

“DEVELOPMENT OF CURCUMIN NANO-EMULSION FOR THE TREATMENT OF VITILIGO”

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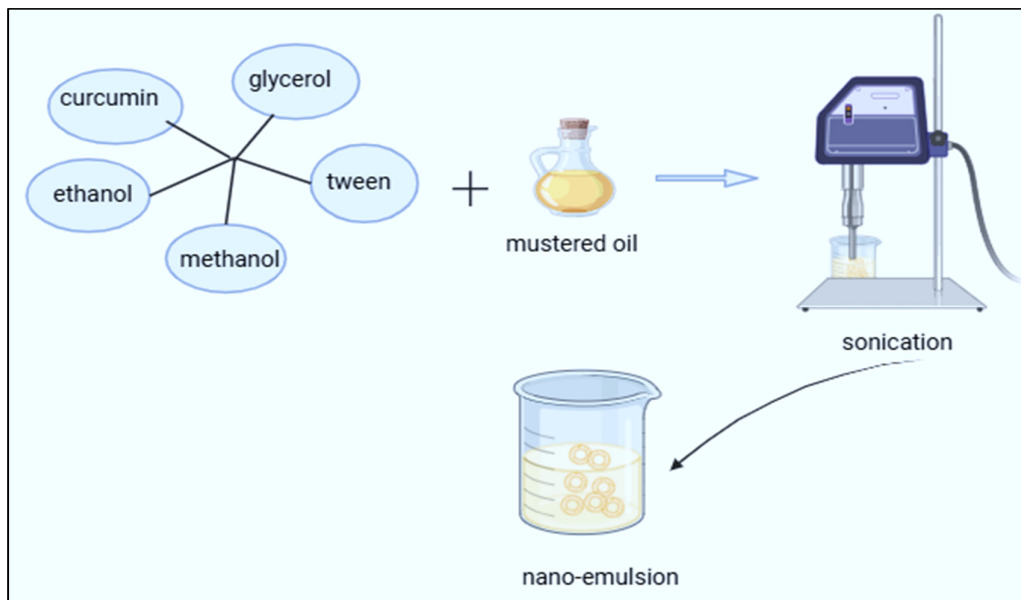
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Abstract

In vitiligo, a common autoimmune disease that lasts for a significant amount of time, areas of skin that are growing begin to lose their colour. There is currently no treatment for vitiligo that the FDA has authorized. To produce an acceptable formulation of curcumin, the study intends to use mustard oil as a component of the formulation. This formulation has the potential to be applied to body surfaces to achieve increased penetration and extended administration. To create nano-emulsion formulations, a number of different medications and mustard oil have been mixed. The current investigation was carried out to determine whether nano-emulsion preparation using sonification may promote the healing of vitiligo. An investigation of the solubility of curcumin was carried out in a variety of aqueous and non-aqueous solvents. It has been determined that curcumin is insoluble in water, however it is highly soluble in methanol and ethanol, readily soluble in propylene glycol, soluble in tween 80 and span 80, and soluble in between these two solvents. Characterizations of the improved formulation include visual inspection, surface morphology, viscosity, pH, particle size and zeta potential distribution, in-vitro investigation, and stability study. These are only some of the characteristics that can be evaluated. There is no alteration in the formulation that occurs with storage.

Keywords: Vitiligo, Curcumin, Mustard Oil, Nano-emulsion

Graphical Abstract:**INTRODUCTION:**

A common acquired pigmentary condition is vitiligo ⁽¹⁾. 'Leukoderma, another name for vitiligo, is a hypo-pigmentary autoimmune disease'. The autoimmune and autoinflammatory disease vitiligo comes from the immune system fighting itself and destroying melanocytes. It results in patches of white colour. It occurs in people of all skin types and colours. Additionally, the skin's hair turns white. Additionally, the insides of the nose and mouth could be affected ⁽²⁾. causes a slow death of the skin's melanocytes, which results in patchy depigmentation. This deformity can have horrible psychological implications, and it often impacts the face and other body parts that are exposed. Typically beginning in childhood, vitiligo deteriorates over time, resulting in a high disease burden and a lower quality of life for those who have it. There are several ways that vitiligo manifests itself, and recognizing these patterns can provide both diagnostic and predictive insights ⁽³⁾. The various There are two forms of vitiligo: segmental and non-segmental ⁽⁴⁾. The most typical kind of vitiligo is non-segmental vitiligo. It is subdivided into 5 types: Generalized, Acrofacial, Mucosal, Universal, and Focal ⁽⁵⁾.

Due to its many health advantages, Turmeric another name as *Curcuma longa*, has been utilized both internally and externally for many years. The primary active component of turmeric, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), this polyphenolic molecule which can be collected from the plant's rhizome. Curcumin, its main active yellow pigment, has reported hypoglycaemic, anti-inflammatory, antitumor, hepatoprotective, neuroprotective, Antioxidant, and cardioprotective effects. Furthermore, the active element in *C. longa* has been proven in several studies to be capable of effectively treating various dermatological conditions. For example, it can successfully treat atopic dermatitis, vitiligo, psoriasis, and acne ⁽⁶⁾. Curcumin exhibits its antioxidant qualities by acting as a free radical trap when low semiquinone radicals are present. However, the production of reactive oxygen species (ROS) takes precedence when concentrations are high ⁽⁷⁾.

Commonly used dosage compositions in cosmetics have a poor penetration ability and a restricted affinity for the skin. Because the active ingredient in traditional cosmetics cannot reach the target site, the intended effect cannot be obtained when they are used as cosmeceuticals. By efficiently lowering the magnitude of active compounds moving to the nanoscale and changing their properties, such as surface characteristics, water solubility, and permeability, the drawbacks of traditional dosage forms may be addressed via nanotechnology. One important technique that could lead to fresh product advancements in nanotechnology is nano emulsions (NE). Among the nanotechnologies that are now attracting the greatest attention is NE. Because of its small particle size, which reduces light scattering, the thermodynamically unstable system with the size of the interior particles ranging from 20 to 200 nm appears translucent or transparent. The size of the particles determines how an NE looks. The product will seem translucent if the particle is less than 100 nm ⁽⁸⁾. Compared to traditional topical treatments such as lotions, creams, ointments, and gels, these systems' higher surface area (nanosized globules), solubilization capacity, and the introduction of extra amphiphilic emulsifying agents (surfactants and co-surfactants) allow for improved drug entrapment and penetration.

Among its advantages include longer-term stability, increased drug solubility, better drug partitioning, and enhanced penetration through the skin's layers ⁽⁹⁾.

The goal of this work was to prepare a NE with active ingredients. The stability test and physicochemical characteristics were assessed following the loading of the active compounds into the water and oil phases. Those who are interested in developing new therapy products will find the study's findings useful.

METHODS

1. Materials

The drug curcumin was provided as a gift sample from Arjuna Natural Pvt Ltd, Kerala, India. Span 80 and Glycerol were purchased from Shreya Scientifics and Chemicals, Bilaspur (C.G.). Mustered oil was obtained from pure mustard seed from home. Other chemicals- Ethanol, methanol, glycerol, tween 80, Propylene Glycol, potassium dihydrogen phosphate, and sodium hydroxide were obtained from the department's chemical store.

2. Pre-formulation study

2.1. **Physical inspection** - Organoleptic properties like colour, odour, and physical properties were analysed as a drug sample of Curcumin.

2.2. **Identification by UV spectroscopy** - UV absorption spectroscopy is one of the broadly utilized strategies for the determination of contaminations in natural compounds. It additionally measures the absorbance at a particular wavelength. Extra peaks can be seen because of impurities in the specimen and they can be contrasted with that of standard crude material. Thus, UV spectral examination is an imperative parameter for the subjective

recognizable proof of the drug. For the spectral analysis of the drug sample, the solvent system used to make the standard solution was ethanol: PBS buffer at a concentration of 1 mg/ml. UV scanning was done between 400-800 nm using a UV-visible spectrophotometer (UV - 1800, Shimadzu, Japan).

2.2.1. Preparation of buffer (pH 7.4) media for standard curve ⁽¹⁰⁾

- **Phosphate buffer (pH 7.4):** After adding 50 ml of 0.2 M potassium dihydrogen phosphate and 39.1 ml of 0.2 M sodium hydroxide, distilled water was added until the 200 ml volumetric flask was filled. For quantitative estimations, a pH meter was used to modify the pH.
- **Preparation of standard stock solution**
 - **Standard stock-I (1000µg/ml):** A precisely weighed 10 mg dose of the substance curcumin was dissolved to make the stock solution in a 10 ml volumetric flask, which had a 1000 µg/ml concentration of curcumin. The capacity was then filled with PBS: with ethanol (1:1).
 - **Standard stock-II (100µg/ml):** Standard stock-I was further diluted by placing 1 ml of it in a volumetric flask with a capacity of 10 ml, and the amount of solution was then added with PBS: Ethanol to obtain the running standard stock solution containing 100 µg/ml of the substance.
 - **Preparation of standard calibration curve - 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml aliquots of standard stock-II (100µg/ml)** were transferred into a 10 ml volumetric flask, and the volume was adjusted to the appropriate level using buffer solution to create a set of standard dilutions of 1, 2, 3, 5, 6, 7, 8, 9, and 10 µg/ml of the drug. After that, the aliquots were examined using a UV spectrophotometer, and the amount of absorption was noted.

2.2.2. Preparation of Phosphate buffer (pH 5.6) media for standard curve ⁽¹¹⁾

- **Solution- I:** After dissolving 2.730 g of potassium dihydrogen phosphate in DW, the volume was adjusted within a 250 ml volumetric flask filled with distilled water.
- **Solution- II:** Distilled water was included in a 250 ml volumetric flask containing 2.902 g of dipotassium hydrogen phosphate after it had been dissolved in DW. After mixing Solution-I (94.4 ml) and Solution-II (5.6 ml), the pH was measured quantitatively with a pH meter.
- **Preparation of standard stock solution**
 - **Standard stock-I (1000µg/ml):** A precisely weighed 10 mg dose of the substance curcumin was dissolved in a volumetric flask with a capacity of 10 ml, and the capacity was then filled with PBS pH 5.6 to create the stock solution, which had a 1000 µg/ml concentration of curcumin.
 - **Standard stock-II (100µg/ml):** The working standard stock solution with a concentration of 100 µg/ml was obtained by further diluting standard stock-I by adding 1 ml of it to a 10 ml volumetric flask and adjusting the volume with phosphate buffer pH 5.6.
 - **Preparation of standard calibration curve -** A set of standard dilutions of 1,2,3,4,5,6,7,8,9 and 10 µg/ml of the drug transferring 0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0.9 and 1 ml aliquots of standard stock-II (100µg/ml) in 10 ml volumetric flask and volume were made up to the mark with buffer solution. Then the aliquots were observed under a UV spectrophotometer and absorption was recorded. The regression equation was calculated and the calibration curve was produced.

2.3. **Identification by FTIR spectroscopy** - FTIR spectroscopy is usually utilized as a part of the examination of pharmaceutical chemicals and drugs to distinguish the useful gatherings

exhibited in the compound. Absorption spectroscopy is the foundation of infrared spectroscopy. When doing infrared spectroscopy, a device called an infrared spectrometer is utilized to produce an infrared spectrum ⁽¹²⁾.

2.4. Determination of solubility - One of the key components of chemical characterization during the formulation process of development is a measurement of the drug's solubility in various solvents. The drug's solubility influences its bioavailability and rate of release, which in turn impacts the drug product's therapeutic efficacy. The maximum quantity of a solute dissolved in every unit of solvent is used to express the drug's solubility ⁽¹³⁾ ⁽¹⁴⁾. Curcumin's solubility in a variety of common solvents was investigated. The equilibrium solubility method ⁽¹⁵⁾.

- At room temperature, 10 millilitres of each solvent under investigation were used to dissolve a specific amount (10 mg) of the material.
- The drug was gradually added to each test tube until the saturation point was reached and undissolved particles were visible.
- After filtering, the solvent was examined using a UV spectrophotometer (Shimadzu, Japan; UV-1800).

2.5. Determination of partition coefficient - The partition coefficient (log P), is the best measure of the lipophilicity of drugs, it has been an important parameter in the complex process of formulation development and drug delivery studies. Numerous biological phenomena, including solubility, absorption potential, membrane permeability, plasma protein binding, volume of distribution, and renal clearance, can be correlated using the partition coefficient. The partition coefficient is the proportion of the unionized compound's concentration between the organic and aqueous phases at equilibrium ⁽¹⁶⁾.

$$P_{O/W} = C_{\text{Oil}} / C_{\text{Water}} \text{ at equilibrium}$$

Method

- To find the curcumin partition coefficient, 10 mg of the precisely weighed medication was obtained and dissolved at 25 ml funnel for separating with 10 ml of each solvent (buffer and n-octanol).
- After six hours of gentle shaking, the flask was left for twenty-four hours until equilibrium was achieved. Phases were separated using a separating funnel, and the drug's partition coefficient was computed after the aqueous phase was analyzed spectrophotometrically ⁽¹⁶⁾.

2.6. Melting point determination - Melting point is the thermal analysis most used to characterize solid crystalline material and is a characteristic technique to determine the drug's purity. By monitoring the substance's melting point within a specific range, one may evaluate the drug's quality. The transition from the solid to liquid phase occurs at this temperature. The melting point was determined using digital melting point equipment, and the measured melting point was then compared to the standard ⁽¹⁷⁾.

Method

- A small amount of an organic solid can be added to a tiny capillary tube to ascertain its melting point.
- Heated one end of the capillary in the flame for two to three minutes to close it.
- After dipping the capillary tube's open end into the finely ground curcumin, gently tap the tube against the table to fill it with the compound until it is between one and two centimetres long.
- The temperature at which curcumin began to melt and melted fully was monitored when the filled capillary tube was placed within a digital melting point instrument.

3. Formulation and optimization - After successful authentication is made on the drug sample (curcumin) and other ingredients that are found to comply with the standards as per

the official and other standard books. The nano-emulsion containing curcumin with emulsion was prepared and developed (Figure 1) (Table 1).

3.1. Preparation method of NE by high-energy method

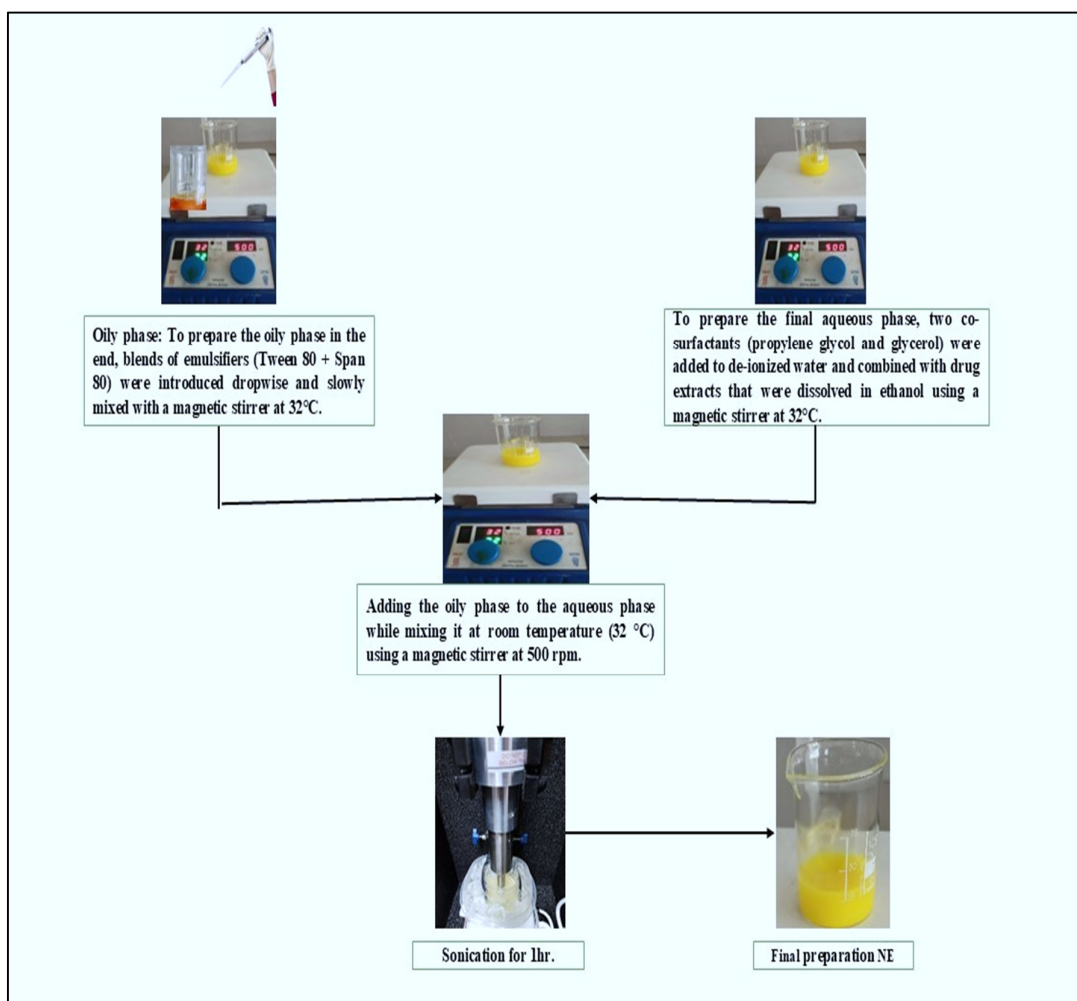


Figure 1: Preparation method of curcumin NE

Table 1: Factors of optimization of formulations variable

Formulation code	Composition variables						Processing variables sonication time (hr)	Particle size (nm)
	Drug (mg)	Mustard oil (ml)	Surfactants		Co-surfactants			
			Tween 80 (ml)	Span 80 (ml)	Propylene Glycol (ml)	Glycerol (ml)		
F1	10	1.289	2.5	3.25	0.3	0	1	2290
F2	10	1.289	3.0	3.25	0.3	0.5	1	281
F3	10	1.289	2.5	2.85	0.3	1.0	1	320
F4	10	1.289	2.5	2.85	0.3	1.5	1	163
F5	10	1.289	2.5	3.25	0.5	0	1	96.4
F6	10	1.289	2.83	3.16	0.4	2.0	1	83.08
F7	10	1.289	3.0	2.85	0.3	0	1	110
F8	10	1.289	3.0	2.85	0.3	0.5	1	74.26

F9	10	1.289	2.5	3.25	0.3	1.0	1	91.8
F10	10	1.289	2.5	2.85	0.5	1.5	1	108

[Drug 10 mg present in 25 ml of formulation]

4. Characterization of optimized formulation - Characterization is a vital step in formulation development. It reveals all the characteristic properties of the developed formulation, whether the formulation is showing the desired outcome or not.

4.1. **Visual inspection** - Curcumin NE was observed in the appearance and inspected for organoleptic properties ⁽¹⁸⁾.

4.2. **Surface morphology** - Light microscopy (photomicroscope with Axiocam-Lab-1A-ERC-5S Camera, Zeiss, Primo star, Germany) was used to determine the surface morphology of drug-loaded NE at a greater resolution ⁽¹⁹⁾.

4.3. **Viscosity** - The viscosity of a fluid indicates how resistant it is to deformation at a certain pace. It is comparable to the informal concept of "Thickness" to liquids. Spindle 63 was used to do the measurement on the sample at 100 RPM. A viscosity determination of optimized formulation was performed using LV Brookfield Viscometer. Spindle 63 was used to do the measurement on the sample at 100 RPM ⁽¹⁸⁾.

4.4. **pH measurement** - The pH of the optimized formulation was measured using a digital pH meter. For this curcumin, NE was dissolved in 25 millilitres of double-distilled water. Three different readings of the constant were then taken when the pH electrode was immersed in the dissolved NE solution ⁽²⁰⁾.

4.5. **Particle size and zeta potential distribution** - Using dynamic light scattering (DLS), the zeta potential and droplet size of optimized Curcumin NE F6 utilizing Lite-sizer 500 (Anton Paar) with Omega cuvette Mat. No. 155765. Automatic settings were used to do the observations, and light scattering was observed at 25°C. Before analysis, the samples were mixed for one minute and diluted with distilled water (1:100) ⁽²¹⁾.

4.6. **In-vitro study**

4.6.1. **In-vitro drug release studies** - The release kinetics of curcumin NE were evaluated by counting the amount of curcumin that was released from NE and then passed through rat skin into the receiver of a Franz diffusion cell. After the rat skin was removed, it was clamped between the receptor chamber and the donor. After placing the stratum corneum-facing skin on the receiver chamber, the donor chamber was clamped into position. 1.25 ml of curcumin-loaded NE was positioned within the donor chamber, whereas PBS buffer (pH 5.6) was placed in the receptor chamber compartment. The rat skin's outermost layer was oriented toward the donor compartment. when it was positioned between the two compartments. The receptor chamber used a recirculating water bath to maintain a thermostatic temperature of 37°C. For six hours, aliquots of the 5 ml were taken out at regular intervals, and to keep the sink conditions stable, an equivalent volume of the freshly warmed medium was added. The samples were analysed for the drug content using a UV spectrophotometer (Shimadzu-1800, Japan) at 426 nm ⁽²²⁾ ⁽¹¹⁾.

4.7. **Mathematical modelling** ⁽²³⁾ - To understand the drug release kinetics, data was analyzed using

A. **Zero-order model** - A method in which the drug's concentration does not affect its rate of release is commonly explained using this kind of framework.

$$Q_t = k_0 t$$

where k_0 is the release rate constant for zero-order and Q_t is the quantity of medication released in time t .

B. **First-order model** - The model clarifies the framework by showing that drug release through the swelling matrix and concentration both affect the rate of drug release.

$$Q_t = q_0 (1 - e^{-k_1 t})$$

Q_0 is the drug's starting dosage, and k_1 is the first-order release rate constant.

C. **Higuchi matrix model** - According to this concept, the drug's release from an insoluble matrix is explained by the squared roots of a time-dependent process based on Fickian diffusion.

$$Q_t = k_h \sqrt{t}$$

The Higuchi release rate constant is k_h , while the quantity of medication released at time t is denoted by Q_t .

D. **Korsmeyer-peppas model** - Using a straightforward exponential relation, this approach is frequently used to explain how pharmaceuticals are released from matrix systems with varying geometries.

$$E. K t n = M_t / M_\infty$$

where the diffusion exponent, K , the release rate constant of the control release device, and M_t / M_∞ represent the percentage of the release of drugs at time t .

Plots were created as follows:

- Calculative percentage of the drug about time (Zero order kinetic model)
- Log cumulative percentage of drug release vs time in a first-order kinetic model
- The cumulative percentage of drug release as a function of time squared in the Higuchi kinetic model
- The Korsmeyer-Peppas kinetic model, which compares log time to log cumulative percentage of drug release.

- **Stability study** - Pharmaceutical stability testing's primary goal is to offer a reasonable level of assurance that a product will maintain a suitable level of quality for the duration that it is on the market, available for patient supply, and suitable for ingestion up to the patient uses up the entire quantity of the product. The stability of any preparation is described as the degree to which its formulation retains its characteristics and properties under specified limits throughout the storage and usage, the same as those it possessed at the time of its packing. The characteristics include physical, chemical, therapeutic, and toxic properties. All the formulations should maintain a toxic content lesser than the specified limit before releasing in the market. Adequate quality data about the drug and its dosage form is crucial for the following three reasons.
 - Safety of patients.
 - The drug's identity, potency, purity, and quality are all impacted by legal regulations⁽²⁴⁾.

4.7.1. **Stability study of formulation** - The pH, viscosity, particle size, zeta potential, and PDI measured form the basis for the optimization operations of different batches. Therefore, the optimized formulation F6 was chosen for the stability analysis to ensure the optimized product's integrity, quality, and efficacy in storage.

4.7.2. **Effect on storage of formulations (short-term stability studies)** - Real-time and accelerated stability tests were performed on the refined formulation for 1 -3 months in storage conditions after packing in a rubber stoppered glass vial properly. After every 7 days, the formulation sample was analysed^{(25) (26)}.

RESULTS AND DISCUSSION

1. Pre-formulation studies

1.1. **Physical inspection** - The observed data were compared with standard parameters (Table-2).

Table 2: Curcumin's organoleptic characteristics

S. No.	Parameters	Observation
1.	State	Powder
2.	Colour	Yellowish
3.	Odor	Characteristics
4.	Taste	Slightly pungent bitter

1.2. **Identification by UV spectroscopy** - For the UV spectroscopy determination, a standard solution of curcumin was prepared for PBS 7.4 pH: Ethanol solution and PBS pH 5.6 was scanned using the spectrum mode, and the 400–800 nm UV visible range overlap spectra were obtained from the overlain spectra of the drug, maximum absorption (λ max) was observed at wavelength 428 nm and 426 nm. The linearity was found under 1-10 μ g/ml and the Correlation coefficient (R^2) was 0.9998 and 0.9991 estimated (Figure-2&3, Table-3&4).

Table 3: Standard calibration curve in PBS: ethanol

S. No.	Concentration (μ g/ml)	Absorbance (at λ max = 428 nm)
1	0	0
2	1	0.0865
3	2	0.1853
4	3	0.2799
5	4	0.3712
6	5	0.4654
7	6	0.5621
8	7	0.6552
9	8	0.7585
10	9	0.8551
11	10	0.9494

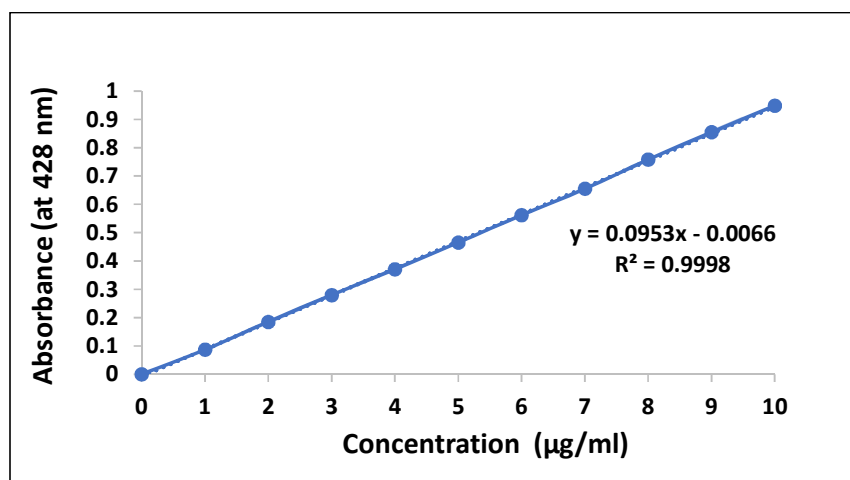
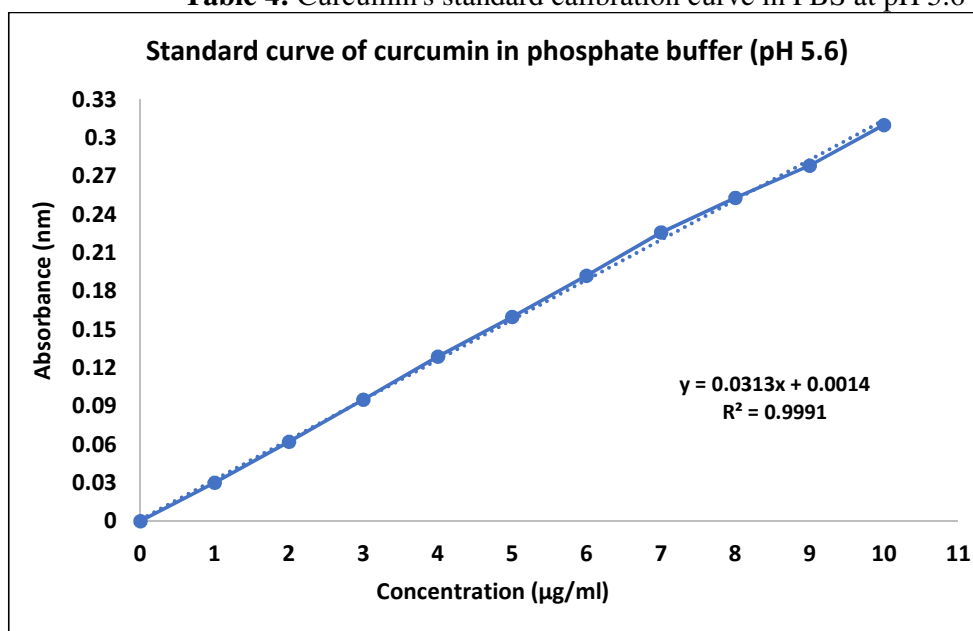


Figure 2: Curcumin's standard calibration curve in PBS at pH 7.4

S. No.	Concentration($\mu\text{g/ml}$)	Absorbance (at λ max = 426 nm)
1	0	0
2	1	0.0301
3	2	0.0621
4	3	0.0951
5	4	0.1287
6	5	0.1599
7	6	0.1921
8	7	0.2259
9	8	0.2531
10	9	0.2783
11	10	0.31

Table 4: Curcumin's standard calibration curve in PBS at pH 5.6**Figure 3:** Curcumin's standard calibration curve in PBS at pH 5.6

1.3. Identification by FTIR spectroscopy - As seen in the picture, the drug sample's FTIR interpretation was compared to the reference IR spectra. All the noticeable peaks of the functional group contained in the drug sample were visible in the drug's obtained infrared spectrum (Table-5-11, Figure.4-10).

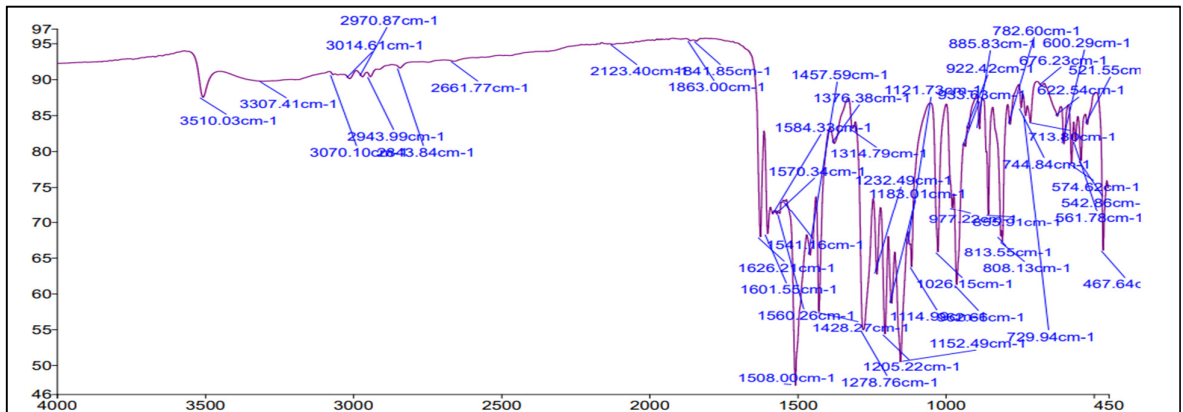


Figure 4: FTIR spectra of curcumin

Table 5: Interpretation of FTIR spectra of drug (curcumin)

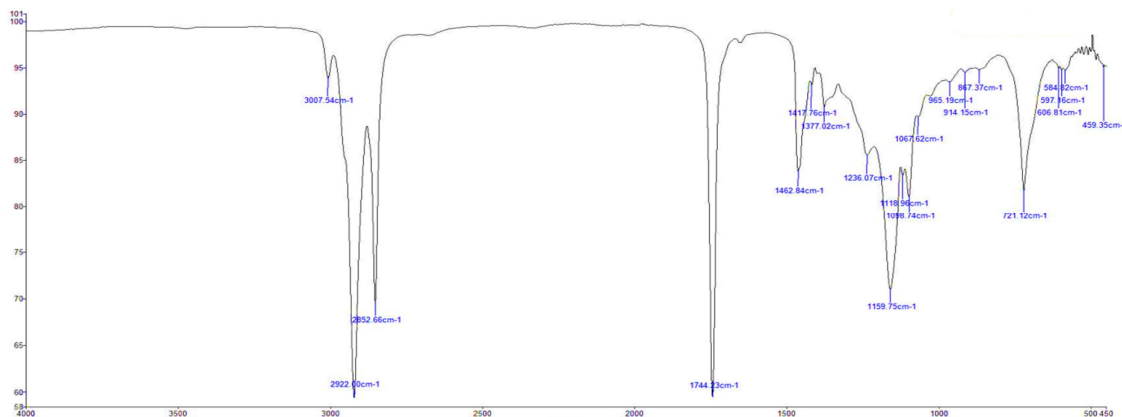


Figure 5: FTIR spectra of mustard oil

Table 6: Interpretation of FTIR spectra of mustard oil

S.no.	Wave number (cm-1)	Functional group
1.	3007.54	CH ₃ Stretching
2.	2922	N-H Stretching
3.	2852.66	N-H Stretching
4.	1744.23	C=C stretching
5.	1462.84	C-H bending
6.	1417.76	O-H stretching
7.	1377.02	O-H bending
8.	1236.07	C-N stretching
9.	1159.75	C-O stretching
10.	1098.74	C-S and C=C stretching
11.	1067.62	S-O stretching
12.	459.35	Alkybenzene ring stretching
5	1415.82	CH stretching and bending
6	1263.89	Aromatic C-H stretching
7	1044.34	C-O-C stretching

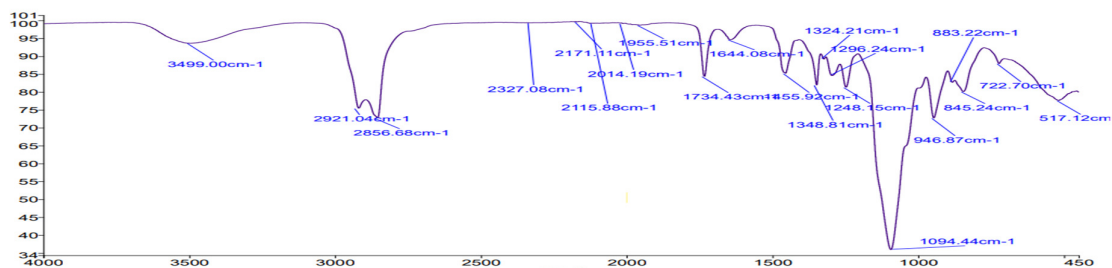
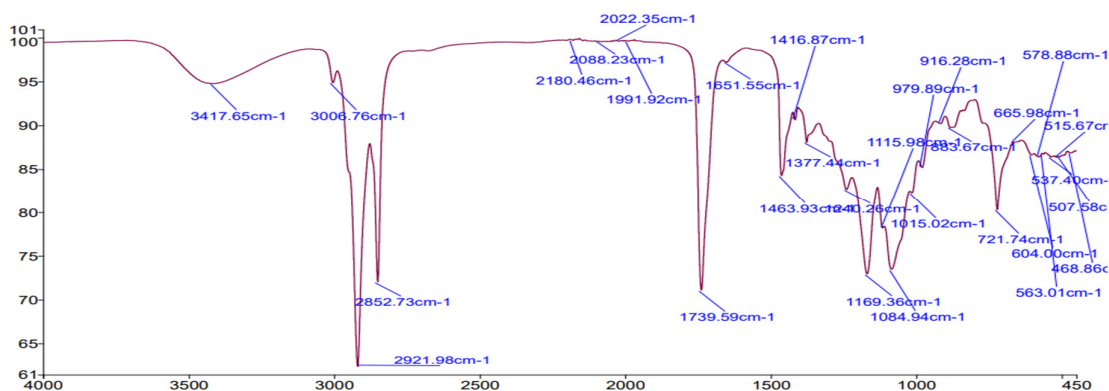


Figure 6: FTIR spectra of tween 80

Table 7: interpretation of FTIR spectra of tween 80

S.no.	Wave number (cm-1)	Functional group assigned
1.	3499	O-H stretching
2.	2921.04	N-H stretching
3.	2856.68	N-H stretching
4.	2115.88	C≡C stretching
5.	1734.43	C=O stretching
6.	1256.24	C-O stretching
7.	1094.44	C-O stretching
8.	517.12	C-Br stretching

**Figure 7:** FTIR spectra of span 80**Table 8:** Interpretation of FTIR spectra of span 80

S.NO.	WAVE NUMBER (cm-1)	FUNCTIONAL GROUP ASSIGNED
1.	3417.65	O-H stretching
2.	3006.76	C-H stretching
3.	2921.98	N-H stretching
4.	2852.73	N-H stretching
5.	1739.59	C=O stretching
6.	1651.52	C=C stretching
7.	1463.93	C-H bending
8.	1416.87	O-H bending
9.	1377.44	O-H bending
10.	1084.94	C-O stretching
11.	1015.02	C-N stretching

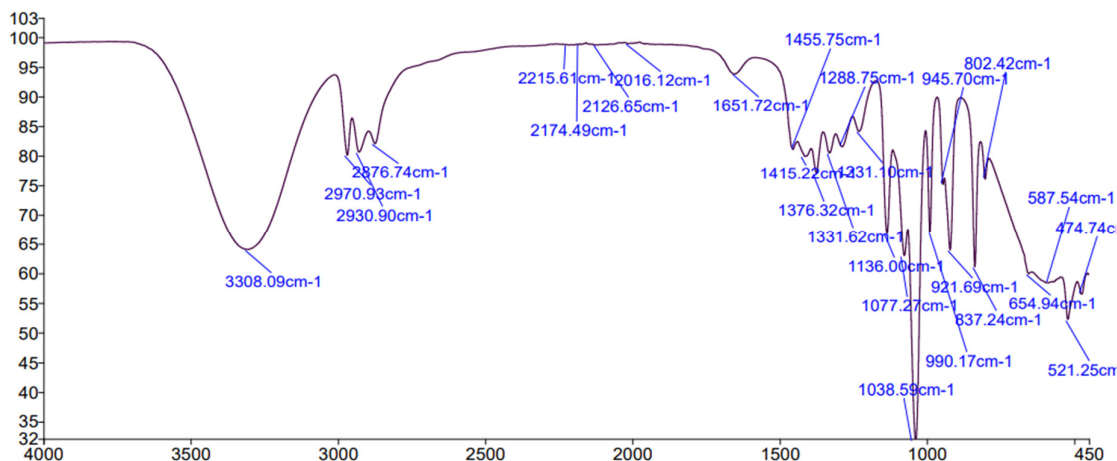


Figure 8: FTIR spectra of propylene glycol

Table 9: interpretation of FTIR spectra of propylene glycol

S.no.	Wave number (cm-1)	Functional group
1.	3308.09	O-H and N-H stretching
2.	2930.90	O-H and N-H stretching
3.	2126.65	C≡C stretching
4.	1651.22	C=C stretching
5.	1415.22	S=O stretching and O-H bending
6.	1288.75	C-N stretching
7.	1136	C-O stretching
8.	1038.59	S=O stretching
9.	474.74	Alkyl halides

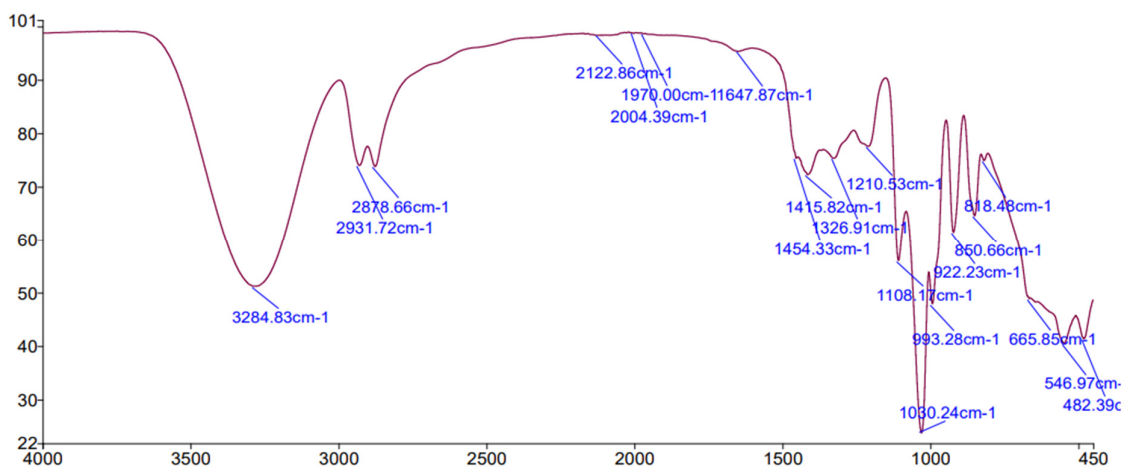


Figure 9: FTIR spectra of glycerol

Table 10: Interpretation of FT-IR spectra of glycerol

S.No.	Wave number (cm-1)	Functional group assigned
1.	3284.83	O-H stretching and C-H stretching
2.	2931.72	O-H stretching and N-H stretching

3.	2122.86	N=N=N stretching
4.	1454.33	C-H bending
5.	1415.82	S=O stretching and O-H bending
6.	1326.91	O-H bending
7.	1108.17	C-O stretching
8.	1030	S=O stretching
9.	482	Alkyl halides

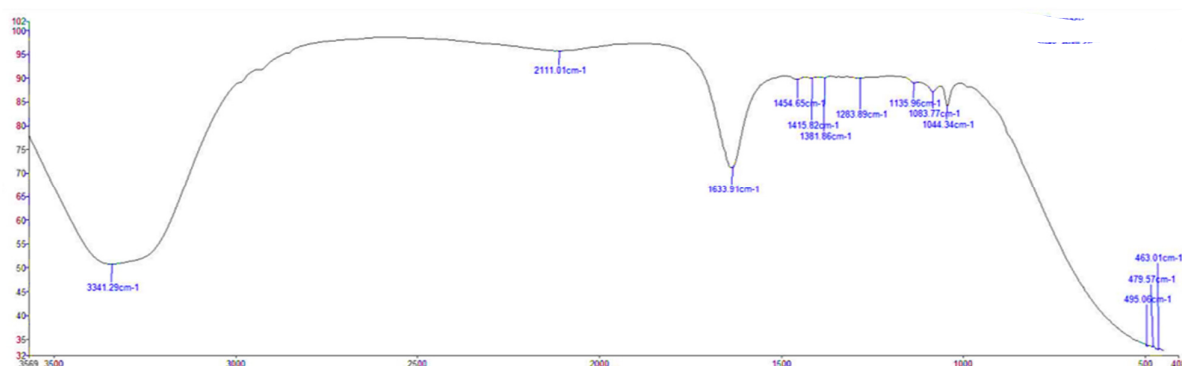


Figure 10: FTIR spectra of formulation

Table 11: interpretation of FTIR spectra of formulation

S.No.	Wave number (cm-1)	Functional group
1.	3341.29	O-H and N-H stretching
2.	2111.01	N=N=N stretching
3.	1633.91	C=O and C=C stretching
4.	1454.65	Benzene ring stretching, C-H bending
5.	1415.82	S=O stretching and O-H bending
6.	1381.86	O-H bending
7.	1135.96	C-O stretching
8.	1083.77	C-O stretching
9.	1044.34	S=O stretching

1.4. Determination of solubility – Curcumin's solubility in a range of aqueous and non-aqueous solvents was assessed. It was discovered that the medication was insoluble in water, easily soluble in propylene glycol, soluble in tween 80 and span 80, and highly soluble in methanol and ethanol (Table-12).

Table 12: solubility study of curcumin in different solvents

S.no.	Solvent	Solubility
1.	Ethanol	+++
2.	Methanol	+++
3.	Water	---

4.	Propylene glycol	++
5.	Tween 80	+
6.	Span 80	+

+++ **Highly soluble (<1 part)**

++ **Freely soluble (1-10 part)**

+ **Soluble (10-30 parts)**

- **Sparingly soluble (30-100parts)**

-- **Slightly soluble (100-1000parts)**

--- **Very slightly soluble (1000-10000)**

1.5. **Determination of partition coefficient** – medication lipophilicity and hydrophilicity are measured by the partition coefficient, which also shows how well a medication may pass across biological membranes. It was discovered that curcumin has a partition coefficient value of 3.21, indicating that the medication is lipophilic.

1.6. **Melting point determination** - Melting point determination is the most widely used thermal analysis technique for describing solid crystalline materials. Curcumin was found to have a melting point of 183°C.

2. EVALUATION OF PREPARED NE

2.1. **Visual inspection** – The formulation was observed for organoleptic properties which show that the formulation has a viscous liquid yellow transparent appearance (Table-13).

Table 13: visual inspection of optimized formulation

S.No.	Properties	Observation
1.	Appearance	Viscous liquid
2.	Color	Yellow Transparent

2.2. **Surface morphology** - The behavior and stabilizing mechanism of NE can be discovered through real-time observation; however, this is still very difficult because regular emulsifier particles are invisible under a light microscope (mostly because of their small size and lack of fluorescence). We were able to perform dynamic monitoring of the curcumin-stabilized emulsion system in our work. These findings support those above, which show that to achieve high stability, curcumin particles are best positioned near the interfaces of NE droplets (Figure-11).

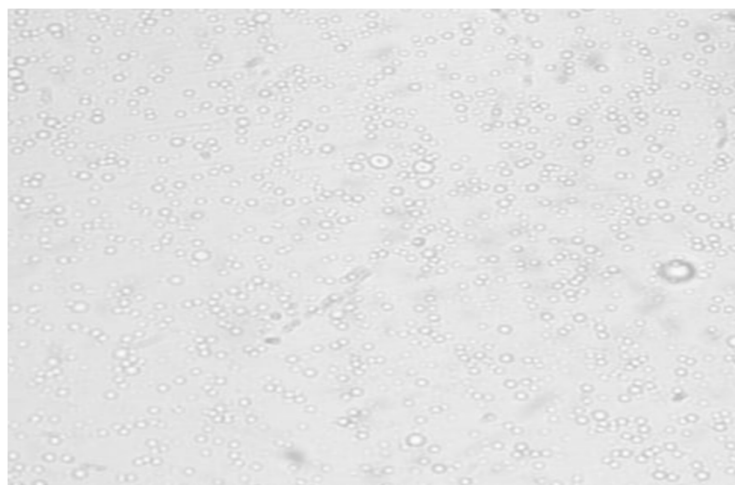


Figure 11: Surface morphology

2.3. **Viscosity** - The viscosity was observed using a Brookfield viscometer that shows a result of 98.33 cps at spindle size 63. (Table-14& figure;12)

Table 14: Viscosity

S.No.	Spindle size	Viscosity (cps)	RPM
1.	63	97	100
2.	63	98	100
3.	63	100	100
MEAN = 98.33			

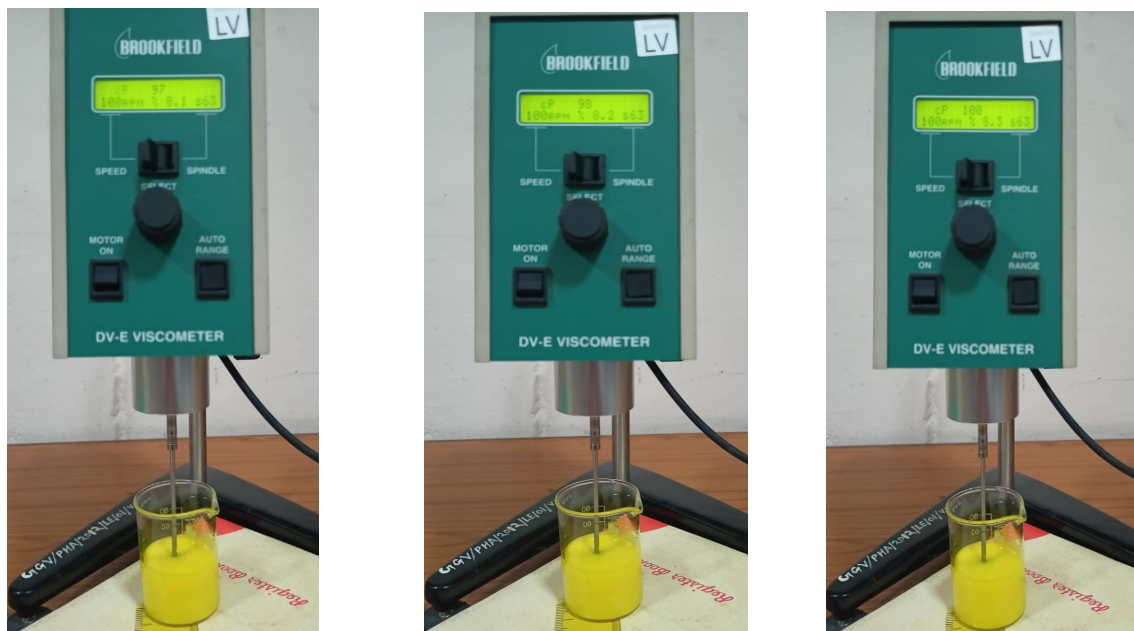


Figure 12: viscosity measurement

brook-field viscometer

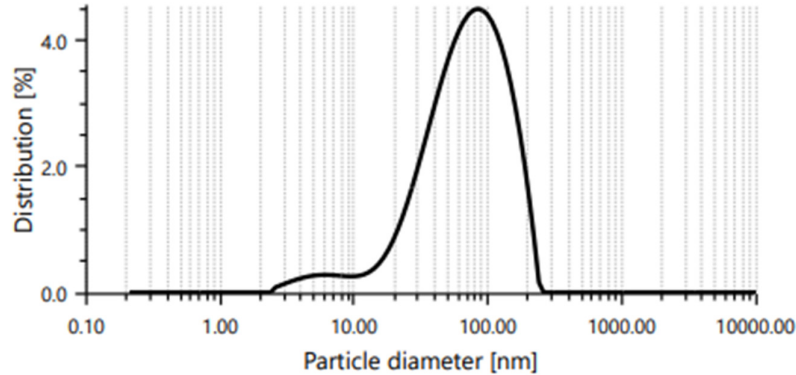
2.4. **pH measurement** - The pH of the optimized F6 was determined using a digital pH meter that showed a result of pH 5.6 (Table-15)

Table 15: pH measurement

S.No.	pH	Mean
1.	5.7	5.6
2.	5.5	
3.	5.6	

2.5. **Particle size and zeta potential distribution** – A drug loading dose of 15 mg/mL was used to create curcumin NE, which was then tested for zeta potential (–0.5 mV) and droplet size distribution (83.08 nm with PDI 27.8).(Figure-13a&b)

a. Particle size distribution (intensity)



b. Zeta potential distribution

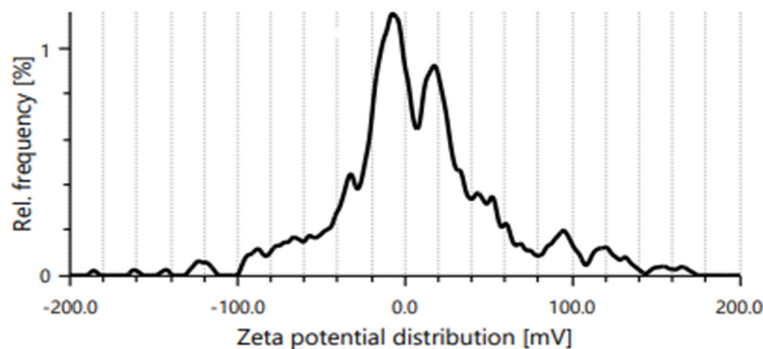


Figure 13: A. Particle size distribution (intensity)
B. Zeta potential distribution

2.6. In-vitro study

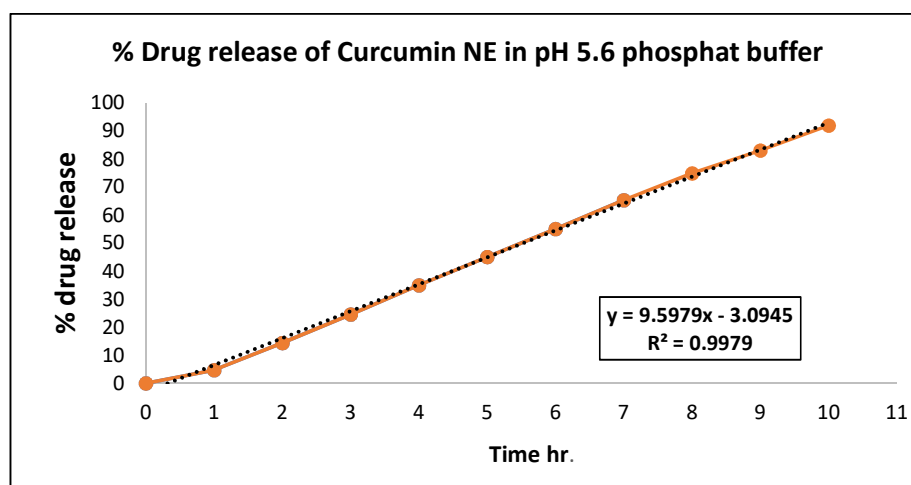
2.6.1. In vitro drug release studies – A Franz diffusion cell was used to conduct an in vitro drug release investigation. The cumulative percentage drug release of prepared Curcumin NE F1, F2, F3, F4, F5, F6, F7, F8, F9, and F10 was determined. Formulation F6 released 95% drug in 10 hours. Based on the above results batch, F6 was selected as the optimized batch among all formulations. Results of release studies retard the

demonstrated that NE can release of Curcumin (Figure.14&15; Table;16).



Figure 14: FRANZ diffusion cell arrangement for in vitro drug release study**Table 16:** dissolution data

S.no.	Time(h)	% Drug release
1.	0	0
2.	1	4.703125
3.	2	14.40625
4.	3	24.5625
5.	4	34.96875
6.	5	45.09375
7.	6	55.0000
8.	7	65.3125
9.	8	74.84375
10.	9	83.03125
11.	10	91.921875

**Figure 15:** % drug release of curcumin NE in pH 5.6 phosphate buffer

2.7. Mathematical modelling – Five kinetic models, including the zero, first, Higuchi, and Korsmeyer–Peppas equations, were used to evaluate and depict the data. The kinetic constant and goodness of fit for the kinetic models are listed in the table using the correlation coefficient, or r². According to the findings, the Korsmeyer–Peppas and Zero-order models were able to effectively match the data. For curcumin NE, the model has the highest correlation coefficient (>0.9) (Table-17&18; Figure-16-19).

Table 17: Release kinetics calculated by different method

Time(h)	Cumulative % drug release	Square root of time	Log time	log cumulative % drug release
0	0	0	-	-

1	4.703125	1	0	0.67
2	14.40625	1.41	0.3	1.15
3	24.5625	1.73	0.47	1.39
4	34.96875	2	0.6	1.54
5	45.09375	2.23	0.69	1.65
6	55	2.44	0.77	1.74
7	65.3125	2.64	0.84	1.81
8	74.84375	2.83	0.9	1.87
9	83.03125	3	0.95	1.91
10	91.921875	3.16	1	1.96

A. Zero-order model

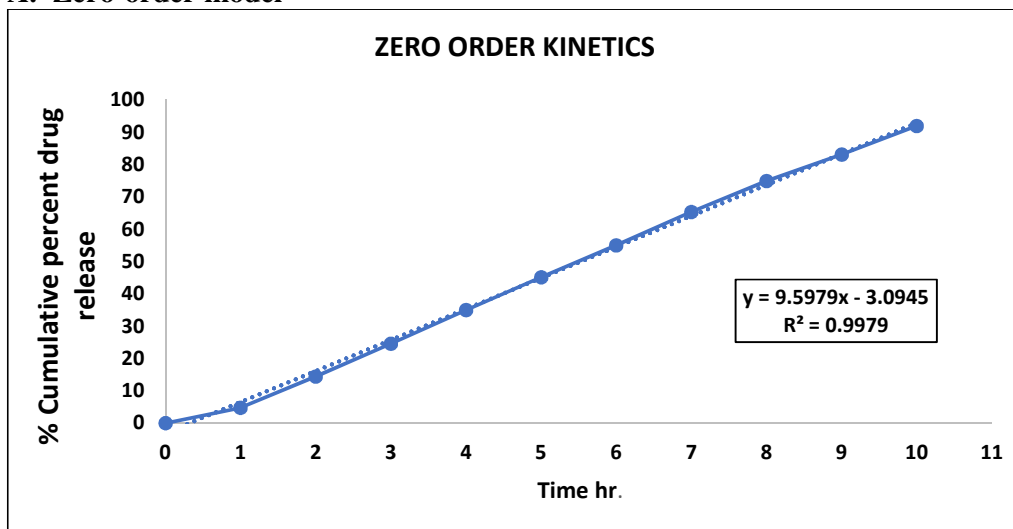


Figure 16: time vs cumulative percent drug release

B. First-order model

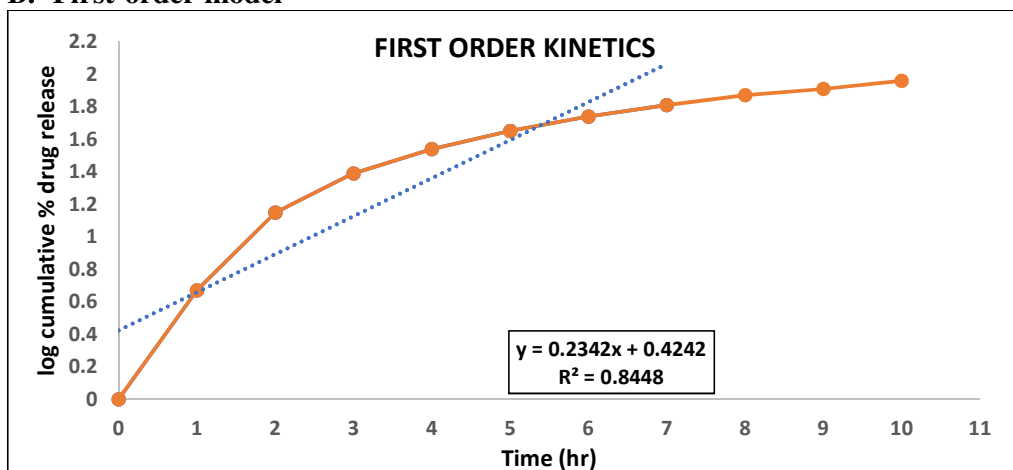
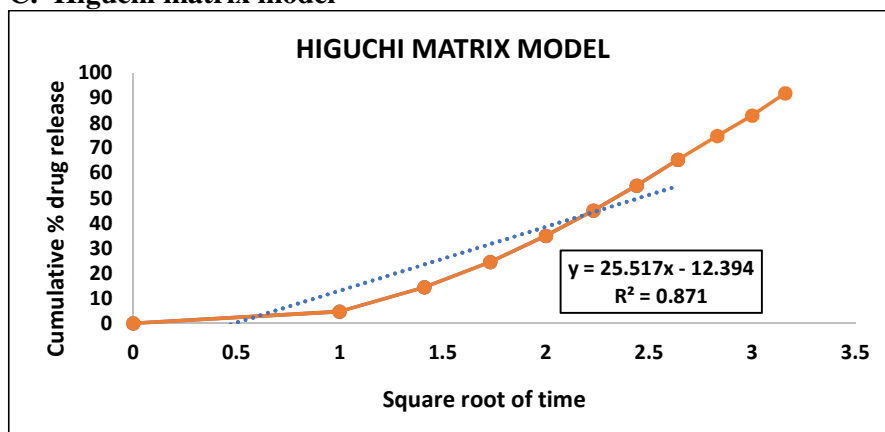
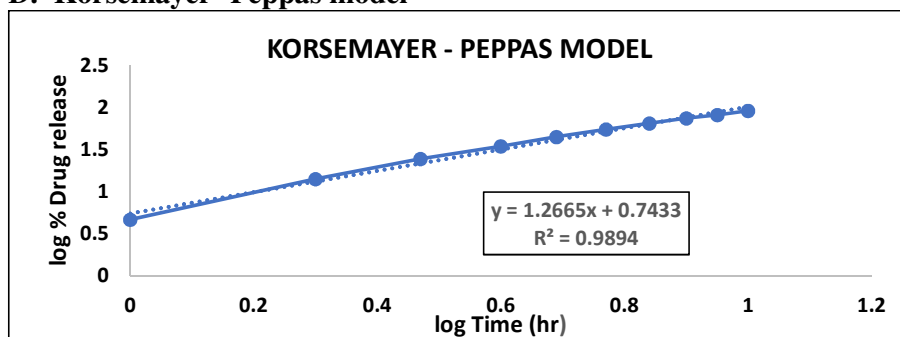


Figure 17: time vs log cumulative percent drug release**C. Higuchi matrix model****Figure 18:** square root of time vs percent drug release**D. Korsmeyer- Peppas model****Figure 19:** log time vs percent drug release**Table 18:** kinetic analysis data for formulation (f6)

Formulation	Zero Order	First Order	Higuchi matrix model	Korsmeyer-Peppas model
F6	R^2	R^2	R^2	R^2
	0.9979	0.8448	0.871	0.9894

2.8. Stability study

- 2.8.1. **Stability study of formulation** - the NE investigations that have been reported. Physical stability, findings, and stabilizing processes are provided together with information on the composition of NE, including oil type, oil concentration, and aqueous phase content, as well as preparation procedures, storage temperature, and testing techniques.
- 2.8.2. **Effect on storage of formulations: short-term stability studies** - The formulation property does not significantly change after storage, according to the data. Three months were spent testing the formulation's stability. The properties showed minor but acceptable adjustments (Table-19).

Table 19: stability studies

Where, RT = Room temperature 25° C, Cold condition = 4-8° C.

DISCUSSION

Parameters	Time (months)						
	0	1		2		3	
Stability conditions	-	RT	Cold condition	RT	Cold condition	RT	Cold condition
Particle size(nm)	83.08	83.20	82.1	85	82.55	85.3	
PDI	27.8	28	26	29	26.7	30	33
Zeta potential	-0.5	-0.6	-0.5	-0.6	-0.8	-0.8	-0.9
pH	5.6	5.62	5.6	5.55	5.59	5.63	5.6
Visual observation	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent

An autoimmune skin condition termed vitiligo affects 0.5–2% of people. Usually, the patient has well-defined, chalky-white skin patches that may appear places. Patients with vitiligo may consequently experience a lower quality of life and severe psychological effects ⁽²⁷⁾. Numerous metabolic and genetic variables, oxidative stress, melanocyte adhesion, and innate and adaptive immunity are thought to impact its development ⁽²⁸⁾. These many processes cause melanocytes to be assaulted by the immune system and lose their intercellular connections, ultimately resulting in cell death ⁽²⁷⁾. Although vitiligo cannot be cured at this time, several treatments can help control the illness and enhance the appearance of the skin that is affected. The severity and extent of the condition, along with individual traits like age and overall health, can influence the treatment strategies ⁽²⁹⁾. Three objectives are pursued in vitiligo treatment: immune suppression to stop the disease's progression, pigmentation regeneration, and recurrence avoidance. To accomplish these objectives, both nonsurgical and surgical approaches are possible. While nonsurgical approaches offer a variety of alternatives that target the many mechanisms involved in the formation of vitiligo, surgical methods seek to implant fresh melanocyte reservoirs into vitiligo lesions. Additionally, current and forthcoming nonsurgical treatments for vitiligo are in the spotlight due to the active pre-clinical and clinical research on numerous medications ⁽³⁰⁾.

The reason behind the selection of the drug Curcumin is an herbal extract derived from rhizomes of *Curcuma longa* as a drug of choice for the proposed study because of its easy low-cost, and non-toxic nature, significant effect on melanocyte growth, and fewer negative side effects compared to synthetic medications. The expected outcome of this research was to develop an effective formulation to treat vitiligo through curcumin-based NE.

During the pre-formulation studies, the drug sample was found to be yellowish powder, which was a characteristics order and slightly pungent bitter. Drugs are soluble in an organic solvent. The standard curve of Curcumin was prepared at UV absorption maxima in ethanol: phosphate buffer (pH 7.4) is obtained at 428 nm. The regression coefficient derived ($R^2=0.9998$) from the calibration curve clearly shows that the drug estimation method adhered to Beer Lambert's Law in the concentration range of 1–10 $\mu\text{g/ml}$ with good accuracy. For another UV spectroscopy determination, a standard solution of curcumin was prepared for PBS pH 5.6 and was scanned using the spectrum mode, and the overlaid spectra were captured within the 400–800 nm UV visible range. The drug's overlain spectra showed that the wavelength at which maximum absorption (λ max) occurred was 426 nm. The linearity was found under 1-10 $\mu\text{g/ml}$ and the Correlation coefficient (R^2) was 0.9991 estimated. The figure shows that the drug sample's FTIR interpretation was compared to the reference IR spectra. All the noticeable peaks of the functional group in the drug sample were visible in the drug's obtained infrared spectrum. Curcumin's solubility in a range of aqueous and non-aqueous solvents was assessed. It was discovered that the medication was insoluble in water, easily solubility in propylene glycol, solubility in tween 80 and span 80, and highly soluble in methanol and ethanol. The partition coefficient is a measure of a drug's lipophilicity or hydrophilicity and an indicator of its capacity to pass through biological membranes. The partition coefficient value of Curcumin was found to be 3.21 which is an indication that the drug is lipophilic. The most widely used technique for thermal analysis for characterizing and determining the melting point of solid crystalline. Curcumin was found to have a melting point of 183°C.

Following optimization, the best formulation was chosen to be characterized based on several factors. The formulation's thick liquid yellow translucent look was determined by observing its organoleptic qualities. Using a Brookfield viscometer, the viscosity was measured; at spindle size 63, the result was 98.33 cps. A digital pH meter was used to measure the pH of the optimized F6, which came out to be 5.6. Curcumin NE was made with a drug loading concentration of 15 mg/mL, and its droplet size distribution (83.08 nm with PDI 27.8) and zeta potential (–0.5 mV) were described. An in vitro drug release study was carried out using a Franz diffusion cell. The cumulative percentage drug release of prepared Curcumin NE F1, F2, F3, F4, F5, F6, F7, F8, F9, and F10 was determined. Formulation F6 released 95% drug in 10 hours. Based on the above results batch, F6 was selected as the optimized batch among all formulations. Results of release studies demonstrated that NE can retard the release of Curcumin. Five kinetic models, including the zero, first, Higuchi, and Korsmeyer–Peppas equations, were used to evaluate and depict the data. The kinetic constant and goodness of fit for the kinetic models are listed in the table using the correlation coefficient, or r^2 . According to the findings, the Korsmeyer–Peppas and Zero-order models were able to effectively match the data. For curcumin NE, the model has the highest correlation coefficient (>0.9). After being properly sealed in a rubber-stoppered glass vial, the optimized formulation was put through real-time stability testing and accelerated stability testing for three months in storage conditions. The formulation sample was examined every seven days, and the monthly average of the findings was recorded. The formulation property does not significantly change after storage, according to the data. Three months were spent testing the formulation's stability. In the attributes stated, minor but acceptable alterations were noted.

CONCLUSION:

Using a high-energy approach (ultra-sonication), we developed curcumin NE in a simple and reproducible manner that could be scaled up for commercial production. Curcumin in the form of NE was a boosting nanomedicine to improve bioavailability with good physical stability and an acceptable release profile, according to our physicochemical investigation.

According to the findings of the safety investigation, curcumin NE was a safe carrier for use as a cutting-edge treatment for vitiligo.

FUTURE PROSPECTIVE - After the characterization of all formulations, found the Curcumin NE F6 formulation is complete with all requirements for further pharmacological study, which could be better for anti-vitiligo, and further *in vivo* specialized study is required for the developed system. A preclinical study in humans is necessary to establish the developed system as a better delivery method for vitiligo therapy.

AUTHOR CONTRIBUTION - Priyanjna Bhagat- Writing original manuscript; Princy Kashyap- editing original manuscript; Rajesh Choudhary- data collection and curation; Ravi Shankar Pandey- editing and analyzing the manuscript; Manoj Kumar- supervision.

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DECLARATION STATEMENT - The authors show no conflicts of interest.

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