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Development and Evaluation of Curcumin Loaded Nanoparticles for Treatment of Diabetes

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Abstract

A nanometer is one billionth of a metre, hence nanotechnology is an intersection of science, engineering, and technology that works with structures and materials at the nanoscale scale, often in the range of 1 to 100 nanometers. Materials frequently display distinctive and innovative features at this scale that are distinct from those at the macroscopic or even microscopic levels. Nanotechnology is the manipulation, design, and control of materials and devices at the nanoscale to produce new products, technologies, and applications. Nanotechnology is essential to the development of tailored medication delivery systems, imaging agents, and diagnostic instruments in medicine. It offers the promise for more targeted treatments that are also less likely to cause negative effects. Since ancient times, turmeric (Curcuma longa L.) has been widely used as a spice and a remedy. Curcumin, a polyphenol that aids in the prevention and management of neurological, pulmonary, cardiovascular, metabolic, inflammatory, and autoimmune illnesses as well as some malignancies, is the primary active component of turmeric. Curcumin does have certain disadvantages, though, including limited water solubility, poor absorption, rapid metabolism, rapid systemic elimination, inadequate bioavailability subpar pharmacokinetics, low stability, and subpar penetration targeting effectiveness. A typical approach is to encapsulate curcumin in nanocarriers for targeted distribution to get over these disadvantages. Concerns have been raised about the degradation of nanocarrier products. In this study, curcumin nanoparticles and nanocurcumin were created without the aid of nanocarriers. To do this, raw turmeric rhizome was soxhlet extracted to obtain curcumin. The stock solutions of various curcumin concentrations made in dichloromethane were sonicated for varying lengths of time and included in boiling water at various flow rates. With 5.00 mg/mL of stock solution concentration, 0.10 mL/min flow rate, and 30 minutes of sonication, an average particle size of 82 04 nm was produced. Particle size seems to decline with sonication time but tends to increase with flow rate and curcumin content in the stock solution. Although nanocurcumins are amorphous, X-ray diffraction

reveals crisp and powerful diffraction peaks for curcumin, suggesting its integrity and high crystallinity. The presence of all the functional groups of curcumin in nanocurcumin is confirmed by Fourier-transform infrared spectroscopy spectra. Images obtained using transmission and scanning electron microscopy display the morphology of completely spherical objects.

Keywords: *Nanotechnology, curcumin, nanoparticles, diabetes*

1. Introduction

Millions of people throughout the world are afflicted by diabetes, which is a common and chronic metabolic illness. Elevated blood glucose (sugar) levels result when the body's capacity to control blood sugar levels is compromised. If this ailment is mistreated or badly managed, it could have detrimental effects on one's health. Diabetes is a complicated, multifaceted condition that necessitates constant monitoring and care (16). Diabetes comes in a variety of forms; however the two most prevalent ones are:

- **Type 1 Diabetes:** This autoimmune disorder occurs when the immune system unintentionally targets and kills the pancreatic beta cells that produce insulin. Because they cannot generate insulin, persons with type 1 diabetes need lifelong insulin replacement medication. Though it can happen at any age, type 1 diabetes most frequently manifests in childhood or adolescent (6).

- **Type 2 Diabetes:** This is the most common kind of diabetes, and lifestyle elements including obesity, inactivity, and a poor diet are frequently linked to it (7). In type 2 diabetes, the body's cells develop a resistance to insulin's actions, and the pancreas may not generate enough insulin to keep blood sugar levels within normal range. Type 2 diabetes is normally managed with dietary changes, oral medicines, and occasionally insulin therapy.

Type 2 diabetes mellitus (T2DM) is a group of metabolic disorders that have become a global pandemic. Over 400 million individuals worldwide are affected by diabetes, which has epidemic proportions. According to the Global Burden of Diseases study from 2016, T2DM and its consequences were to blame for the 22% rise in disability over the previous ten years, having a substantial effect on public health. The creation and implementation of programmes for managing diabetes prevention in clinical settings is one of the issues facing clinical diabetology today (8-10). A bioactive substance called curcumin, which is present in curcuma longa, has a number of physiological and pharmacological properties, including antioxidant, anti-inflammatory, anti-cancer, neuroprotective, and anti-diabetic effects. The turmeric plant, Curcuma longa, which is frequently used as a spice in culinary preparation, has been acknowledged by the scientific community. Sadly, a number of oral hypoglycemic medications showed anti-diabetic benefits via various modes of action with negative side effects. Due to their low cost, simplicity of availability, and little side effects, the use of medicinal plants has therefore emerged as the most important component of all currently used therapies. The most potent polyphenol found in turmeric (Curcuma longa) rhizomes is curcumin. Southeast Asia is well-known for and actively cultivates this plant, which is distinguished by orange tuberous rhizomes. Since ancient times, it has been employed in these areas as a natural medicinal medication for a number of pathological disorders. The inclusion of curcumin, which has anti-inflammatory and antioxidant effects, is this plant's distinguishing feature. Curcumin is a well-known potential therapeutic natural substance that has been shown to have super antioxidants, hypoglycemic , antitumor , antihypertensive, anti-inflammatory, anti-scleroderma, antipsoriatic, and antibacterial activity that can treat a number of chronic disorders (11). Due to its polar structure and low molecular mass, it can also efficiently cross the blood-brain barrier. In diabetic rats, curcumin has been shown to boost insulin sensitivity, increase pancreatic -cell antioxidant capacity, and improve hyperlipidemia, all of which are associated with a decrease in plasma free fatty acids (FFA). Numerous research has been published recently on the development of curcumin-loaded nanoparticulate systems (CUR), which display better biological activity than traditional treatments.

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2. Materials And Methods

Chemical Requirements

Tetrahydrobiopterin (BH4) (HPLC grade), streptozotocin (STZ), iodine, potassium iodide (KI), trichloroacetic acid (TCA), sodium hy- droxide (NaOH), ascorbic acid, and curcumin (≥94 % (curcuminoid content), ≥80 % (Curcumin), CAS Number 458-37-7) were purchased from Sigma-Aldrich Co. USA. Tween 80 was obtained from R&M (UK) polyethylene glycol (PEG) and Sodium hydrogen phosphate (Na_2HPO_4) Sarabhai M. Chemicals, dimethyl sulfoxide (DMSO), Dichloromethane (DCM), Ethyl acetate was obtained from Merck specialities Pvt. Ltd. For experimental tools and characteristics of the resultant nanoparticles, deionized water (DI) was utilized. All chemicals were used without further purification.

Preparation of Curcumin nanoparticles (CUR-NPs)

CUR-NPs were prepared by dissolving 200 mg of curcumin powder in 45 mL of dichloromethane (DCM) under magnetic stirring for 15-20 min. The prepared solution was then added dropwise to 150 mL of deionized warm water containing 200 mg of Tween 80 as stabilizing agent with continuous stirring then 200mg of polyethylene glycol (PEG) was added. After complete addition, the solution was conducted to ultrasonication waves of 100 kHz for 1.5 h. The sonicated solution was magnetically stirred at 1300 rpm for another 1.5 h. The yellowish orange coloured precipitate was obtained by centrifugation at 5000 rpm for 45 min. Afterwards, the sample was kept at -80 ˚C for few hours followed by freeze drying at 0.2 mbar for 3-4 days for complete drying. The orange dried solid of CUR-NPs was obtained and kept at room temperature for further study; characterization and application.

UV–visible spectroscopy

UV-Vis Spectroscopy gives much insight about synthesised CuNP. UV-Visible spectroscopy (UV-Vis) measures the extinction (scatter + absorption) of light passing through a sample. Nanoparticles have unique optical properties that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV-Vis a valuable tool for identifying, characterising, and studying nanomaterials.

FTIR analysis

The samples were combined with potassium bromide, and the mixtures of the samples and potassium bromide were pressed into a transparent tablet. The FTIR spectra for the samples were recorded on an FTIR Perkin Elmer 1720 (Perkin Elmer) in the transmission mode with the wave number ranging from 4,000 to 450 cm 1.

Particle Size Analysis. The mean particle diameter of curcumin nanoparticles was measured by dynamic light scattering (DLS) performed on Malvern Zetasizer S90 series. The sample was prepared by taking 1.5 mg of the sample in 20 mL of distilled water.

Transmission electron micrograph (TEM)

On a Morgagni 268 D from FEI, transmission electron microscopy (TEM) investigation was carried out. A drop of the aqueous dispersion of curcumin nanoparticles was applied to the copper grid to create the sample, which was then allowed to air dry. While the majority of CuNPs have spherical or quasi-spherical geometries with smooth edges, some nanoparticles have anisotropic nanostructures and asymmetrical shapes (12-14). Additionally, other NP shapes like decahedral, ellipsoidal, oblong spherical, and triangular can be seen. With an average size of 0.5 nm and a size distribution of nearly 75% between 10 and 40 nm, NPs were primarily in the size range of 5-40 nm.

A scanning electron micrograph (SEM)

The nanoparticle dispersion was spread across a carbon tape and dried under a nitrogen stream to produce a scanning electron micrograph (SEM) of the aqueous dispersion. The sample was subsequently coated with a gold layer in a vacuum environment using an EMITECH K 550 x sputter coater.

Preparation of Microorganism Suspension

Staphylococcus aureus (G-) MTCC 96, Bacillus subtilis (G-) Pseudomonas aeruginosa (G-) MTCC 741, P. notatum, and Aspergillus Niger (G-) were used as test organisms for curcumin's antimicrobial properties. The experimental bacteria and test fungus were cultured on agar with nutrients (NA) and potatoes with dextrose (PDA), accordingly. Each strain was moved from maintained slants at 5 C to a 15 mL nutritional broth or a potato dextrose broth tube, where it was then grown for an entire night at 40 C.

Determination of MIC

The agar dilution method was used to test the MIC of curcumin and nanocurcumin. 2.5 mg of the substance was dissolved in 5 mL of distilled water to create a stock solution of nanocurcumin, which resulted in an orange-hued, transparent nanodispersion. The stock solution was diluted serially to produce concentrations between 100 and 1000 g/mL. A similar aqueous stock solution could not be made for curcumin since it is totally insoluble in water. So, 2.5 mg of curcumin were dissolved in 5 mL of DMSO to create the stock solution. Various amounts of curcumin (DMSO) and nanocurcumin (water) solutions were introduced separately to flasks containing 50 mL of melted agar. The control plates were filled with an equal volume of DMSO and allowed to set. The plates were incubated at 45 C for 24 hours in the case of bacteria and at 25 C for 5-6 days in the context of fungus using a total of 200 L of culture that was inoculated under aseptic conditions.

3. Results and Discussion

UV–visible spectroscopy

The prepared nanocurcumin sample was subjected to UV–visible spectroscopy analysis in the 200–900 nm range. The absorption spectra of nanogel displayed a characteristic peak of 418.

Determination of Curcumin Calibration Curve

The absorbance range of standard concentrations were plotted (fig.1) and linearity was evaluated with an $r^2 = 0.9987$ when analysed at 418 nm.

S. No	Conc. (µg/ml)	Absorbance (nm)
1	0	0
2	0.5	0.049
3	1.0	0.088
4	1.5	0.139
5	2.0	0.185
6	2.5	0.224

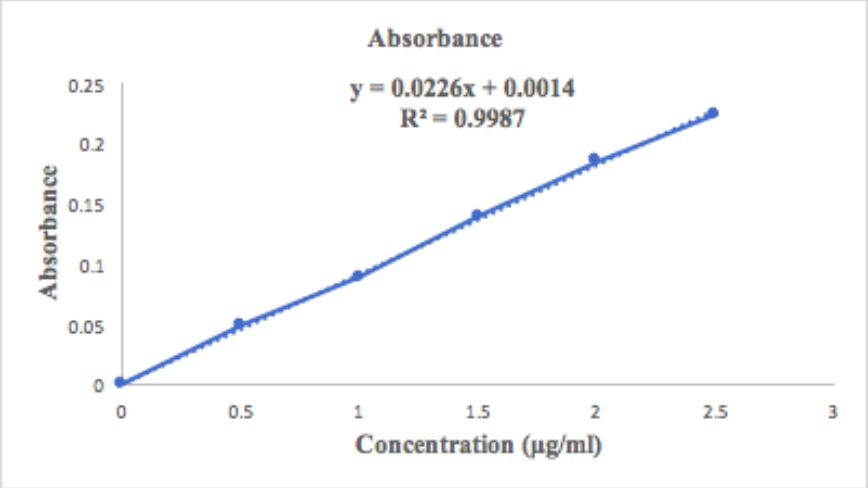

Fig.1 Calibration curve of Curcumin

FTIR analysis

In FTIR spectrum of Nano-curcumin, peaks were observed at 1733, 1442, 1164 and 1143 cm -1 spectrum of curcumin in DMSO (control) and Nanocurcumin (experimental) was recorded (Figure 2).

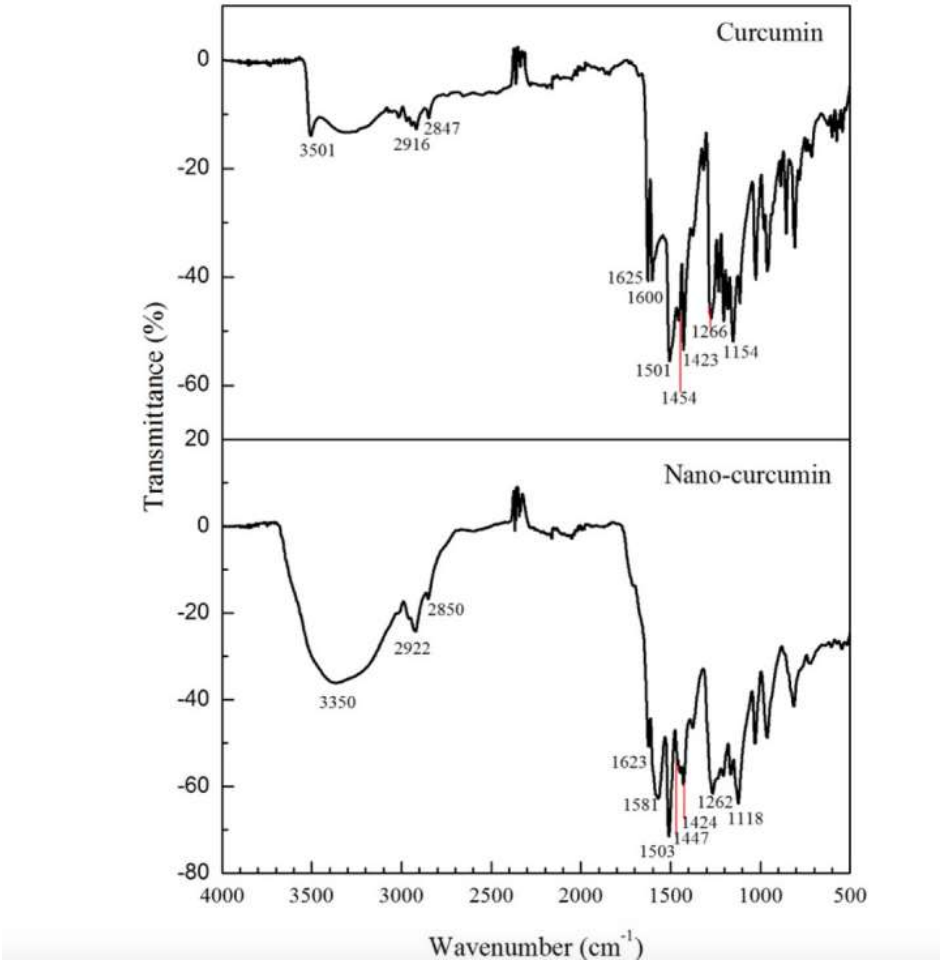

Fig.2: FTIR Analysis of Curcumin nanoparticles

Table. 1 Functional Groups and corresponding IR Peaks of Curcumin nanoparticle

S. No.	Functional group	Peak (cm-1)
1.	C-H	2976.09
2.	C=O	1867.5
3.	O-H	1265.05
4.	C-F	876.65
5.	C=C	-
6.	N-H	1657.16

XRD analysis

The XRD pattern of curcumin displayed sharp and intense peaks between 10° and 50°, representing a crystalline arrangement and 31.09, 49.90, 26.12 degrees, diffraction peaks were observed (Fig.3).

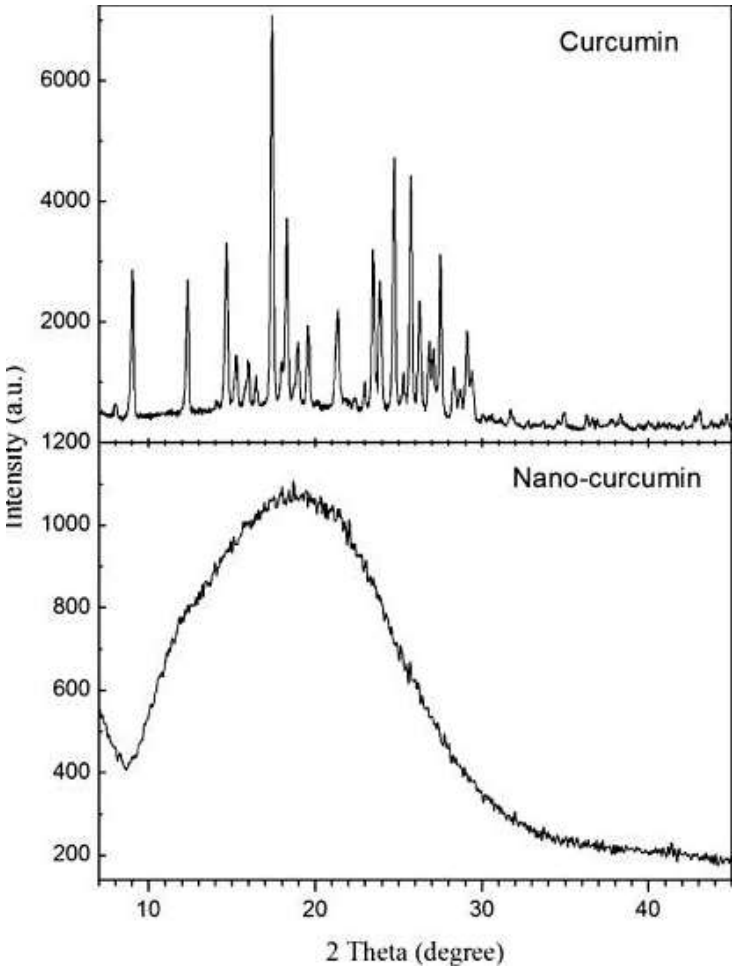

Fig. 3: XRD **analysis of Curcumin nanoparticle**

The particle size analysis:

Dynamic light scattering (DLS) was used to examine the particle size of the synthesised nanocurcumin, and Figure 4 displays the distributions of the hydrodynamic dimension of the synthesised particles. It can be presumed that spherical particles have been synthesised because the diameter of the hydrodynamic distribution only has one peak. A statistical analysis was done on the average hydrodynamic radius of every single treatment. The analysis of variance (ANOVA) approach was used to compare mean values. The null hypothesis is dismissed since the P-value (P = 0.0001, so P 0.05) is lower than the alpha P value.

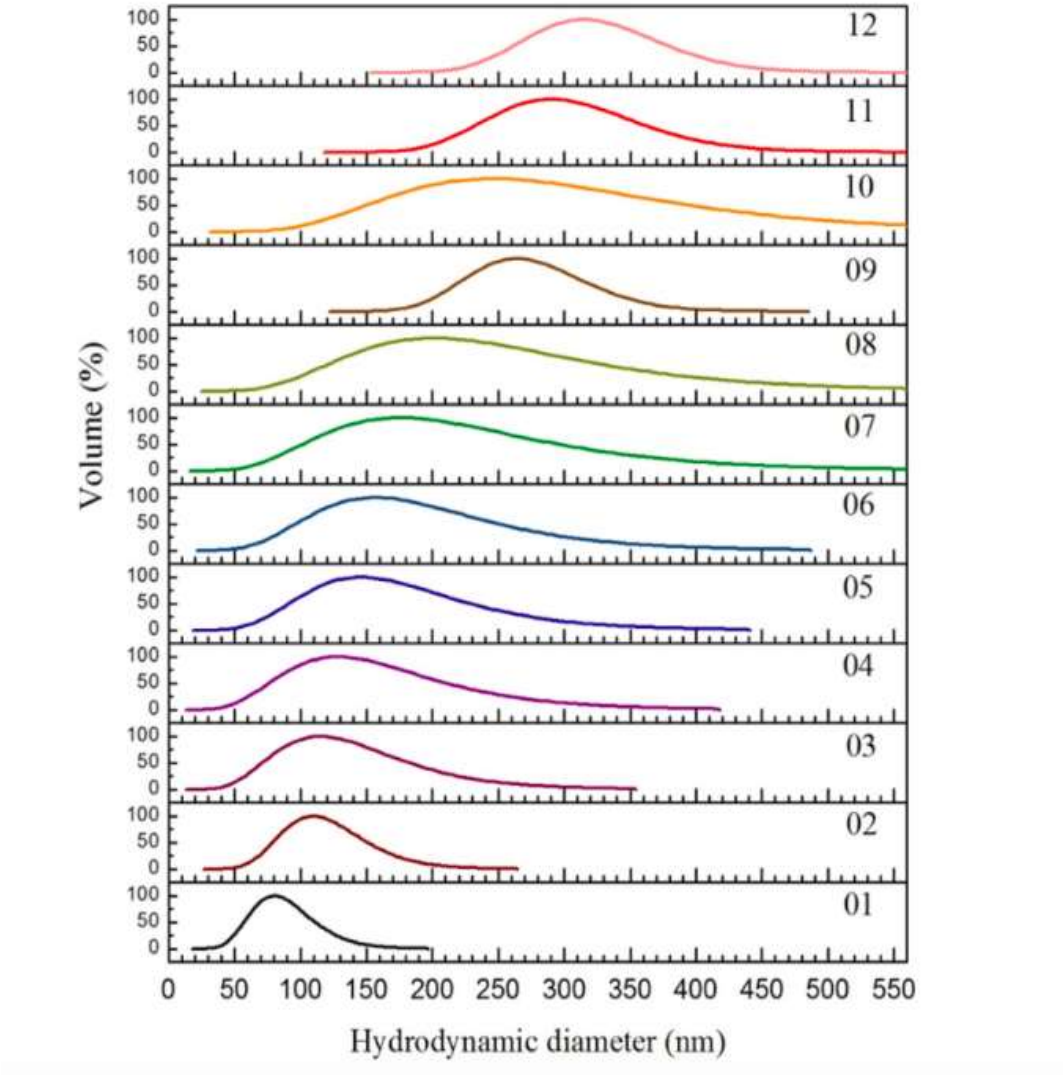

Fig. 4: The particle size analysis of curcumin nanoparticles

TEM and SEM

The particle size of the aqueous dispersion was revealed by TEM to be between 2 and 50nm (Fig. 5-7), while the powdered sample was revealed by SEM to have particles that were roughly 50 nm in size. It was discovered that dry, lyophilized nanocurcumin powder had good physical and chemical stability (Fig. 8), was easily dissolved in water, and could be kept at room temperature for more than 6 months without experiencing any breakdown or aggregation. The higher surface area of nano-sized curcumin particles, which encourages dissolution, may be the cause of their increased aqueous solubility. Similar outcomes have been reported in earlier research where active compounds' potency, solubility, and bioavailability were improved by downsizing them to nanoparticle size.

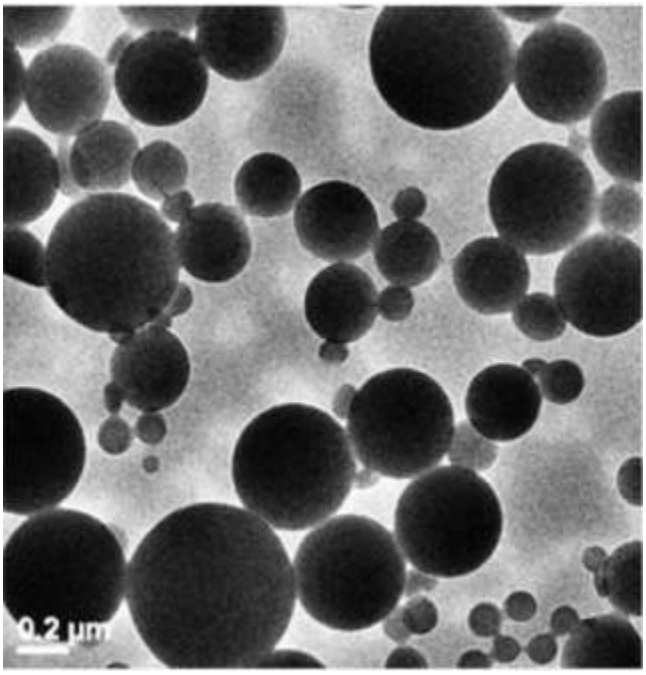

Fig 5: TEM image of curcumin nanoparticle at 400 nm

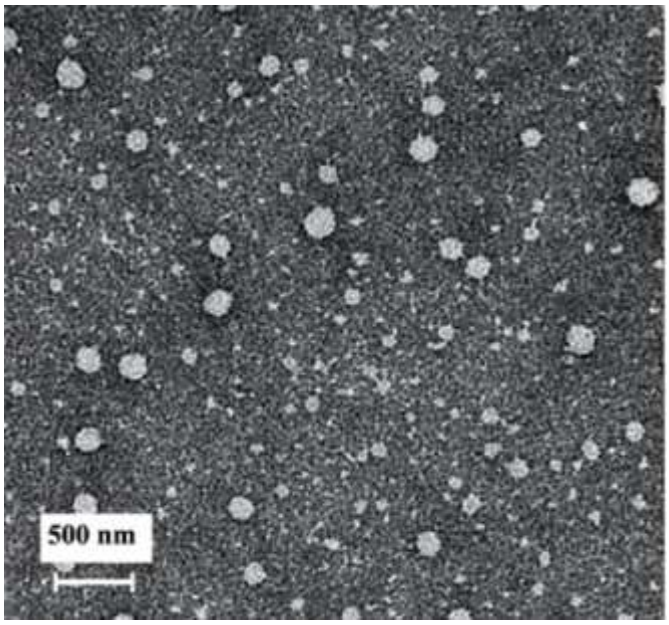

Fig 6: TEM image of curcumin nanoparticle at 100 nm

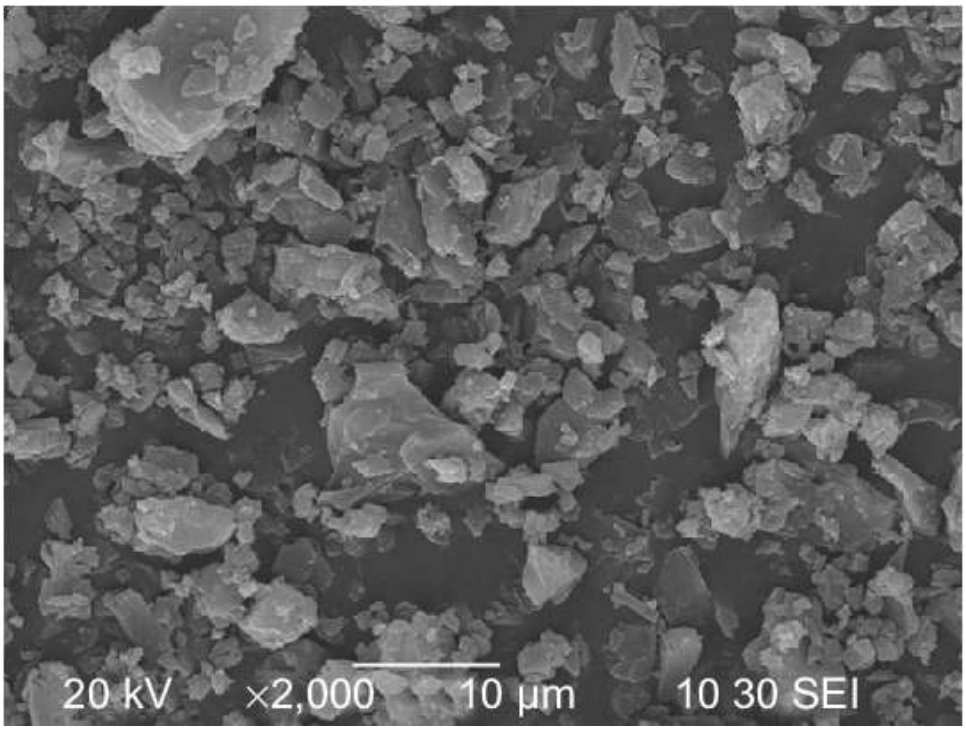

Fig 8: SEM of curcumin nanoparticle at magnification 2.0kx

***In vitro* drug release of curcumin nanoparticle**

The cumulative amount of drug permeated per unit area was plotted as a function of time. The steady-state permeation rate (J_{ss}) and lag time (LT, hrs) were calculated from the slope and X-intercept of the linear portion, respectively. The permeability coefficient (Kp) was calculated by dividing transdermal flux values (Jss) by the initial concentration of drug in the donor cell (Co) (Fig.10).

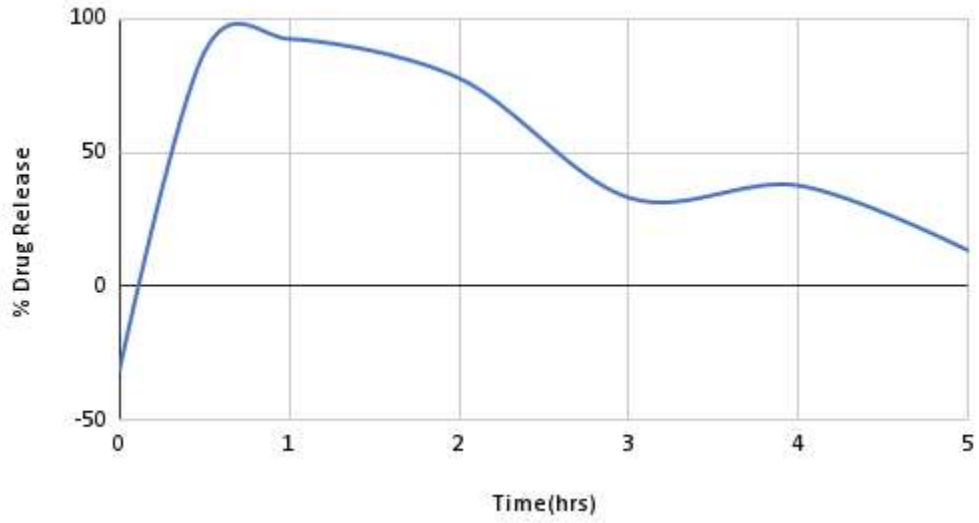

Fig.10: In vitro drug release of curcumin nanoparticle

Antibacterial and Antifungal Assay

The outcomes of testing the MIC of curcumin and nanocurcumin over two bacterial strains (S. aureus and B. subtilis), two bacterial strains (E. coli and P. aeruginosa), and two strains of fungi (P. notatum and A. niger) are shown in Table 1. Nanocurcumin has been shown to have antibacterial activity against S. aureus, B. subtilis, E. coli, and P. aeruginosa, demonstrating that it has a broad-spectrum inhibitory

impact on all microbes. MIC of nanocurcumin was 100, 150, 250, and 300 g/mL for S. aureus, B. subtilis, E. coli, and P. aeruginosa, compared to 75, 100, 300, and 250 g/mL for curcumin. The order of the distinct bacteria's % suppression by 200 g/mL solutions of nanocurcumin (water) and curcumin (DMSO) was B. subtilis > S. aureus > P. aeruginosa > E. coli. The standard deviations were discovered to be between 0.8 and 3.2. Additionally, the size of the inhibitory zones at 500 g/mL of curcumin demonstrated the highest level of effectiveness for B. subtilis (Fig.9). Overall, the findings suggested that the chosen Gram-positive bacteria were more sensitive than the chosen Gram-negative bacteria. This might be the result of variations in the components and architecture of their cell membranes. Gram-positive bacteria have an exterior peptidoglycan level, whereas Gram-negative bacteria have an outer phospholipidic membrane; since curcumin encounters either of these, distinct types of interactions occur. Regardless of high concentrations up to 1000 g/mL, nanocurcumin and curcumin were found to be useless against P. notatum by the two fungal strains' MIC tests. Although the MIC value was greater above the MIC range (150-300 g/mL) for the bacteria tested, they nevertheless demonstrated some antifungal action for A. niger (350 g/mL for nanocurcumin). We emphasise that we have contrasted nanocurcumin solutions in aqueous and DMSO solutions. This kind of comparison was purposefully carried out to investigate the impact of particle reduction on the bioavailability and solubility of curcumin.

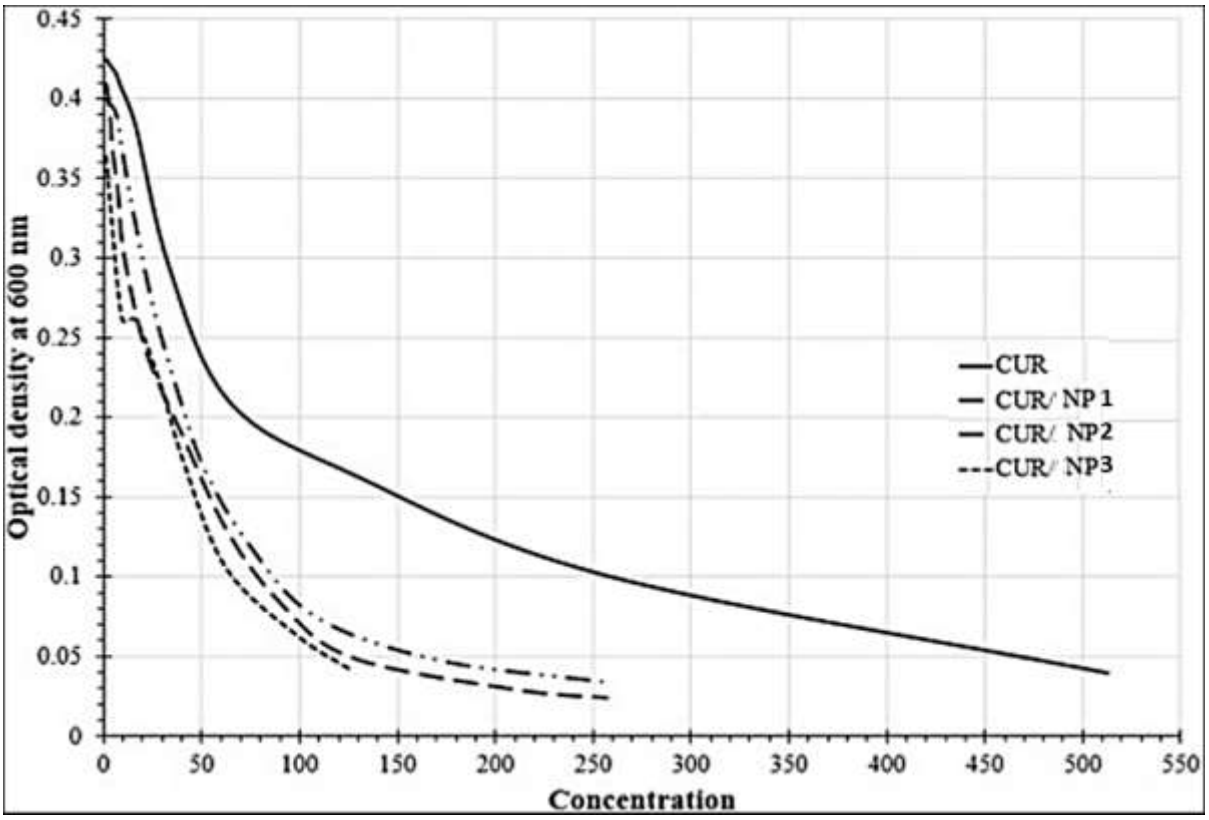

Fig 9: MIC of curcumin nanoparticles

We demonstrate for the very first time that curcumin, in its nano form, can be dissolved in water and is just as potent as curcumin in DMSO. The reason why nanocurcumin exhibits greater activity than curcumin in DMSO is due to the particle size. The important thing to remember is that when curcumin forms nanoparticles, its size decreases to 4–40 nm, which is significantly smaller than the size of curcumin particles dispersed in DMSO (600–800 nm) and is what allows for improved cell penetration and absorption. When comparing to DMSO solution of curcumin, the anti-microbial and anti-fungal assay of nanocurcumin's aqueous solution showed comparable or superior in vitro antimicrobial effectiveness. The antibacterial effect was substantially stronger than the antifungal effect and much more apparent over Grampositive germs than against Gram-negative ones. The in vitro biological studies unmistakably showed that curcumin's water solubility and effectiveness as an antibacterial agent

are considerably enhanced by conversion to the nano state. It would be fascinating to examine how particle reduction affects toxicity to macroorganisms as studies to identify the whole toxicological assessment of curcumin nanoparticles advance.

4. Conclusion

Nanocurcumins were created using a physico-chemical technique. The process employed to create Nano curcumins is straightforward, easy to use, and natural. Curcumin nanoparticles showed spherical and compact shapes with smooth surfaces, and they were stable in artificial gastric and intestinal fluids. Their mean particle size was 281.91 ±1 nm, their zeta potential was +20.05 mV, and their entrapment efficiency ranged from 87.28 to 93.41%. Synthesized nanoparticles showed its efficacy against S. aureus, B. subtilis, E. coli, and P. aeruginosa. The results indicate that the activity of bacteria was suppressed by Nano curcumin The created nanoparticle is a novel type of antiseptic cream that may be applied to infections brought on by P., S. aureus, and E. coli. It also showed anti-inflammatory activity.

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