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Formulation, development and evaluation of organogel loaded with resveratrol

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ABSTRACT:

Present study involved the formulation and development of resveratrol loaded organogel and its characterization studies, including the stability and in vitro dissolution study. To begin, preliminary studies of resveratrol was done, which included solubility and melting point determination, further the solubility with excipients were considered. Different oil ratios and surfactant ratios were taken to form the emulsion loaded with resveratrol. Out of the various batches prepared, and characterization (freeze-thaw, stability test, precipitation test), stable batch was taken to form the resveratrol loaded organogel. The characterization studies of the formulated organogel, including the spreading coefficient, entrapment efficiency, stability studies and in vitro dissolution studies showed significant results proving the efficacy of the prepared organogel.

Introduction

1. Gels

Gels are defined as semi-solid systems with liquid solvent phase immobilized within a three-dimensional (3D) networked structure formed by the gelator molecules via physical and/or chemical manners. Ordinarily, the formation of thermodynamically and dynamically stable gels depends on the physicochemical property of gelator molecules and their resulting affinity to solubilizing solvents. Therefore, depending on the nature of their dispersed solvent phases, gels can be typically classified into hydrogels and organogels (Lin, Kelly et al. 2020).

2. Gels: Classification

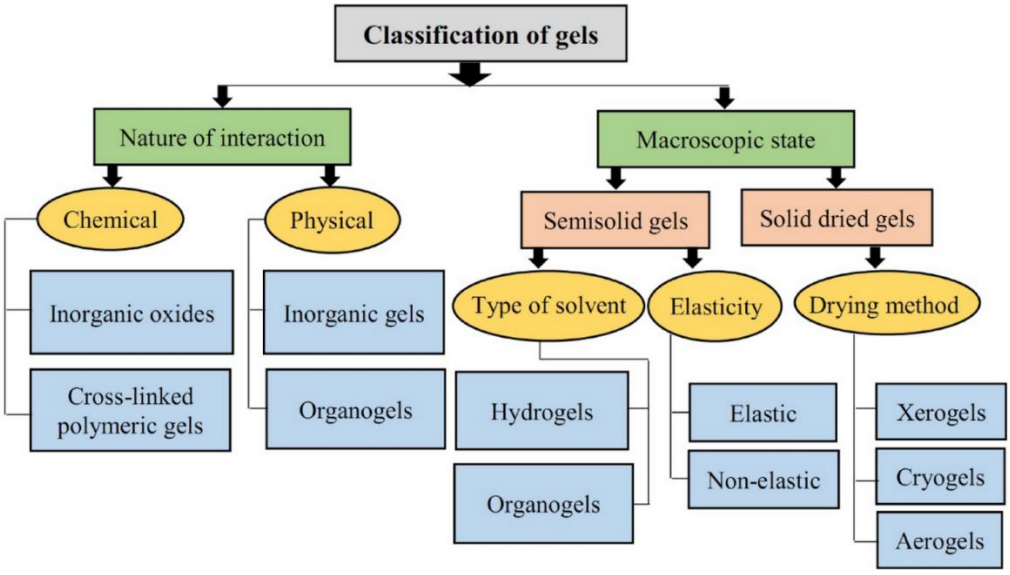


Figure 1: Classification of gels (Izadi, Mahinroosta et al. 2023)

3. Organogels

Organogels, the focus of this review, can be distinguished from hydrogels by their predominantly organic continuous phase and can then be further subdivided based on the nature of the gelling molecule: polymeric or low molecular weight (LMW) organogelators. Polymers immobilize the organic solvent by forming a network of either crosslinked or entangled chains for chemical and physical gels, respectively. The latter is possibly further stabilized by weak inter-chain interactions such as hydrogen bonding, van der Waals forces, and π-stacking. Likewise, the self-assembly of LMW organogelators depends on physical interactions for the formation of aggregates sufficiently long to overlap and induce solvent gelation. Depending on the kinetic properties of aggregates, an important distinction amongst LMW organogels is made between those composed of solid (or strong) *versus* fluid (or weak) fiber networks (Kuzina, Kartsev et al. 2023).

4. Advantages of organogels

- Ease of preparation.
- More stable than other types of gel.
- Enhanced the drug penetration through the skin.
- Avoid first pass metabolism.
- Organogels are moisture insensitive.
- Cost reduction due to less number of ingredients.
- Thermodynamically stable.
- Short half life drug used
- Controlled release of drug, longer shelf life and for prolonged action used.
- Reduces frequency of drug dosing.
- They are less greasy and can be easily removed from the skin.
- Organogel can diminish the diffusion rate of drug because the drug is dissolved in polymer & transported between chains.
- Since it consists of both hydrophobic and hydrophilic components, both Liphophilic and hydrophilic drugs can be incorporated (Zeng, Lin et al. 2021).

Review of Literature

1. **Resveratrol**: Natural polyphenols like resveratrol (RES) can be found in red wine, peanuts, and grapes, among other plant species. These plants accumulate this chemical as a result of UV radiation, damage, and fungal infections, among other environmental stressors. The biological protective effects of resveratrol, such as its anti-aging, anti-inflammatory, antioxidant, antidiabetic, cardioprotective, neuroprotective, and anticancer properties, have recently attracted a lot of interest. Acting as a primary and secondary antioxidant, resveratrol (trans-3,4',5-trihydroxystilbene, MW 228.24) is renowned for its strong antioxidant capabilities. Resveratrol functions as a major antioxidant by scavenging free radicals and converting them into less dangerous compounds. It works as a secondary antioxidant by blocking the actions of enzymes such as xanthine oxidase, lipoxygenase, and cyclooxygenase, which helps to prevent a number of ailments (Diaz-Gerevini, Repossi et al. 2016).

2. **Sources of resveratrol:** Synthetic, semi-synthetic and natural: Commercially, resveratrol is available in both pure forms in native as well as artificial combinations with polyphenols. Trans- resveratrol is overall stable compound but may become unstable when it comes in contact with light, and it is the only trans-isomer that has been consistently associated to multifarious health benefit in pharmacological as well as clinical studies while cis-resveratrol has limited success as an anti-platelet. Resveratrol for nutritional and wellness supplements is predominantly resourced from the plant *P. cuspidatum (syn. Fallopia japonica*) root extracts. The leading producer of trans-resveratrol from root extracts is China using an extraction process. The level of trans-resveratrol purity in these extracts varies between 49%-99%. Alternative sources are therefore being extensively explored to meet the rising demands. Researchers have attempted several methods for resveratrol production like chemical synthesis, semi-synthesis route using natural precursor, and in vitro cell culture based production (Thapa, Pandey et al. 2019).The drawbacks of these methods are that they required a large number of reaction steps which is challenging to pursue, and extraction of precursor for semi-synthesis of resveratrol is cost intensive proposition hence novel microorganisms are being screened as potential substitute for an environment friendly and relatively simple and economical method of resveratrol production (Jeandet, Clément et al. 2014). The resveratrol biosynthesis in plants is through the phenylpropanoid pathways, p-coumaric acid happens to be an essential intermediate which gets converted into p-coumaryl CoA through the enzyme 4 coumarate: coenzyme A ligase subsequent to which stilbene synthase or resveratrol synthase condenses 4 coumaryl co A with 3 molecules of malonyl co A to form resveratrol (Abo-Kadoum, Abouelela et al. 2022). To meet the rising global demand of resveratrol, plant extraction is the classical method currently being used, however its major 2 Introduction 2019 drawback is low abundance in natural material which results due to seasonal, environmental or regional variations in the source. Yet another constraint is the complexity of the synthetic pathway and low total yield by chemical synthesis. Hence biotechnological approaches are explored as they offer the option of scalability using low cost or renewable resources which hold a great promise in commercial production of low volume, high value products (Almagro, Belchi-Navarro et al. 2013).

1. Uses of resveratrol:

1. **Cardioprotective effects of resveratrol:**

Resveratrol has demonstrated a protective effect that enhances cardiovascular function in diabetic mice by preserving the functionality of stem/progenitor cells and mature heart cells. It improves the cardiovascular environment by reducing inflammation and minimizing ventricular remodeling associated with heart disease, resulting in a significant restoration of ventricular function. Additionally, resveratrol positively impacts heart failure by enhancing left ventricular function, decreasing cardiac hypertrophy, alleviating dysfunction and remodeling, reducing interstitial fibrosis, and lowering plasma BNP levels. Its molecular mechanisms of action include inhibiting prohypertrophic signaling molecules, improving myocardial calcium handling, promoting phosphorylation of prosurvival pathways (like Akt-1 and GSK-3β), and reducing oxidative stress and inflammation by decreasing the activity of NOS, COX-2, and ROS formation (Kazemirad and Kazerani 2020).

2. Anti cancer potential:

Resveratrol has gained significant attention as a promising preventive and therapeutic agent against breast cancer. Its potential to enhance chemotherapy arises from its ability to inactivate the protein NF-κB, a transcription factor produced by cancer cells that regulates the expression of specific genes. By blocking this transcription factor, resveratrol allows chemotherapeutic agents to more effectively target their intended sites. Additionally, resveratrol shows promise in combination therapy, especially in carcinoma, as it has been found to reverse drug resistance in various in vitro cell systems, sensitizing tumor cells to the effects of other chemotherapeutic agents (Elshaer, Chen et al. 2018). Notably, resveratrol enhances the sensitivity of carcinoma cells to gemcitabine therapy. Furthermore, it reduces the nephrotoxicity risk associated with cisplatin, a chemotherapy drug used for ovarian, bladder, testicular, and other cancers. Silver nanoparticles loaded with resveratrol have also exhibited antioxidant properties in cancer cells. Overall, resveratrol's beneficial effects are evident when used as an adjunctive treatment alongside chemotherapy and radiotherapy (Wang, Zhang et al. 2017).

3. Anti-inflammatory effect:

Stilbenoids that include resveratrol are polyphenols, both nitrogenous and non-nitrogenous, characterized by their acidic and amphiphilic properties, which contribute to their anti-inflammatory effects. Their main targets include cyclooxygenase (COX), 5-lipoxygenase (5-LOX), and protein kinase B, which relate to the inhibitory activity on COX-1 and COX-2, as well as transcription factors that regulate COX activity (de Sá Coutinho, Pacheco et al. 2018). Research has shown that resveratrol can decrease fluid accumulation and the presence of inflammatory markers. Its anti-inflammatory properties help mitigate acute pharyngitis by inhibiting NF-κB, tumor necrosis factor-α, interleukin-6 serum levels, macrophage-2 inflammatory proteins, cyclooxygenase-2, the production of reactive oxygen species, and caspase 3/9 in rabbit models (Meng, Xiao et al. 2021).

4. Neuroprotective effect:

Resveratrol plays a significant role in preventing dysfunction associated with various neurodegenerative disorders, including Alzheimer's, Huntington's, Parkinson's, and amyotrophic lateral sclerosis. Its protective effects are attributed not only to its anti-inflammatory and antioxidant properties but also to its ability to enhance mitochondrial activity and biogenesis through the SIRT1/AMPK/PGC1α pathway and vitagenes, thereby mitigating oxidative stress. Resveratrol has been shown to regulate neurotrophic factor expression, support cholinergic neurotransmission, reduce oxidative stress, promote the clearance of β-amyloid peptides, facilitate anti-amyloidogenic cleavage of APP, and decrease neuronal apoptosis (Rao, Ganaraja et al. 2020). Meta-analyses indicate that resveratrol significantly reduces scores on the Profile of Mood States (POMS), particularly in terms of energy and fatigue, though it does not have a notable impact on memory and cognitive function. Certain compounds found in resveratrol, such as heyneanol A and vitisin A, have demonstrated superior dose-dependent effects compared to standard inhibitors like galantamine in inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities. Additionally, resveratrol has been shown to improve the strength of rat models and activate the neuroinflammatory response following intracerebral hemorrhage, suggesting its potential as a novel therapeutic agent for treating intracerebral bleeding (Bastianetto, Ménard et al. 2015).

5. Antiaging and antioxidant:

Resveratrol is recognized as a potent antioxidant, but its effectiveness is limited by low bioavailability. To address this, researchers have been exploring resveratrol derivatives through esterification processes to enhance their lipophilicity and suitability for lipid-based foods and biological environments. Approximately 12 different esterified acyl chlorides have been identified, including caproyl, butyryl, myristoyl, capryloyl, docosahexaenoyl, capryl, eicosapentaenoyl, oleoyl, palmitoyl, lauroyl, propionyl, and stearoyl chlorides (De La Lastra and Villegas 2007). These derivatives have shown effectiveness in inhibiting copper ion-induced oxidation of low-density lipoprotein (LDL) and preventing hydroxyl radical-induced DNA damage. These findings suggest that resveratrol derivatives could serve as potential antioxidants in food and biological systems (Zhou, Luo et al. 2021).

6. Antimicrobial effects:

In addition to its previously mentioned benefits, resveratrol has been investigated for its ability to inhibit the growth of certain bacteria, including both Gram-positive and Gram-negative strains, as well as fungi. Notably, resveratrol has been effective against *Candida albicans*, with studies showing that dimethoxy resveratrol exhibits lethal activity against it at low inhibitory concentrations (MIC) ranging from 29 to 37 μg/mL, along with effectiveness against 11 other *Candida* species. However, the candidacidal properties of resveratrol remain debated, as some research suggests it does not inhibit *C. albicans* and other non-*C. albicans* species (Vestergaard and Ingmer 2019).

Furthermore, *Campylobacter jejuni* and *Campylobacter coli*, which are major causes of bacterial gastroenteritis, along with *Arcobacter* species linked to human and animal diseases, have been studied in relation to resveratrol. A formulation of resveratrol with hydroxypropyl-γ-cyclodextrin has been shown to enhance resveratrol's solubility and exhibit anti-*Campylobacter* and anti-*Arcobacter* effects. This formulation also inhibited biofilm formation and enhanced biofilm dispersion, even at sub-MIC levels, suggesting its potential as a new anti-biofilm agent to improve food safety and shelf life (Klančnik, Šikić Pogačar et al. 2017).

Resveratrol has demonstrated antibacterial activity against Gram-positive bacteria, and experiments indicate that this effect may be due to its bacteriostatic properties. However, the exact mechanisms behind its antimicrobial activity are not well understood. Additionally, resveratrol has been found to impact cells with alterations in morphology and DNA content (Chalal, Klinguer et al. 2014).

1. Materials and methods:

1.1 Preformulation studies:

Understanding a drug's physical and chemical characteristics prior to incorporating it into a formulation requires preformulation research. These characteristics have a big impact on processing aspects like compatibility, loading efficiency, pharmacokinetic behaviour, and preparation techniques. They are essential for creating stable, safe, and effective dosage forms. The basis of formulation development is preformulation research, which necessitates the ability to gather information, carry out scientific investigations, and evaluate experimental outcomes. In order to conduct targeted research on pertinent medication qualities, formulators need to be aware of the features of each dosage form. Preformulation research's main goal is to set the stage for formulation process design by offering focused answers to problems that come up during formulation development. The qualities of the drug material have a major impact on the final drug product's quality attributes. Enhancing quality and process reliability can frequently be achieved more successfully by controlling these attributes than by using other strategies. Current trends in product development place a strong emphasis on taking early control of the process.

Preformulation studies encompass

- An evaluation of the drug's physiochemical properties and their applicability to the final formulation.
- The drug's physical and chemical stability.
- The API's physical and/or chemical compatibility with possible excipients.

1.2 Characterization of the drug

For the current study, resveratrol was acquired from Yucca Enterprises in Mumbai, India. The drug's specific characteristics were then noted, and the outcomes were then contrasted with established benchmarks found in the literature. The following formulation studies were carried out.

a**) Organoleptic properties**: The drug powder was physically examined to determine its colour, state, and other physical characteristics.

b) **Solubility studies in solvents and excipients**: In 1 millilitre of solvent (methanol, ethanol, Dimethyl sulfoxide (DMSO)) and excipients (oleic acid, castor oil, tween 20, tween 80, propylene glycol, and polyethylene glycol 400), 10 milligrams of excess resveratrol was added. At room temperature, all of the samples were shaken for 24 hours. Subsequently, the samples underwent a 10-minute centrifugation at 10,000 rpm, and the supernatant was taken out and filtered using a 0.45-micron filter. At 304 nm, samples were examined using UV spectroscopy after appropriate dilutions were made using appropriate solvents. Solubility was computed using the noted absorbance (Herneisey M et al., 2016; Balata GF et al., 2016).

c) **Identification of drug and analytical methodology:** UV spectrophotometry was used as an analytical method of Resveratrol for identification and to quantify resveratrol for different studies including solubility studies, partition coefficient, in vitro drug release and ex vivo skin permeability studies. Methanol and PBS (pH 5.5) were used.

1) Determination of Absorption maxima (λmax) and construction of Calibration curve of Resveratrol

1.1) Preparation of stock solution and dilutions: 10 mg of resveratrol were dissolved in 10 ml methanol to create a stock solution containing one milligram of resveratrol per millilitre that is 1000 ppm. The stock solution was then diluted ten times to create a working standard of 100 ppm. It was then diluted once more to create a solution with a concentration of 10 ppm. Using this method, further dilutions were made using the ten times dilution technique by taking suitable quantities (i.e., 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml) from 10 ppm solution and adding in 9 ml, 8 ml, 7 ml, 6 ml, and 5 ml, methanol respectively, to achieve the resultant dilutions of 1, 2, 3, 4, and 5 ppm, respectively.

1.2) Determination of absorption maxima (λmax) and Calibration curve construction

- Using a UV spectrophotometer, the produced solution was scanned between 200 and 400 nm to determine the absorption maxima (λmax) of resveratrol, which was then compared with published data.
- The produced dilutions were then scanned using a UV spectrophotometer at the determined absorption maxima (λmax), which is 304 nm, to determine the absorbance. The absorbance and concentration of resveratrol were correlated to create a calibration curve, and linear regression analysis was used to determine the best-fit line.

2) Construction of calibration curve using Phosphate Buffer Solution(PBS) (pH 5.5)

2.1) Preparation of PBS (pH 5.5): Using Sorenson's phosphate buffer technique, a PBS solution with a pH of 5.5 was created. To make a 0.2 M solution of disodium hydrogen phosphate, 3.56 g of the substance was first dissolved in 100 ml of distilled water. Likewise, a 0.2 M solution of sodium dihydrogen phosphate was created by dissolving 2.76 g of the compound in 100 ml of distilled water. 4 ml of the 0.2 M disodium hydrogen phosphate solution was added to a beaker and 46 ml of 0.2 M sodium dihydrogen phosphate solution was then added to it. Next, using distilled water, the mixture was diluted to a final volume of 100 ml. A very small amount of sodium dihydrogen phosphate solution was added to fine tune the pH to a desired level.

2.2) Preparation of stock solution and dilutions 10 mg of resveratrol were dissolved in 10 ml of prepared PBS of pH 5.5 to create a stock solution containing one milligram of resveratrol per millilitre that is 1000 ppm. The stock solution was then diluted ten times to create a working standard of 100 ppm.

It was then diluted once more to create a solution with a concentration of 10 ppm. Using this method, further dilutions were made using the ten times dilution technique by taking suitable quantities (i.e., 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml) from 10 ppm solution and adding in 9 ml, 8 ml, 7 ml, 6 ml, and 5 ml, PBS (pH 5.5) respectively, to achieve the resultant dilutions of 1, 2, 3, 4, and 5 ppm, respectively.

2.3) Construction of calibration curve with PBS pH 5.5: The produced dilutions were then scanned using a UV spectrophotometer at 304 nm, to determine the absorbance. The absorbance and concentration of resveratrol were correlated to create a calibration curve, and linear regression analysis was used to determine the best-fit line.

d) Drug – excipient interaction by visual observation:

Several important factors that should be considered while doing visual observations to identify drug-excipient and excipient-excipient interactions include whether there are any physical changes, such as colour changes, phase separation, precipitation, or crystallisation. Next, texture and consistency should be noted, including any changes in the mixture's consistency and whether it becomes sticky, hard, or brittle. There are factors related to physical states, such as efflorescence, deliquescence, and hygroscopicity. There should not be the creation of clumps or aggregates. Odour changes or the creation of new smells may be signs of chemical breakdown or reactions. There should not be any signs of sedimentation or phase separation. Potential interactions that could impact the final pharmaceutical product's stability, efficacy, and safety can be found by keeping account of these factors.

e) Melting point: The melting point of RES was determined using the capillary method. It was filled in a small quantity in a capillary which was inserted in melting point apparatus (MR-VIS, Labindia, Mumbai, India) and as the temperature raised the melting point of the drug was noted. The temperature at which the drug started melting was noted and a temperature at which the drug was completely melted was noted. The melting point was reported as a temperature range.

f) Partition coefficient: To evaluate the drug's hydrophilicity and lipophilicity, the partition coefficient must be determined. For this, the Shake Flask method was applied. A particular quantity of the medication that is RES was dissolved in a mixture of equal parts of octanol and distilled water in a flask. After agitating the flask to reach equilibration, phase separation was accomplished by allowing it to rest. UV spectrophotometry was used to determine the resveratrol amounts in both phases upon separation. The samples were scanned at 304 nm. With the obtained absorbance, the concentration was calculated. Using the following formula, the partition coefficient (K) was determined: (Cumming H and Rucker C, 2017)

$$K_{o/w} = C_o / C_w \qquad \text{(Eq 4.1)}$$

Where, K- partition coefficient

Co - concentration of resveratrol in organic solvent

Cw - concentration of resveratrol in distilled water.

1.4 Formulation of Resveratrol loaded emulsion:

The emulsion was prepared using oleic acid as oil, Tween 20 as surfactant, Propylene Glycol as co-surfactant and water as continuous phase. The emulsion prepared was an O/W emulsion. Tween 20 and Propylene Glycol were mixed in equal ratio that is 1:1 to get a mixture of surfactant: co-surfactant which is called as Smix. The oleic acid was taken first and Smix was added in it in a certain quantity and mixed properly. Then the water was added slowly in the required quantity with continuous magnetic stirring at 500 rpm to make a emulsion. Care must be taken during formulation development so that no turbidity is seen and foam formation has to be avoided. In the above-mentioned method, the quantities of ingredients that needs to be added was decided on the basis of ratios selected from the developed Pseudo – Ternary Plot. According to it five different ratios were selected as given in **table** and blank formulations were developed that is without adding drug. After detecting the stability, of these formulations three formulations were selected which were F1, F4 and F5 and they were developed again by adding drug. Oleic acid was taken and drug was added into it and was mixed properly after which the Smix was added following which the water in the required quantity was added slowly with continuous magnetic stirring at 500 rpm. (Patel SK et al., 2013; Afzal O et al., 2023; Herneisey M et al., 2016; Balata et al., 2016).

Table 3: Different batches of the formulation made

Formulation	Oil (Oleic acid) (%)	Smix (1:1) (%)	Water (%)
F1	4.3	30	65
F2	4.5	40.9	54.54
F3	4.34	47.8	47.8
F4	13	39	47.8
F5	13	47.8	39.13

1.5 Characterization of Resveratrol loaded emulsion (F1 to F5)

1.5.1 pH determination:

The pH of the RES emulsion was determined with a digital pH meter. The electrode was fully immersed in the emulsion to ensure complete coverage. Measurements were conducted in triplicate, and the average value of these readings was recorded.

1.5.2 Thermodynamic Stability and Precipitation assessment

1.5.2.1 Centrifugation test:

Accelerated ageing was done using centrifugation and freeze-thaw cycles to evaluate thermodynamic stability. The formulations that had "good" dispersion properties were centrifuged for 30 minutes at 5,000 rpm, and any indications of phase separation, including creaming or cracking, were noted. For the ensuing freeze-thaw testing, only the formulations that passed the centrifugation test were chosen (Afzal O et al., 2023, Harshal M et al., 2011).

1.5.2.2 Freeze – Thaw test:

The chosen formulations were made to go through four freeze-thaw cycles, each lasting 24 hours, with the freezing phase lasting at -4°C and the thawing phase lasting at 40°C and the changes were noted (Afzal O et al., 2023, Harshal M et al., 2011).

1.5.2.3 Precipitation assessment:

After 24 hours, precipitation was detected by visually examining the resultant emulsion. The formulations were categorised as non-clear (turbid), clear (transparent or transparent with a bluish tinge), stable (no precipitation after 24 hours) and unstable (precipitation detected within 24 hours) (Afzal O et al., 2023, Kumar Gupta S, nd.).

4.2.4.4 Entrapment efficiency (EE):

Entrapment efficiency of the RES emulsion was determined using centrifugation method. Here the formulation was developed by adding 400 mg of drug to make a 10mg/ml concentration. Then this formulation was centrifuged at 10,000 rpm for 5 minutes. Supernatant was separated and added in sufficient quantity of methanol to dissolve the drug in methanol. This sample was then tested in UV spectrophotometer to get the absorbance and concentration was calculated from it. The detected concentration was of un associated drug. Using the below formula the entrapment efficiency (EE %) was calculated. Experiment was performed in triplicate (Hamzawy et al., 2017).

Entrapment Efficiency (EE%) = Total amount of drug – amount of unbound drug X 100 Total amount of drug

4.2.5 Formulation of Resveratrol loaded organogel:

To prepare the resveratrol-loaded organogel, a systematic procedure was followed. Initially, a emulsion containing resveratrol was prepared in a volume of 40 ml. Subsequently, 400 mg of Carbopol 940 was incorporated into the emulsion to achieve a 1% gel solution. This mixture was then homogenized using a mechanical mixer for a minimum duration of one hour to ensure thorough blending and consistency. As the mixture began to thicken, 3-4 drops of triethanolamine were added, which were then mixed properly to transform the mixture into the desired gel form. This method ensured the emulsion was effectively integrated into the gel matrix, achieving the intended consistency and properties (Prajapati et al., 2021; Gupta et al., 2022; Nawaz et al., 2022).

4.2.6 Characterization of Resveratrol loaded organogel

4.2.6.1 Measurement of pH:

The pH of the RES organogel was determined with a digital pH meter. The electrode was fully immersed in the to ensure complete coverage. Measurements were conducted in triplicate, and the average value of these readings was recorded.

4.2.6.2 Viscosity measurement:

The viscosity of the developed gel was measured at varying shearing rates at room temperature using a MCR 102e, Modular Compact Rheometer, Anton Paar India Pvt. Ltd (Nawaz et al., 2022).

4.2.6.3 Spreadability:

The parallel plate method was used to assess the Resveratrol-loaded organogel's spreadability. A precisely weighed 0.5 g sample was spread into a circle with a designated diameter of 1 cm on a glass plate. Subsequently, the gel sample was carefully covered with a second glass plate. The gel was then allowed to spread out on the plates by applying a 500 g weight for five minutes. The increase in diameter brought on by the gel spreading was measured after the allotted amount of time. To guarantee that the results could be repeated and are accurate, this experiment was carried out in triplicate. A linear scale was used to measure the extension in order to quantify the spreadability. The following formula was used to calculate the spreadibility of organogel (Nawaz et al., 2022).

Spreadibility (S) = m . l t (Eq 4.3) Where, S represents spreadibility m is mass applied l is length of the spread of gel in cm (average diameter) t is time in seconds

4.2.6.4 Stability studies:

In order to make sure that the formulation created is stable and safe, three samples of Resveratrol loaded organogel were prepared and all the samples were subjected to a high temperature of 40°C for a period of 1 month. The samples were withdrawn from each of the three developed organogels at regular intervals of time that is at 0,2 and 4 weeks, to check for appearance, pH and concentration of drug to ensure that there are no major changes in these characteristics. UV Spectrophotometry was used to perform the drug assay of formulations using methanol as solvent. Appearance was checked visually and 81 the pH was checked using a digital pH meter (IG-10PH, PH Meter, IGene Labserve Pvt. Ltd.) (Afzal O et al., 2023, Harshal M et al., 2011).

Results and Discussion:

5.1 Preformulation studies

5.1.1 Organoleptic properties The drug powder was physically examined and the following observations were recorded. The recorded observations of physical state, colour and powder odour of the drug were found to be similar to the reference reported in official literature.

Table 5.1: Observed organoleptic properties of Resveratrol

Properties	Resveratrol
Physical form	Amorphous powder

Colour	Off - white
Odor	Odourless – slight earthy smell

5.1.2 Solubility studies: In solvents: It was experimentally found that resveratrol is highly soluble in methanol and the resultant order of solubility is methanol>ethanol>DMSO. In excipients: Using UV/VIS spectroscopy, the solubility of resveratrol in a variety of oils, surfactants, and co-surfactants was ascertained. Drug was found to be highly soluble in oleic acid, tween 20 and propylene glycol and these excipients are selected to be used in formulation development.

Table 5.2: Experimentally obtained solubility values of resveratrol in different excipients

Excipients	Concentration
Oleic acid	6.23 ± 0.06 mg/ml
Castor oil	3.14 ± 0.05 mg/ml
Tween 80	7.259 ± 0.49 mg/ml
Tween 20	8.984 ± 0.58 mg/ml
Propylene glycol	13.011 ± 0.28 mg/ml
PEG 400	10.894 ± 0.83 mg/ml

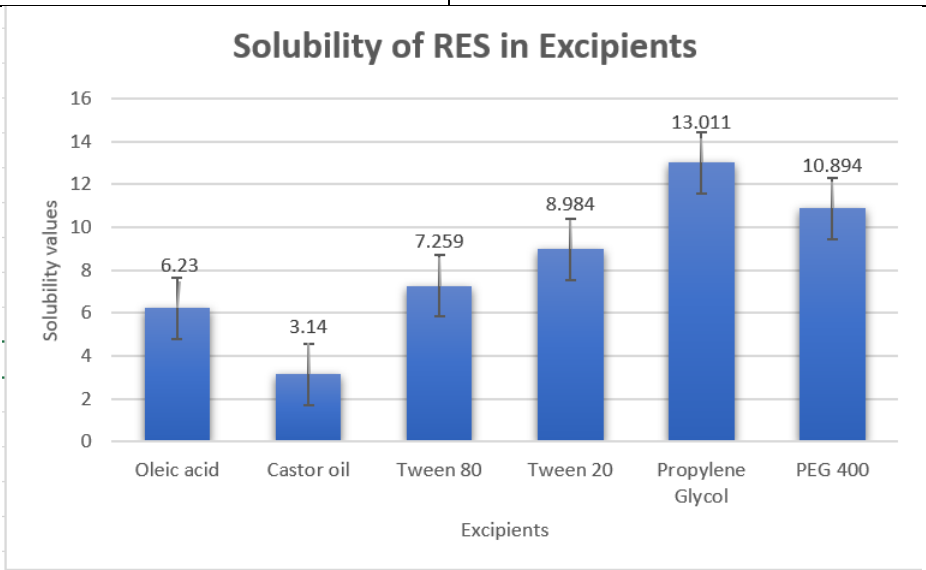


Figure 5.1: Solubility of Resveratrol in different Excipients

5.1.3 Identification of drug and analytical method

5.1.3.1 Determination of Absorption maxima (λmax) and construction of Calibration curve of Resveratrol:

5.1.3.1.A Determination of Absorption maxima (λmax) of Resveratrol:

Lambda max of resveratrol was identified using UV-Vis spectroscopy and was found to be 304 nm as depicted by the figure 5.2. The wavelength of 304 nm 88 was chosen for the λmax because it is the point on a bell-shaped peak where the maximum absorption occurs. Selecting a peak with a bell shape is beneficial since the absorbance of a solution changes quickly with small wavelength differences on its steep sides. If there is even a slight variation in the wavelength setting of the instrument, this quick change can result in significant measurement inaccuracies. As a result, a bell-shaped peak reduces the possibility of appreciable errors in absorbance readings, guaranteeing more accurate and consistent observations.

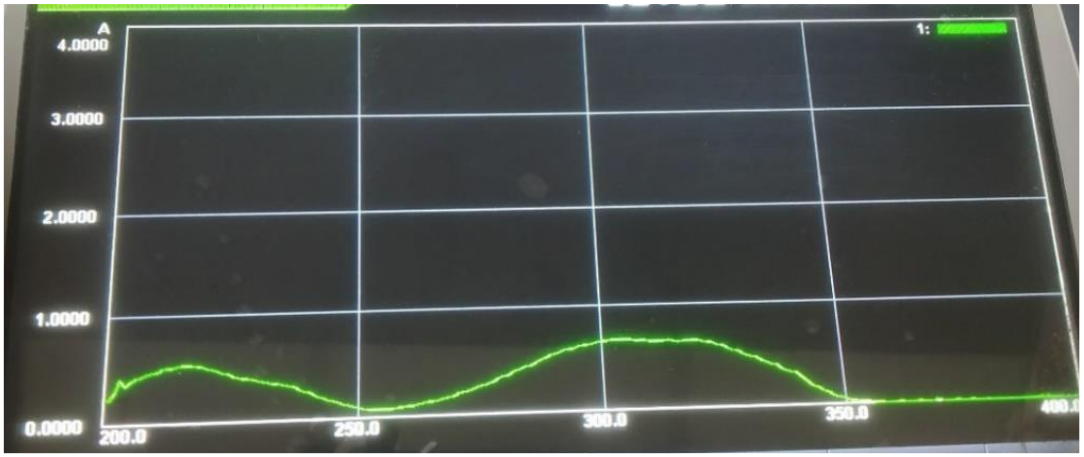


Figure : Absorption maxima of resveratrol in Methanol Solvent Absorption maxima (λ max) (Xu, Hu et al.) Methanol 304

5.1.3.1.B Construction of calibration curve of Resveratrol:

Using the different dilutions that were made, absorbance values of Resveratrol at different concentrations were determined and these values along with concentration values were plotted on a graph to get the calibration curve. The regression value was calculated and was found to be 0.991.

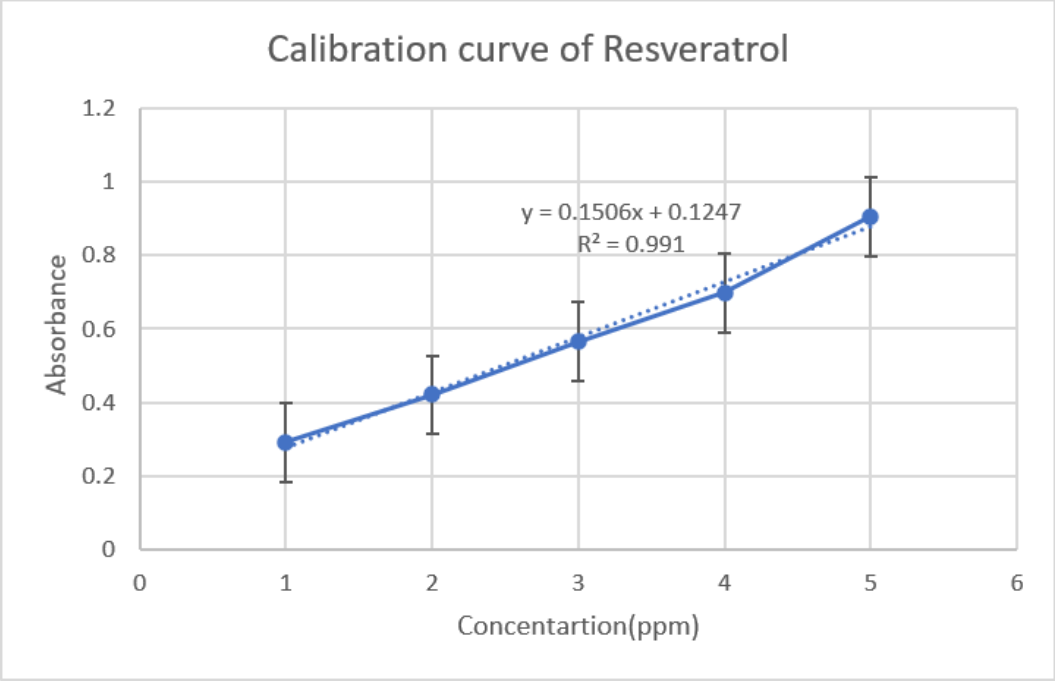
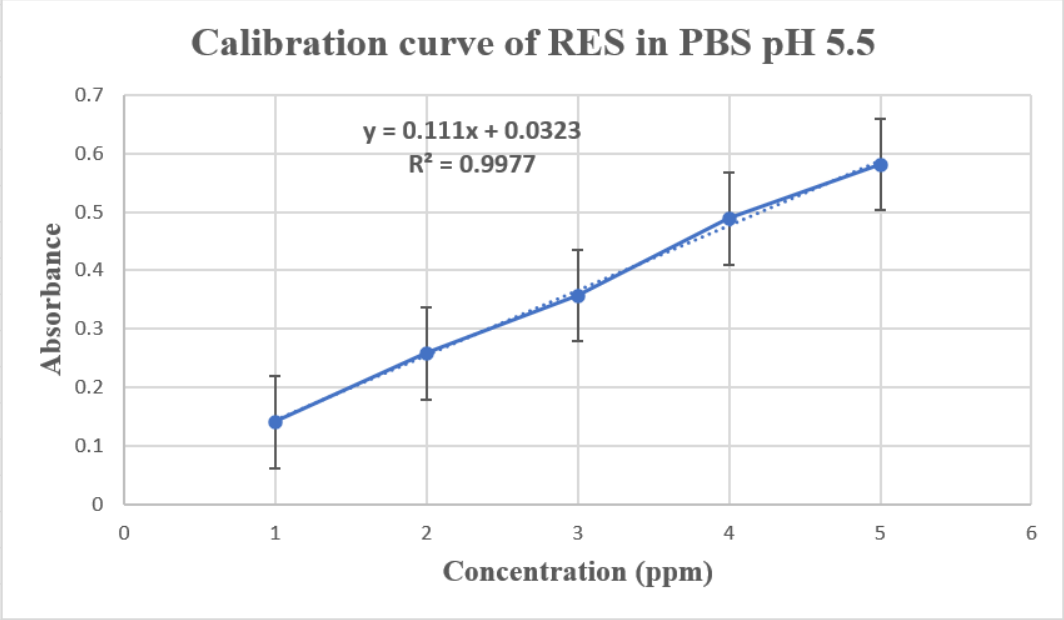


Figure 5.3 depicts the generated calibration curve of Resveratrol in methanol.

5.1.3.1.C Construction of calibration curve of Resveratrol in PBS (pH 5.5):

Using the different dilutions that were made in PBS of pH 5.5, absorbance values of Resveratrol at different concentrations were determined and these values along with concentration values were plotted on a graph to get the calibration curve. The regression value was calculated and was found to be 0.9977. Figure 5.4 depicts the prepared calibration curve of Resveratrol in PBS (pH 5.5).



5.1.4 Drug – excipient interaction by visual observation:

There were no physical changes, such as colour changes, phase separation, precipitation, or crystallisation. Texture and consistency were also same. No aggregates formation was there. Any new odour was not developed and there was no change in odour with absence of sedimentation or phase separation. These results suggested that the developed formulation is free of any kind of potential interactions and is stable and safe.

5.1.5 Melting point:

The melting point of pure Resveratrol was found to be 252 ± 1°C. Since the experimentally obtained melting point is found to be near the actual melting point of Resveratrol, it can be suggested that the compound is pure and this is also likely to be confirming the identity of the compound.

5.1.6 Partition coefficient:

To evaluate the drug's hydrophilicity and lipophilicity, the partition coefficient was determined because it is a fundamental parameter which can influence various parameters of formulation development like, solubility, permeability, stability, distribution and more. Absorbance values were determined using UV spectrophotometer of Resveratrol in octanol and Resveratrol in water, which was used to calculate concentration of Resveratrol in these solvents and the formula was used to determine partition coefficient, which was found to be 2.98 ± 0.053.

Table 5.6: Mean absorbance with obtained concentration and calculated partition coefficient

Solvents	**Mean absorbance ± SD(N=3)**	**Concentration ± SD(N=3)**
Octanol	0.1920 ± 0.0002	4.47 ± 0.013
Water	0.1473 ± 0.0004	1.5 ± 0.027
Calculated partition coefficient (P) = 2.98 ± 0.053		

5.2 Preparation of resveratrol loaded emulsion:

The emulsion containing the resveratrol was prepared and 5 different batches were prepared. The three batches, F1, F4 and F5 were stable, but F2 and F3 showed phase separation, hence these batches were rejected here itself. Characterization of F1, F4 and F5 were only done.

Formulation	**Oil(Oleic acid) (%)**	**Smix (1:1) (%)**	**Water (%)**	**Drug**
F1	4.3	30	65	10 mg/ml
F2	4.5	40.9	54.54	10 mg/ml
F3	4.34	47.8	47.8	10 mg/ml
F4	13	39	47.8	10 mg/ml
F5	13	47.8	39.13	10 mg/ml

5.3 Characterization of Resveratrol loaded emulsion

5.3.2 pH determination

The pH of the Resveratrol loaded emulsion are shown in the following table. All the values are in range of 5-7, which suggests that this can be used for development of gel.

5.3.3 Thermodynamic Stability and Precipitation assessment

5.3.3.1 Centrifugation test

The formulation F4 showed phase separation after the centrifugation test and hence was not considered further. Whereas, the formulations F1 and F5 did not display any phase separation and were found to be stable as a result of which these were considered for freeze – thaw test.

5.3.3.2 Freeze – Thaw test

The formulation F5 after the freeze – thaw test was found to be unstable at changing temperatures and was showing visible precipiation, as a result of which, it was not considered further and formulation F1 was stable after completion of test.

5.3.3.3 Precipitation assessment

It was found after completion of this test that the formulation F1 was not showing any precipitation and was categorized as stable and it's appearance was somewhere between transparent to translucent due to colour of the drug. Hence according to results of particle size and stability F1 formulation was finalized to be developed further.

5.3.4 Entrapment Efficiency (EE)

The experiment was performed and using the equation entrapment efficiency of the developed and finalized Resveratrol loaded emulsion was determined. The Entrapment Efficiency was found to be 80.72 ± 2.37%. This suggests that a good amount of drug was entrapped in the emulsion formulation.

After development and testing of the F1, F4 and F5 ratios for important properties; it was found that the F1 formulation was giving better results and can be further worked upon to get best results which can improve the particle size, drug release and penetrability. The F4 and F5 were found to be unstable in Centrifugation test and Freeze-Thaw test of stability respectively; hence were not taken for further development.

Table : Stability test observations for formulation F1, F4, and F5 Formulation

	Observation	**Inference**
F1	No change suggesting stability problem was observed in Centrifugation, Freeze-Thaw and Precipitation stability test	Stable
F4	Phase separation after Centrifugation test	Unstable
F5	Visible precipitation after Freeze-Thaw test	Unstable

Final selected ratio

Formulation	**Oil (Oleic acid) (%)**	**Smix (1:1) (%)**	**Water (%)**	**Drug**
F1	**4.3**	**30**	**65**	**10 mg/ml**

Development of Resveratrol loaded organogel

The F1 formulation was used to develop the gel. The gelling medium was prepared and the F1 emulsion was incorporated into it. The developed gel after following the procedure was of appropriate characteristics as were desired. The appearance, texture, spreadibility and consistency were all as per requirement.

Characterization of Resveratrol loaded organogel

5.5.1 Measurement of pH

The pH of the developed Resveratrol loaded organogel was found to be 5.5 and it is the desired pH for the formulation as per its applications.

5.5.2 Viscosity measurement

The viscosity of the developed RES loaded organogel was determined using MCR 102e, Modular Compact Rheometer, Anton Paar India Pvt. Ltd and was found to be **29.21 Poise**. The observed viscosity was then compared with standard range of viscosity for topical gels given in the standard literature and was found to be in the range and appropriate. In this experiment it was observed that the viscosity was decreasing with the increasing shear rate, which suggests that the gel demonstrates a Pseudoplastic (Shear-Thinning) flow behavior which suggests that the developed gel will be relatively easy to spread on the skin due to low viscosity on spreading, providing a smooth application experience. The obtained viscosity is likely to facilitate good skin absorption, as it becomes a bit more viscous when shear force is removed and it is not too thick to impede the release and penetration of Resveratrol, which ensures effective delivery of active ingredient to the skin. Being more viscous when not under shear force also gives a sustained release of drug to provide a sustained therapeutic effect and stability will also be ensured. Being not too thick or greasy, it will ensure improved user compliance and satisfaction.

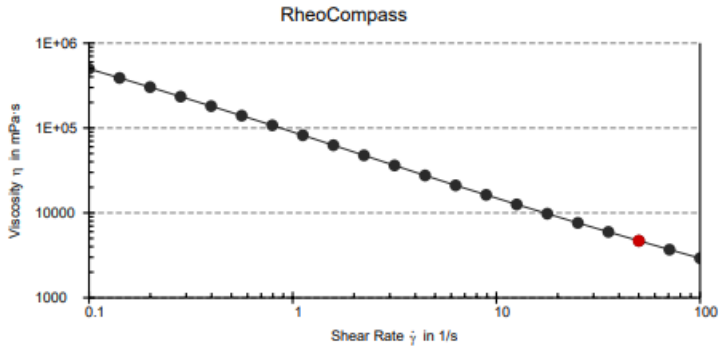


Figure: Viscosity report of Resveratrol loaded organogel

Spreadibility

When the experiment was completed successfully after the allotted time, the final diameter was noted and the average was found out. This diameter was then used to calculate the spreadibility using the equation 4.3. The calculated spreadibility was found to be 5.1 g.cm/s and was compared to the available standard literature to be well within a range so that it can be said to be having good spreadibility.

Table 5.13: Average diameter and spreadibility of RES loaded organogel

Formulation	**Average diameter**
RES loaded organogel	3.1
Calculated Spreadibility (S) = 5.1 g.cm/s	

Stability study

Stability study of three samples of developed Resveratrol loaded organogel was done at a high temperature of 40°C and it was found that the pH of the formulation was consistent with negligible change, appearance does not changed in any manner to suggest any instability in the developed formulation as it was consistent throughout the test period and no change in colour, odour and consistency. Assay of drug was done and it was found that a very minor variation in the concentration of drug was there which did not suggest any degradation of the drug. The results suggests that all the samples of developed formulation did not show any major variations in pH, appearance and drug concentration which provides us evidence to report that the developed formulation is stable and it can be concluded that it is safe to use and its efficacy is also ascertained.

Table: Results of stability studies

Parameter	**Time point**	**Formulation**
Appearance	**Week 0**	Clear
	Week 2	Clear
	Week 4	Clear
pH ± SD (N=3)	**Week 0**	5.5 ± 0.1
	Week 2	5.5 ± 0.2
	Week 4	5.4 ± 0.1
Assay [Drug concentration ± SD (N=3) (%)]	**Week 0**	99.33 ± 0.54
	Week 2	99.26 ± 0.44
	Week 4	99.23 ± 0.48

***In vitro* release test**

5.6.1 *In vitro* release test of RES loaded nanoemulsion

The nanoemulsion with Resveratrol was tested for release of drug and the medium used was PBS of pH 5.5. It was found after the experiment that the release of drug from the nanoemulsion is good and the drug is being released at a constant rate. The time of release was extended and about **81.49 ± 0.062%** of drug was calculated to be released in eight hours. It was also noted that about 50 ± 0.0062% of drug was released within 3-4 hours which was suggesting that a gel formulation can be considered to further sustain the release of drug from formulation.

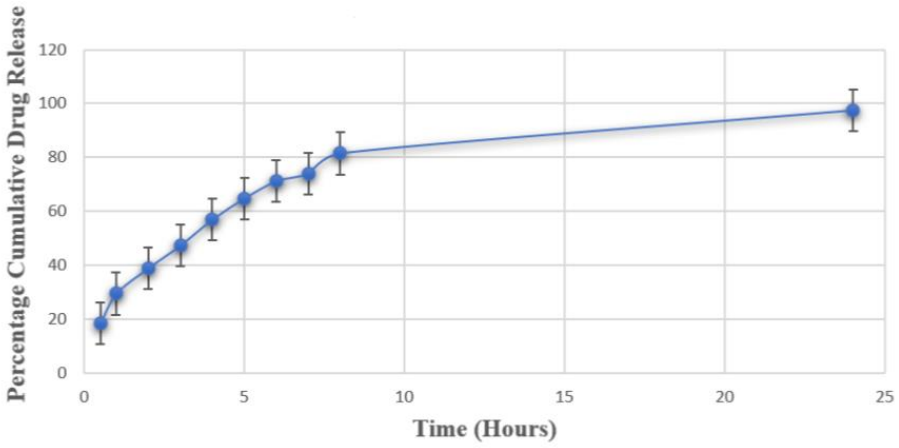


Figure : *In vitro* drug release from RES loaded organogel

Summary and Conclusion:

Many carrier systems, such as ethosomal creams (Kaundal & Kumar, 2024), solid lipid nanoparticles (Shrotriya *et al*., 2017), nanoethosome hydrogels (Arora & Nanda, 2019), and nanostructured lipid carriers (Marques *et al*., 2023), have been developed to solve these problems. Potential treatments for ICD may come from these formulations because of their
encouraging outcomes in terms of particle size, entrapment efficiency, controlled drug release, skin targeting, and therapeutic efficacy.

In this study a similar approach was followed to develop a Resveratrol loaded organogel for the future benefits of skin related disorders. Due to its many protective qualities, such as anti-aging, anti-inflammatory, antioxidant, antidiabetic, cardioprotective, neuroprotective, and anticancer effects, Resveratrol—which is present in red wine, peanuts, and grapes—has drawn attention (Wu CF *et al*., 2013; Pangeni R *et al*., 2014; Amri A *et al*., 2012). It is especially helpful for skin health, since it lowers the incidence of skin cancer and also protects against UV-induced oxidative stress, treats inflammatory illnesses like psoriasis (Kjaer TN *et al*., 2015), and guards against infections. Skin thickness, elasticity, and attractiveness are all improved by its use in cosmetics (Singh G & Pai RS, 2014). Resveratrol is a promising candidate for the management of irritant contact dermatitis (ICD) despite pharmacokinetic challenges due to its anti-inflammatory and potent antioxidant properties, which include scavenging free radicals and inhibiting harmful enzyme activities (Acquaviva R *et al*., 2002; Bhat KP & Pezzuto JM, 2002; Deostri IE, 2000; Fauconneau B *et al*., 1997; Li ZD *et al*., 2006).

The goal of the current study was to develop a novel Resveratrol-based formulation that would be easy to use, improve skin permeability, improved efficacy and guarantee non-toxicity. The study showed various advantages of using organogel to administer Resveratrol. Pre-formulation investigations depicted that all the values were within the optimal range and that there were no incompatibilities between formulation ingredients.
In order to optimise RES emulsion, different ratios were considered and the best among these was then chosen based on the characteristic tests. This led to the development of the final formulation that was F1; which was then used to make organogel.
Further, the Spreadibility and viscosity were also tested and were found to be 5.1 g.cm/s and 29.21 Poise, respectively; which were well within the range values considered as appropriate for a organogel according to literature. The observed value of viscosity suggested that the developed formulation demonstrated a Pseudoplastic (Shear-Thinning) flow behaviour which suggests that the developed gel will be relatively easy to spread on skin when shear is applied and when the application shear is removed the viscosity increases which will facilitate good skin absorption of drug. The developed formulation was found to be stable on testing for stability at a high temperature of 40°C for appearance, pH and drug Concentration. An 81.49 ± 0.062% drug release over an 08-hour period was observed from organogel, respectively, in the *in vitro* release experiments.

REFERENCES:

1. Abo-Kadoum, M. A., M. E. Abouelela, et al. (2022). "Resveratrol biosynthesis, optimization, induction, bio-transformation and bio-degradation in mycoendophytes." Front Microbiol **13**: 1010332.

2. Acquaviva R, Russo A, Campisi A, Sorrenti V, Di Giacomo C, Barcellona ML, Avitabile M, Vanella A (2002) Antioxidant activity and protective effect on DNA cleavage of resveratrol. J Food Sci 67:137–141. doi:10.1111/j.1365-2621.2002.tb11373.x.

3. Afzal O, Altamimi ASA, Alamri MA, Altharawi A, Alossaimi MA, Akhtar MS, et al. Resveratrol-Loaded Chia Seed Oil-Based Nanogel as an Anti-Inflammatory in Adjuvant-Induced Arthritis. Gels. 2023 Feb 1;9(Tumati and W/W).

4. Almagro, L., S. Belchi-Navarro, et al. (2013). Bioproduction of trans-Resveratrol from Grapevine Cell Cultures**:** 1683-1713

5. Amri A, Chaumeil JC, Sfar S, Charrueau C. Administration of resveratrol: What formulation solutions to bioavailability limitations? J Control Release [Internet]. 2012;158(Tumati and W/W):182–93.

6. Balata GF, Essa EA, Shamardl HA, Zaidan SH, Abourehab MAS. Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol. Drug Des Devel Ther. 2016 Jan 5; 10:117–28.

7. Bastianetto, S., C. Ménard, et al. (2015). "Neuroprotective action of resveratrol." **1852**(6): 1195-1201.

8. Bhat KP, Pezzuto JM (2002) Cancer chemopreventive activity of resveratrol. Ann NY Acad Sci 957:210–229. doi:10.1111/j.1749-6632.2002.tb02918.x.

9. Chalal, M., A. Klinguer, et al. (2014). "Antimicrobial activity of resveratrol analogues." **19**(6): 7679-7688.

10. Cumming H, Rücker C. Octanol-Water Partition Coefficient Measurement by a Simple 1H NMR Method. ACS Omega. 2017 Sep 30;2(9):6244–9.

11. De La Lastra, C. A. and I. J. B. S. T. Villegas (2007). "Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications." (Tumati and W/W): 1156-1160.

12. de Sá Coutinho, D., M. T. Pacheco, et al. (2018). "Anti-inflammatory effects of resveratrol: Mechanistic insights." **19**(6): 1812.

13. Diaz-Gerevini, G. T., G. Repossi, et al. (2016). "Beneficial action of resveratrol: How and why?" **32**(Tumati and W/W): 174-178.

14. Elshaer, M., Y. Chen, et al. (2018). "Resveratrol: An overview of its anti-cancer mechanisms." **207**: 340-349.

15. Gupta, R., Garg, T., & Sharma, R. B. (2022). Preparation and Characterization of Luliconazole Nanoemulsion Based Hydrogel. International Journal of Pharmaceutical Sciences and Medicine, 7(9), 10–19.

16. Harshal M, Tanvir S, Dheeraj B, Rajendra W. Design and development of solid self-micro-emulsifying drug delivery system (SEDDS) of fenofibrate. Int J Pharm Pharm Sci. 2011;3(4):163–166.

17. Herneisey M, Williams J, Mirtic J, Liu L, Potdar S, Bagia C, et al. Development and characterization of resveratrol nanoemulsions carrying dual-imaging agents. Ther Deliv. 2016 Dec 1;7(12):795–808.

18. Izadi, R., M. Mahinroosta, et al. (2023). "An inclusive review on inorganic gels: classifications, synthesis methods and applications." Journal of the Iranian Chemical Society **20**(8): 1757-1779.

19. Jeandet, P., C. Clément, et al. (2014). "Resveratrol production at large scale using plant cell suspensions." Engineering in Life Sciences **14**.

20. Kazemirad, H. and H. R. J. M. B. R. Kazerani (2020). "Cardioprotective effects of resveratrol following myocardial ischemia and reperfusion." **47**(8): 5843-5850.

21. Klančnik, A., M. Šikić Pogačar, et al. (2017). "Anti-Campylobacter activity of resveratrol and an extract from waste Pinot noir grape skins and seeds, and resistance of Camp. jejuni planktonic and biofilm cells, mediated via the CmeABC efflux pump." **122**(1): 65-77.

22. Kumar Gupta S. Formulation and Evaluation of Nanoemulsion Based Nanoemulgel of Aceclofenac.

23. Kuzina, M. A., D. D. Kartsev, et al. (2023). "Organogels versus hydrogels: advantages, challenges, and applications." **33**(27): 2301421

24. Lin, D., A. L. Kelly, et al. (2020). "Preparation, structure-property relationships and applications of different emulsion gels: Bulk emulsion gels, emulsion gel particles, and fluid emulsion gels." **102**: 123-137.

25. Meng, T., D. Xiao, et al. (2021). "Anti-inflammatory action and mechanisms of resveratrol." **26**(1): 229

26. Nawaz, A., Latif, M. S., Alnuwaiser, M. A., Ullah, S., Iqbal, M., Alfatama, M., & Lim, V. (2022). Synthesis and Characterization of Chitosan-Decorated Nanoemulsion Gel of 5-Fluorouracil for Topical Delivery. Gels, 8(7).

27. Patel SK, Zhang Y, Pollock JA, Janjic JM. Cyclooxgenase-2 Inhibiting Perfluoropoly (Ethylene Glycol) Ether Theranostic Nanoemulsions-In Vitro Study. PLoS One. 2013 Feb 7;8(Tumati and W/W).

28. Rao, Y. L., B. Ganaraja, et al. (2020). "Neuroprotective effects of resveratrol in Alzheimer's disease." **12**(1): 139-149.

29. Thapa, S. B., R. P. Pandey, et al. (2019). "Biotechnological Advances in Resveratrol Production and its Chemical Diversity." Molecules **24**(14).

30. Vestergaard, M. and H. J. I. J. o. A. A. Ingmer (2019). "Antibacterial and antifungal properties of resveratrol." **53**(6): 716-723.

31. Wang, W., L. Zhang, et al. (2017). "Anticancer effects of resveratrol-loaded solid lipid nanoparticles on human breast cancer cells." **22**(11): 1814.

32. Zeng, L., X. Lin, et al. (2021). "Recent advances of organogels: from fabrications and functions to applications." Progress in Organic Coatings **159**: 106417.

33. Zhou, D.-D., M. Luo, et al. (2021). "Effects and mechanisms of resveratrol on aging and age-related diseases." **2021**(1): 9932218.