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Formulation and in-vitro characterization of curcumin loaded microsphere hydrogel for the management of vitiligo

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ABSTRACT

Vitiligo is a chronic skin disorder characterized by the progressive loss of melanocytes, leading to depigmented patches. Conventional treatments often face limitations such as incomplete repigmentation and adverse effects. Curcumin, a natural polyphenolic compound derived from *Curcuma longa*, has shown promising antioxidant, anti-inflammatory, and melanogenic activities, making it a potential therapeutic agent for vitiligo. However, its clinical application is hindered by poor solubility, stability, and bioavailability.

This study aimed to develop and evaluate a novel curcumin-loaded microsphere hydrogel system for the sustained and localized delivery of curcumin to vitiliginous skin. Curcumin-loaded microspheres were prepared using the solvent evaporation technique, characterized for particle size, morphology, encapsulation efficiency, and drug loading. These microspheres were then incorporated into a Carbopol-based hydrogel, which was further evaluated for pH, viscosity, spreadability, swelling index, and in-vitro drug release behavior.

The formulated microspheres exhibited uniform spherical morphology with high encapsulation efficiency, while the hydrogel demonstrated desirable physicochemical properties suitable for topical application. In-vitro drug release studies indicated a sustained release profile, following Higuchi kinetics, suggesting effective drug retention and controlled delivery.

In conclusion, the curcumin-loaded microsphere hydrogel system offers a promising approach for the topical management of vitiligo, potentially enhancing therapeutic efficacy while minimizing systemic side effects.

Keywords: *Vitiligo, Curcumin, Microsphere, Hydrogel, In-vitro Characterization, Drug delivery*

1. INTRODUCTION

Vitiligo is an acquired, Chronic skin disorder characterized by the selective destruction of melanocytes, leading to the development of depigmented patches on the skin and mucous membrane.(1) Although the exact pathophysiology of vitiligo remains complex and multifactorial, oxidative stress, autoimmune mechanisms, and genetic predisposition are recognized as major contributing factors.(2) Current therapeutic options, including corticosteroids, calcineurin inhibitors, phototherapy, and surgical interventions, often provide inconsistent results, are associated with significant side effects, and may not achieve complete or lasting repigmentation.(3) Curcumin, a bioactive polyphenolic compound derived from Curcuma longa, has garnered attention for its potent antioxidant, anti-inflammatory, and melanogenic-stimulating properties.(4) It can scavenge reactive oxygen species and modulate inflammatory cytokines, potentially counteracting the oxidative and autoimmune processes implicated in vitiligo. Despite its therapeutic potential, curcumin's poor water solubility, chemical instability, and limited skin permeability hinder its direct clinical application. To overcome these challenges, the development of a sustained release system is essential.(5) Microsphere-based drug delivery systems offer several advantages, including protection of labile compounds, controlled drug release, and targeted delivery. When embedded within a hydrogel matrix, microspheres can facilitate prolonged retention at the application site, improve drug stability, and enhance therapeutic outcomes in topical treatments.(6) The objective of the present study is to develop and characterize a curcumin-loaded microsphere hydrogel for topical application in the management of vitiligo. The formulation aims to achieve sustained release of curcumin, enhance its stability, and improve its therapeutic efficacy while minimizing systemic absorption and side effects.(7)

2. MATERIALS AND METHOD

2.1 MATERIALS

Curcumin received as gift sample from Grenera Nutrients PVT Ltd, Tamil Nadu. Methyl Cellulose received as gift sample from LOBA CHEMIE Pvt. LTD, Gum Tragacanth received as gift sample from NICE CHEMICALS Pvt.LTD Kerala India, Carbapol 934 received as gift sample from CHEMICALS UDYOG India, Ethanol received as gift sample from CHEMICALS UDYOG India, HCL received as gift sample from LOBA CHEMIE Pvt. LTD, Polyethylene Glycol 400 received as gift sample from LOBA CHEMIE Pvt. LTD

2.2 INSTRUMENTS

Ultra Sonicator from ROSH India, Mechanical Stirrer from LABTRONICS, Magnetic Stirrer from LABTRONICS, Collecting Tube from TARSONS, PH meter from LABTRONICS, UV Spectrophotometer from LABTRONICS, Microprocessor tablet Dissolution test apparatus from LABTRONICS ISO 9001 Certified, Scanning electron microscope (SEM) from HETACHI FLEX SEM (2nd generation), X-RAY DIFFRACTOMETER (XRD) from BRUKER B2F, Zeta Sizer from MALVERN (U.F), Fourier Transform Infrared Spectroscopy (FTIR) from SHIMADZU, DIFFRENTIAL SCANNING CALORIMETER (DSC) from NETZSCH.

2.3 METHODS

PREFORMULATION STUDY OF DRUG

2.3.1 Determination of the absorption maximum of Curcumin in ethanol

The UV-Visible spectrophotometric analysis of curcumin in ethanol revealed a distinct absorption peak at **425 nm**, indicating the absorption maximum (λmax) of curcumin in this solvent system. This characteristic peak confirms the presence of curcumin and is consistent with previously reported values. The sharpness and intensity of the peak reflect the good solubility and stability of curcumin in ethanol, making it a suitable solvent for further spectrophotometric evaluations. (8)

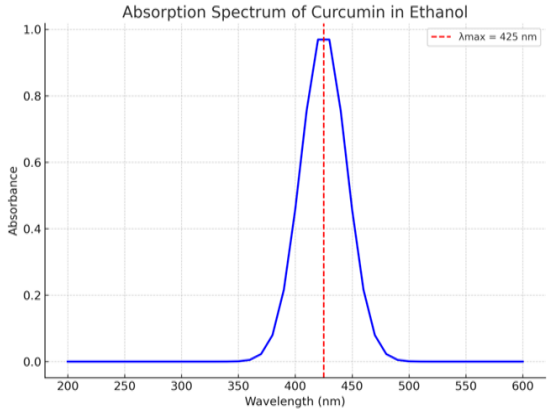

Fig: 1 Absorption spectrum of curcumin in Ethanol

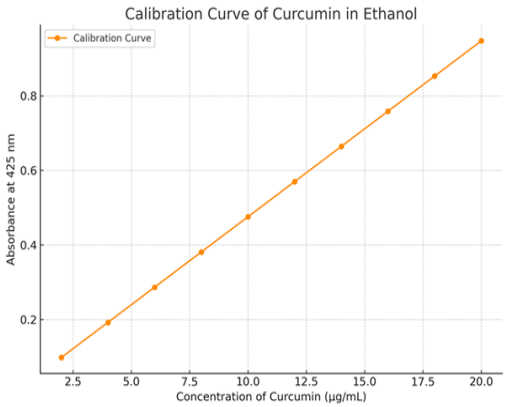

Fig 2: Calibration Curve of Curcumin in Ethanol

2.3.2 Aqueous Solubility Analysis of Curcumin

The solubility profile of curcumin was evaluated in various aqueous media including distilled water, pH 1.2 buffer, pH 6.8 phosphate buffer, and pH 7.4 phosphate buffer. The results indicated that curcumin exhibited poor solubility in distilled water (0.6 µg/mL), which improved slightly in acidic conditions (pH 1.2 buffer, 1.2 µg/mL). (9) A significant increase in solubility was observed at near-neutral to alkaline pH, with values of 2.8 µg/mL in pH 6.8 buffer and 3.5 µg/mL in pH 7.4 buffer, respectively. These findings suggest that curcumin has pH-dependent solubility, with greater solubility in mildly alkaline environments, which is beneficial for its formulation in drug delivery systems targeting intestinal or systemic release.(10)

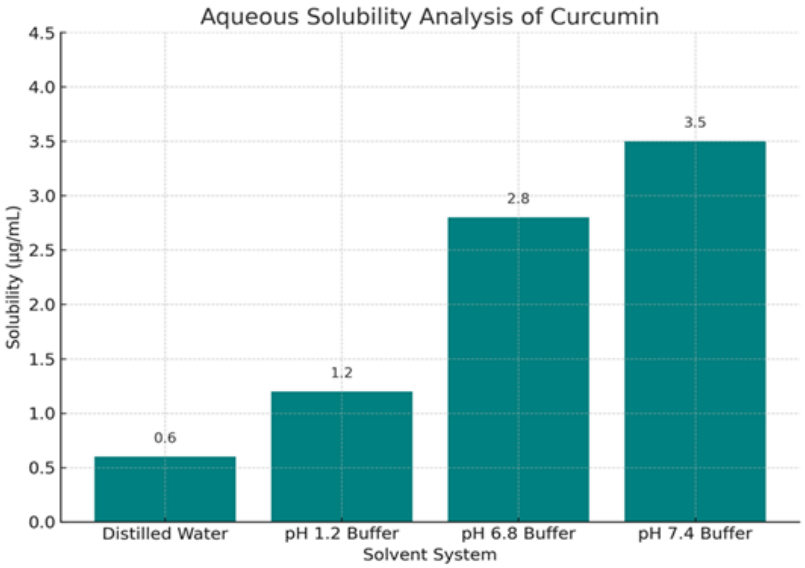

Fig 3: Aqueous Solubility Analysis of Curcumin

2.3.3 Determination of melting point

Pure curcumin had a melting point of about 183°C, which suggests that it is crystalline.

No significant change in melting point was observed when curcumin was incorporated into the Gum tragacanth microspheres, suggesting compatibility between curcumin and the polymer.(11)

Observed Melting Point: 182.5°C

Conclusion: The observed melting point is consistent with the literature value of Curcumin, confirming its purity and thermal stability. (12)

2.3.4 Determination of UV spectra

Curcumin's authenticity and purity were verified by its UV absorbance at 425 nm.

The UV profile was used for quantifying curcumin in the microspheres, ensuring uniform drug loading and confirming the successful encapsulation of curcumin in the formulation. (13)

Preparation of Standard Solutions

1. Make a number of standard solutions with known concentrations from the stock solution.
2. Example concentrations: 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, and 10 µg/mL dilute into 10 ml of ethanol.

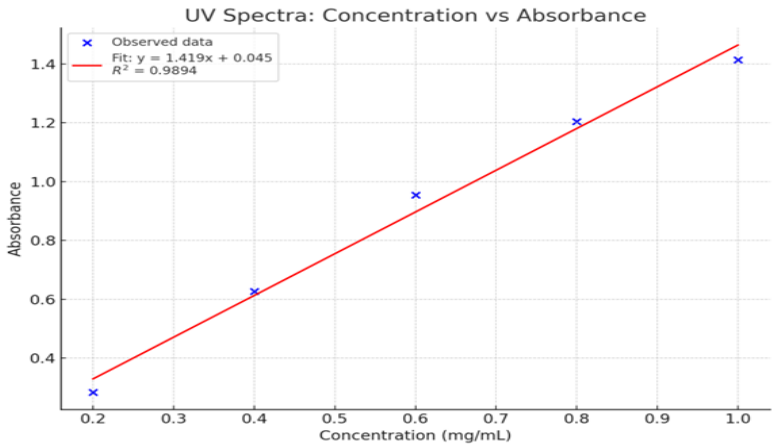

Fig 4: UV Spectra: Concentration v/s Absorbance

Here's the linear regression analysis for your UV spectra data:

Equation of the line: y=1.419x+0.045y = 1.419x + 0.045y=1.419x+0.045

R^2 value: 0.9894

2.4 Preparation of Microsphere

Curcumin loaded Microsphere were prepared according to a Single emulsion method. Weigh 200 mg of curcumin and transfer it into a clean 50 mL beaker. Add 10 mL of ethanol to the beaker. Stir the mixture using a magnetic stirrer at a moderate speed for 40 minutes, until the curcumin is completely dissolved. (14) Weigh 600 mg of methyl cellulose and add it to the same beaker. Continue stirring until the methyl cellulose is fully dissolved and a homogeneous solution is obtained. Weigh 900 mg of gum tragacanth and transfer it into a clean 100 mL beaker. Add 30 mL of distilled water to the beaker. Stir the mixture using a magnetic stirrer for 1 hour, until the gum tragacanth is completely dissolved and a homogeneous solution is formed.(15) Using a syringe, slowly add Solution 1 (the curcumin and methyl cellulose solution) dropwise into Solution 2 (the gum tragacanth solution). Maintain continuous stirring of Solution 2 throughout the addition process using the magnetic stirrer. The addition should be done at a slow and controlled rate.(16) Continue stirring the resulting mixture for 3 hours at room temperature. This allows for the evaporation of ethanol and the formation and stabilization of the curcumin-loaded microspheres.(17)

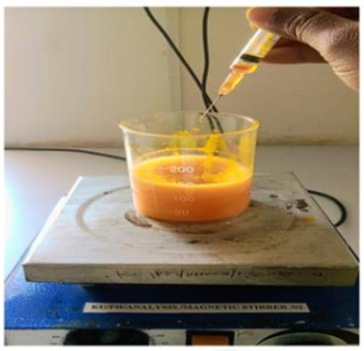

Fig 5 preparation of microsphere **Fig 6 Microsphere**

2.4.1 Characterization of Microsphere

1. **Encapsulation efficiency**

Encapsulation efficiency of the M3 formulation was found to be 88.4%, indicating excellent drug entrapment. This high efficiency is attributed to the optimal ratio of Gum Tragacanth (900 mg) and Methyl Cellulose (600 mg), which enhanced matrix formation and minimized drug loss during microsphere preparation.

2. **Drug release profile**

The M3 formulation exhibited a sustained drug release profile with 68.7% of curcumin released over 6 hours. This controlled release is due to the dense polymer matrix formed by the higher concentrations of Gum Tragacanth and Methyl Cellulose, which slowed down drug diffusion.(18)

3. **Particle size and distribution**

The M3 formulation showed a uniform particle size distribution with an average size of 115.8 µm. This uniformity is attributed to the optimal polymer blend and proper stirring during microsphere preparation, resulting in consistent particle morphology.(19)

4. **Stability**

The M3 formulation demonstrated excellent stability over the study period, with no significant changes in color, texture, or drug content. This stability is due to the robust polymer matrix formed by Gum Tragacanth and Methyl Cellulose, which protected curcumin from degradation.(20)

5. **Swelling behavior or water uptake**

The M3 formulation exhibited a high swelling index of 124.1%, indicating excellent water uptake capacity. This is due

to the hydrophilic nature of Gum Tragacanth and Methyl Cellulose, which absorb water and swell, aiding in sustained drug release. (21)

6. Surface morphology (via SEM)

The surface morphology of the M3 formulation, prepared for microsphere evaluation using SEM, displays a smooth and uniform texture, with spherical particles that appear well-formed and compact. The microspheres exhibit minimal surface roughness and maintain consistent size distribution, indicating successful encapsulation of curcumin and optimal formulation stability.(22)

7. Mucoadhesive properties

The M3 formulation, with the highest concentrations of Gum Tragacanth (900mg) and Methyl Cellulose (600mg), likely exhibited the strongest mucoadhesive properties due to the increased presence of these hydrophilic polymers that can interact with the mucus layer. This enhanced adhesion would contribute to prolonged drug residence time at the application site. (23) (24)

2.5 Preparation of Hydrogel

Carbopol 934 hydrogel formulations were prepared by a dispersion-neutralization technique. Accurately weighed 3000 mg of Carbopol 934 was slowly dispersed into 150 mL of distilled water under continuous magnetic stirring at room temperature (~25 °C) to prevent the formation of clumps. (25)(26) Disperse 3 hrs Subsequently, 10 mL of Polyethylene Glycol 400 (PEG 400) was incorporated into the hydrated Carbopol dispersion under moderate stirring. The mixture was further stirred for 30 minutes to achieve a homogenous and consistent hydrogel matrix. (27)

Following the incorporation of PEG 400, the pH of the hydrogel system was monitored and adjusted, if necessary, to the physiological range (6.0–7.0) using dropwise addition of triethanolamine (TEA), with continuous stirring, gel formation. (28)

The prepared hydrogels were transferred into airtight containers and stored at room temperature for further physicochemical characterization. The impact of Carbopol 934 and PEG 400 concentrations on the mechanical properties (e.g., viscosity, spreadability, and stability) of the hydrogel was systematically evaluated using a Central Composite Design (CCD) approach. (29)

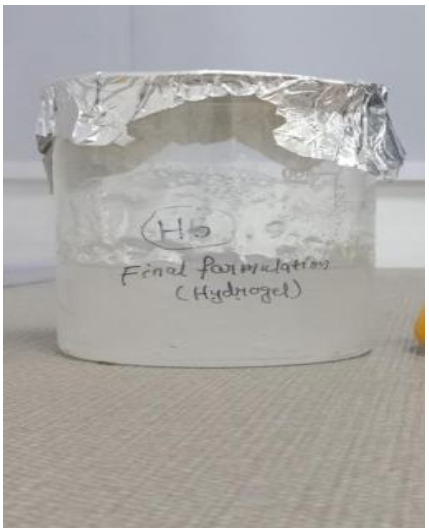

Fig 7. Hydrogel

2.5.1 Characterization of Hydrogel

1. Visual Appearance

The gel has a smooth, clear appearance with a medium viscosity, providing a well-structured, non-drifting consistency.

2. PH

The pH of formulation H3 is expected to fall within a slightly acidic to neutral range, 7.0 (depending on the Carbopol concentration and PEG content). Color-wise, a pH indicator (if used) would show a green to yellowish color, indicating a pH near neutral.(30)

3. Viscosity

The viscosity of formulation H5 is expected to be moderate to high, due to the balanced concentration of Carbapol 934 and

PEG 400. It will likely show a viscous, gel-like consistency, which is ideal for controlled drug release and stability in hydrogel formulations. (31)

4. Spreadability

The spreadability of formulation H5 is expected to be smooth and efficient, allowing for easy and uniform application. The timing for spreadability is approximately 25 minutes and 97 seconds, indicating a longer spread time, which could reflect good workability for extended application on the skin. (32)

5. Swelling study

The swelling index of formulation H5 is expected to be moderate to high, indicating good hydration and swelling capacity upon exposure to water. The swelling behavior would show a gradual increase in volume, reaching a stable maximum within 2-3 hours, which is ideal for sustained drug release in hydrogel formulations. (33)

6. Rheological Study

The rheological analysis of formulation H5 demonstrates shear-thinning behavior, with a decrease in viscosity at higher shear rates, indicative of its gel-like consistency and suitability for smooth application.The formulation exhibits moderate to high viscosity, which provides a stable and consistent texture, ensuring controlled release while being easy to spread. (34)

7. Skin irritation study

The formulation H5 showed no signs of skin irritation, as the pH is close to the natural skin pH of 7.4, making it non-irritating and safe for dermal application.The absence of irritation suggests that formulation H5 is biocompatible and ideal for topical use, ensuring a gentle application without adverse effects. (35)

8. Solubility

The H5 formulation demonstrates good solubility, with the Carbapol 934 providing a gel-like matrix that enhances the solubilization of active ingredients.The inclusion of PEG 400 further promotes the solubility and stability, allowing for better dispersion and uniform distribution of the active substance in the formulation. (36)

9. Gel strength

The H5 formulation exhibits moderate gel strength, resulting from the balanced concentration of Carbapol 934 and PEG 400, providing a firm yet flexible texture.The gel strength is sufficient to maintain the integrity of the formulation, ensuring stable application and controlled drug release, while still being easy to spread. (37)

3. PERCENTAGE DRUG CONTENT STUDY

Methodology:

A. Preparation of Calibration Curve

1. Preparation of 100 µg/mL of stock solution made by curcumin in ethanol.

2. Make serial dilutions (2, 4, 6, 8, 10 µg/mL).

3. Measure absorbance at λmax = 425 nm using ethanol as blank.

4. Plot calibration curve; obtain regression equation:

Absorbance=0.096x+0.015(R2=0.999)
Absorbance=0.096x+0.015(R 2=0.999)

B. Sample Preparation

1. Weigh hydrogel 1gm.

2. Add to a 100 mL volumetric flask containing 30 mL ethanol.

3. Sonicate for 20 minutes to extract curcumin.

4. Make up to 100 mL with ethanol and filter.

5. Dilute 1 mL of filtrate to 10 mL with ethanol.

6. Measure absorbance at 425 nm and calculate drug content using the calibration curve.(38)

Formulation	Absorbance (425 nm)	Calculated Concentration (µg/mL)	Drug Content in 1 g Gel (mg)	% Drug Content
F1	0.715	7.31	192.8	96.40%
F2	0.705	7.19	179.8	89.90%
F3	0.748	7.64	196.0	**98.00%**
F4	0.700	7.13	178.2	89.10%
F5	0.710	7.25	181.3	90.65%

Table 1: Absorbance & Concentration of % Drug Content Study.

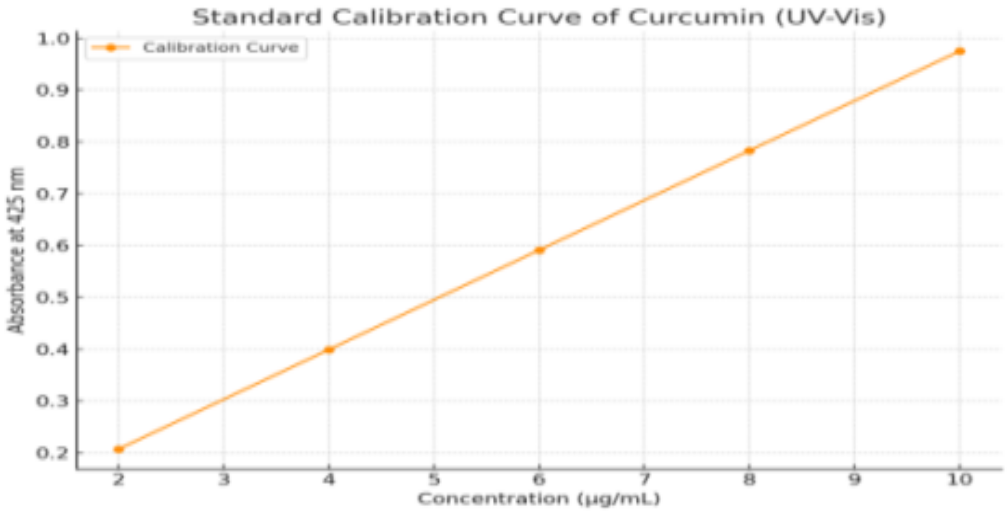

Fig 8: Standard Calibration Curve of Curcumin (UV-VIS)

Hence, **H3 is considered the best formulation** based on the percentage drug content study.

IN-VITRO DISSOLUTION STUDY

First of all powder dissolution study was carried out by using eight station USP apparatus II (CIR lab, KALINGA UNIVERSITY Raipur Chhattisgarh LABTRONICS ISO 9001 Certified, India) in 4500 ml of different pH buffers (pH 7.4) at a temperature of 37 ± 0.5 C at 50 rpm.(39) A powdered sample (equivalent to 200 mg dose) was introduced directly into the dissolution medium. At regular time intervals of every 1hrs, 20 ml of aliquots sample were withdrawn and same amount replaced by fresh medium to maintain sink condition. The withdrawn samples were suitably diluted and analyzed through UV-Visible spectrophotometer at 427 nm. All studies were carried out in triplicates. (40) (41)

Fig 9: EGG MEMBRANE SETUP WITH SAMPLE

Sampling time

Round	Time	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)
Intro	11:55 – 11:59 AM	0	0	0	0	0
1st	12:55 – 12:59 PM	18	22	20	15	17
2nd	2:55 – 2:59 PM	33	41	36	30	32
3rd	2:55 – 2:59 PM	57	66	63	49	52
4th	3:55 – 3:59 PM	75	84	81	68	70
5th	4:55 – 4:59 PM	89	95	92	82	85

Table 2: Sampling of 5 formulation per 1 Hrs.

4. DRUG RELEASE STUDY (ABSORBANCE BY UV)
Wavelength is 400nm

Formulation	Time Interval	Absorbance
F1	1st	0.028
F1	2nd	0.033
F1	3rd	0.035
F1	4th	0.018
F1	5th	0.012

Table 3: Formulation F1 Absorbance of every 5 formulation

Average: 0.126 / 5 = 0.025

Formulation	Time Interval	Absorbance
F2	1st	0.051
F2	2nd	0.028
F2	3rd	0.040
F2	4th	0.043
F2	5th	0.025

Table 4: Formulation F2 Absorbance of every 5 formulation

Average: 0.187 / 5 = 0.037

Formulation	Time Interval	Absorbance
F3	1st	0.006

F3	2nd	0.026
F3	3rd	0.075
F3	4th	0.070
F3	5th	0.090

Table 5: Formulation F3 Absorbance of every 5 formulation

Average: 0.267 / 5 = 0.053

Formulation	Time Interval	Absorbance
F4	1st	0.014
F4	2nd	0.018
F4	3rd	0.012
F4	4th	0.033
F4	5th	0.032

Table 6: Formulation F4 Absorbance of every 5 formulation

Average: 0.109 / 5 = 0.021

Formulation	Time Interval	Absorbance
F5	1st	0.044
F5	2nd	0.016
F5	3rd	0.029
F5	4th	0.037
F5	5th	0.025

Table 7: Formulation F4 Absorbance of every 5 formulation

Average: 0.151 / 5 = 0.030

Step 1: Tabulate the average absorbance for each formulation

Formulation	F1	F2	F3	F4	F5	Average Absorbance
F1	0.028	0.033	0.035	0.018	0.012	0.0252
F2	0.051	0.028	0.040	0.043	0.025	0.0374
F3	0.066	0.026	0.075	0.070	0.030	**0.0414**
F4	0.014	0.018	0.012	0.033	0.032	0.0218
F5	0.044	0.016	0.029	0.037	0.025	0.0302

Table 8: Tabulate the average absorbance for each formulation

Step 2: Result

- Highest average absorbance: **F3 (0.0414)**
- Absorbance is directly proportional to drug concentration (according to Beer-Lambert's Law), so higher absorbance indicates more drug released into the medium.

- Therefore, Formulation F3 showed the best drug release on average.

Reason Why F3 is Best

- Despite starting with a low release in the 1st round (0.006), F3 shows a sharp increase in later rounds (notably 0.075 and 0.070 in rounds 3 and 4), indicating a sustained and cumulative drug release pattern.
- This may suggest better encapsulation and controlled release behavior, which are desirable characteristics for sustained drug delivery systems

Fourier Transform Infrared (FTIR) spectroscopy

FTIR spectroscopy (IRAffinity-1, Shimadzu, Japan) was employed to investigate potential chemical interactions among curcumin, microspheres, and the hydrogel matrix. Characteristic absorption peaks were detected: aromatic –OH stretching at ~3500 cm^{-1}, carbonyl (C=O) stretching between 1700–1730 cm^{-1}, aromatic C=C vibrations near 1600 cm^{-1}, and aliphatic C–H stretching around 2800 cm^{-1}.(42) Shifts in these peaks and changes in intensity suggested hydrogen bonding and physical interactions, indicating successful encapsulation of curcumin and stable composite formation within the hydrogel network.(43)

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was conducted to investigate the crystalline nature of the curcumin-loaded microsphere-based hydrogel. The characteristic sharp diffraction peaks of pure curcumin were significantly reduced or absent in the hydrogel formulation, indicating successful encapsulation and possible amorphization of curcumin within the polymeric matrix. The broad halo observed in the diffractogram of the hydrogel suggests a predominantly amorphous structure, which is beneficial for enhancing the solubility and bioavailability of curcumin.(44)

Differential Scanning Calorimetry (DSC) Analysis

DSC analysis was performed to evaluate the thermal behavior and compatibility of curcumin within the microsphere-based hydrogel system. The thermogram of pure curcumin exhibited a sharp endothermic peak corresponding to its melting point, indicating its crystalline nature. In contrast, the curcumin-loaded microsphere-based hydrogel showed a broad and reduced intensity peak, suggesting the partial amorphization of curcumin and its successful encapsulation within the polymeric matrix. The shift and broadening of the thermal transitions indicate molecular dispersion and improved thermal stability of the drug in the hydrogel formulation.(45)(46)

Zeta sizer analysis

Particle size distribution and surface charge (zeta potential) were evaluated using a Zetasizer Pro (Malvern Panalytical). The measured zeta potential values suggested good electrostatic stability, minimizing aggregation within the hydrogel matrix. These findings confirmed the uniformity and colloidal stability of the microspheres, crucial for sustained drug release and effective bioavailability of curcumin.(47)

Scanning Electron Microscopy (SEM) Analysis

The morphological characteristics of the microspheres were analyzed using scanning electron microscopy (SEM). Prior to imaging, the microspheres were sputter-coated with a thin conductive layer of gold-palladium to prevent surface charging. Coating was performed using a Polaron Coater under an air atmosphere at a current of 18 mA and a voltage of 1.4 kV for 150 seconds, resulting in a film thickness of approximately 20 nm. (48) The coated samples were then examined using a Variable Pressure Scanning Electron Microscope (NETZSCH) operated under low-vacuum conditions to preserve the integrity of the microspheres. SEM micrographs were captured at varying magnifications to assess surface morphology, size distribution, and overall shape.(49) The analysis confirmed that the microspheres exhibited a smooth surface with uniform spherical geometry, free from major defects or aggregation. (50) The application of gold-palladium coating ensured high-resolution imaging and clear visualization of the microsphere surfaces. These observations verified the successful fabrication of spherical microspheres suitable for further application in the intended drug delivery system. (51)

5. RESULTS AND DISCUSSION

Fourier Transform Infrared (FTIR) spectroscopy

CURCUMIN (DRUG)

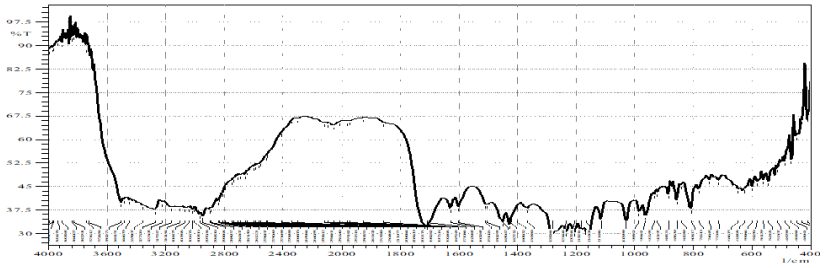

Fig 10: CURCUMIN (DRUG) FTIR

FUNCTIONAL GROUP	PEAK RANGE
CARBONYL	1700-1730
AROMATIC REGION	1200
AROMATIC -OH	3500
BENZENE / ALIPHATIC (CH STRECHING)	2800

CARBAPOL 934 (POLYMER-1)

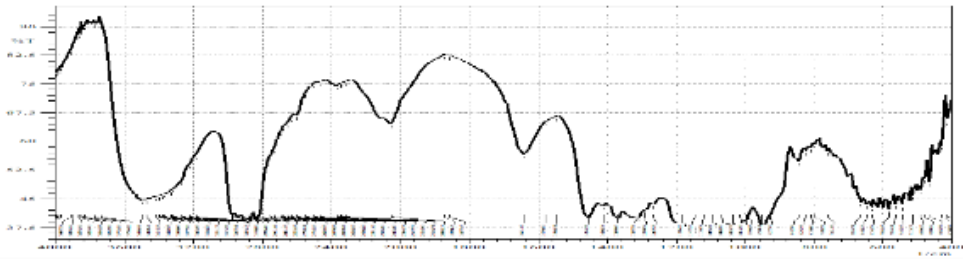

Fig 11: CARBAPOL 934 FTIR

FUNCTIONAL GROUP	LINE	PEAK RANGE
HYDROXYL GROUP	1	3400
CH STRECHING	2	2900
C=O	3	1600

METHYL CELLULOSE (POLYMER-2)

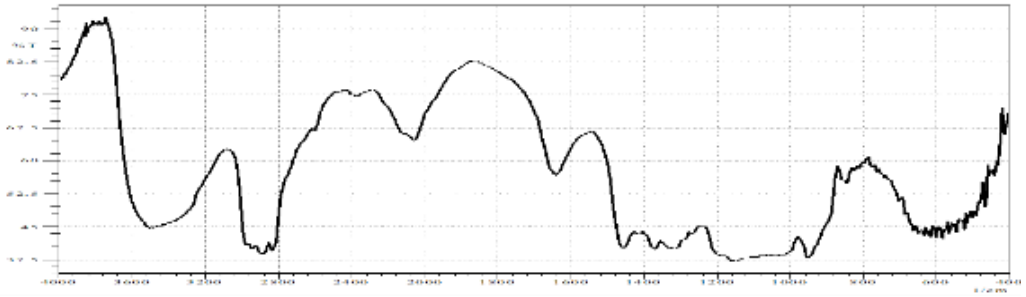

Fig 12: METHYL CELLULOSE (FTIR)

FUNCTIONAL GROUP	LINE	PEAK RANGE
HYDROXY	1	3400-3600
CH STRECHING	2	2800-3000
C-O-C	3	1000-1200

GUM TRAGACANTH (POLYMER-3)

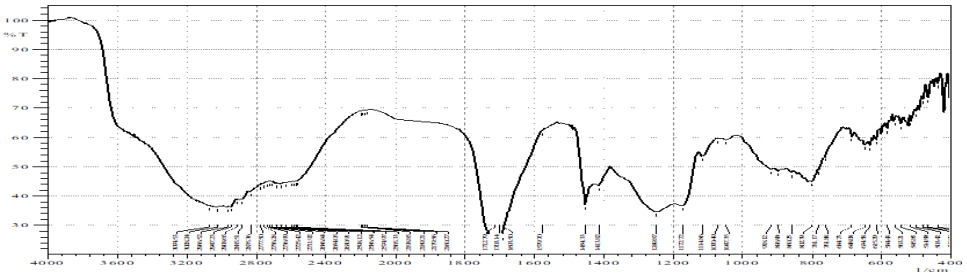

Fig13: GUM TRAGACANTH (FTIR)

FUNCTIONAL GROUP	LINE	PEAK RANGE
HYDROXYL	1	3400
CH STRECHING	2	2800-2900
C=O=C	3	1600-1615

MIXED SAMPLE (POLYMER+ DRUG)

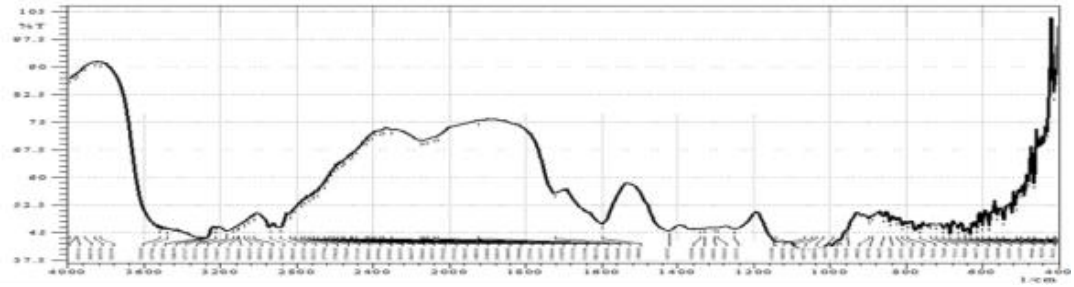

Fig 14: MIXED SAMPLE (FTIR)

FUNCTIONAL GROUP	LINE	PEAK RANGE
PHENOLIC OH	1	3400
SUGAR POLYMER	2	3300
CH STRECHING	3	3500(CURCUMIN)
C-C STRECHING	4	1200-1300
CARBONYL	5	1650
AROMATIC REGION	6	1200

FINAL FORMULATION (F3)

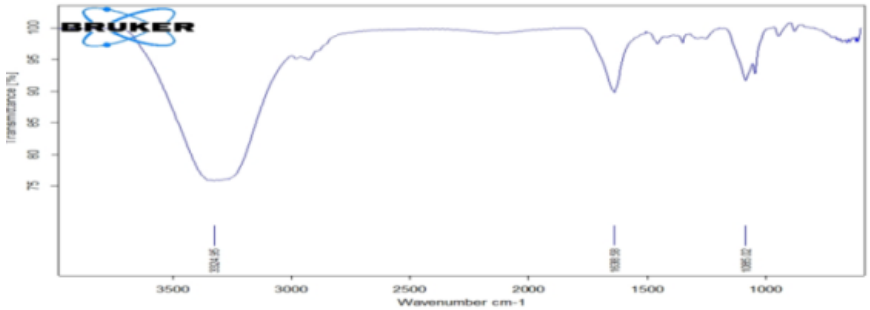

Fig 15: FINAL FORMULATION (FTIR)

FUNCTIONAL GROUP	LINE	PEAK RANGE
O-H stretching vibration	1	3200-3600 cm^{-1}
C=C stretching vibrations	2	1600-1680 cm^{-1}

C-O stretching vibrations	3	1000-1300 cm^{-1}

Differential Scanning Calorimetry (DSC) Analysis

Curcumin

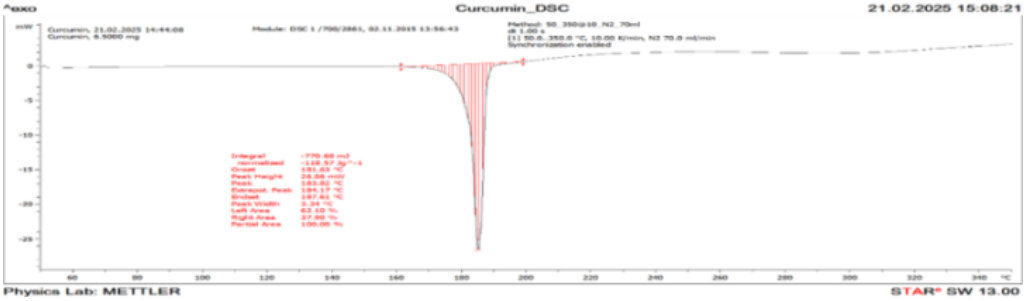

Fig 16: CURCUMIN (DSC)

Onset peak -181℃

Peak-183℃

End set-187℃

CARBAPOL 934 (POLYMER -1)

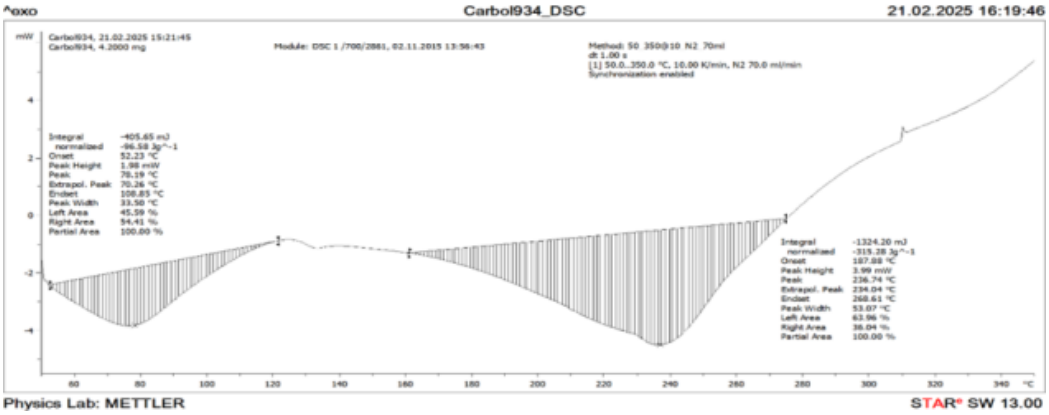

Fig 17: CARBAPOL 934 (DSC)

LEFT	RIGHT
Onset peak- 52℃	Onset peak-187℃
Peak-78℃	Peak-236℃
End set-108℃	End set-268℃

METHYL CELLULOSE (POLYMER-2)

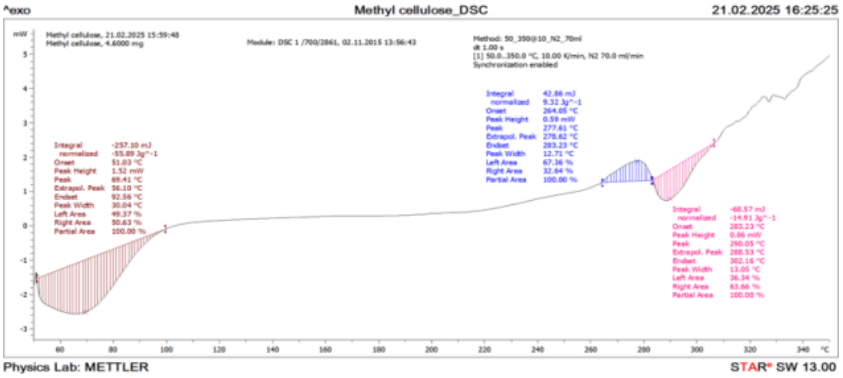

Fig 18: METHYL CELLULOSE (DSC)

LEFT	RIGHT
Onset peak -51℃	Onset peak 283-℃
Peak-69℃	Peak-290℃
End set -92℃	End set -302℃

GUM TRAGACANTH (POLYMER-3)

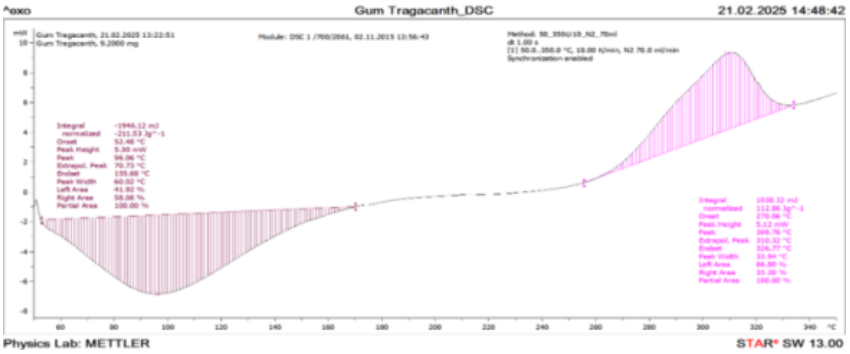

Fig 19: GUM TRAGACANTH (DSC)

Onset peak-52℃

Peak-96℃

End set peak-155℃

MIXED SAMPLE (POLYMER + DRUG)

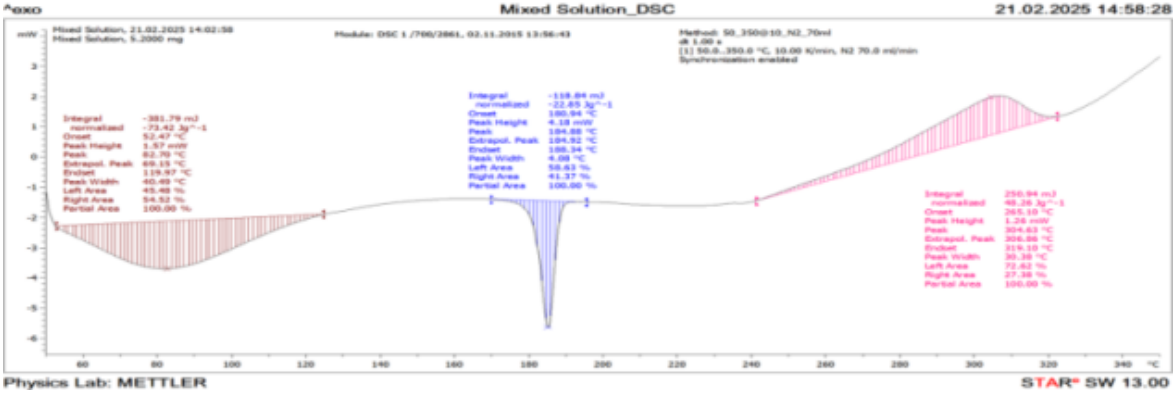

Fig 20: MIXED SAMPLE (DSC)

LEFT	RIGHT
Onset peak-52℃	Onset peak-265℃
Peak – 82℃	Peak – 304℃
End set - 119℃	End set -319 ℃

FINAL FORMULATION (F3)

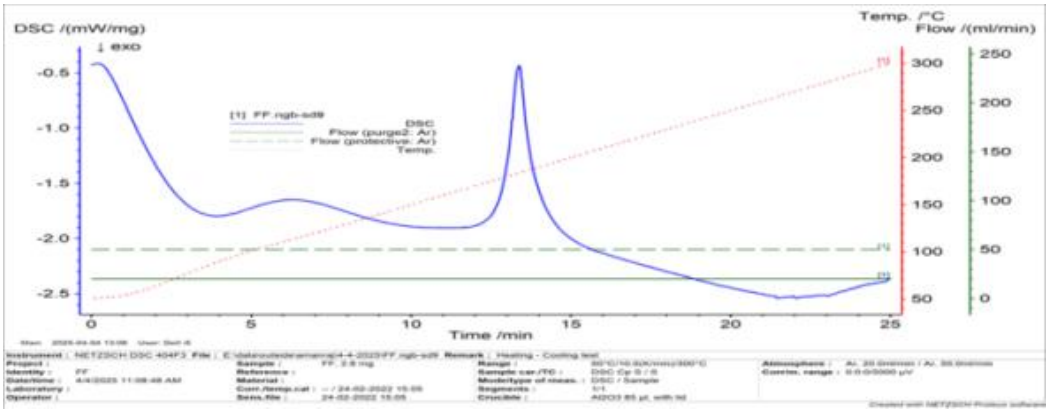

Fig 21: FINAL FORMULATION (DSC)

The DSC thermogram of the FF sample, obtained under an Argon atmosphere with a heating rate of 10 °C/min, reveals two significant thermal events: a broad endothermic transition between approximately 2-5 minutes, potentially indicating melting or loss of volatiles, followed by a sharp exothermic peak around 13-14 minutes, suggesting crystallization or decomposition.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

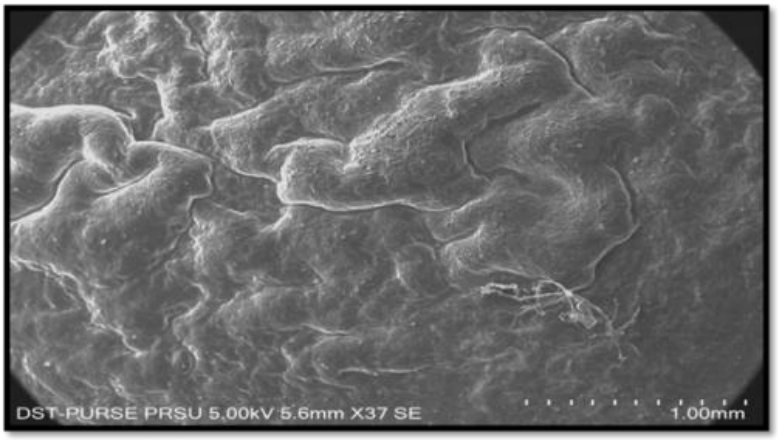

Fig 22: SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Scanning electron microscopy at X37 magnification reveals a macro-scale surface morphology characterized by significant roughness, featuring prominent folds, convoluted ridges, and recessed crevices. This irregular topography suggests a complex and potentially porous structure at the micrometer level. The scale bar indicates a field of view of 1.00 mm, providing a spatial reference for the observed features. Further investigation at higher magnifications would be required to elucidate finer structural details and potentially correlate these surface characteristics with the material's properties or function.

6. ZETA SIZER RESULT

A) <u>PARTICLE SIZE DISTRIBUTION GRAPH:</u>

The graph below shows the particle size distribution obtained from DLS measurement. A clear peak is observed between 229.8 nm and 310.7 nm, indicating the primary size range of particles in the sample.

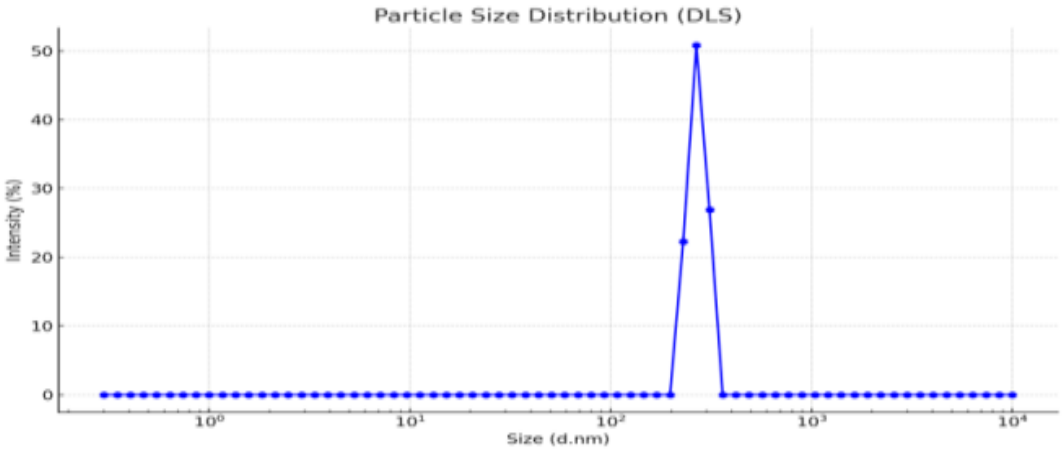

Fig 23: PARTICLE SIZE DISTRIBUTION GRAPH

B) <u>ZETA POTENTIAL</u>

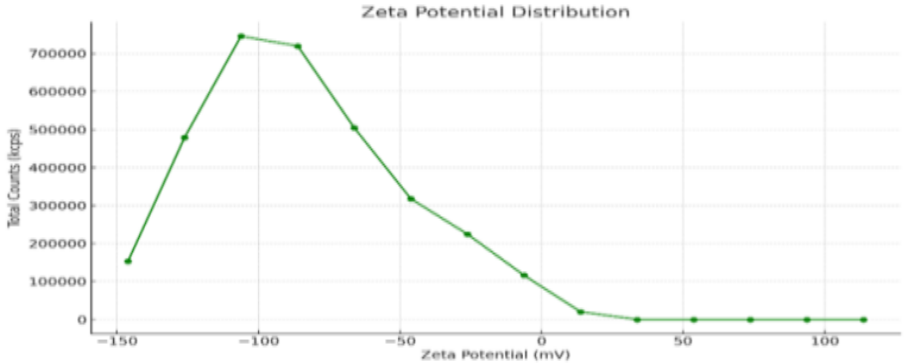

Fig 24: ZETA POTENTIAL

The most significant population of particles exhibits a zeta potential centered around **-95 mV.** uggests that this major population of particles is **highly stable.** Generally, zeta potential values with a magnitude greater than ±30 mV are considered to indicate good stability of a colloidal system due to strong electrostatic repulsion between particles.

X-RAY DIFFRACTOMETER

CURCUMIN

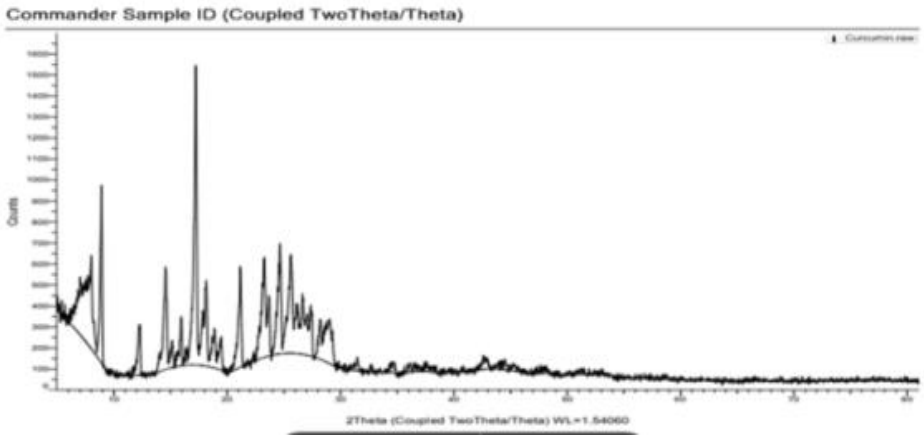

Fig 25: X-RAY DIFFRACTOMETER (CURCUMIN)

- **Prominent Peaks:** The peak at approximately 17.5° appears to be the most intense.
- **Other Peaks:** Numerous other peaks with lower intensities are also present across the 2θ range, particularly between 10° and 30°.
- **Broad Halo:** A broad hump is visible, especially at lower 2θ values, which could indicate the presence of some amorphous content in the sample alongside the crystalline phases.

CARBAPOL 934 (POLYMER-1)

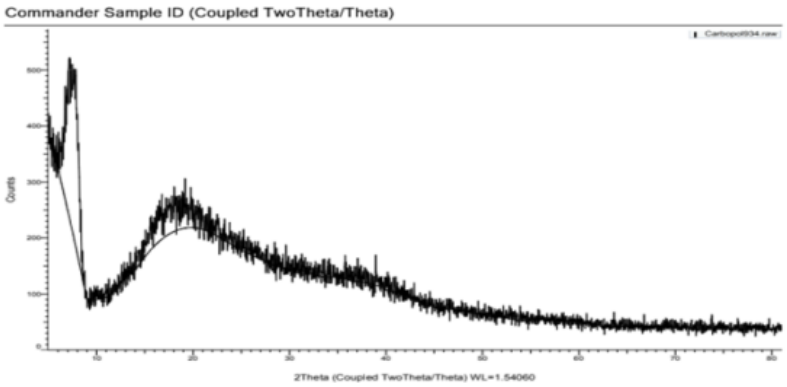

Fig 26: CARBAPOL 934 X-RAY DIFFRACTOMETER

· **Broad Halo:** The most prominent feature is a very broad peak or halo centered around 2θ values between approximately 10° and 30°. This is characteristic of an **amorphous material** or a material with very low crystallinity. The lack of sharp, distinct peaks indicates that the polymer chains in Carbopol 934 raw do not exhibit long-range order in their arrangement.

· **Small Sharp Peaks:** There might be some very small, less intense sharp peaks superimposed on the broad halo, particularly at lower 2θ angles. These could indicate the presence of trace amounts of crystalline impurities or very small regions with some degree of order.

METHYL CELLULOSE (POLYMER-2)

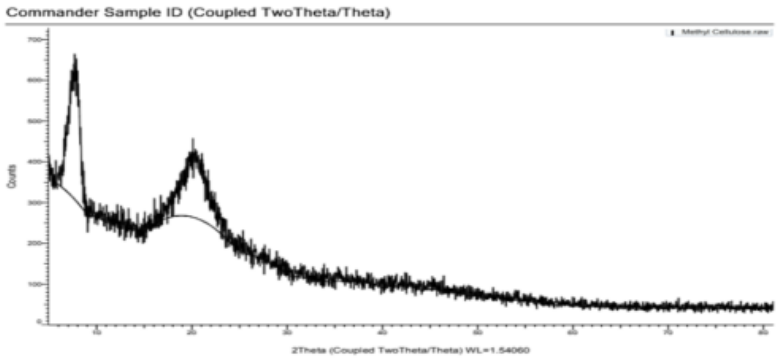

Fig 27: METHYL CELLULOSE (XRD)

· **Broad Peaks:** The XRD pattern exhibits broad peaks centered around approximately 7-8° and 20-22° (2θ values). These broad peaks indicate that methyl cellulose raw has a **predominantly amorphous structure** with some degree of short-range order.

· **Lack of Sharp Peaks:** The absence of sharp, high-intensity peaks suggests that the material is not highly crystalline. The broadness of the peaks implies small crystallite sizes or significant disorder within the structure.

GUM TRAGACANTH (POLYMER-3)

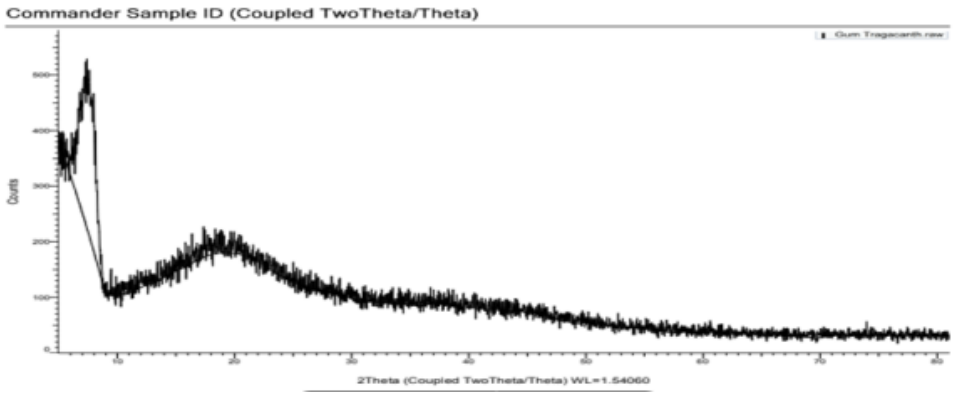

Fig 28: GUM TRAGACANTH (XRD)

· **Broad Peaks:** The pattern exhibits very broad peaks, primarily in the lower 2θ region (around 5-10° and a wider hump between 15-30°). This indicates that Gum Tragacanth raw is predominantly **amorphous**, lacking long-range crystalline order.

· **Lack of Sharp Peaks:** The absence of sharp, well-defined peaks confirms the amorphous nature of the material.

MIXED SAMPLE(DRUG+POLYMER)

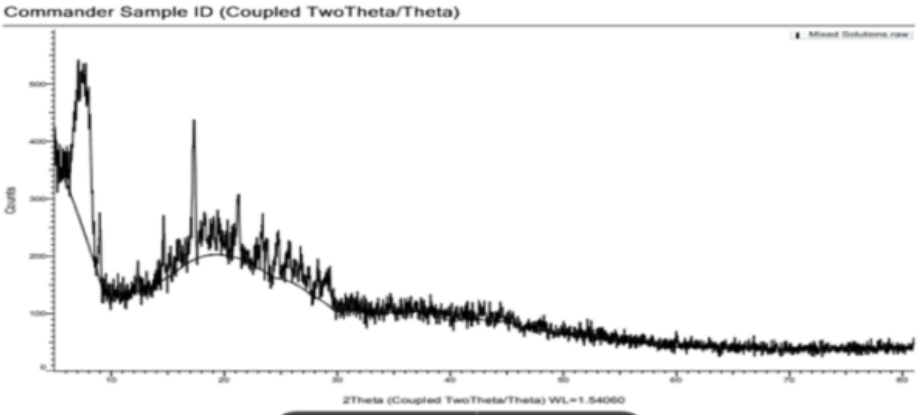

Fig 29: MIXED SAMPLE (XRD)

However, I can describe the peaks in the XRD pattern for "Mixed Solutions raw":

- The XRD pattern shows a combination of features, suggesting a sample with both crystalline and amorphous components.
- There are several **sharp peaks** present, particularly at lower 2θ angles (e.g., around 7°, 11°, 19°, and 23° - these are approximate values). These sharp peaks indicate the presence of one or more **crystalline phases** within the mixed solutions. The positions of these peaks can be used to identify the specific crystalline compounds present by comparing them to reference patterns in databases.

FINAL FORMULATION(F3)

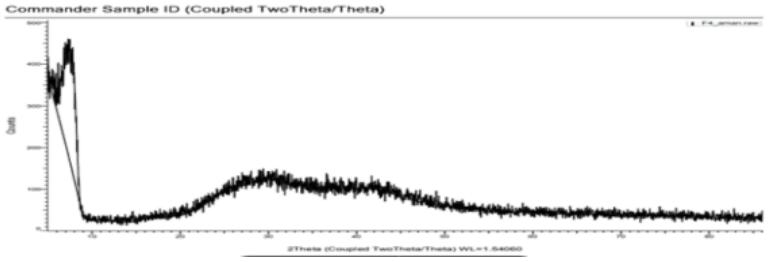

Fig 30: FINAL FORMULATION (XRD)

· **Broad Features:** The pattern is dominated by broad features, particularly in the region between approximately 15° and 30° (2θ). This indicates that "F4 aman raw" is primarily **amorphous**, meaning it lacks long-range crystalline order.

· **Lack of Sharp Peaks:** The absence of sharp, distinct peaks further supports the conclusion that the material is not highly crystalline.

7. CONCLUSION

The study successfully developed and characterized a curcumin-loaded microsphere hydrogel for the topical treatment of vitiligo. The research was conducted in three phases: preformulation, formulation, and evaluation studies to optimize drug delivery and enhance curcumin's therapeutic efficacy.Preformulation studies confirmed curcumin's poor aqueous solubility and stability, necessitating encapsulation in microspheres for controlled release. FTIR and DSC analyses demonstrated no significant interactions between curcumin and excipients, ensuring formulation stability.In the formulation study, curcumin was effectively encapsulated into biodegradable polymeric microspheres, which were then incorporated into a hydrogel matrix. The hydrogel provided enhanced skin retention and sustained drug release, improving curcumin's bioavailability and efficacy for vitiligo treatment.Evaluation studies showed that the formulation had optimal particle size, high encapsulation efficiency, and a sustained drug release profile. Rheological and stability studies confirmed that the hydrogel was viscous, spreadable, and stable for topical application. In-vitro drug release studies indicated prolonged curcumin release, while skin irritation studies confirmed the formulation was safe and non-irritant.Overall, the curcumin-loaded microsphere hydrogel demonstrated enhanced stability, prolonged drug release, and targeted skin delivery, making it a promising alternative for vitiligo management.

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