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Formulation, Development and Characterization of Microemulgel Containing Plant Bioactive with Antimicrobial Activity

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ABSTRACT

Chrysin, the natural bioactive flavonoids compound under class IV drug according BCS has poor permeability and solubility in water. So, the main goal was to overcome this problem. The present work aims to create and evaluate a gel based on microemulsions for enhanced solubility and permeability. The best alternatives to synthetic medications are believed to be flavonoids, alkaloids, glycosides, terpenoids, phenolic acid, peptides, tannins, and other plant bioactive compounds. The current study aims to formulate and characterise a microemulgel containing plant bioactive with antibacterial activity in order to improve the therapeutic impact. It was looked at how soluble chrysin was in a variety of oils, surfactants, and cosurfactants. Additionally, DMSO was used as a solubilizing agent and permeability booster. An adjustment to the surfactant to cosurfactant ratio (Smix) produced a pseudo-ternary phase diagram. Sodium alginate and Carbopol 940 were used as gelling agents. Optimized microemulgel was prepared and characterized for drug content, viscosity, spreadability, *in vitro* permeation study, antimicrobial study and stability study. The optimized microemulgel (F5) showed acceptable physical properties with lowest viscosity value (0.896±0.06 mPa·s) and highest spreadability value (23.96 gm.cm/s), high drug permeation (90.11%) after 8hr. Optimized formulation F5 represents greater zone of inhibition as compared with test sample and showed acceptable stability profile. Conclusion: The produced microemulgel improved the permeability and solubility of chrysin while being stable and effective.

Key words :- Chrysin, Microemulgel, Antimicrobial, Bioactive, DMSO.

Introduction

Skin has long served as a crucial barrier between the outside world and the body's inside, protecting the internal organs from the outside environment. The very active organ known as the skin adapts to mechanical pressures, prevents excessive water loss, promotes transpiration cooling, shields the body from the sun's damaging rays, promotes skin cell regeneration, and guards against ingesting foreign objects.

Although skin is a well-known channel for drug administration, its uses are limited to local effects. Researchers today view the topical distribution of medications or the targeting of medications to certain areas for systemic effects as a challenge. Due to a number of benefits, transdermal medication administration has been acknowledged as one of the most promising methods for both local and systemic drug delivery. While limiting the therapeutic effect to certain tissues (targeting a specific spot), topical administration of bioactive chemicals is a potent method for lowering their systemic toxicity.[1,2,3]

The avoidance of hepatic first-pass metabolism, salivary and stomach drug degradation, and related toxicity consequences are just a few of the benefits of topical medication administration over other approaches.[4]

Medicinal plants have been used widely in nutraceuticals and cosmeceuticals in which phytochemicals from plants represent natural sources of compounds with several biological benefits.[5] Some plant bio-actives have antioxidant, antimicrobial, anticancer, antiviral, and antitumor and many more activities to a greater or lesser extent. Amongst naturally occurring plant bioactives are glycosides, alkaloids, tannins, triterpenes, flavonoid, phenol, alcohol shows antimicrobial activity. Flavonoid such as kaempferol, quercetin, rutin, catechin, chrysin, naringin, hesperidins and apigenin that contribute to the total phenolic content can also be found in plant like *Centella Asiatica, Brachychiton Popolneus, Mentha Piperita* which are having a well reported antimicrobial potential.[6]

Novel drug delivery system have several advantages over conventional ones, including enhanced bioavailability, therapeutic activity, strength, tissue distribution, prolonged delivery, physical and chemical degradation resistance and higher solubility. Microemulsion is a clear, stable, isotropic mixture of oil, water and surfactant, frequently used in combination with a co-surfactant. Micro-emulsion-based drug delivery consists of delivering a drug dissolved in a mixture of one or more excipient which may be a mono, di and tri-glycerides, lipophilic and hydrophilic surfactants and a co-surfactant. The particle size of microemulsions ranges from about 10 nm to 300 nm. Because of the small particle sizes, microemulsions appear as clear or translucent solutions.

Although microemulsion are advantages in many ways, their stability may alter due to low viscosities. To overcome this problem microemulsion convert into microemulgel. Microemulgel is a prepared formulation that combines microemulsion and gel for use in dosage forms. It has the benefits of both emulgel and micro-emulsion by incorporating both hydrophilic and hydrophobic drugs into dosage forms as well as providing a large surface area for drug absorption. Additionally, the oil portion increases bioavailability by increasing drug permeability. Additionally, adding microemulsion to gel increases its stability.

In comparison to microemulsions, Microemulgel have a certain level of elegance and are simple to wash when necessary.[7]

MATERIAL AND METHOD

Materials

Chrysin was procured from Ottochemie Pvt. Limited, Mumbai. Dimethyl Sulfoxide (DMSO) was obtained from LOBA Chemie Pvt. Ltd. Mumbai. Propylene Glycol and Tween 80 were obtained from Unijules life sciences Pvt. Ltd. Nagpur. All remaining chemicals were obtained from Samar Chemicals, Nagpur.

Methods

Solubility studies

The solubility of chrysin in various oils (oleic acid, olive oil, IPM), surfactants (tween20, tween80, span 20), cosurfactants (ethanol, propylene glycol, PEG 400), water and DMSO was assessed in order to identify suitable oils that have a good solubilizing capacity for chrysin as well as the ability to yield systems with a larger microemulsion area. For this solubility study, excess amount of chrysin was dissolved in each oil, surfactant, cosurfactant, water and DMSO. In order to achieve equilibrium, the mixture of vials were then maintained at 37 ± 0.5 °C in an isothermal shaker (Jyoti Instrument Industry, M.P., India) for 72 hours. After that, the shaker was shut off, and the samples were centrifuged for 10 minutes at 3000 rpm. The supernatant was separated, filtered and after appropriate dilution with ethanol, Solubility was determined by UV spectrophotometer at λmax 269 nm.[8]

Pseudo ternary phase diagrams

Chrysin showed maximum solubility in DMSO as compared to other organic solvent; hence, it was selected for further studies. Tween 80, as a surfactant, and propylene glycol, as cosurfactants, showed better solubility for Chrysin and good emulsifying properties with oleic oil. Pseudo ternary phase diagrams were constructed using water titration method. Surfactant and cosurfactant (Smix) were mixed in different weight ratios 1:1 Oil and Smix mixture were mixed thoroughly in different weight ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1). Distilled water was added drop wise to the different mixtures of oil/Smix until cloudiness was disperse. Pseudo ternary plots were constructed using Chemix School Software, and microemulsions were prepared based on ternary phase diagram.[9] Different surfactant-Cosurfactant mixture (Smix) ratios were used to generate the phase diagram. These phase diagrams give the necessary concentration range to the microemulsion region.

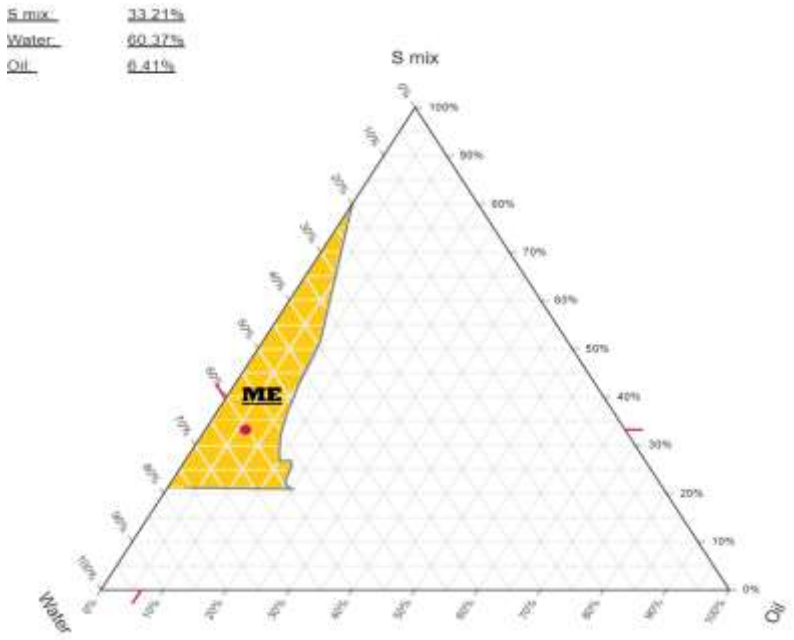

Figure No. 1 Pseudo ternary phase diagram of microemulsion

Determination of λ max

An accurately weighed Chrysin (50mg) was dissolved in the 100 ml of pH 6.8 phosphate buffer. From this solution pipette out 1ml (100µg/ml) and was diluted to make it 10µg/ml solution. The resultant solution of 10µg/ml was scanned over the range of 200-400 nm against pH 6.8 phosphate buffer as a blank using double beam UV Spectrophotometer.

Standard Calibration Curve for Chrysin

- **Preparation of pH 6.8 Phosphate Buffer**

  Dissolve 28.80g of disodium hydrogen phosphate and 11.45g of potassium dihydrogen phosphate in small amount of distilled water and make up the volume up to 1000ml using distilled water.

- **Preparation of Standard Calibration Curve in pH 6.8 Phosphate Buffer**

  From the stock solution 2, 4, 6, 8, 10 and 12 ml were withdrawn and further diluted up to 100 ml volumetric flasks to obtain a concentration range of 2-12µg/ml. The absorbance of the solutions was measured at 269 nm by using a UV-spectrophotometer. A graph of Concentration vs. Absorbance was plotted.[10]

- **Preparation of Phosphate Buffer Solution of pH 7.4**

  Dissolve 2.38g disodium hydrogen phosphate and 0.19g of potassium dihydrogen phosphate and 8g sodium chloride in small amount of distilled water and make up the volume upto 1000ml using distilled water. This prepared buffer solution was used for *In vitro* Drug Diffusion Study of Chrysin.

DRUG – EXCIPIENT COMPATIBILITY STUDY

The FTIR study performed to detect any suspicious interactions which affect stability, efficacy of drug and excipients chosen for the preparation of microemulgel, over the range of 4000-400 cm-1 in the FTIR spectrophotometer (FTIR-8400S, Shimadzu, Japan).

IR has been the method of choice to probe the nature and extent of interactions in excipient blends. The premise of using an IR to study excipient blends is that the mixing of the two components at molecular level will cause changes in oscillating dipoles of the molecules. This will manifest itself as changes in frequency and bandwidth of interacting group in the spectrum if the drug and polymer interact then functional groups in FTIR spectra will show band shifts and broadening compared to the spectra of pure drug.[11]

FORMULATION METHOD OF CHRYSIN MICRO-EMULSION

The ratio of the surfactant and cosurfactant was chosen from the pseudo-ternary phase diagrams, and the o/w microemulsion region was identified. The microemulsions were prepared at specific component ratios, namely oil, Smix, and water. The surfactant, cosurfactant, oil, and DMSO as a solubility and permeability enhancer in which the drug had maximum solubility were mixed with continuous stirring using magnetic stirrer (Remi, India). Then fresh distilled water was added dropwise with the help of a syringe to this uniform blend and mixed well for 30 minutes to form a microemulsion.[12]

FORMULATION OF MICROEMULSION LOADED GEL

The various gelling agents (sodium alginate, carbopol 934, carbopol 940) were selected for preparation of gel base. The gelling agent were weighed accurately and dispersed in water with stirring. Then it was allowed to swell for 24 hours to obtain homogeneous gel base. The pH of the gel was adjusted to 6-7 by the dropwise addition of triethanolamine. Optimized ME was added slowly to the prepared gel base, stirring on a high-speed homogenizer at 1200rpm to get ME loaded gel.[13]

CHARACTERIZATION AND EVALUATION MICROEMULGEL

Physical Appearance

Microemulgel were evaluated for their visual appearance, consistency and phase separation with naked eyes.

Determination of Viscosity

To determine viscosity 10 gm of micro-emulgel was filled in a 25 ml beaker and the beaker is subjected to Brookfield viscometer assembled with spindle number S6 at a speed of 50 rpm.

pH Determination

1% aqueous solution of the prepared microemulgel was made by dissolving 1gm of formulation in 100 ml distilled water and kept it a side for 2 hr. After stabilization pH of the formulation is measured using digital pH meter in triplicate manner at room temperature. The pH meter was calibrated before to each utilizing buffer solution (pH 4.0, 7.0 and 9.2). pH was adjusted to 6-7 by the dropwise addition of triethanolamine.

Spreadability

Spreadability was measured on the basis of ‘‘Slip and Drag’’ characteristics of microemulgel. A ground glass slide (7.3 × 2.5 cm) was fixed on the wooden box. microemulgel formulation under the study, 1 gm, was placed on this ground slide. The formulation was then sandwiched between ground slide and upper glass slide having same dimensions as that of ground slide and it was provided with the hook. Weight of 50 gm was allowed to rest on upper slide for 2 min to expel air and to provide uniform film of formulation. Known weight was placed in the pan attached to the pulley with the help of hook. The time required to cover distance of 7.3 cm was recorded. A shorter interval indicates better spreadability.

It is calculated using the formula:

$$S = M \times L/T$$

where M is the weight tied to upper slide, L is length of glass slide and T is time taken to separate the slide.

***In vitro* Drug Diffusion Study**

In vitro drug release study of chrysin loaded microemulsion based gel was carried out using a modified Franz diffusion cell with an active diffusion area of 4.91 cm^2. The membrane which was previously soaked overnight in phosphate buffer pH 7.4 was mounted between the donor and receptor compartment. The 1 g of the drug loaded microemulsion based gel equivalent to 10 mg of chrysin was placed in contact with the dialysis membrane of the donor compartment. The receiver compartment contained 18 mL of phosphate buffer pH 7.4 and was stirred at 100 rpm using a magnetic stirrer throughout the duration of the experiment. The temperature of the assembly was maintained at 37±2ºC. Then, 1 mL of the fluid from the receptor compartment was periodically withdrawn every one hour up to 8 h and was immediately replaced by an equal volume of receiver fluid. The concentration of the drug in the receptor compartment was determined spectrophotometrically at 269 nm after suitable dilution with phosphate buffer pH 7.4 using the same as the blank.

Antimicrobial activity

Antimicrobial activity of formulation as a test sample microemulgel was checked by cup plate method (diffusion method) in comparison with standard antibiotic with increasing concentrations as 2ug/ml, 4ug/ml, 6ug/ml and 8ug/ml as S1, S2, S3 and S4 respectively. The cultures were grown in nutrient broth and incubated at 37^0C for 24 hrs. After incubation periods definite volume of the *Escherichia coli* suspension (inoculum) was poured into the sterilized antibacterial assay media (cooled at 40^0C) and mixed thoroughly. About 30 ml of this suspension was poured aseptically in the petri plates and kept till the solidification. The well was bored with 6mm borer in seeded agar. The prepared well were filled with equal volume of drug containing microemulsion based gel. After that plate were incubated at 37^0C for 18 to 24 hrs. After incubation period was over, the microbial growth was observed and the zone of inhibition was measured.

Stability studies

The purpose of stability testing is to provide evidence on how the quality of the drug substance or drug product varies with time under the influence of variety of environmental factor such as temperature, humidity, light, labels recommended storage conditions and shelf-lives.

The stability studies were performed in accordance with the ICH guidelines. The stability of the optimized microemulsion was monitored at 40°C/75% RH for 3 months. It was inspected and evaluated for physical stability, percent transmittance, viscosity, pH, drug content and in vitro drug release.

RESULTS AND DISCUSSION

Solubility

Based on solubility studies, it was determined that for the preparation of a microemulsion of chrysin, solubility in the oils, surfactants, and cosurfactants. The chrysin was found soluble in oil, surfactant and co surfactants such as oleic acid, Tween 80, DMSO, Tween 20, and propylene glycol. In olive oil, IPM, Span 20, ethanol, and PEG400, it was found to be slightly soluble. Various physicochemical characteristics of the chosen oils were examined, and it was discovered that they were advantageous for a topical microemulsion drug delivery system. The chosen oils fall under the category of "Generally Recognised as Safe," and they are regularly used in a variety of food products.

Table 1: Solubility of Chrysin in different oils, surfactants and co-surfactants

Sr. No.	Samples	Solubility(mg/ml)
1	Olive oil	0.25±0.010
2	Oleic Acid	1.56±0.010
3	IPM	0.19±0.006
4	Tween 80	18.23±0.012
5	Tween 20	16.12±0.006
6	Span 20	11.27±0.012
7	Propylene Glycol	0.46±0.006
8	PEG 400	0.24±0.010
9	Ethanol	0.23±0.023
10	DMSO	30.02±0.002

UV Scanning of Chrysin

An accurately weighed Chrysin (50mg) was dissolved in the 100 ml of pH 6.8 phosphate buffer. From this solution pipette out 1ml (100µg/ml) and was diluted to make it 10µg/ml solution. The resultant solution of 10µg/ml was scanned over the range of 200-400 nm against pH 6.8 phosphate buffer as a blank using double beam UV Spectrophotometer.

From the scanning of Chrysin in pH 6.8 phosphate buffer λmax of 269nm was found.

Standard Calibration Curve for Chrysin

Table 2: Standard calibration curve of chrysin in pH 6.8 phosphate buffer

Sr. No	Concentration (ug/ml)	Absorbance at 269nm
1	2	0.221±0.0006
2	4	0.408±0.0006
3	6	0.628±0.001
4	8	0.836±0.006
5	10	0.994±0.006

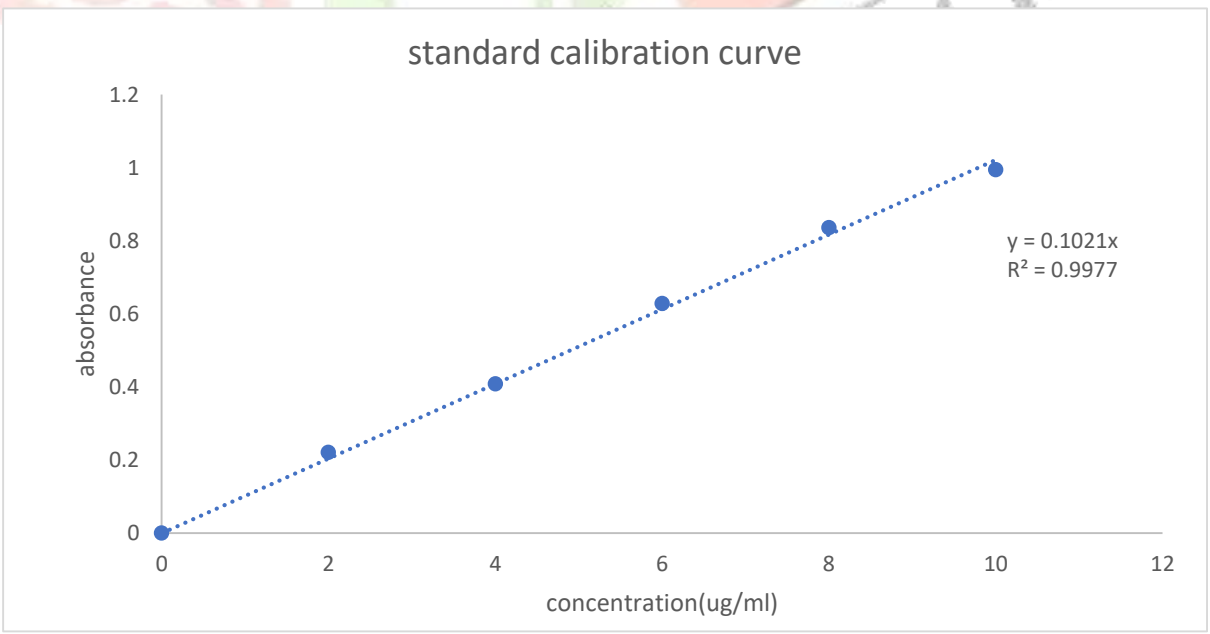

Figure no. 2 Standard calibration curve of chrysin

From the standard curve of Chrysin in pH 6.8 Phosphate buffer (Figure No.13) it was observed that the relationship between drug concentration and absorbance is linear and the curve obeys Beer-Lambert's law in the concentration range 2-10µg/ml.

The linear equation was, y=0.1021x and R^2 = 0.9993 in pH 6.8 phosphate buffer.

DRUG – EXCIPIENT COMPATIBILITY STUDY

Chrysin and chrysin loaded formulation was characterized by FTIR. FTIR of the drug confirmed the presences of all prominent peaks are at wave numbers 3344.34 cm-1 (O-H Stretching), 2977.89, 2939.31and 2927.74 cm-1 (Aromatic C-H Stretching), 1650.95 cm-1 (C=O Stretching), 746.40 and 730.97 (Aromatic C-C bending), 1575.73 and 1465.08 (Aromatic C=C Stretching), 1124.42 (C-O Stretching in C-O-C bond) indicating its authenticity (Table No.8 and Table No.9).

From results, it was concluded that there was no interference in the functional group as the principle peaks of Chrysin were found to be unaltered in the drug loaded formulation. (Figure No. 3 and Figure No.4).

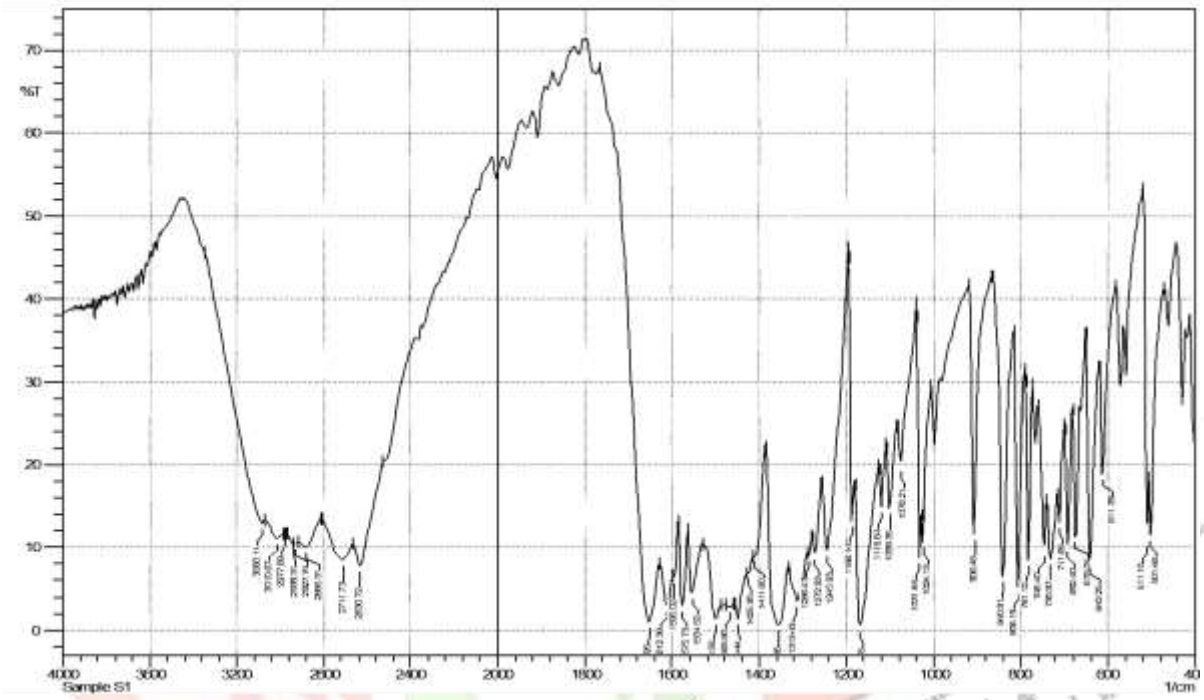

Figure No.3 : FTIR spectrum analysis of Chrysin

Table 3: FTIR Spectral Analysis of Chrysin

Sr. No	Standard range (cm^{-1})	Observed Frequency (cm^{-1})	Functional Groups
1	3100-2900	2977.89, 2939.31, 2927.74	Aromatic C-H stretching
2	3400-3200	3344.34	Phenolic O-H Stretching
3	1600-1400	1575.73, 1465.08	Aromatic C=C Stretching
4	1870-1650	1650.95	α, β-unsaturated C=O Stretching 6 membered
5	770-735	746.40, 730.97	Aromatic C-C bending
6	1150-1070	1124.42	C-O Stretching in C-O-C bond

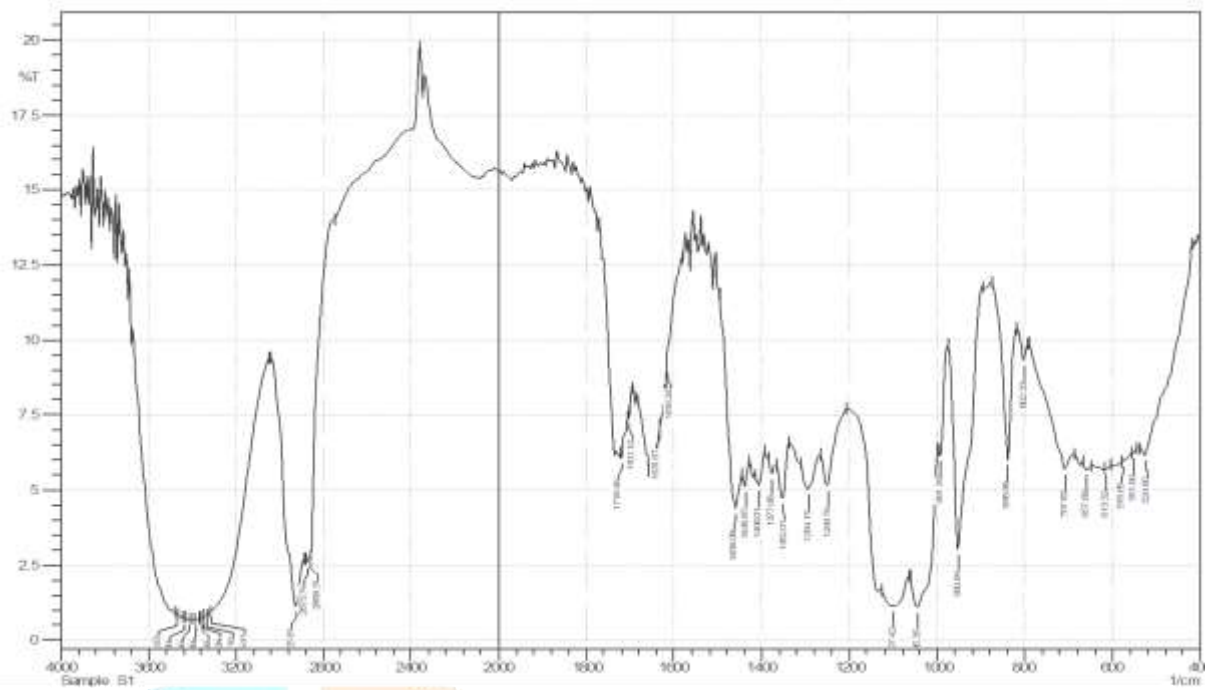

Figure No. 4: FTIR spectrum analysis of Chrysin loaded microemulsion

Table 4: FTIR Spectral Analysis of Chrysin loaded microemulsion

Sr. No	Standard range (cm^{-1})	Observed Frequency (cm^{-1})	Functional Groups
1	3100-2900	2925.81	Aromatic C-H stretching
2	3400-3200	3344.34, 3353.98, 3380.98	Phenolic O-H Stretching
3	1600-1400	1436.87, 1458.08	Aromatic C=C Stretching
4	1870-1650	1701.1, 1718.46	α, β-unsaturated C=O Stretching 6 membered
5	770-735	730.97	Aromatic C-C bending
6	1150-1070	1097.42	C-O Stretching in C-O-C bond

FORMULATION OF MICROEMULSION LOADED GEL

The low viscosity of microemulsions restricts its application and this disadvantage can be overcome by incorporating them in gelling agents. The optimized drug loaded microemulsion was selected to formulate the chrysin microemulsion based gel. The various gelling agents (sodium alginate, Carbopol 940) was selected for preparation of gel base.

Ingredients (% w/w)	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8	MG9
Chrysin (mg)	100	100	100	100	100	100	100	100	100
Oil	4.5	4.5	4.5	6.0	**6.0**	6.0	7.5	7.5	7.5
S_{mix}	25.75	27.50	29.66	30.00	**33.33**	34.00	36.30	36.70	39.50
DMSO	10	10	10	10	10	10	10	10	10
Carbopol 940 (%)	0.5	0.5	0.5	1.0	**1.0**	1.0	1.5	1.5	1.5
Sodium alginate (%)	1.0	1.0	1.0	2.0	**2.0**	2.0	3.0	3.0	3.0
Methyl Paraben	0.03	0.03	0.03	0.03	**0.03**	0.03	0.03	0.03	0.03
Propyl Paraben	0.01	0.01	0.01	0.01	**0.01**	0.01	0.01	0.01	0.01
Triethanol-amine	2	2	2	2	**2**	2	2	2	2
Distilled water	q.s.	q.s.	q.s.	q.s.	**q.s.**	q.s.	q.s.	q.s.	q.s.

Table 5: Formulation table of Microemulsion based gel

Centrifugation

This parameter was measured to evaluate physical stability. Micro-emulsion was centrifuged at ambient temperature and 3000 RPM for 15 minutes to evaluate the system for creaming or phase separation. System was observed visually for appearance. From observation of centrifugation, drug loaded microemulsion did not show any sign of phase separation or precipitation thereby conforming the stability of the microemulsions.

Dilution Test

Dilution test was performed and the result shows that microemulsion was stable. After dilution of micro-emulsion was carried out there are no phase separation and clear microemulsion.

Conductivity

All the prepared microemulsions were checked for Conductivity**.** For this 1ml of the microemulsion was diluted 10-100 times using continuous phase i.e, distilled water and then its conductivity was recorded using conductometer. (Table No. 14)

% Transmittance

All the prepared microemulsions were checked for % Transmittance. For this 1ml of micro-emulsion was diluted 50–100 times with continuous phase (i.e, distilled water) for the % Transmittance measurement. By using UV-Visible spectrophotometer at a specified wavelength of 630 nm and continuous phase as a blank, percentage transmittance of the formulation was assessed.

The characterization result showed 95.00±0.11 that the formulation batch ME5 has good % transmittance.

Drug content determination

The drug content of all batches was found to range from 87.64±0.001 to 99.62±0.005, with the MG5 formulation having the greatest drug content. MG5 formulation was therefore regarded as an optimized batch.

Table 6: Physical appearance, % transmittance, conductivity and drug content

Formulation	**Physical Appearance**	**% Transmittance**	**Conductivity (mS/cm)**	**Drug content (%)**
ME1	Transparent	90.73±0.12	0.369±0.06	98.32±0.005
ME2	Translucent	92.63±0.15	0.548±0.12	87.64±0.001
ME3	Translucent	92.63±0.12	0.442±0.12	96.86±0.001
ME4	Transparent	93.77±0.06	0.253±0.04	93.25±0.002
ME5	Transparent	**95.00±0.11**	**0.161± 0.04**	**99.62±0.005**
ME6	Transparent	88.37±0.06	0.284±0.06	90.41±0.002
ME7	Transparent	92.47±0.06	0.366±0.21	95.28±0.001
ME8	Transparent	90.47±0.06	0.457±0.07	97.16±0.002
ME9	Translucent	91.54±0.02	0.185±0.06	97.70±0.001

Particle size and Zeta potential

The concentration of the oil phase in the formulated microemulsion affected the globule size. The average particle size increased with the increase in the concentration of the oil. PDI varies from 0.0 to 1.0 and is a measure of particle homogeneity. The particles are more homogenous if the PDI value is closer to zero. The PDI of the formulations was found to be less than 1 and indicated acceptable homogeneity. The zeta potential of the formulations was found to have a negative charge. Due to the slight negative charge of the

droplets, aggregation is less likely to happen.

The results of particle size, zeta potential and polydispersity index are reported in Table no.13 Formulation ME5 was found to have satisfactory results.

Table 7: Particle size, PDI and zeta potential of chrysin microemulsion

Formulation	Particle size(nm)	PDI	Zeta potential(mV)
ME1	156.2±23.6	0.445	-24.9
ME2	161.1±37.7	0.365	-26.8
ME3	170.6±0.52	0.321	-42.5
ME4	160.2±0.16	0.412	-33.4
ME5	**154.9±15.5**	**0.246**	**-22.3**
ME6	168.5±12.3	0.466	-36.6
ME7	175.4±21.4	0.503	-23.4
ME8	178.7±16.2	0.385	-28.5
ME9	159.8±14.3	0.336	-25.2

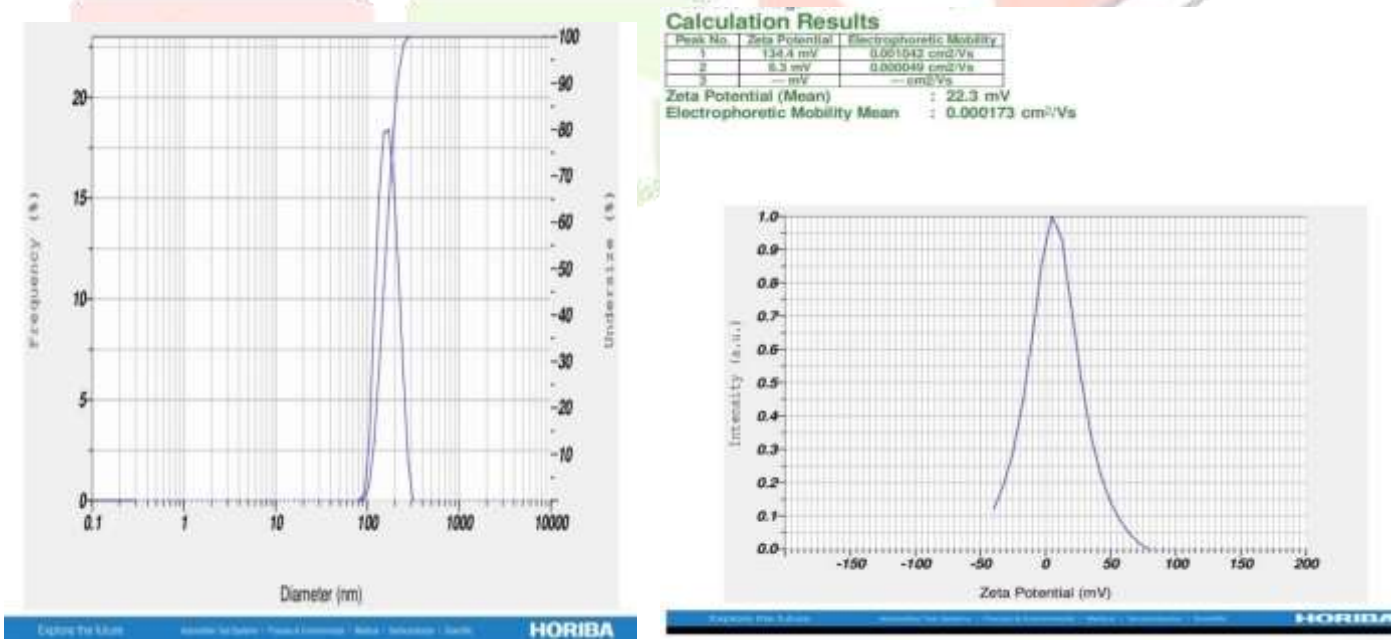

Figure No. 5: Particle size, PDI and Zeta potential of optimized microemulsion batch

Determination of Viscosity

The viscosity of the all the prepared microemulgel was found to be in range of 0.892±0.02 to 0.924±0.12 and increased with the increase in concentration of the surfactant and cosurfactant.

pH Determination

The pH range accepted for dermal preparation 4-7 i.e, close to the pH of the skin. The pH of the system lied within the limit.

Spreadability

The term "spreadability" is used to describe the size of the area to which the gel spreads easily when applied to the skin. A miroemulgel must meet several basic requirements, including having good spreadability. The spreadability of a more viscous formulation would be poor. Table no. 16 provided the spreadability values for each formulation. Better spreadability is indicated by a shorter interval of time.

The spreading coefficient for formulation MG5 was higher than that of the other formulations.

Table 8: Viscosity, pH and Spreadability

Sr. No	Formulation	Viscosity (mPa·s)	pH	Spreadability (gm.cm/s)
1	MG1	0.892±0.02	6.52±0.03	16.07
2	MG2	0.893±0.04	5.86±0.02	22.26
3	MG3	0.894±0.06	6.84±0.04	19.85
4	MG4	0.895±0.04	6.58±0.02	20.12
5	**MG5**	**0.896±0.06**	**6.82±0.02**	**23.96**
6	MG6	0.898±0.12	6.24±0.06	18.50
7	MG7	0.906±0.02	6.56±0.02	17.76
8	MG8	0.924±0.12	5.64±0.01	14.60
9	MG9	0.986±0.14	6.75±0.04	16.17

***In vitro* Drug Diffusion Study**

In vitro drug release study of optimized formulation was carried out using modified Franz diffusion cell. The optimized formulation MG5 showed the maximum 90.11% drug release in 8hrs. The results of stability studies of MG5 formulation reveled negligible changes at $40^0C \pm 2^0C$ and 75%±5% RH the prepared gel was found stable at elevated temperature and humidity.

Table 9: % Cumulative Drug Diffused

Time(hrs)	MG1	MG2	MG3	MG4	**MG5**	MG6	MG7	MG8	MG9
0	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
1	8.58	5.28	5.724	6.165	**8.35**	6.86	6.81	5.05	7.28
2	17.59	11.7	12.581	13.463	**18.42**	15.74	15.83	11.86	16.98
3	27.1	18.49	19.808	21.572	**29.42**	25.38	25.34	20.93	29.37
4	37.09	25.76	27.944	30.148	**40.82**	35.59	35.1	30.7	38.76
5	47.04	35.88	37.623	40.268	**52.71**	46.55	45.63	41.22	47.84
6	57.91	46.16	47.897	50.984	**64.98**	57.87	56.5	52.09	57.77
7	69.67	57.16	58.903	62.872	**77.33**	69.23	67.82	63.41	70.33
8	83.12	68.41	70.157	75.007	**90.11**	80.74	79.32	74.91	79.86

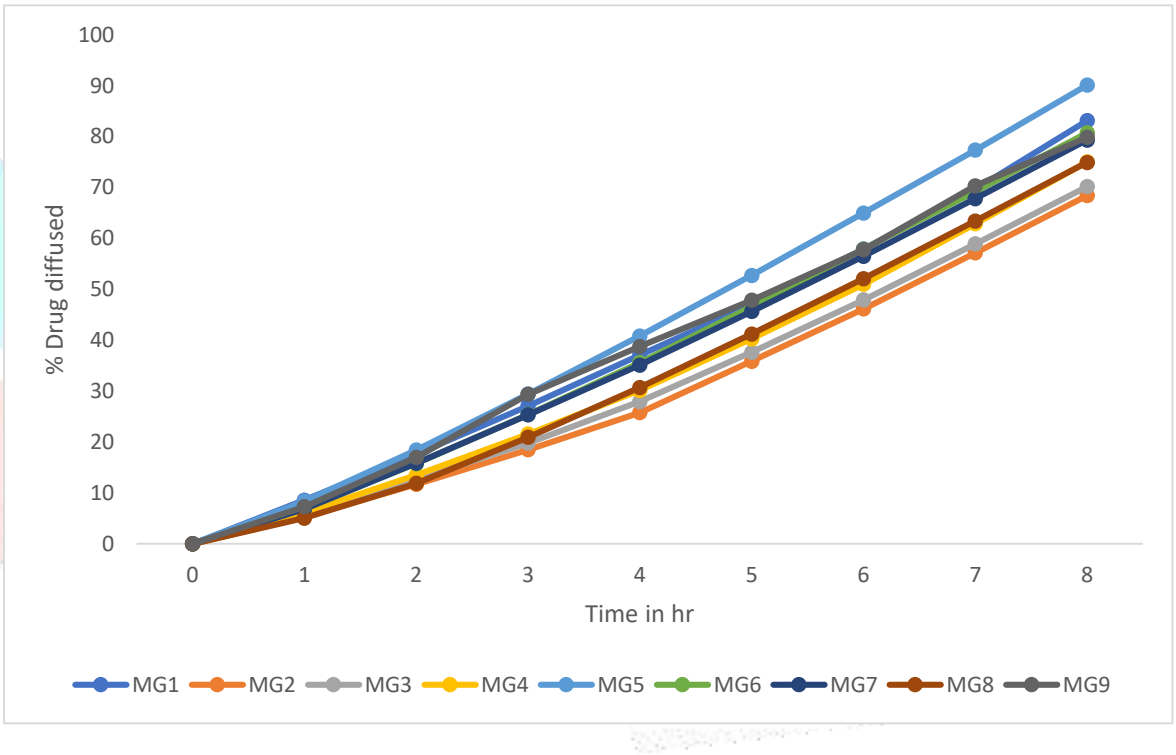

Figure No. 6: Drug diffusion of all formulations

Antimicrobial activity

Antimicrobial activity of chrysin microemulgel was evaluated by cup plate method using *Escherichia coli* strain for optimized formulation MG5. The zone of inhibition of optimized formulation MG5 was greater than all other formulation.

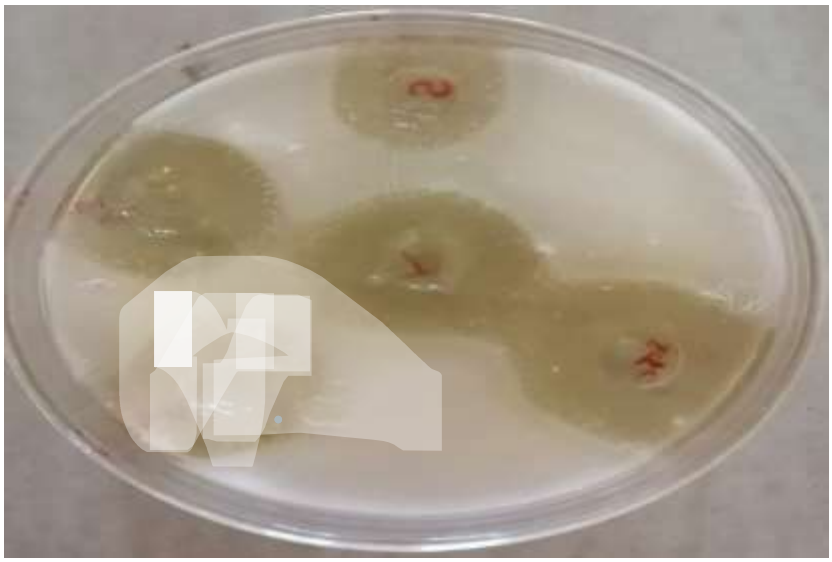

Figure No. 7: Zone of inhibition of optimized batch MG5

Table 10: Zone of Inhibition of Formulation MG1-MG9

Formulation	Zone of Inhibition (ZOI) in mm				
	S1	**S2**	**S3**	**S4**	**Test**
MG1	12	14	16	19	17
MG2	10	15	17	20	11
MG3	15	18	20	21	19
MG4	11	13	15	17	16
MG5	**22**	**24**	**26**	**28**	**27**
MG6	16	18	20	22	19
MG7	11	13	14	17	12
MG8	11	12	15	17	11
MG9	12	14	16	18	13

Stability Study

Stability study of chrysin microemulgel were carried out by storing the formulation at 40°C ± 2° and 75% ± 5% humidity for 3 months. The results of the stability studies of the microemulsion based gel showed no significant change in physical appearance, viscosity, pH, drug content and *in vitro* release after 3 months of storage at accelerated conditions of temperature and humidity.

Table 11: Stability study of optimized formulation MG5

Sr. No.	Parameters	Duration			
		0 Month	1 Month	2 Months	3 Months
1	Physical appearance	Yellowish white gel	Yellowish white gel	Yellowish white gel	Yellowish white gel
2	Viscosity	0.896±0.06	0.895±0.05	0.866±0.04	0.864±0.02
3	pH	6.82±0.02	6.82±0.04	6.80±0.03	6.78±0.01
4	% Transmittance	95.00±0.11	95.00±0.08	94.85±0.10	95.74±0.12
5	Drug content	99.62±0.005	99.52±0.004	99.12±0.006	98.95±0.004
6	*In vitro* drug release	90.11%	90.06%	89.75%	89.66%

Conclusion

Chrysin, class IV drug according to BCS has poor permeability and solubility in water, so the main goal was to overcome this problem. On the basis of solubility of chrysin, we have selected various ingredients such as oils, surfactants and cosurfactants were chosen. For solving the solubility issue and to enhance penetration of drug into skin, DMSO was added. The formulated microemulsion based gel of chrysin has a potential for the topical delivery of the drug thereby overcoming the complications associated with the oral administration of the drug. From the results it is clear that, the prepared miroemulgel is found to have enhanced solubility and permeability of chrysin through skin. From antimicrobial study it can be concluded that developed formulation has good antimicrobial potential. Even after three months of stability, the microemulsions physical appearance, viscosity, pH, % transmittance, drug content and *In vitro* drug release did not alter. Thus, the prepared microemulsion has shown good stability for 3 months.

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