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Research Article

Nanoemulsion Formulation for Enhanced Delivery of 5-Fluorauracil and Resveratrol with Advanced Techniques

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ABSTRACT
This study focuses on the formulation and optimization of a nanoemulsion-based lipid nanosystem for the dual delivery of 5-fluorouracil (5-FU) and resveratrol (RSV) to enhance dermal drug permeation and therapeutic efficacy. A systematic approach was employed to evaluate excipient screening, lipid selection, emulsifier efficiency, and formulation techniques. Labrasol® (LBR) was identified as the optimal medium-chain oil (MCO) due to its high drug solubility and P-GP inhibitory activity, while Emulcire® 61 WL 2659 (EML) was chosen as the solid lipid for its excellent emulsification properties and stability.
Among the various formulation methods tested (S, M, H, K), Method-K, involving double emulsification, demonstrated superior stability, smaller particle sizes, lower polydispersity index (PDI), and higher drug entrapment efficiency (%EE) compared to other methods. Drug-excipient interaction studies using DSC and Fourier-transform infrared spectroscopy (FTIR) confirmed the compatibility and stability of 5-FU and RSV within the optimized formulation. The developed nanoemulsion system exhibited promising potential for dermal drug delivery, with enhanced drug solubility, stability, and bioavailability. These findings provide a strong foundation for further clinical investigations into the use of lipid-based nanosystems for targeted skin cancer therapy.
Binary mixture (BM) miscibility studies confirmed LBR and EML as the ideal lipid combination, further optimized using differential scanning calorimetry (DSC) analysis. Tween® 80 emerged as the most effective emulsifier due to its superior emulsification properties and enhancement of membrane fluidity, facilitating drug permeation.

Keywords: Nanoemulsion, 5-Fluorouracil (5-FU), Resveratrol (RSV), Dermal Drug Delivery, Lipid Nanoparticles, Nano Lipid Carriers (NLC).

INTRODUCTION

Cancer remains a formidable global challenge, spurring relentless research into more effective therapeutic approaches. Conventional cancer treatments have evolved significantly, increasingly favoring targeted drug delivery systems to improve treatment efficacy while mitigating systemic side effects.1-2 Nanotechnology has emerged as a transformative tool in cancer therapy, offering precise delivery of therapeutic agents through nanocarrier systems. These nanoscale vehicles include nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), polymeric nanoparticles, metallic nanoparticles, nanoemulsions, carbon nanotubes, and dendrimers, each meticulously designed to optimize drug delivery and therapeutic outcomes.3-5

Emulsifier selection and optimization are pivotal in stabilizing lipid-water interfaces during nanoemulsion preparation, crucial for achieving efficient drug delivery. Long-term compatibility between 5-FU, RSV, and selected excipients is assessed through rigorous drug-excipient interaction studies using advanced analytical techniques like DSC and Fourier-transform infrared spectroscopy (FTIR). Formulation techniques are then compared and optimized—such as solvent diffusion, melt dispersion, hot high-pressure homogenization, and modified emulsiosonication— based on critical parameters like particle size, polydispersity index (PDI), % transmittance, and drug entrapment efficiency (%EE).
Comprehensive characterization of the selected formulations includes evaluating optical clarity, assessing phase separation, and examining particle morphology using transmission electron microscopy (TEM). Furthermore, structural integrity is analyzed using thermal analysis methods (DSC and FTIR), ensuring the formulation's stability and suitability for targeted drug delivery applications.

METHODOLOGY

Design, Development, and Optimization of Dual Drug-loaded NLC

Selection of Excipients for Combinatorial NLC

To develop a stable dual-drug loaded NLC containing 5-FU and RSV, excipients including solid lipids, liquid lipids, and emulsifiers were selected from the GRAS (Generally Recognized as Safe) category.

Screening and Selection of Liquid Lipids (Oils) 13

Suitable oils were chosen based on their maximum affinity with 5-FU and RSV. Various herbal or long-chain oils (LCO) such as linseed oil, olive oil, hemp oil, oleic acid, wheat germ oil, jojoba oil, castor oil, and fish oil, as well as medium-chain oils (MCO) such as Capryol PGMCTM, Capmul MCM®, Labrasol® (LBR), Sefsol 288, Captex® 355, and Capmul® PG 8 NF were screened for solubility of the drugs.

Drugs were added in excess (2 mg) to 1 mL of each oil in separate glass vials, followed by continuous vortexing. The mixtures were then incubated in a mechanical shaker-incubator (Lab-Therm, Kuhenr, Switzerland) for 72 hours at 25 ± 1°C to reach equilibrium. After 72 hours, the oil samples were centrifuged (REMI Groups Laboratory Instruments, Mumbai, India) at 5000 rpm for 30 minutes. Supernatants were collected from the vials, dissolved in methanol, and analyzed using a spectrophotometer at 266 nm and 307 nm to identify the oil containing the highest quantities of 5-FU and RSV.

Screening and Selection of Solid Lipids

For selecting solid lipids, increments of 2 mg of 5-FU and RSV were added to different glass vials containing various molten solid lipids. Continuous stirring was performed on a magnetic stirrer (REMI Groups Laboratory Instruments, Mumbai, India) at 200 rpm, maintaining the temperature at 5 ± 0.5°C above their melting points. The solid lipids tested included Tefose® 1500, Gelucire® 43/01, Emulcire™ 61WL2659 (EML), Labrafil® M 2130 CS, Apifil®, Compritol® 888 ATO, Precirol® ATO 5, and GeleolTM. The process continued until saturation, which was observed visually. Afterward, the amount of drug dissolved in each solid lipid was calculated, and the lipid that dissolved the maximum amount of drugs was selected.14-15

Miscibility Study of Solid and Liquid Lipids16

To select the binary mixture (BM) of solid and liquid lipids, miscibility studies were conducted. Selected molten solid lipids were mixed with the optimized liquid lipid in glass vials and kept for 48 hours at 25 ± 1°C. After this period, the mixtures were visually inspected for phase separation or precipitation. The mixture that exhibited homogenous and solid characteristics was selected as the optimized lipid BM.

Screening and Selection of Emulsifier17

Emulsifier selection was based on its emulsification capacity with the selected BM. Various 5% emulsifier solutions were prepared and added to 100 mg of the selected BM, liquified in 2 mL of methylene chloride, with continuous stirring using a magnetic stirrer. The mixture was heated to 50 ± 1°C and stirred at 500 rpm for 30 minutes to remove the methylene chloride. The remaining mixtures were then diluted with milli-Q water. The emulsification capacity was determined by measuring the % transmittance (%T) of the mixture spectrophotometrically at 510 nm at 25 ± 1°C.

Optimization of Binary Mixture (BM) Using DSC Thermal Analysis

The ratio of BM for developing the combinatorial lipid-nanosystem was optimized using DSC thermal analysis. Various ratios of BM (1:0, 9:1, 8:2, 7:3, and 6:4) were prepared, incorporating an increasing concentration of solid lipid. The lipids were heated together in a water bath at 80°C, agitated for 1 hour, and stored at room temperature for 72 hours. These BMs were then subjected to thermal scanning to determine the percentage change in crystalline nature, using 100% of the previously selected solid lipid as a control. The instrument was set to a heating rate of 10°C per minute within a temperature range of 20-100°C.

Drug-Excipients Interaction Studies

A mixture of 5-FU and RSV in a 1:1 ratio was accurately weighed and mixed with selected excipients in the same ratio. This mixture was placed in a closed glass vial and stored for 3 months at 40°C ± 2°C and 75 ± 5% relative humidity in a stability chamber. Compatibility between the drugs and excipients, as well as between the drugs themselves, was assessed by visual observation for any physical changes such as changes in physical state or odor. Additionally, DSC thermograms and FTIR

spectra of the various mixtures were recorded to identify any chemical interactions.

Implementation of Various Formulation Techniques for Development of a Stable Combinatorial NLC[18-19]

Based on preformulation studies and extensive trials, a 7:3 ratio of BM (EML) was selected for the preparation of the combinatorial lipid-nanosystem, with concentrations varying between 1-2% w/w (i.e., 1, 1.5, and 2% w/w). The concentration of the selected emulsifier, Tween 80®, was varied between 1-5% w/w (i.e., 1, 2, 3, 4, and 5% w/w). Trials indicated that BM concentrations below 1% w/w were insufficient to entrap the desired quantity of drugs, while concentrations above 2% w/w led to phase separation and flocculation. Several established techniques for NLC nanosystem preparation were employed, each impacting the formulation's characteristics and stability differently. To develop a robust lipid-nanosystem formulation with maximum stability, four different techniques were used: solvent diffusion method (S), melt dispersion method (M), hot high-pressure homogenization (H), and modified emulsiosonication method (K).

Method S - Solvent Diffusion Method

Optimized BM at 1, 1.5, and 2% w/w was dissolved in a 1:1 ratio of acetone at 75°C with stirring at 600 rpm on a digital magnetic stirrer. Five mg of 5-FU and RSV were added to the dissolved lipid-organic phase with continuous stirring. Simultaneously, an aqueous phase was prepared by dissolving various percentages of Tween 80® (1, 2, 3, 4, and 5% w/w) in half the quantity of milli-Q water at 75°C with stirring at 600 rpm, and the final volume was adjusted to 10 mL with milli-Q water. The lipid-organic solution was quickly dispersed into the aqueous phase under continuous mechanical stirring at 75°C with 700 rpm. The mixtures were then stirred at 1000 rpm for 1 hour at 75°C, sonicated for 13 minutes at 40% amplitude for two cycles using a probe sonicator (Hielscher, Germany), and cooled to room temperature.

Method M - Melt Dispersion Method

Optimized BM at 1, 1.5, and 2% w/w was melted at 75°C with stirring at 600 rpm on a digital magnetic stirrer. Five mg of 5-FU and RSV were added to the melted lipid phase with continuous stirring. Concurrently, an aqueous phase was prepared by dissolving various percentages of Tween 80® (1, 2, 3, 4, and 5% w/w) in half the quantity of milli-Q water at 75°C with stirring at 600 rpm, and the final volume was slowly adjusted to 10 mL. The aqueous solution was then dispersed into the oily phase dropwise with continuous mechanical stirring at 700 rpm at 75°C. The mixtures were stirred for 2 hours at 75°C with 1000 rpm, sonicated for 13 minutes at 40% amplitude for two cycles using a probe sonicator (Hielscher, Germany), and cooled to room temperature.

Method H - Hot High-Pressure Homogenization

Optimized BM at 1, 1.5, and 2% w/w was melted at 75°C with stirring at 600 rpm on a digital magnetic stirrer. Five mg of 5-FU and RSV were added to the melted lipid phase with continuous stirring. Simultaneously, an aqueous phase was prepared by dissolving various percentages of Tween 80® (1, 2, 3, 4, and 5% w/w) in a small quantity of milli-Q water at 75°C with stirring at 600 rpm, and the final volume was adjusted to 10 mL. The aqueous solution was then dispersed into the lipid phase dropwise with continuous mechanical stirring at 700 rpm at 75°C. The mixtures were stirred for 1 hour at 75°C with 1000 rpm, passed through a high-pressure homogenizer at 1000 bars for seven cycles, and cooled to room temperature.

Method K - Modified Emulsiosonication Method

In this method, modifications were made in the pre-emulsion stage: the emulsifier was divided into two parts, one part incorporated into the lipid phase and the other dissolved in the aqueous phase (10 mL). Both mixtures were heated to 75°C with stirring at 500 rpm on a digital magnetic stirrer. Five mg of 5-FU and RSV were added to the first emulsified solution of lipid-emulsifier with continuous stirring. The emulsified phase was then mixed with the aqueous phase and stirred for 30 minutes at 800 rpm at 75°C. The double-emulsified solutions were sonicated for 10 minutes at 20% amplitude for two cycles using a probe sonicator (Hielscher, Germany) with 1-minute intervals on ice and then cooled to room temperature.

Optimization and Selection of Formulation Technique

In order to optimize the best-suited formulation technique for the preparation of stable combinatorial FR-NLCs (5-FU and RSV loaded nanostructured lipid carriers), the prepared

formulations were characterized for optical clarity, percent transmittance (%T), size of the particle, PDI, and percent entrapment efficiency (%EE). After the assessment of different parameters, an optimized formulation was selected for further examination.

Observation of Clarity, Phase Separation, and Homogeneity

All the prepared formulations were kept at 25 ± 1°C undisturbed for 24h. Subsequently, formulations were observed visually for clarity as well as for any phase separation. The selection of the best stable FR-NLC was done for the formulation that showed clarity and uniformity without phase separation. In order to confirm the result of visual observation, %T was determined by diluting 1 mL of prepared FR-NLC dispersions into milli-Q water (10 mL), and the %T of the FR-NLC mixture was observed spectrophotometrically in triplicate at 615 nm (Khan et al., 2016). The formulations which exhibited maximum %T were selected as homogenous and stable lipid-nanosystem.

Particle Size and Polydispersity Index (PDI)

The size of the particle, as well as PDI of the formulations, were estimated by using Nano Zetasizer ZS90 (Malvern Instruments Ltd., Worcestershire, UK) equipped with Malvern software v7.12. The sample of NLC was diluted in the ratio of 1:100 by adding milli-Q water to get uniform dispersion. The condition of analysis was standard laser 4mW He-Ne, 633 nm, room temperature (25 ± 2°C) at a fixed angle of 90°. The instrument analyzed the changes in the light intensity scattered by NLC, which constantly underwent Brownian motion (Cristiano et al., 2019). All measurement was performed in triplicate at 25 ± 2°C.

Entrapment Efficiency (EE)

The %EE of FR-NLC formulations were estimated by centrifugation where samples were exposed to high-speed centrifuge (15,000 rpm) (Sigma-3K30, Osterode am Harz, Germany) at 4 ± 1°C for 1h. The supernatant was separated after centrifugation and filtered using a 0.25 µm pore size nylon syringe-driven membrane filter. The filtered sample was further diluted and examined in triplicate by a previously developed and validated HPLC method at 272 nm to determine the unentrapped drug.

Stability Study for Optimization and Selection of Formulation Technique

To further shortlist the formulation methodology, FR-NLCs prepared by method-H and K were subjected to stability studies. Both the formulations were stored at 4 ± 2°C for 3 months in different sealed glass vials. After 1,2, and 3months these formulations were examined in triplicate for homogeneity via %T, particle size, PDI, %EE, zeta potential, and particle morphology through TEM. Along with this, visual inspection was also performed to find out any precipitation and turbidity.

RESULTS AND DISCUSSION

Design, Development, and Optimization of Dual Drug-loaded NLC

Solid lipid, liquid lipid, and emulsifier are fundamental components of NLCs that contribute to drug entrapment and release. In dermal drug delivery, these components also enhance drug permeation through the stratum corneum. The selection of solid and liquid lipids was based on their ability to solubilize the maximum amount of drugs, while the selection of the emulsifier was based on its emulsification efficiency with the selected lipids.

Selection of Oils for the Development of Combinatorial NLC

High solubility of drugs in lipids increases the entrapment efficiency of NLCs and reduces drug leakage, thereby enhancing stability. Solubility studies revealed that 5-FU had the highest solubility in LBR, a medium-chain oil (MCO) (9.967 ± 0.197 mg/mL) compared to other long-chain oils (LCOs). Given 5-FU's maximum solubility in MCO, RSV's solubility analysis was conducted only in various MCOs, showing that RSV also had the highest solubility in LBR (54.990 ± 0.480 mg/mL).

LBR, chemically known as caprylocaproyl polyoxyl-8 glycerides, possesses excellent solubilizing properties and acts as a permeation enhancer for dermal applications. Additionally, LBR has been reported to have P-GP inhibitory activity, making it potentially useful as an adjuvant in the treatment of skin tumors.

Table 1: Solubility Of 5-FU in Various Oils (Mean ± SD, N = 3)

Long chain oils (LCO)	Mean of drug dissolved ($mg.mL^{-1}$)	Medium chain liquid lipids	Mean of drug dissolved ($mg.mL^{-1}$)

Linseed Oil	3.130 ± 0.036	Capryol™ PGMC	4.183 ± 0.159
Olive Oil	0.543 ± 0.068	Capmul® MCM	5.177 ± 0.176
Hemp oil	0.937 ± 0.043	Labrasol®	9.967 ± 0.197
Oleic Acid	1.753 ± 0.045	Sefsol 288	2.797 ± 0.109
Wheat Germ oil	2.053 ± 0.129	Captex® 355	3.777 ± 0.130
Jajoba Oil	0.247 ± 0.021	Capmul® PG 8 NF	1.727 ± 0.117
Castor Oil	0.550 ± 0.030		
Fish Oil	5.180 ± 0.020		

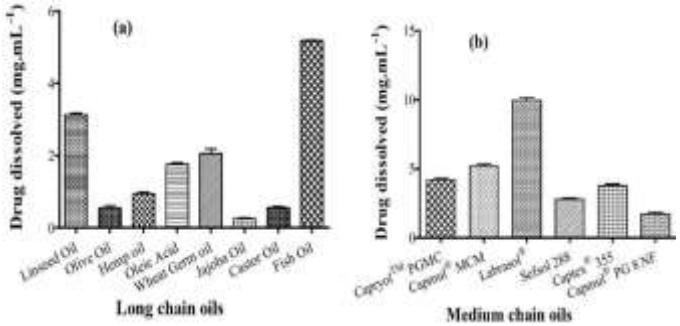

Figure 1: Solubility of 5-FU in (A) Long-Chain Oils (Lcos), and (B) Medium-Chain Oils (Mcos).

Table 2: Solubility of RSV in various oils (Mean ± SD, n = 3).

Liquid lipids (Oils)	**Mean of drug dissolved (mg.mL^{-1})**
Capryol™ PGMC	3.080 ± 0.318
Capmul® MCM	29.733 ± 0.575
Labrasol®	54.990 ± 0.480
Sefsol 288	1.693 ± 0.277
Captex® 355	1.960 ± 0.342
Capmul® PG 8 NF	0.997 ± 0.329

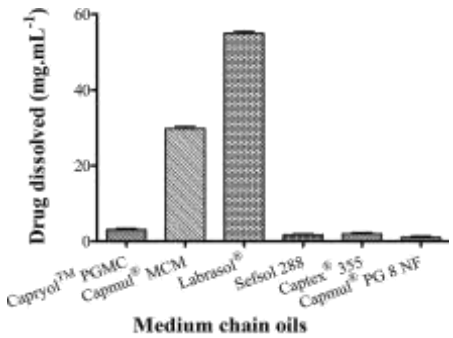

Figure 2: Solubility of RSV in oils.

Selection of Solid Lipid for the Development of Combinatorial NLC

To select the solid lipid for the development of combinatorial NLC, a solubility study of 5-FU in various solid lipids was conducted. The study showed solubility values of 5.23 ± 0.46 mg/g in Apifil®, 6.433 ± 0.493 mg/g in EML, and 6.700 ± 0.179 mg/g in Tefose® 1500. Based on these results, RSV's solubility study was performed only in Apifil® (34 ± 0.89 mg/g), EML (34.67 ± 1.0 mg/g), and Tefose® 1500 (25.33 ± 1.37 mg/g). EML was selected as the solid lipid due to its composition of cetyl alcohol and ethoxylated fatty alcohols, which provide excellent emulsification and stability to the formulation, even in the presence of poorly lipophilic drugs and under heat.

Table 3: Solubility of 5-FU and RSV in solid lipids (Mean ± SD, n = 3).

5-FU in solid lipids	Solubility (mg.gM^{-1})	RSV in solid lipids	Solubility (mg.gM^{-1})
Tefose® 1500	6.700 ± 0.179	Tefose® 1500	34.000 ± 0.894
Gelucire® 43/01	2.267 ± 0.137	Emulcire™ 61 WL 2659	34.667 ± 1.033
EmulcireTM 61 WL 2659	6.433 ± 0.493	Apifil®	25.333 ± 1.366
Labrafil® M 2130 CS	3.200 ± 0.155		
Apifil®	5.233 ± 0.459		
Compritol® 888 ATO	1.600 ± 0.268		
Precirol® ATO 5	2.067 ± 0.137		
GeleolTM	1.867 ± 0.186		

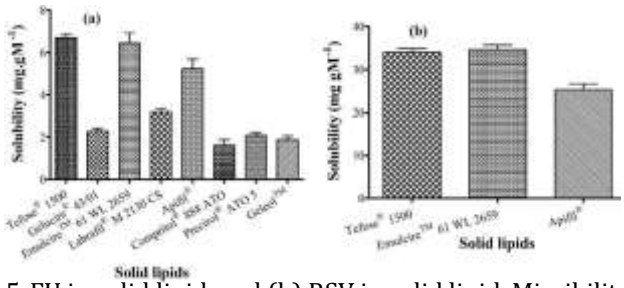

Figure 3: Solubility of (a) 5-FU in solid lipid, and (b) RSV in solid lipid. Miscibility studies for the selection of BM of oils and solid lipid

In order to check for any possible incompatibility between the selected oil with various selected solid lipids such as Apifil®, Tefose® 1500, and EML, miscibility studies were carried out.

Figure 4: Miscibility study of selected oil (labrasol) with various solid lipids.

Miscibility Studies for the Selection of BM

The miscibility studies conducted for the selection of the binary mixture (BM) showed good miscibility and homogeneity between EML and LBR. In contrast, the mixtures of Apifil® and Tefose® 1500 exhibited turbidity and phase separation after 48 hours of storage. Consequently, EML and LBR were chosen as the optimized solid lipid and liquid lipid, respectively, for the BM in the formulation development.

Optimization of BM Ratio Using DSC Thermal Analysis

The ratio of solid lipid to oil in the BM was optimized using DSC thermal analysis, focusing on crystallinity index (CI), enthalpy of fusion, and the width of thermal phenomena.

Table 4: DSC parameters of various ratio of BM.

Percent of labrasol® (w/w)	Onset temperature (°C)	Melting point (°C)	Enthalpy ($J.g^{-1}$)	Crystallinity index (CI %)
0	46.58	55.63	752.99	100
10	40.75	52.01	584.09	77.56
20	39.16	51.11	443.49	58.89
30	38.39	50.31	399.37	53.03
40	38.12	50.03	399.4	53.04

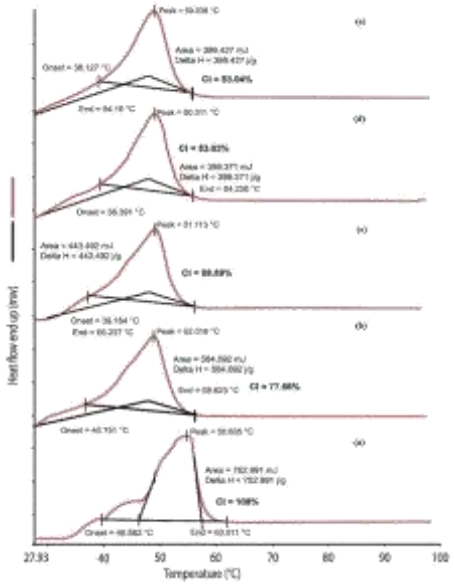

Figure 5: DSC Thermograms (a) EML 100% w/w, (b) BM with 10% w/w LBR, (c) BM with 20% w/w LBR, (d) BM with 30% w/w LBR, and (e) BM with 40% w/w LBR. (CI: Percentage crystallinity index; EML: Emulcire® 61 WL 2659; BM: Binary mixture; LBR: Labrasol®).

Selection of Emulsifier

To evaluate the emulsification capability, several emulsifiers including Cremophor® RH 40, PEG-400, Poloxamer® 188, Tween® 20, Tween® 80, Transcutol® P, and sodium tauroglyocholate (bile salt) were screened. The assessment was based on the percent transmittance (%T) of the dispersion medium, which correlates with particle size and reflects the effectiveness of emulsification. Emulsifiers that resulted in smaller particle sizes within the dispersion medium exhibited higher %T, indicating superior emulsification properties.

Table 5: Selection of emulsifier based on maximum %T of aqueous dispersion of BM (Mean ± SD, n = 3).

Name of emulsifiers	Transmittance (%)	Observation
Bile salt	84.82 ± 1.06	No phase separation
Cremophore® RH 40	69.13 ± 0.93	Phase separation after 24 h
PEG-400	36.14 ± 0.95	Phase separation after 24 h
Poloxamar® 188	24.11 ± 0.47	Phase separation after 24 h
Transcutol® P	26.80 ± 0.93	Phase separation after 24 h
Tween® 20	91.78 ± 1.15	Phase separation after 36 h
Tween® 80	96.02 ± 0.97	No phase separation

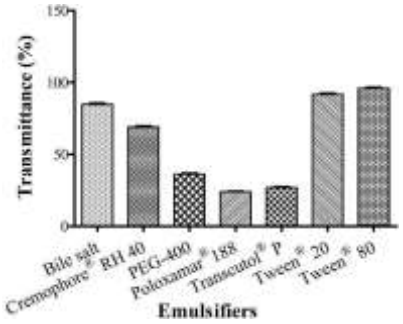

Figure 6: %T of aqueous dispersion of BM of LBR: EML emulsified with various emulsifiers.

Selection of Emulsifier

Tween® 80 exhibited the highest transmittance (96.02 ± 0.97) in the emulsion, indicating superior emulsification properties suitable for the selected BM. Therefore, Tween® 80 was chosen for further formulation development. Its non-ionic nature was particularly advantageous for dermal formulations, mitigating concerns related to irritation often associated with other surfactants. Additionally, Tween® 80 is known to enhance membrane fluidity, facilitating increased permeation of poorly soluble drugs. Its medicinal utility extends to cancer treatment, where it has demonstrated antiangiogenic properties that inhibit tumor growth. The selected excipients not only facilitated formulation development but were also expected to enhance the dermal delivery of both drugs, ensuring optimal availability of 5-FU and RSV in the epidermal and dermal layers of the skin.

Drug-Excipients Interaction Studies

For the formulation of NLC, the selected drugs and excipients were combined in a 1:1 ratio and stored at 40 ± 2°C and 75 ± 5% RH. The samples were monitored for any physical changes such as alterations in the physical state of the mixture or changes in odor.

Table 6: Observation for drug-drug and drug-excipient interaction studies.

Drug/ Excipients	Initial Description	Visual observation (after 3-month storage at 40 ± 2°C and 75 ± 5% RH)	
		Color	Physical form
5-FU	White crystalline powder	No change	No change
RSV	Off-white crystalline	No change	No change
	powder		
5-FU + RSV	White solid crystalline powder	No change	No change
Drug mixture + BM	White semisolid mixture	No change	No change
Drug mixture + emulsifier	Off-white semisolid	No change	No change
	mixture		

To assess the compatibility of the selected excipients with 5-FU and RSV, visual inspection was followed by thermal and spectral analyses using DSC and FTIR, respectively. The DSC thermograms of various mixtures were recorded. In these thermograms, characteristic peaks of 5-FU and RSV were identified at 289.462°C and 271.756°C, respectively. The mixture of 5-FU and RSV exhibited an exothermic peak at 286.263°C and 267.571°C, respectively. The presence of EML, with a maximum enthalpy of 2691.939 J.g-1, altered the intensity of the drug peaks but did not affect their melting points. In contrast, Tween® 80, with a lower enthalpy of 33.012 J.g-1 and a melting point of 110.594°C, showed a reduced intensity of peaks compared to the drugs. Overall, no significant changes were observed in the thermal characteristics of 5-FU and RSV when mixed with the screened excipients in the thermograms.

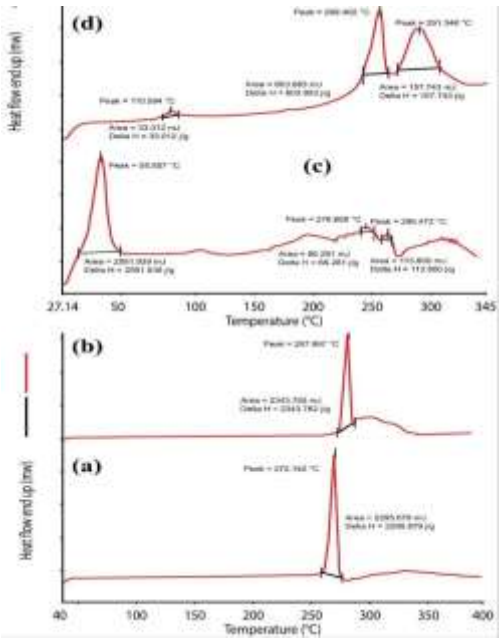

Figure 7: DSC thermogram of (a) 5-FU, (b) RSV, (c) drug mixture and BM of lipids, and (d) mixture of drug and Tween® 80.

FTIR spectra were simultaneously recorded for both drugs and their mixtures with selected excipients. 5-FU exhibited characteristic absorption peaks at (a) 3183.39 cm-1 (N-H Stretch), (b) 1720.85 cm-1 (C=O Stretch), (c) 1586.38 cm-1 and 1237.95 cm-1 (C-N stretch), and (d) 1176.36 cm-1 (C-O). RSV showed characteristic absorption peaks at (a) 3196.40 cm-1 (O-H stretching),

(b) 1583.65 cm-1 (C=C aromatic double-bond stretch), (c) 1502.49 cm-1 (C–C olefinic stretch),

(d) 1378.68 cm-1 (C–O stretch), and (e) 972.37 cm-1 (trans olefinic band).

The FTIR spectra of the binary mixture (BM) with the drugs and Tween® 80 did not exhibit significant peak shifts, indicating the absence of chemical interactions between the drugs themselves and between the drugs and excipients. This absence of changes in peak positions suggests the compatibility of 5-FU and RSV with each other and with the selected excipients. Thus, the drugs demonstrated compatibility both with each other and with the chosen excipients.

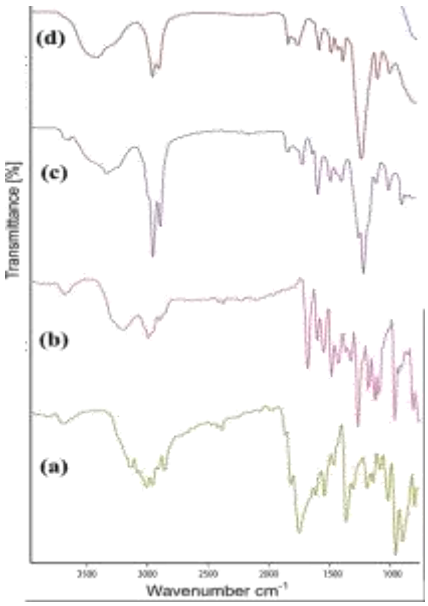

Figure 8: FTIR spectra of (a) 5-FU, (b) RSV, (c) drug mixture and BM of lipid, and (d) mixture of drug and Tween® 80.

Optimization and Selection of Formulation Technique

To determine the most suitable technique for developing a stable dual drug-loaded lipid-nanosystem, formulations were prepared using four different methods and subjected to various characterization parameters:

Observation of Clarity, Phase Separation, and Homogeneity

Formulations prepared by method-S (S-5) and method-M (M-5) appeared homogenous and optically clear initially, but phase separation was observed after 24 hours of storage. In contrast, formulations prepared using method-H (H-5) and method-K (K-5) remained optically clear without phase separation after 24 hours. The percent transmittance (%T) for formulations prepared by methods S, M, H, and K ranged from 48.97 ± 1.34 to 96.77 ± 0.72, indicating good optical clarity and stability over time.

The results indicated that formulations prepared by methods H and K demonstrated better stability and homogeneity compared to methods S and M. Method-K, which involved double emulsification, likely reduced interfacial tension between the lipid phase and drugs, enhancing drug affinity and minimizing phase separation between aqueous and lipid phases.

Particle Size and Polydispersity Index (PDI)

Particle size analysis showed that formulations prepared by methods S, M, H, and K had particle sizes ranging from 178.97 ± 2.54 to 472.17 ± 3.51 nm, with corresponding PDIs ranging from 0.29 ± 0.073 to 0.79 ± 0.082. Methods H and K consistently produced smaller particle sizes and lower PDIs, indicating more uniform particle distribution compared to methods S and M. The use of organic solvents and high heat in methods S and M likely contributed to larger particle sizes and higher PDIs, possibly due to particle aggregation and increased drug leakage.

Entrapment Efficiency

Entrapment efficiency (%EE) varied depending on the method and the concentrations of lipids and emulsifiers used. %EE for 5-FU ranged from 23.47 ± 3.722 to 71.62 ± 2.324, and for RSV from 63.73 ± 2.311 to 98.23 ± 1.663 across different methods. Method-K consistently showed higher %EE for both drugs compared to other methods, indicating superior drug entrapment efficiency. This was attributed to the optimized ratio of emulsifier to lipid, which played a crucial role in stabilizing the formulation.

Selection of Optimized Formulation Technique

After 24 hours of storage, formulation number 5 (S-5, M-5, H-5, and K-5) from each technique was selected for comparative analysis. Methods S and M exhibited phase separation, whereas methods H and K maintained stability. Comparative analysis of %T, particle size, PDI, and %EE confirmed that methods H and K outperformed methods S and M in terms of formulation stability and quality attributes.

CONCLUSION

This study comprehensively explored the formulation development of a nanoemulsion-based delivery system for dual drugs, 5-fluorouracil (5-FU) and resveratrol (RSV), focusing on enhancing their efficacy in dermal drug delivery. The research systematically addressed key aspects including excipient screening, lipid optimization, emulsifier selection, drug-excipient interaction studies, and formulation technique optimization using various methods (S, M, H, K). Excipient screening identified Labrasol® (LBR) as an optimal medium-chain oil (MCO) for solubilizing 5-FU and RSV due to its high drug solubility and P-GP inhibitory activity, which may aid in skin tumor treatments. Solid lipid selection favored Emulcire® 61 WL 2659 (EML) for its excellent emulsification properties, contributing to stable formulations even with heat and poorly lipophilic drugs. The miscibility studies highlighted the compatibility of EML with LBR, guiding the selection of a binary mixture (BM) for further optimization using DSC thermal analysis. Method-K, involving double emulsification, emerged as the preferred technique for formulating stable lipid-nanosystems. This method facilitated smaller particle sizes, lower polydispersity indices (PDIs), and higher entrapment efficiencies (%EE) for both drugs compared to methods S and M, which exhibited phase separation and larger particle sizes. Drug-excipient interaction studies using DSC and FTIR confirmed the compatibility of 5-FU and RSV with selected excipients, validating the formulation's stability over extended storage periods. Tween® 80 was identified as an effective emulsifier, enhancing membrane fluidity and facilitating drug permeation, crucial for dermal drug delivery applications. In conclusion, this research provides a robust framework for developing nanoemulsion formulations that optimize drug solubility, stability, and therapeutic efficacy. The findings support the potential of these formulations to enhance the delivery and bioavailability of 5-FU and RSV in dermal cancer treatments, offering promising avenues for future clinical applications in oncology.

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