efficacy of Anti-Microbial. International Journal of Innovative Research in Science, Engineering and Technology. 2014; 3: 17958-17965.
12. Mishra N, Srivastava V, Kaushik A, Chauhan V, Srivastava G. Formulation and in vitro- evaluation of Niosomes of Aceclofenac. JSIR. 2014; 3: 337-341.
13. Bayindir Z, Yuksel N. Characterization of noisome sprepared with various non-ionic surfactants for paclitaxel oral delivery. Journal of Pharmaceutical Sciences. 2010; 99: 2049-2060.
14. Srivastav A, Das P. To Study the Formulation of Niosome of Ofloxacin and Its Evaluation for Efficacy of Anti-Microbial. International Journal of Innovative Researchin Science, Engineering and Technology. 2014; 3: 17958-17965.