

FORMULATION AND EVALUATION OF MICROEMULSION BASED GEL FOR HYPERPIGMENTATION

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Abstract:

Objective: Aim of this study is to formulate and evaluate Cinnamic Acid microemulsion-based gel for topical use to reduce hyperpigmentation.

Methods: The gel was prepared using oleic acid as oil phase, tween 80 as a surfactant, propylene glycol as co-surfactant, carbopol 934 as a gelling agent and distilled water as the aqueous phase. Then, skin pH (6.5-7) was maintained by dropwise addition of triethanolamine. Then the prepared microemulsion-based gel was characterized for pH, spreadability, viscosity, and in vitro studies.

Results: Results indicated that the gel showed good appearance, homogeneity, and spreadability. Viscosity is between 34720 ± 1254 centipoises. The in vitro release studies also indicated that the formulation was of desirable release pattern, Drug release microemulsion within 5 h was observed at 52.75%.

Conclusion: In the present study, a satisfactory attempt was made to formulate a microemulsion-based gel of Cinnamic Acid and acceptable for topical delivery.

1. INTRODUCTION:

Hyperpigmentation is defined as the harmless condition where the skin becomes darker due to an increased concentration of melanin. Melanin is a pigment present in the topmost layer of skin i.e. epidermis, and it gives color to hair, eyes, and skin [1]. The most common types of melanin are I) brown and black eumelanin and II) Reddish yellow pheomelanin [2] [3]. Melanin is produced by a biosynthetic pathway called melanogenesis using a group of cells known as melanocytes which are present in the basal layer of the epidermis. Melanin synthesis is regulated by the rate-limiting enzyme tyrosinase. Melanogenesis involves the conversion of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and then oxidation which leads to the formation of L-dopaquinone. Both steps are catalyzed by the tyrosinase enzyme which is located in melanosomes and further, the pathway is cleaved to form eumelanin and pheomelanin [3] [4]. Therefore, tyrosinase enzyme inhibitors are necessary for treating hyperpigmentary disorders. Hyperpigmentation is usually caused due to an increased number of tyrosinase and melanocytes. Common causes of hyperpigmentation include melasma and post-inflammatory hyperpigmentation. Melasma primarily occurs on the face with brown-grey patches. Melasma is generally associated with pregnancy and exposure to sunlight [3] [5] whereas post-inflammatory hyperpigmentation is a common consequence of inflammation but frequently affects patients with pigmented skin.

Tyrosinase is a key factor in melanin synthesis. Thus the melanin synthesis act by inhibiting the tyrosinase for the treatment of hyperpigmentation. Most of the commercially available cosmetics and or skin lightening agents are tyrosinase inhibitors. Several tyrosinase inhibitors have been identified from both natural and synthetic sources. Many tyrosinase inhibitors such as hydroquinone, kojic acid, arbutin, azelaic acid, L-ascorbic acid, ellagic acid, resorcinol, quercetin, and ascorbic acid have been used as skin whitening agents with limitations [9].

Hydroquinone is known as the gold standard for skin lightening and also it has been for decades. But it has got some disadvantages, long term use can lead to carcinogenicity and nephrotoxicity [10].

The microemulsion-based topical gel has considered a promising technology amid a novel drug delivery system as it has both microemulsion and gel. Topical drug delivery is most common in cosmetic fields also It avoids first-pass metabolism and easy termination of treatment. In a topical drug delivery system, the drug will diffuse out and reach the site of action, and get absorbed by the skin. Microemulsion has sufficient solubility and permeability into the skin, low skin irritation, and improves the rate and

absorption of lipophilic drugs [6] [7]. It has small-sized droplets of oil, water, and surfactant which provide a large area for drug absorption. It has been reported that the ingredients of microemulsion may decrease the diffusion barrier of the stratum corneum and enhance the permeation of the drug. Therefore, it is likely for both transdermal and dermal delivery of drugs as an effective route of drug administration.

Microemulgels have properties like less greasiness, high surface area, easily spreadable, and longer shelf life.

We worked on the drug Cinnamic Acid which is a phenylpropanoid derivative found in *Cinnamomum cassia*. This compound has various biological activities like anti-oxidant, anti-inflammatory, and anti-cancer. It shows tyrosinase inhibitory effect as well as depigmenting activity on UV-B induced hyperpigmentation [8].

The present study is aimed to formulate and evaluate the microemulsion-based gel of Cinnamic Acid to enhance its solubility and permeability for a better depigmentation effect. There is no reported study regarding Cinnamic Acid topical formulation, this study could give an understanding of the possibility of Cinnamic Acid topical formulation.

2. EXPERIMENTAL:

2.1 Materials and methods:

Chemicals:

Cinnamic Acid, Oleic acid, Tween 80, Propylene glycol, Phosphate buffer (pH- 6.7, 7.4, and 5.5), Carbopol 934, Triethanolamine, Methanol, Acetone.

Table no. 1: List of Instruments used for an experiment

S. No.	INSTRUMENT	MODEL
1.	pH Meter	LABTRONICS
2.	Digital weighing balance	WENSAR DAB 120
3.	Oven	BIOMEDICA
4.	UV-Spectrophotometer	Jasco V-730
5.	Franz diffusion cell	Orchid scientifics FDC-03
6.	Brookfield viscometer	DV-II+ Pro
7.	Water bath	BIOMEDICA
8.	Particle size analyser	ZETASIZER Nano ZS 90
9.	Texture analyser	Brookfield CT3

2.2 Formulation of Emulsion:

A Weighted amount of Carbopol 934 was dispersed in 80 ml distilled water and stirred magnetically at a high speed. Stirring was continued until a thin dispersion, without lumps, was obtained, Triethanolamine was slowly added to the dispersion with continuous stirring which resulted in a stiff gel. 1 gm of Cinnamic Acid was dissolved in 20 ml of acetone. Then the obtained solution was mixed with a gel base.

2.3 Formulation of microemulsion:

The microemulsion was developed manually by a mixture of a surfactant (tween 80) and Co-surfactant (propyleneglycol) in a 4:1 ratio. 20 ml sample of surfactant was added to 20ml of oleic acid (oil) and assimilated with the magnetic stirrer. Surfactant mixture and Cinnamic Acid (1 g), are added to oleic acid (oil) and mixed vigorously up to dissolve totally. Finally, under continuous stirring, 5 ml distilled water was gradually added at room temperature.

2.4 Formulation of gel Base:

A carbopol gel was developed by gently dissolving 1g of carbopol 934 in 50 ml of distilled water slowly by the application of a magnetic stirrer at room temperature. Till the formation of gel, keep adding triethanolamine to achieve the pH of 4-7.

2.5 Formulation of Microemulsion based gel:

For the formulation of microemulsion gel-based, microemulsion containing Cinnamic Acid would be vigorously mixed with carbopol 934 gel base at room temperature [11].

Table no. 2 Composition of microemulsion based gel

INGREDIENTS	QUANTITY
Cinnamic Acid(Drug)	01 gm
Tween 80 (Surfactant)	20 ml
Propylene glycol (Co-surfactant)	05 ml
Oleic Acid (Oil)	20 ml
Carbopol 934(Gelling agent)	01 gm
Triethanolamine(pH adjuster)	1-2 drops
Methyl Paraben (preservative)	0.02%
Distilled Water (Aqueous phase)	To make 100 ml

2.6 Calibration curve:

The calibration of Cinnamic Acid was carried out by UV spectrophotometry. Cinnamic Acid was dissolved in methanol to produce a stock solution of 1000 µg/ml. From this stock solution, concentrations of 10,20,30,40 and 50 µg/ml were prepared. The absorbance of these concentrations was having λ_{max} of 237 nm. The calibration curve obtained was found to obey the Beer-Lamberts law. The equation of the line $y = 0.0047x + 0.0089$ $R^2 = 0.9972$

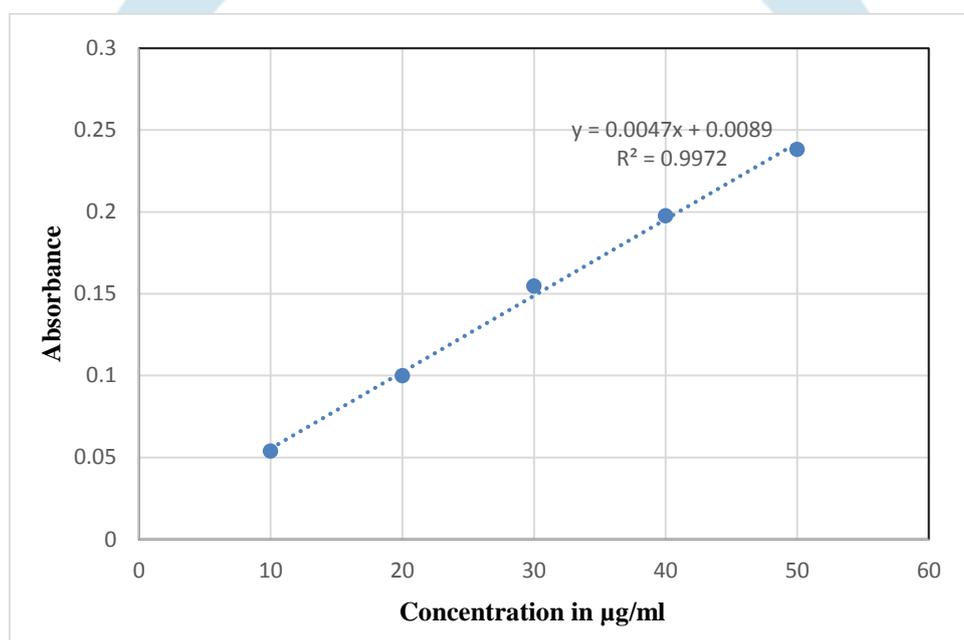


Fig. No. 1. Calibration curve of Cinnamic Acid in methanol

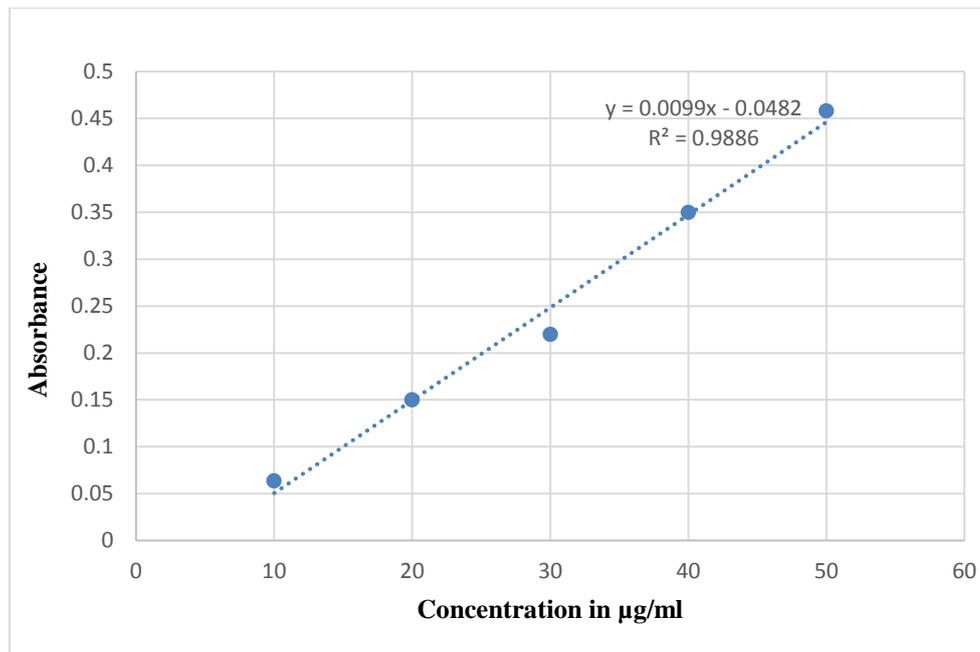


Fig.No. 2. Calibration curve of Cinnamic Acid in water

2.7 PSEUDOPSEUDOTERNARYPHASE DIAGRAM:-

Constructing a pseudoternary phase diagram makes it easy to find out the required concentration range of components and helps to determine whether the formula will provide a good emulsion preparation [17].

With the help of the water titration method, the pseudoternary phase diagram of water, oil, and Surfactant-Co-surfactant to be used in the formulation to produce microemulsion was constructed.

The surfactant-co-surfactant used was Tween 80 and Propylene glycol and the oil was oleic acid.

A mixture of surfactant and co-surfactant and oleic acid were mixed in the ratios 8:2, 6:4, 4:6, 2:8, and 5:5 respectively. This surfactant mixture and oleic acid were titrated with water and the samples were then marked as points in the phase diagram.

The area covered by these points was considered amicroemulsion region of existence

Quantities of all three components were taken in % w/w [16].

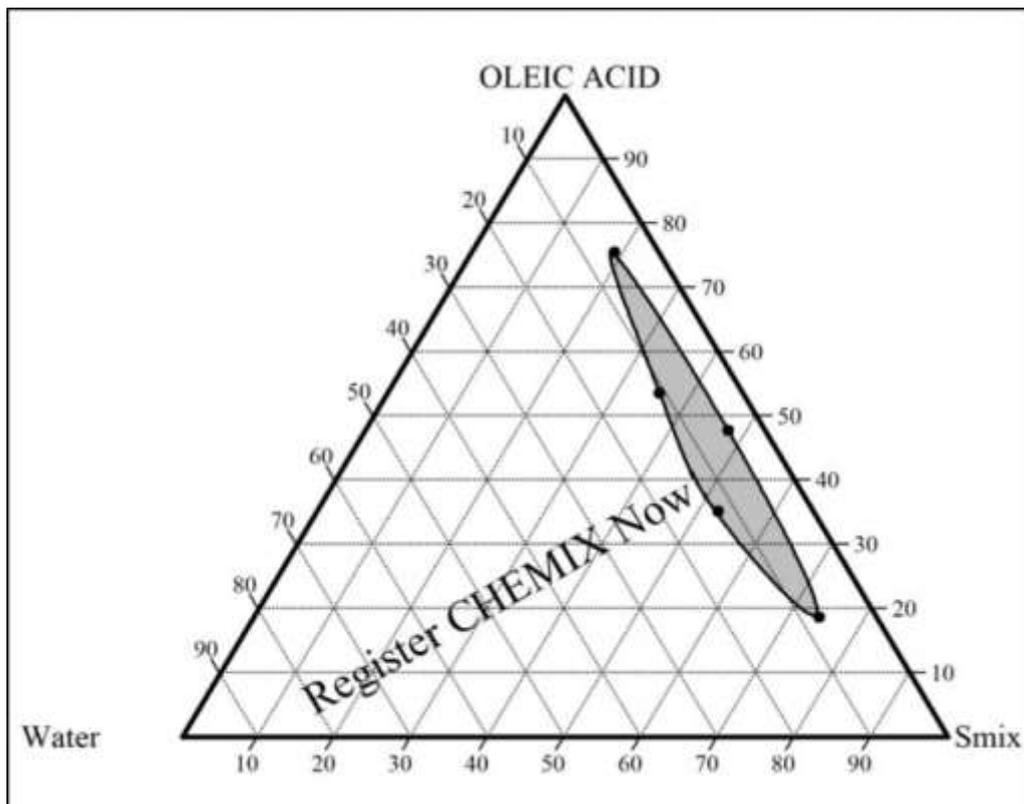


Fig.No.3: The pseudoternary phase diagrams of the oil-surfactant-Water system at 4:1 weight ratio of Tween 80 to co-surfactant Propylene glycol mixture at 20^oc.

3. EVALUATION:-

The microemulsion-based gel was evaluated for appearance, pH, spreadability, viscosity, particle size, and in vitro diffusion study.

3.1 Organoleptic evaluation:

The Cinnamic Acid microemulsion had a transparent yellow color. Cinnamic Acid microemulsion gel was slightly turbid. The microemulsion did not show phase separation before and after the addition of the gel base, which intended that the microemulsion was stable. When applied to the skin, the microemulsion gel preparations get cold [12].



Fig. No. 4: Prepared microemulsion based gel and plain gel

3.2. HOMOGENEITY:

Both microemulsion-based gel and plain gel were packed in containers and then tested for homogeneity by visual inspection. They were tested for their appearance and presence of any agglomeration [13].

3.3. DETERMINATION OF pH:-

The pH of the gel was checked with the help of a digital pH meter at a constant temperature. Using the standard buffer solutions at pH 4, 7, and 9, the pH meter was calibrated and then the electrode was washed with dematerialized water. To take reading at room temperature the electrode was inserted into the gel formulation 10 min prior and constant reading was noted[14][18].

3.4 SPREADABILITY (HARDNESS):-

The spreadability of the gel was measured with the help of a texture analyzer.

Hardness was measured as the maximum force required to achieve a deformation of the gel or defined as the maximum peak force in the first cycle of compression [19].

The apparatus consists of a conical container and conical probe with a matching cone angle of 90 degrees. The gel was spread in the conical container and the sample is forced between the two cone surfaces during the compression cycle, initially penetrating, then extruding the product.

3.5 PARTICLE SIZE:-

The particle size of the sample was measured by a Malvern zeta sizer. Samples were taken in cuvettes following a proper dilution of the formulation in distilled water and the observations were taken [20].

3.6 VISCOSITY:-

The viscosity of gel was measured at room temperature with the help of a Brookfield viscometer and the spindle number used was 92. Corresponding reading was noted down. The viscosity of the formulation is measured in centipoises [6].

3.7. IN VITRO RELEASE STUDY:-

The study was performed by a modified Franz diffusion cell with the usage of a dialysis membrane. Before carrying out the study, the membrane becomes stored and mounted cautiously among the donor and receptor chambers. Both plain gel and microemulsion-based gel become homogeneity spread on the HPLC membrane of Pore Size: 0.45 μ m and 47mm diameter. Phosphate buffer (pH 5.5) remained located in the receptor medium as dissolution media. Both donor and receptor compartments had been in contact with each other and the whole assembly was maintained at a consistent temperature of $20 \pm 0.5^\circ\text{C}$. Magnetic beads become used to stir the solution of the receptor chamber. 1ml of the sample become withdrawn after 0.5, 1, 2, 3, 4, and 5 periods and an equal amount was replaced with fresh dissolution media. Sample absorbance becomes calculated spectrophotometrically at 250 nm and % cumulative drug release is calculated.

The results are mentioned in Table 3.



Fig. No. 5: Franz diffusion cell apparatus

Comparison of In vitro drug release profile of microemulsion based gel and plain gel

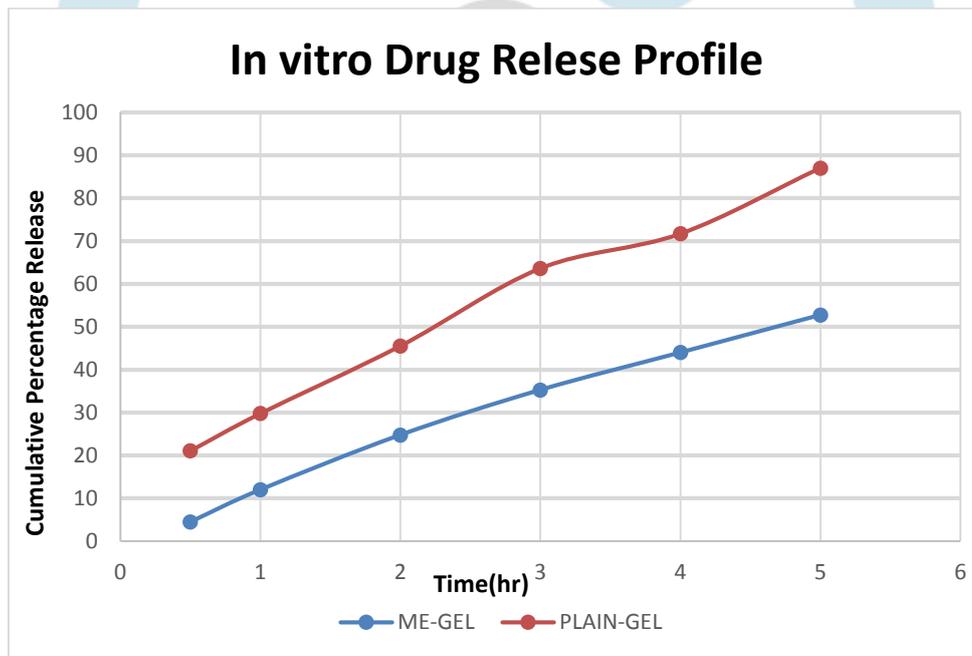


Fig.No. 6: In vitro Dissolution Profile in Franz Diffusion Cell

Table 3. Cumulative % Drug release of microemulsion and plain gel

Time	Microemulsion based gel	Plain gel
0.5	4.5	21.06
1	12	29.78
2	24.775	45.53
3	35.275	63.61
4	44	71.7
5	52.75	87.02

4. RESULT AND DISCUSSION:

4.1 Physicochemical properties:

White crystalline compound that is slightly soluble in water, soluble in oils, and freely soluble in many organic solvents
Solubility in water: 500 mg/L [16].

4.2 UV spectroscopic study:

Cinnamic Acid in methanol solution showed maximum absorbance (λ_{max}) of 260nm and the calibration curve exhibits good linearity.

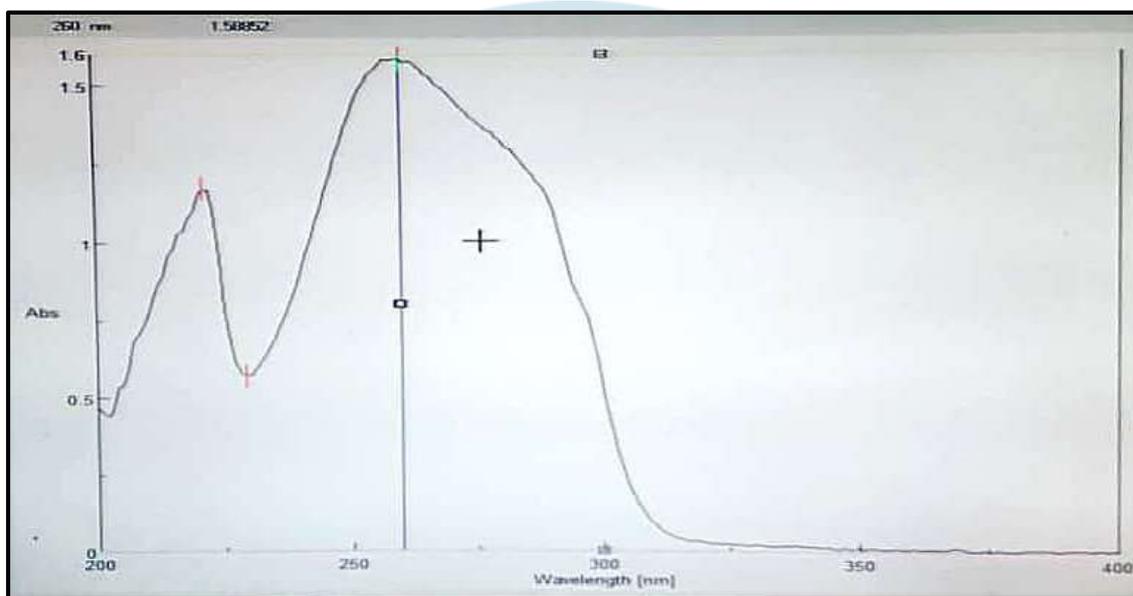


Fig. 7: Ultraviolet spectrum of Cinnamic Acid in Methanol

4.3 pH:-

The pH of both of the formulations was compared.

The pH of the microemulsion-based gel of Cinnamic Acid was found to be 6.5 and for plain gel, the pH was 7.0.

This pH is in accordance with the pH of the skin indicating skin compatibility and avoiding the risk of skin irritation upon application of the gel.

Table 4: pH of formulations:

Sample	pH
Microemulsion gel	6.5
Plain gel	7.0

4.4 SPREADABILITY (HARDNESS):-

Comparing the hardness value of both formulations we conclude that it was higher in microemulsion based gel of Cinnamic Acid with 159.62 gm. than in plain gel it was 18.90 gm.

Spreadability values contribute to the efficacy of the formulation.

When the concentration of gelling agent decreased, the spreadability values were found to be increased. The spreadability and viscosity of formulations are inversely proportional to each other. Therefore the microemulsion-based gel of Cinnamic Acid has good spreadability as compared to plain gel indicating evenly spread of gel on the skin surface.

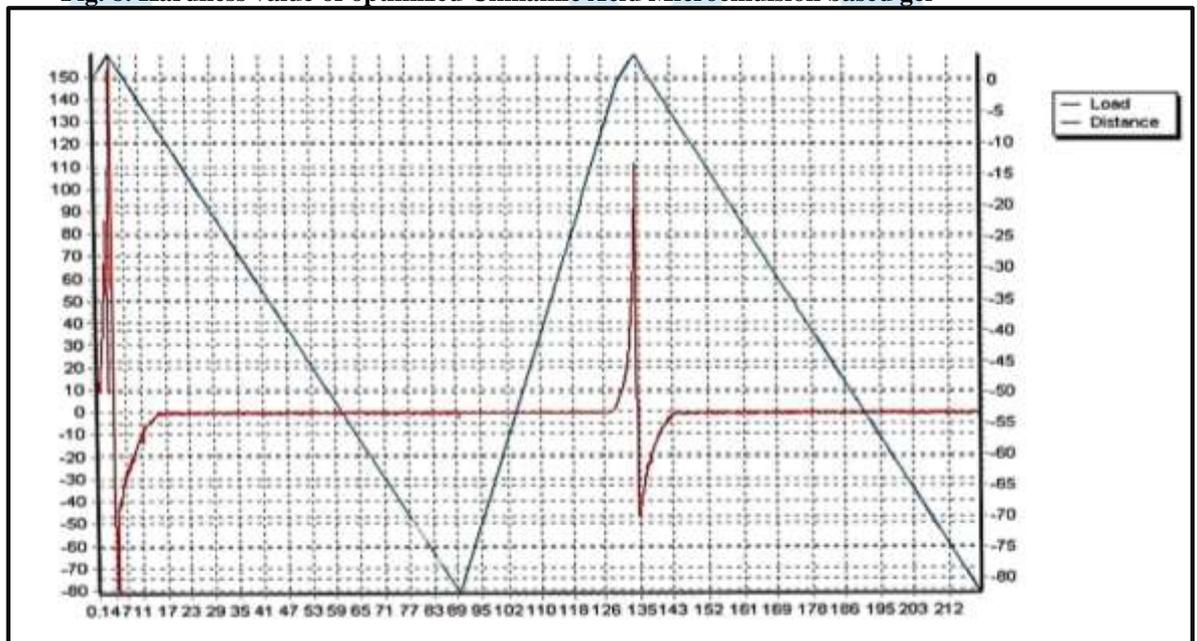
Table 5. SPREADABILITY (Hardness) of formulations:

SAMPLE	SPREADABILITY (Hardness)
Microemulsion gel	159.60 g
Plain gel	18.90 g

MICROEMULSION:

Results		
Hardness Cycle 1:	159.60	g
Hardness Work Cycle 1:	1.92	mJ
Adhesive Force:	81.10	g
Adhesiveness:	2.34	mJ

Fig. 8. Hardness value of optimized Cinnamic Acid Microemulsion based gel



PLAIN GEL:-

Results		
Hardness Cycle 1:	18.90	g
Adhesive Force:	8.50	g
Adhesiveness:	0.34	mJ

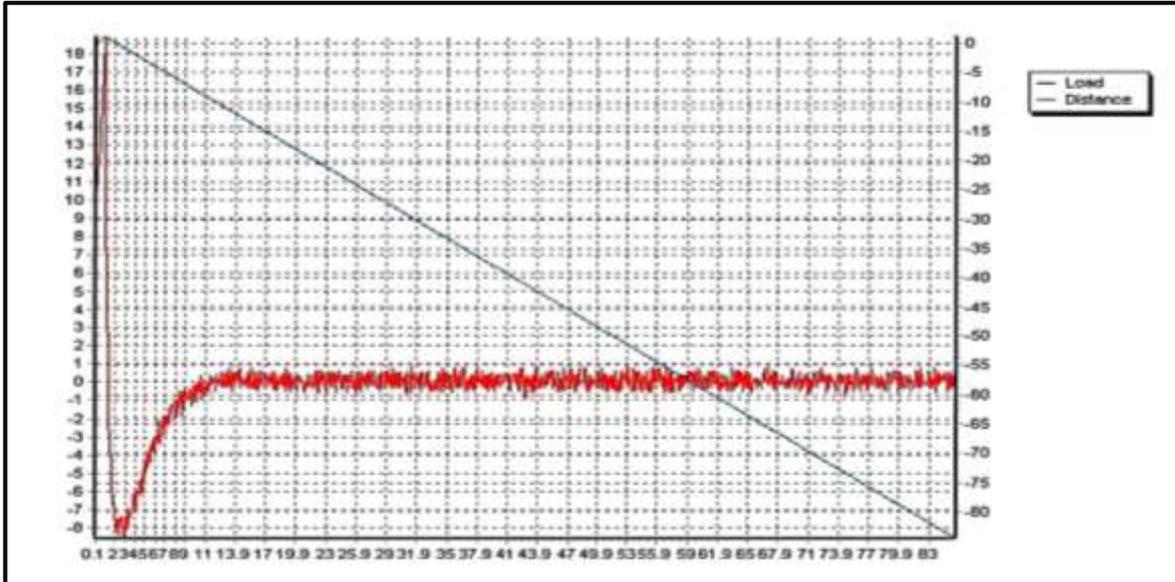


Fig 9: Hardness value of optimized Cinnamic Acid Plain gel

4.5 PARTICLE SIZE:-

The particle size of both the formulations was analyzed. The particle size of microemulsion-based gel of Cinnamic Acid was found to be 236.3 nm and for plain gel, it

was found to be 1973 nm. Hence we conclude that the microemulsion-based gel of Cinnamic Acid has less particle size than plain gel.

Table 6. Particle Size of both Microemulsion and plain gel

SAMPLE	PARTICLE SIZE
Microemulsion gel	236.3 nm
Plain gel	1973 nm

MICROEMULSION:-

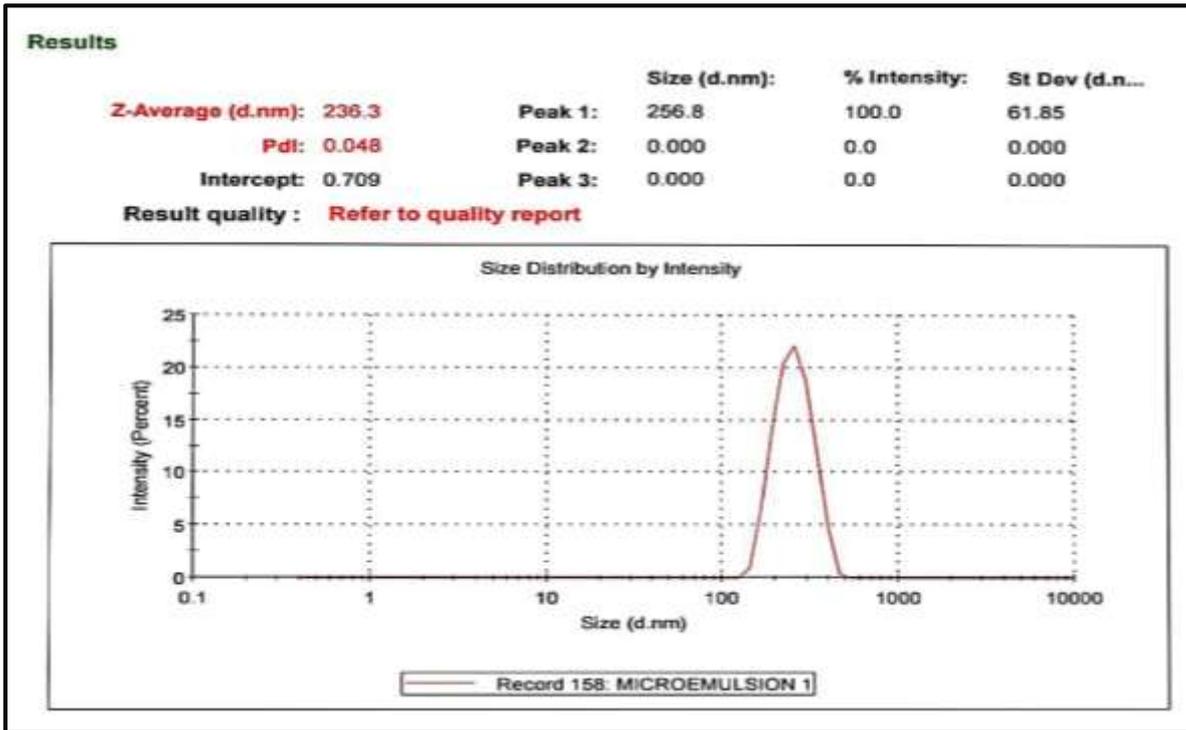


Fig. 10: Particle size of Microemulsion based gel containing Cinnamic Acid

PLAIN GEL:-

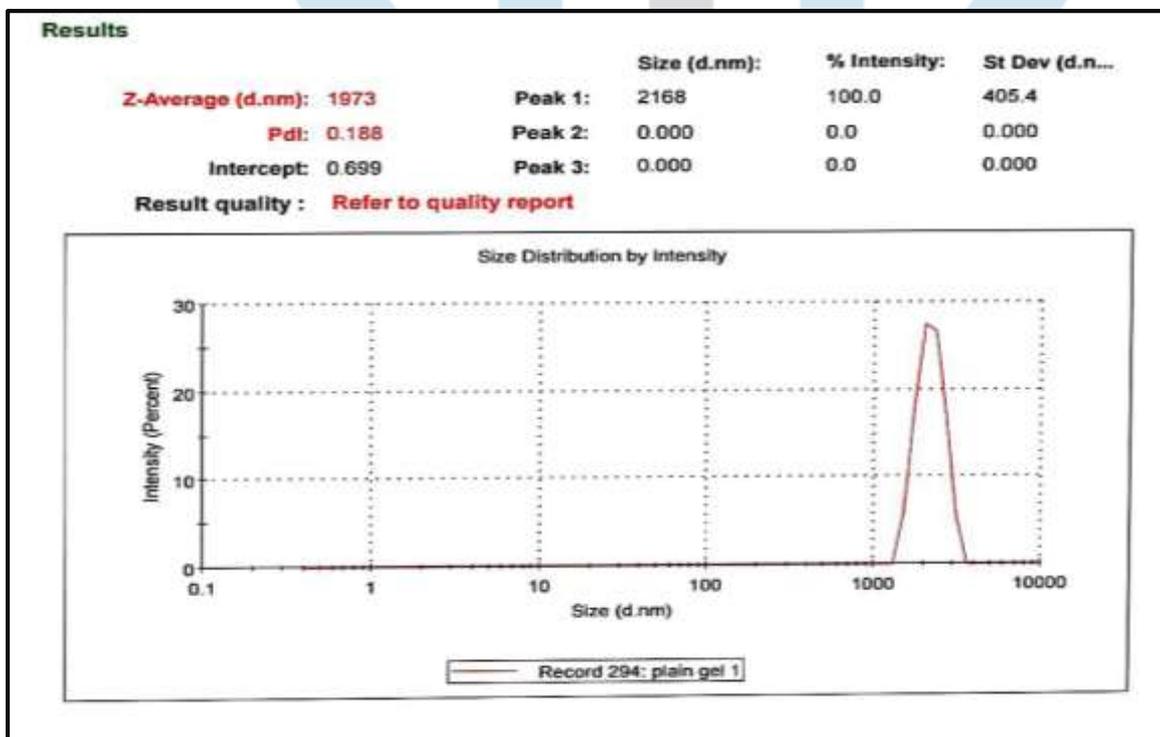


Fig. 11: Particle size of Plain gel containing Cinnamic Acid

4.6 VISCOSITY:-

Microemulsion incorporated with carbopol 934 increases the viscosity of microemulsion-based gel of Cinnamic Acid. For drug permeation viscosity of the gel is responsible.

Viscosity is inversely proportional to the spreadability of the formulation.

Viscosity was determined by Viscometer (Brookfield viscometer DV-II+ Pro) at 10 rpm with spindle no. 92 at room temperature.

The viscosity of microemulsion-based gel of Cinnamic Acid and the plain gel was found to be 34720 cp and 44000 cp respectively. After comparing both the formulation, microemulsion based gel of Cinnamic Acid has less viscosity than plain gel.

Lower viscosity here indicates good spreadability of gel.

Table 7. The viscosity of both Microemulsion and plain gel

SAMPLE	VISCOSITY
Microemulsion gel	34720 cp
Plain gel	44000 cp



Fig. 12: Viscosity determination of Microemulsion Based gel



Fig.13: Viscosity determination of plain gel

CONCLUSION:-

Hyperpigmentation is a common skin condition that occurs when the skin produces more melanin giving skin its color. Spots or patches of skin appear darker than the surrounding areas. Forms of hyperpigmentation include melasma and sunspots and are more likely to affect areas of skin like the face, arms, and legs after skin exposure. It affects people of all skin types [21].

Melanin is essential for skin protection but overproduction of melanin causes skin disorders. In the melanin synthesis pathway, tyrosinase catalyzes the first rate-determining step and is the key enzyme in melanin production. Moreover Cinnamic Acid recently reported an inhibitory effect on tyrosinase activity. Cinnamic Acid is one of the major components of *Cinnamomum cassia*. It has various biological activities such as anti-oxidant, anti-cancer, and anti-inflammatory [8].

The main aim of the study was to formulate and evaluate the microemulsion-based gel of Cinnamic Acid to reduce hyperpigmentation.

Cinnamic Acid has various uses and one of them is it acts as a depigmenting agent by inhibiting tyrosine activity and expression with melanocytes to treat hyperpigmentation.

Cinnamic Acid microemulsion was first prepared and then it was incorporated into gel form to treat hyperpigmentation.

Firstly the oil was selected as oleic acid based on solubility then the surfactant mixture of Tween 80 and Propylene glycol was selected.

A pseudoternary phase diagram was plotted to determine the concentration range of components that can result in a large existence area of the microemulsion.

The ratio of surfactant-Cosurfactant was selected based on the area covered by the microemulsion. Therefore the ratio of 4:1 of Tween 80 and Propylene glycol was selected.

This surfactant mixture is then mixed with the oil phase (Oleic acid) to obtain microemulsion. To convert this Cinnamic Acid microemulsion into gel form Carbopol 934 was added as a gelling agent and then pH was adjusted with the help of Triethanolamine. Also, a plain gel of Cinnamic Acid was formulated.

Both the gels were optimized for evaluation studies, Physical evaluations like colour, appearance, texture, pH, and physicochemical evaluations like viscosity, particle size, and spreadability.

The pH of the microemulsion-based gel of Cinnamic Acid was 6.5 which is in accordance with skin pH and avoids the risk of irritation to the skin. It has less viscosity than plain gel which indicates it has good spreadability and it can even spread over the skin surface. Particle size was also less as compared to plain gel. Drug release within 5 hrs was observed as 52.75% indicating that the formulation was of desirable release pattern and it also showed good appearance and homogeneity.

Thus we conclude that the microemulsion-based gel of Cinnamic Acid was formulated and evaluated successfully and can be acceptable for topical drug delivery.

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