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Original Article

SUBLIMATION-BASED CUTTING-EDGE TECHNOLOGY (SUSTAINABLE DEVELOPMENT GOALS 9 and 15) TO DEVELOP CURCUMIN NANOPARTICLES BY SOLVENT-FREE GREEN CHEMISTRY METHOD FOR THEIR ANTIOXIDANT AND ANTICANCER ACTIVITY

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ABSTRACT

Objective: This research aimed to develop a new, cost-effective, solvent/surfactant/supercritical carbon dioxide-free, sublimation-based method to prepare curcumin nanoparticles. The research objective was to meet Sustainable Development Goals (SDG-3,9 and 15). The problem of poor absorption of curcumin is sorted out by micro or nanonization, solid dispersion, solid solution, β-cyclodextrin complexation, micelle formation, and solution-enhanced dispersion by supercritical carbon dioxide.

Methods: The curcumin, mixed with menthol, was allowed to melt at 29 °C and placed under vacuum for 6 H (h). The menthol sublimates and leaves the curcumin particles as residue. The residual curcumin particles were characterised, and stability studies were also performed.

Results: The curcumin nanoparticles were stable, in the nano-size range (10-300 nm); Fourier Transform Infrared Spectroscopy (FTIR) showed the presence of CH_3 and CH_2 bending, aromatic C=C and C=O stretching, aromatic CC and OH stretching, aliphatic C-H bending, aromatic-OH bending with both pure curcumin and curcumin nanoparticles, that no change in bonds and groups, and differential scanning calorimetry (DSC) showed the temperature (T) =162.03, 185.64 and peak maximum is 177.986 for pure curcumin while T=164.43, 185.68 and peak maximum is 177.784 for curcumin nanoparticles that indicated compatibility between curcumin and menthol. The curcumin nanoparticles showed improved solubility, dissolution, and antioxidant activity by calculating Inhibitory Concentration$_{50}$ (IC_{50}) value 114.51 and *in vitro* cytotoxicity (IC_{50}=165.6±0.084 µg/ml) of curcumin nanoparticles against MCF-7, a human breast cancer cell line with estrogen, progesterone, and glucocorticoid receptors.

Conclusion: It concluded that the sublimation technique can used to prepare the nanoparticles of drugs or might be for thermo-labile drugs.

Keywords: Curcumin, Nanoparticle, Antioxidant, Anticancer, Cytotoxicity

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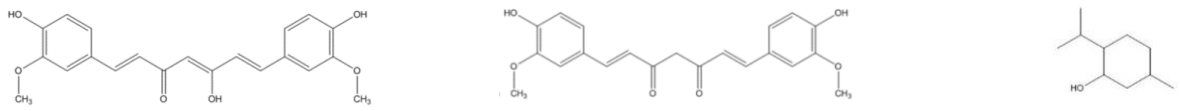
INTRODUCTION

The turmeric (*Curcuma longa*; Family-Zingiberaceae) comprise a yellow-orange polyphenol compound named curcumin. Chemically curcumin is known as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1-6-heptadiene-3,5-dione that exist in keto and enol forms as shown in fig. 1A, fig. 1B. It has various uses like spice, food preservatives, flavouring and colouring agents. It also reveals many therapeutic activities like scavenging hydroxyl radicals, singlet oxygen, superoxide anion, nitric oxide, anti-inflammation, anti-oxidation, inhibit lipid peroxidation, anticancer, and antimicrobial activities. At physiological pH, poor aqueous solubility of curcumin is a basic and major problem in absorption and results in low bioavailability. It shows tautomerism as it shows pH-dependent keto and enol forms [1-4].

Approaches used for improved drug delivery by enhancement of aqueous solubility and bioavailability of curcumin using various drug carriers like phospholipid, liposomes, protein nanoparticles such as silk fibroin, zein, and bovine serum albumin cyclodextrin, chitosan nanoparticles, polymer nanofibers such as poly-(ε-caprolactone), Polyvinyl Alcohol (PVA). Another approach for solubility and bioavailability enhancement is size reduction by micronization or nanonization has a surface area, solubility and dissolution are inversely related to particle size. Various methods are used to prepare the nanoparticles mechanical methods, synthesis of nanomaterials by high energy ball milling, melt mixing method to disperse carbon nanotubes into thermoplastic polymers, laser ablation, chemical methods, colloids synthesis, methods based on evaporation, physical vapour deposition, synthesis of metal nanoparticles by colloidal method, sol-gel method and biological methods synthesis using plant extracts, bio-based method, Tollens's method, irradiation methods, electrochemical synthetic method. A mechanical communication process-based method uses a wet-milling technique to prepare the nanoparticles. Flash nanoprecipitation produces curcumin nanoparticles in a range of 40 Nanometres (nm) utilizing a spray-drying method but requires organic solvent and surfactants and elevated temperature in spray-drying and may promote denaturation of active pharmaceutical ingredients or phytoconstituents. The Supercritical Antisolvent (SAS) process is used to prepare nano-sized particles by use of Supercritical Carbon Dioxide ($scCO_2$) at Critical Pressure (P_c) 7.38 Megapascal (MPa), Critical Temperature (T_c) 304.1 Kalvin (K), with advantage like the use of non-flammability gas, either few or no solvent residues [5-12].

Menthol is a waxy, clear, or white crystalline substance, shown in fig. 1C, chemically a monoterpenoid that is obtained from the oils of corn mint, peppermint, or other mints or made synthetically. It exists in two crystal forms as it exists in a racemic mixture, showing melting points of 28 °C and 38 °C [13].



A B C

Fig. 1: Structures; A) Enol form of curcumin, (B) Keto form of curcumin, (C) Menthol [2, 13]

In the present research, the sublimation utilized to synthesize nanoparticles of curcumin to compare the results with an already reported method to nanoparticles prepared using Solution-Enhanced Dispersion (SEDS) method by using Supercritical Carbon Dioxide ($scCO_2$). The Ultraviolet-Visible (UV) spectrophotometry, Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC) and solubility and dissolution rate performed with developed nanoparticles were prepared first time by this method.

MATERIALS AND METHODS

The curcumin was procured from Indian Glycols Limited, Noida, Utter Pradesh. India. Menthol crystals procured from BIOCHEM Chemo Pharma, France.

Pre-formulation of curcumin

The UV-visible analysis, FTIR and DSC were performed.

UV-Visible spectrophotometer

The 1 mg/ml (stock solution) of curcumin was made of methanol and the dilutions (1,2,3,4,5,6,7 µg/ml) were prepared. The maximum absorbance (λ_{max}) is measured, and dilutions are scanned to determine the absorbance to obtain calibration data and plot a calibration graph between concertation and absorbance. The experiment was conducted in triplicate [14].

FTIR

The FTIR of curcumin was measured by IRAffinity-1S (Shimadzu) to identify the type of bonds and functional groups in each sample [15].

DSC

DSC characterize the thermal response of various materials like melting and crystallization behaviours under optimal conditions. The DSC-60APlus, (Shimadzu) was used and the curcumin sample was placed in an aluminium pan and closed with a lid. The DSC was programmed at heating up to 300 °C with a sample holding temperature of 10 °C in a nitrogen environment and 10 ml/min was the gas flow rate. The DSC thermogram between heat in Milliwatts (mW) vs time in Minutes (min) vs temperature in Degrees Centigrade (°C) was recorded and further turned into a plot of heat (mW) vs temperature (°C) [15].

Pre-formulation of menthol

The FTIR and DSC performed as performed for curcumin in the above section [15].

Preparation of curcumin nanoparticles by sublimation process

The 1 g menthol was placed in a beaker and the temperature was maintained at 50 °C to melt the menthol. Accurately weighed 100 mg drug placed in a beaker and mixed. Curcumin dissolved in molten menthol. Mixture mixed at 500 revolutions per minute (rpm) for 5 min (50 °C) and then cooled at room temperature 25 °C. The temperature was maintained at 40 °C and a vacuum was applied for sublimation as shown in fig. 2. A powdered residual mass obtained characterized.

Evaluation of curcumin nanoparticles

The yield of residual powder was calculated and evaluated by FTIR, Scanning Electron Microscopy (SEM), DSC, particle size analysis, solubility profile and dissolution.

Yield: The yield calculated.

FTIR: The FTIR recorded to identification of bonds and functional groups in pure curcumin and curcumin nanoparticles [15].

DSC: The DSC performed as run for curcumin [15].

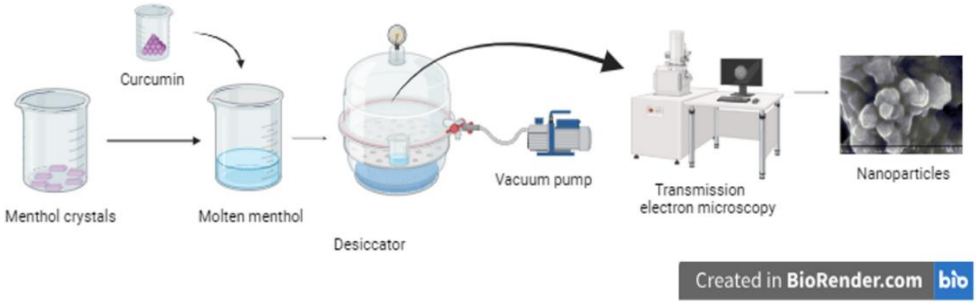


Fig. 2: Synthesis of curcumin nanoparticles

SEM

The curcumin nanoparticles are attached to a carbon tape and placed on an aluminium stub. The sample loaded stub was placed in a chamber for goal coating in an argon environment to make curcumin nanoparticles conductive. The prepared sample was examined for surface morphology by field emission scanning electron microscopy (FE-SEM) (JSM-6490; JEOL, Tokyo, Japan) and particle size distribution was analysed by SmileView software from the SEM photographs [16].

Solubility analysis

Solubility analysis of pure curcumin and nanoparticles of curcumin performed using cellulose nitrate membrane. Accurately weighed 10 mg sample dispersed placed in a cellulose nitrate membrane and further dipped in 5 ml of dissolution media, phosphate buffer saline (PBS pH 6.8). The bag was put into 50 ml of solvent and maintained at 37 °C on a magnetic stirrer at 60 Revolutions Per Minute (rpm). At a specific time, 1 ml solution was removed and centrifuged (10,000×*g*, 10 min) and the absorbance of the supernatant was measured at 425 nm by UV-visible spectrophotometer (Shimadzu, UV-1800) to calculate the concentration. The dissolution rate was calculated, and each experiment was conducted in triplicate [16].

Dissolution study

Dissolution behaviour of pure curcumin powder and developed curcumin nanoparticles studied. The accurately weighed 500 mg powder sample was placed in 900 ml dissolution media i. e., Phosphate buffer saline (PBS) buffer pH 6.8, using Indian Pharmacopoeia (IP) dissolution apparatus II (Paddle type) at 100 rpm rotation. The temperature was 37±0.5°C maintained throughout the experiment. The 10 ml sample was withdrawn and replaced with a 10 ml fresh volume of dissolution media. The absorbance of all collected samples was measured at 425 nm. The percentage of curcumin dissolved at various time intervals was calculated and plotted against time [16].

Drug release kinetic study

The drug release kinetics indicated the order and mechanism of drug release. The BIT software BIT-soft 1.12 calculates the drug release kinetics [16].

Antioxidant activity

The pure curcumin and nanoparticles of curcumin were evaluated for antioxidant activity by free radical-scavenging activity using diphenylpicrylhydrazyl (DPPH method). The decay in colour of the

stable free radical DPPH by interaction with an antioxidant was measured spectrophotometrically at 517 nm. The concentration of a sample required to decrease DPPH absorbance by 50% is termed an inhibitory concentration (IC_{50}) value that can be graphically calculated by plotting absorbance (% inhibition of DPPH radicals) vs log concentration of DPPH and determining the slope of the nonlinear regression. Butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), and ascorbic acid are used as antioxidants and are generally used as a positive control. Assays conducted in triplicate.

The scavenging activity for DPPH free radicals was measured according to the procedure described by Gulcin, 2023. Curcumin at various concentrations was added separately to each 5 ml of 0.1 mmol methanolic solution of DPPH and allowed to stand for 30 min at room temperature. After incubation, the absorbance of each solution was determined at 517 nm using a spectrophotometer (Shimadzu UV-1800). Ascorbic acid is used as standard.

$$\text{RAS}\ (\%) = \left[\frac{\text{Ac} - \text{As}}{\text{Ac}}\right] \times 100 = \left[1 - \frac{\text{Ac}}{\text{As}}\right] \times 100$$

Where A_c is the absorbance at 517 nm of the control sample, and A_s is the absorbance at 517 nm that contains the test sample, including plant extracts or pure compounds.

The IC_{50} was calculated from the graph plotting the scavenging percentage against test sample concentration (µg/ml). DPPH radicals decrease significantly upon exposure to radical remover. The IC_{50} value is correlated with drug potency, i. e., the amount of drug necessary to produce the effect lower the IC_{50} value, the more potent the drug as conducted by Meyer *et al.* 2019 [17].

Anticancer activity

The prepared nanoparticles of curcumin evaluated for *in vitro* cytotoxicity against MCF-7 anticancer cell lines procured from National Centre for Cell Science (NCCS) Pune) using (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assay at Aakar Biotech Labs, Lucknow, India. The concentration of cells was 10000 cells/well cultured in 96 well plates for 24 h in Dulbecco's Modified Eagle Medium (DMEM medium-AT149-1L) supplemented with 10% Fetal Bovine Serum (FBS-HIMEDIA-RM 10432) and 1% antibiotic solution at 37 °C with 5% CO_2. After 24 h, cells were treated with formulations (different concentrations were prepared in incomplete medium) and incubated for 24 h. MTT solution was added to the cell culture and further incubated for 2 h. At the end of the experiment, the culture supernatant was removed, and the cell layer matrix dissolved in 100 µl** Dimethyl Sulfoxide (DMSO-SRL-Cat no.-67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 660 nm. The IC_{50} was calculated by using the software Graph Pad Prism-6. Images captured under an inverted microscope (Olympus EK2) using Camera (AmScope digital camera 10 MP Aptima CMOS) [18].

Stability studies

The samples were kept in amber-coloured, screw-capped glass bottles in triplicate, placed at different temperatures and relative humidity for 1, 3, and 6 mo as per International Conference on Harmonization (ICH) guidelines. The drug content was examined, and the initial drug content was regarded as 100%. The experiment was conducted in triplicate [16].

RESULTS AND DISCUSSION

Pre-formulation of curcumin

UV visible spectrophotometer

The λ_{max} was found to be 424 nm and the line of the equation was Y=0.1503X+0.0011 with r^2 value 0.9997, as shown in table 1 and fig. 3. The calibration data and ANOVA are shown in table 2. The results were significant and $p<0.05$ as tabulated in table 1.

Table 1: UV-visible calibration data of curcumin in methanol at 424 nm

Concentration (µg/ml)	Absorbance
1	0.151±0.003
2	0.298±0.001
3	0.459±0.002
4	0.601±0.001
5	0.748±0.002
6	0.911±0.002
7	1.049±0.003

Data are expressed as mean±SD, n=3

ANOVA Summary

Source	Degrees of freedom	Sum of squares	Mean square	F-stat	P-value
	DF	SS	MS		
Between groups	6	1.8986	0.3164	69220.3438	0
Within groups	14	0.0001	0		
Total	20	1.8987			

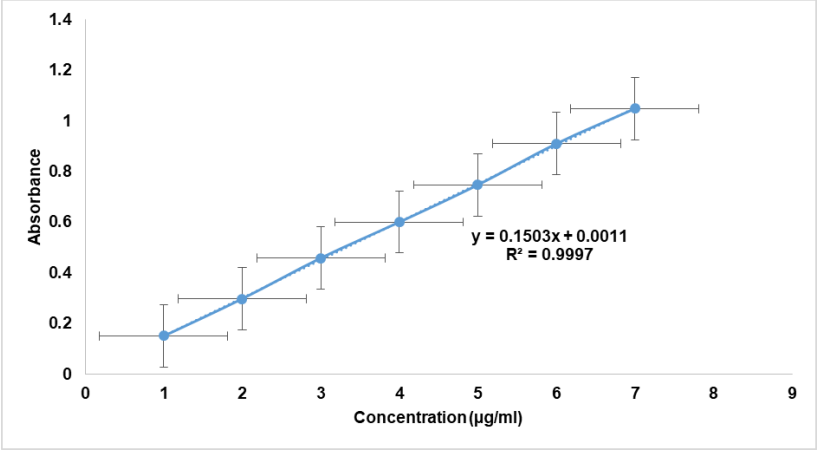


Fig. 3: UV-visible calibration curve of curcumin in methanol at 424 nm, data are expressed as mean±SD, n=3

FTIR

The FTIR of curcumin showed CO stretch at 1122 cm^{-1}, C-O bending vibration of the phenolic group at 1277 cm^{-1}, CH_3 bending at 1377 cm^{-1}, CH_2 bend at 1423 cm^{-1}, aromatic C=C stretching vibration at 1427 cm^{-1}, stretching vibrations of the C=O at 1509 cm^{-1}, benzene ring bend at 1516 cm^{-1}, aromatic CC stretch at 1589 cm^{-1}, aromatic ring stretch at 1597 cm^{-1}, CO stretch at 1624 cm^{-1}, C=O asymmetric stretch 1627 cm^{-1}, C=C at 1597 cm^{-1}, overlap stretch vibrations of alkenes C=C and carbonyl C=O character, 1637 cm^{-1}, C=O at 1512 cm^{-1}, OH stretching vibration between 3500-3200 cm^{-1} as a broad peak, C-H bending vibrations of aliphatic C-H bonds at 1400-1200 cm^{-1}, 1000-1300 cm^{-1}aromatic-OH Bending vibrations of aromatic hydroxyl groups, C-O Stretching vibrations of C-O bonds at 1200-1000 cm^{-1}, aliphatic C-H stretch vibrations at 3000-2800 cm^{-1} as showed in fig. 4.

DSC

The DSC of curcumin showed an endothermic peak maximum at 177.986 °C as shown in fig. 5.

Pre-formulation of menthol

FTIR

The FTIR of menthol showed OH stretch at 3300-3500 cm^{-1}, CH_3 stretch at 2800-3000 cm^{-1}, alkene stretch C=C around 1630-1680 cm^{-1}, C-H bending around 1300-1450 cm^{-1} as shown in fig. 4.

DSC

The DSC of menthol showed the presence of three endothermic peak maximums at 46.11 °C, 127.269 °C, and 161.147 °C as shown in fig. 5.

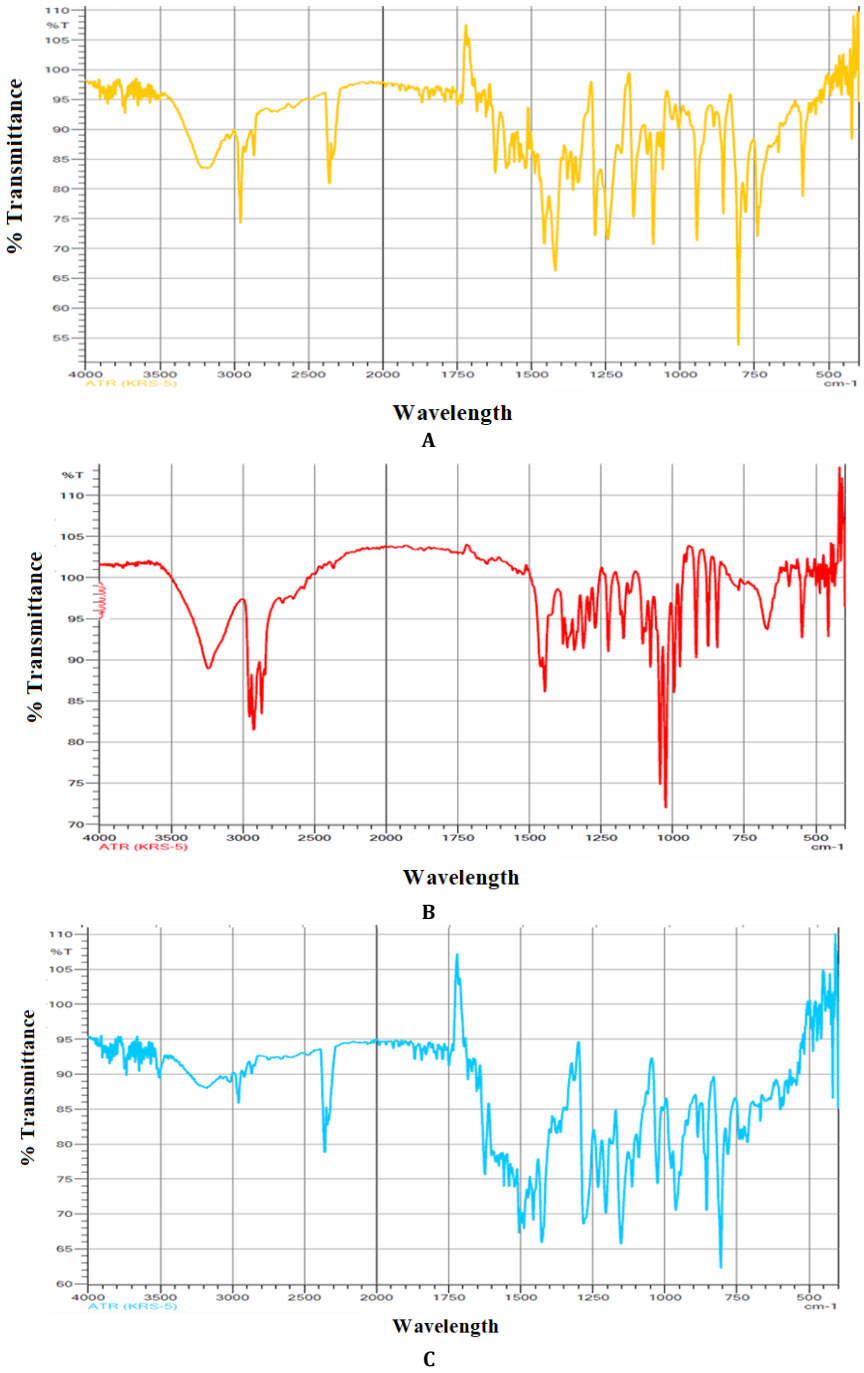


Fig. 4: FTIR of (A) Curcumin, (B) Menthol, (C) Curcumin nanoparticles

Evaluation of curcumin nanoparticles

Yield: The yield was found to be 98.97±1.02%.

FTIR

The FTIR of curcumin nanoparticles showed CO stretch at 1125 cm^{-1}, C-O bending vibration of phenolic group at 1273 cm^{-1}, CH_3bending at 1375 cm^{-1}, CH_2 bend at 1421 cm^{-1}, aromatic C=C stretch vibration at 1426 cm^{-1}, stretch vibrations of the C=O at 1511 cm^{-1}, benzene ring bend at 1514 cm^{-1}, aromatic CC stretch at 1588 cm^{-1}, aromatic ring stretch at 1596 cm^{-1}, CO stretch at 1625 cm^{-1}, C=O asymmetric stretch 1625 cm^{-1}, C=C at 1599 cm^{-1}, 1626 cm^{-1}stretch overlapping stretch vibration of alkenes C=C and carbonyl C=O character, 1637 cm^{-1} C=C aromatic ring stretch C=O at 1513 cm^{-1} attributed to the mixed vibrations including stretching carbonyl bond, OH stretching vibration between 3500-3200 cm^{-1} as a broad peak, C-H Bending *bend* vibrations of aliphatic C-H bonds at 1400-1200 cm^{-1}, 1000-1300 cm^{-1} at aromatic-OH Bending vibrations of aromatic hydroxyl groups (aromatic-OH), C-O Stretching vibrations of C-O bonds at 1200-1000 cm^{-1}, C-H Stretching vibrations of aliphatic C-H bonds at 3000-2800 cm^{-1} as showed in fig. 4. Research showed no change in the FTIR of original curcumin and curcumin nanoparticles indicated no change in the chemical composition of the samples before and after the solution-enhanced dispersion by SEDS CO_2 process and showed stretching vibrations of the benzene ring, C=C vibrations, aromatic C-O stretching, and C-O-C stretching [12].

DSC

The DSC of developed curcumin nanoparticles showed a peak maximum at 177.784 °C as shown in fig. 5, corresponding to the peak maximum of pure curcumin 177.986 similar.

The melting point of curcumin and curcumin nanoparticles was unchanged, and peaks were sharp. Due to the high crystallization extent, curcumin nanoparticles showed a lower value for the endothermic peak areas than that of original curcumin (145.618 $J{\cdot}g^{-1}$ vs 59.922 $J{\cdot}g^{-1}$). One research showed a lower value for the endothermic peak areas of curcumin nanoparticles than that of original curcumin (705.4 $J{\cdot}g^{-1}$ vs 985.1 $J{\cdot}g^{-1}$) [12].

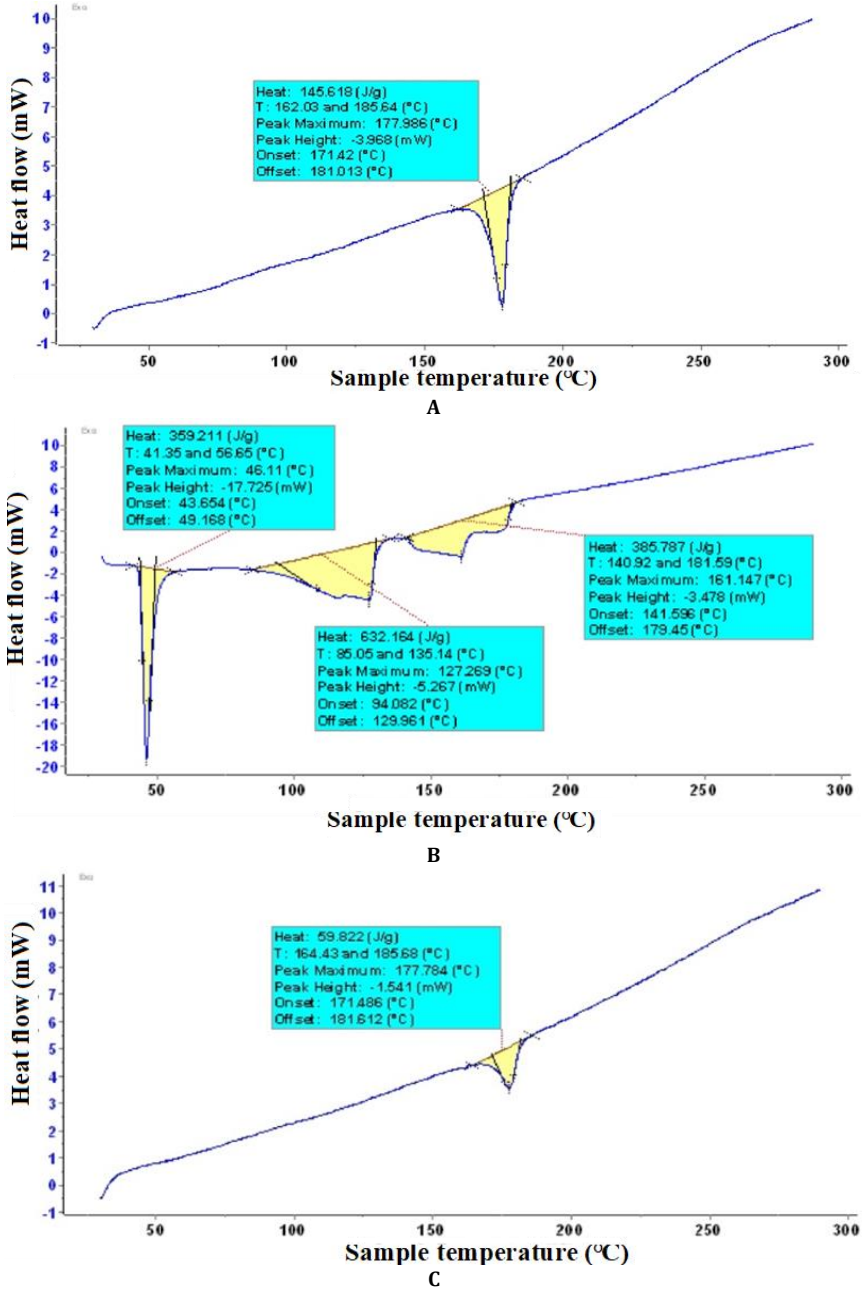


Fig. 5: DSC of (A) Curcumin, (B) Menthol, (C) Curcumin nanoparticles

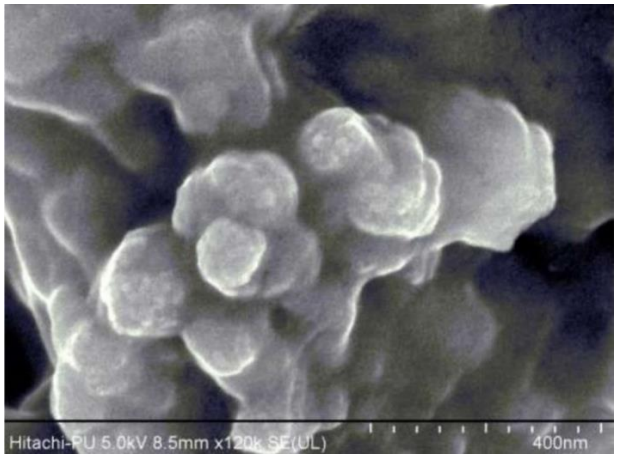


Fig. 6: SEM of curcumin nanoparticles

SEM

The SEM of curcumin nanoparticles were varied from 10-300 nm and particle size distribution analysed by Smile View software from the SEM photographs as shown in fig. 6.

The particle size analysis by Smile View software was performed using SEM images of nanoparticles shown in fig. 7 and data tabulated in table 2. The frequency distribution is shown in fig. 8.

In past years, research showed nano curcumin particles with sizes ranging from 65 to 75 nm, spherical 90-200 nm nanoparticles prepared by physicochemical fabrication method using dichloromethane under ultrasonication conditions followed by precipitate, spherical morphology 325 to 1,024 nm mean particle size curcumin nanoparticles using supercritical carbon dioxide (CO_2) [19-21].

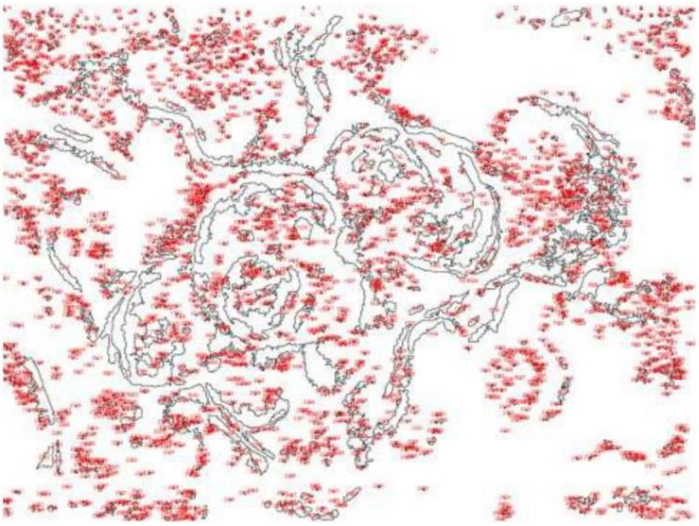


Fig. 7: Particle size analysis by Smile View software using SEM image of curcumin nanoparticles

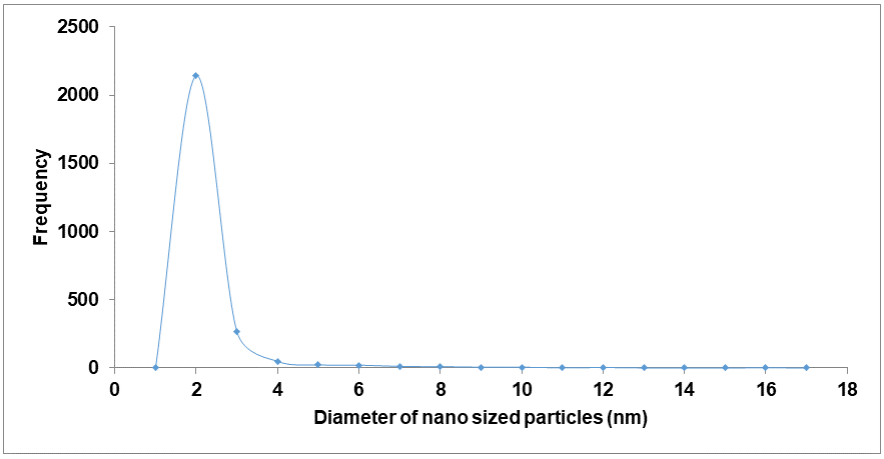


Fig. 8: Particle size distribution curve

Table 2: Particle size data of nano-sized particles

Average diameter (nm)	Frequency
0	0
2.5	2143
7.55	266
12.55	49
17.55	23
22.55	21
27.55	12
32.55	10
37.55	6
42.55	6
52.55	2
57.55	4
62.55	1
67.55	1
72.55	1
77.55	2
82.55	1

Solubility analysis

The solubility of developed curcumin performed in phosphate buffer pH 4.5 and 6.8 showed improved solubility of curcumin nanoparticles than curcumin as shown in table 3 and fig. 9 due to an increase in total surface area. The results show a significant improvement in solubility using curcumin nanoparticles and $p<0.05$. The solubility profile of curcumin and curcumin nanoparticles is shown in fig. 9 and the one-way ANOVA for the solubility profile of curcumin and curcumin nanoparticles data is shown in table 4. The enhancement solubility among curcumin and curcumin nanoparticles is shown in fig. 10. An improved solubility of curcumin nanoparticles prepared using the SEDS technique recorded than pure curcumin particles [12].

Dissolution study

The dissolution graph of curcumin powder and developed curcumin nanoparticles showed an improved dissolution as shown in table 5 and fig. 11. The results of the Paired *t-test* were significant and $p< 0.05$ shown in table 6. The curcumin nanoparticles prepared using the SEDS technique also showed better drug dissolution than pure curcumin [12].

Table 3: Solubility profile of curcumin and curcumin nanoparticles

Solubility	Curcumin	Curcumin nanoparticles	Enhancement solubility
Water	6.53±1.32	14.56±1.75	2.23
0.1 N HCl	1.98±1.54	10.56±2.18	5.33
pH 4.5 Phosphate Buffer Solution	11.43±2.01	19.74±1.67	1.73
pH 6.8 Phosphate Buffer Solution	22.95±1.65	28.69±.1.24	1.25

Data are expressed as mean±SD, n=3

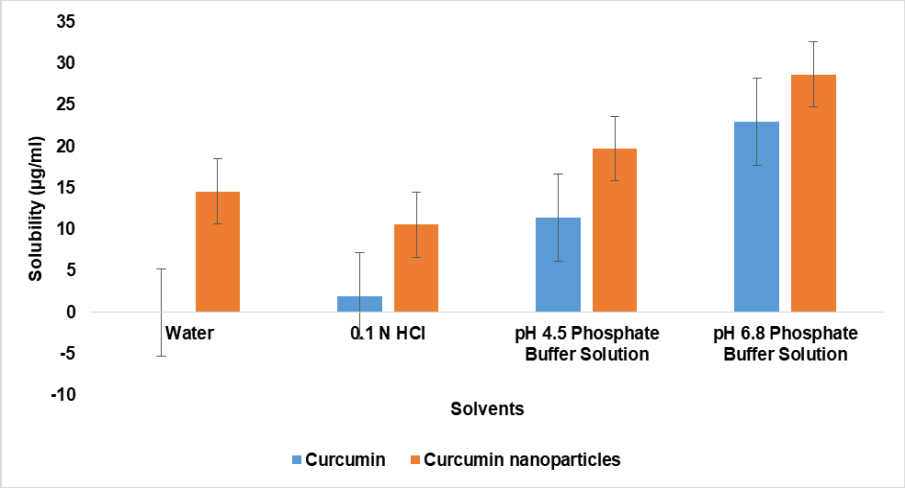


Fig. 9: Solubility profile of curcumin and curcumin nanoparticles. Data are expressed as mean±SD, n=3

Table 4: One-way ANOVA for solubility profile of curcumin and curcumin nanoparticles

ANOVA summary						
	Source	Degrees of freedom (DF)	Sum of squares (SS)	Mean square (MS)	F-stat	P-value
Water	Between groups	1	96.7214	96.7214	40.2595	0.0032
	Within groups	4	9.6098	2.4025		
	Total	5	106.3312			
0.1 N HCl	Between groups	1	181.9425	181.9425	11.3139	0.0282
	Within groups	4	64.3254	16.0814		
	Total:	5	246.2679			
pH 4.5 Phosphate	Between groups	1	102.5894	102.5894	30.0452	0.0054
Buffer Solution	Within groups	4	13.658	3.4145		
	Total:	5	116.2474			
pH 6.8 Phosphate	Between groups	1	49.4214	49.4214	23.202	0.0085
Buffer Solution	Within groups	4	8.5202	2.13		
	Total:	5	57.9416			

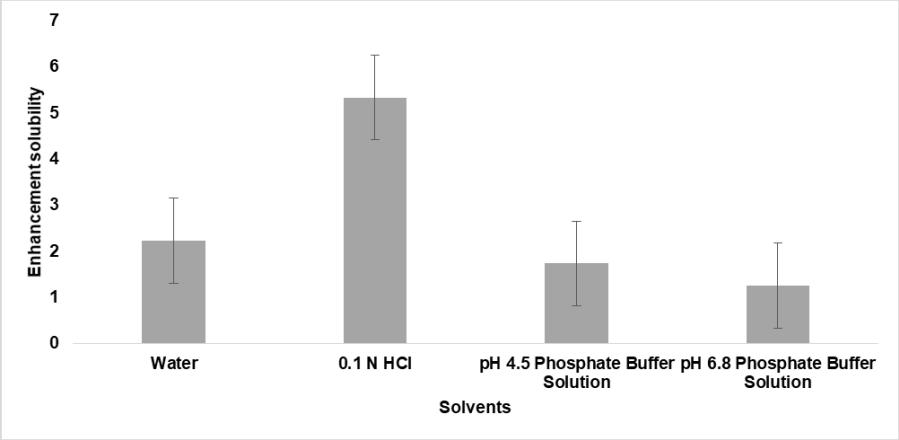


Fig. 10: Solubility enhancement of curcumin and curcumin nanoparticles. Data are expressed as mean±SD, n=3

Table 5: Dissolution data of curcumin and curcumin nanoparticles in PBS pH 6.8

Time (Min)	Curcumin (µg/ml)	Curcumin nanoparticles (µg/ml)
10	0.14±0.02	0.44±0.12
20	0.17±0.06	0.61±0.07
30	0.21±0.01	0.72±0.04
40	0.23±0.02	0.84±0.05
50	0.25±0.03	0.94±0.11
60	0.27±0.07	1.06±0.14
70	0.29±0.04	1.18±0.08
80	0.31±0.02	1.26±0.12
90	0.33±0.05	1.36±0.15
100	0.35±0.04	1.47±0.12
110	0.37±0.01	1.53±0.07
120	0.4±0.04	1.62±0.12

Data are expressed as mean±SD, n=3

The two-tailed paired *t*-test results are shown in table 6 and the P value is less than 0.0001 by conventional criteria; this difference is extremely statistically significant. The mean of curcumin (µg/ml) minus curcumin nanoparticles (µg/ml) equals-0.8092 (Confidence interval), and the95% confidence interval of this difference varied from-1.0010 to-0.6173, t = 9.2832 and degree of freedom (d_f) = 11 were the intermediate values used in calculations. The standard error of difference was 0.087.

Research showed improved solubility of curcumin nanoparticles than original curcumin, where the maximum solubility of original curcumin was about 0.2 µg/ml at 60 min increased to 1.4 µg/ml using curcumin nanoparticles developed by the SEDS process. The dissolution rate of curcumin nanoparticles was higher than the original curcumin. An increased dissolution rate and solubility of curcumin nanoparticles were due to the high specific surface area that promotes the wettability of nano-sized curcumin [12].

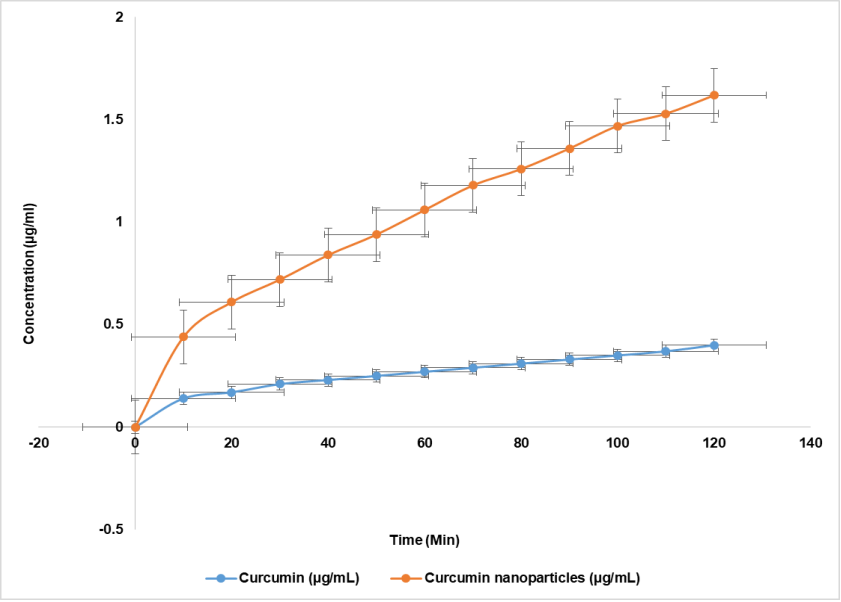


Fig. 11: Dissolution profile of curcumin and curcumin nanoparticles, data are expressed as mean±SD, n=3

Table 6: Paired *t-test* results

Group	Curcumin (µg/ml)	Curcumin nanoparticles (µg/ml)
Mean	0.2767	1.0858
SD	0.0803	0.3819
SEM	0.0232	0.1102
N	12	12

Drug release kinetic study

The curcumin and curcumin nanoparticles followed the Korsmeyer-Peppas equation model as the best-fit model and the mechanism of drug release was Fickian diffusion (Higuchi Matrix) and anomalous transport, respectively. Fickian diffusion refers to the solute transport process in which the polymer relaxation time (t_r) is much greater than the characteristic solvent diffusion time (t_d). When $t_r \approx t_d$, the macroscopic drug release becomes non-Fickian or anomalous transport that can understood as the nonlinear scaling with time of the mean square displacement of transported particles. The drug release kinetics of curcumin and curcumin nanoparticles are shown in table 7.

Table 7: Drug release kinetics of curcumin and curcumin nanoparticles

Model fitting		Curcumin		Curcumin nanoparticles	
		R^2	k	R^2	k
Zero-order		0.9057	0.0027	0.9520	0.0119
1st order		0.9061	0.0000	0.9531	-0.0001
Higuchi Matrix		0.9093	0.6351	0.9068	1.3308
Peppas		0.9871	0.2725	0.9935	0.4039
Hix. Crow.		0.9060	0.0000	0.9527	0.0000
Parameters for Korsmeyer-Peppas Equation	n		0.4197		0.5309
	k		0.2725		0.4039
Best fit model		Peppas Korsmeyer		Peppas Korsmeyer	
Mechanism of release		Fickian Diffusion (Higuchi Matrix)		Anomalous Transport	

Antioxidant activity

The DPPH assay graph of ascorbic acid is shown in fig. 12 and data in table 4. The DPPH assay graph of pure curcumin and curcumin nanoparticles is shown in fig. 13 and table 10. The IC_{50} value of ascorbic acid, pure curcumin, and curcumin nanoparticles was found to be 36.827, 114.517, and 144.308 and ANOVA results are shown in Tables 8 and 9 and fig. 12 and 13. An IC_{50} value of curcumin nanoparticles (IC_{50}=114.51) was lower than pure curcumin (IC_{50}=144.30) which indicates more potency of curcumin nanoparticles over pure curcumin. The value of $p<0.05$ is shown in table 8 for the antioxidant activity of ascorbic acid by DPPH. Significant antioxidant activity of curcumin nanoparticles was reported than pure curcumin in a study [21].

Table 8: Antioxidant activity of ascorbic acid by DPPH

Sample (ml)		Concentration (µg/ml)		% inhibition by ascorbic acid	
1		5		4.23±0.03	
2		10		9.65±0.01	
3		20		17.98±0.02	
4		40		43.13±0.02	
5		50		81.63±0.04	
Data are expressed as mean±SD, n=3					
ANOVA Summary					
Source	Degrees of freedom	Sum of squares	Mean square	F-stat	P-value
	DF	SS	MS		
Between groups	4	12155.9542	3038.9885	4469100.794	0
Within groups	10	0.0068	0.0007		
Total	14	12155.961			

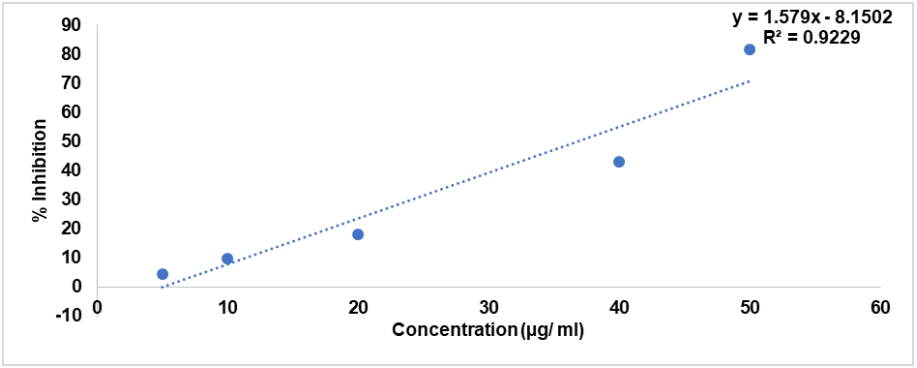


Fig. 12: Antioxidant activity of ascorbic acid by DPPH

The value of $p<0.05$ is shown in table 9 for the antioxidant activity of pure curcumin and curcumin nanoparticles by DPPH. Research showed improved antioxidant activity of curcumin nanoparticles than pure curcumin [21].

Table 9: Antioxidant activity of pure curcumin and curcumin nanoparticles by DPPH

Data summary % inhibition by curcumin					
Sample (ml)	**Concentration (µg/ml)**			**% Inhibition by curcumin**	
1	12.5			6.35±1.22	
2	25			17.56±1.53	
3	50			25.59±1.25	
4	100			36.89±2.53	
5	200			65.57±1.64	
Data are expressed as mean±SD, n=3					
ANOVA summary					
Source	Degrees of freedom DF	Sum of squares SS	Mean square MS	F-Stat	P-value
Between groups	4	6136.3586	1534.0897	529.643	0
Within groups	10	28.9646	2.8965		
Total:	14	6165.3232			
Data summary % inhibition by curcumin nanoparticles					
Sample (ml)	Concentration (µg/ml)		% inhibition by curcumin nanoparticles		
1	12.5		7.79±1.53		
2	25		21.52±1.22		
3	50		32.24±2.14		
4	100		47.14±1.15		
5	200		77.43±1.31		
ANOVA summary					
Source	Degrees of freedom DF	Sum of squares SS	mean square MS	F-Stat	P-Value
Between groups	4	8559.8826	2139.9707	934.6891	0
Within groups	10	22.895	2.2895		
Total	14	8582.7776			

Table 10: IC_{50} value of ascorbic acid, pure curcumin, and curcumin nanoparticles

	Equation of line	**R^2 Value**	**IC_{50}***
Ascorbic acid	y = 1.579x-8.1502	0.9229	36.827±2.43
Curcumin	y = 0.2935x+7.6454	0.9792	144.308±2.61
Curcumin nanoparticles	y = 0.3451x+10.48	0.9705	114.517±1.53

*Data are expressed as mean±SD, n=3

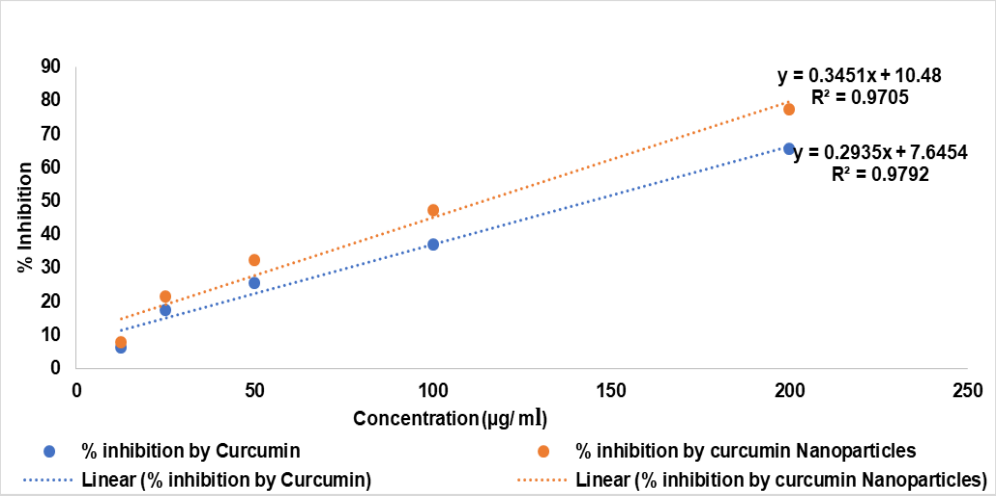


Fig. 13: Antioxidant activity of pure curcumin and curcumin nanoparticles by DPPH

Anticancer activity

The prepared nanoparticles of curcumin evaluated for *in vitro* cytotoxicity against MCF-7anticancer cell lines are shown in fig. 14, the MTT assay graph is shown in fig. 15 and the MTT assay data and ANOVA summary showed in table 11. Research showed better anticancer activity of nano curcumin than curcumin [22].

Stability studies

The developed curcumin nanoparticles showed drug content of 98.01±0.13 to 99.67±0.12% at different temperatures and relative humidity as shown in table 8 and fig. 16. The stability study data is shown in table 12 and showed significant results and $p<0.005$.

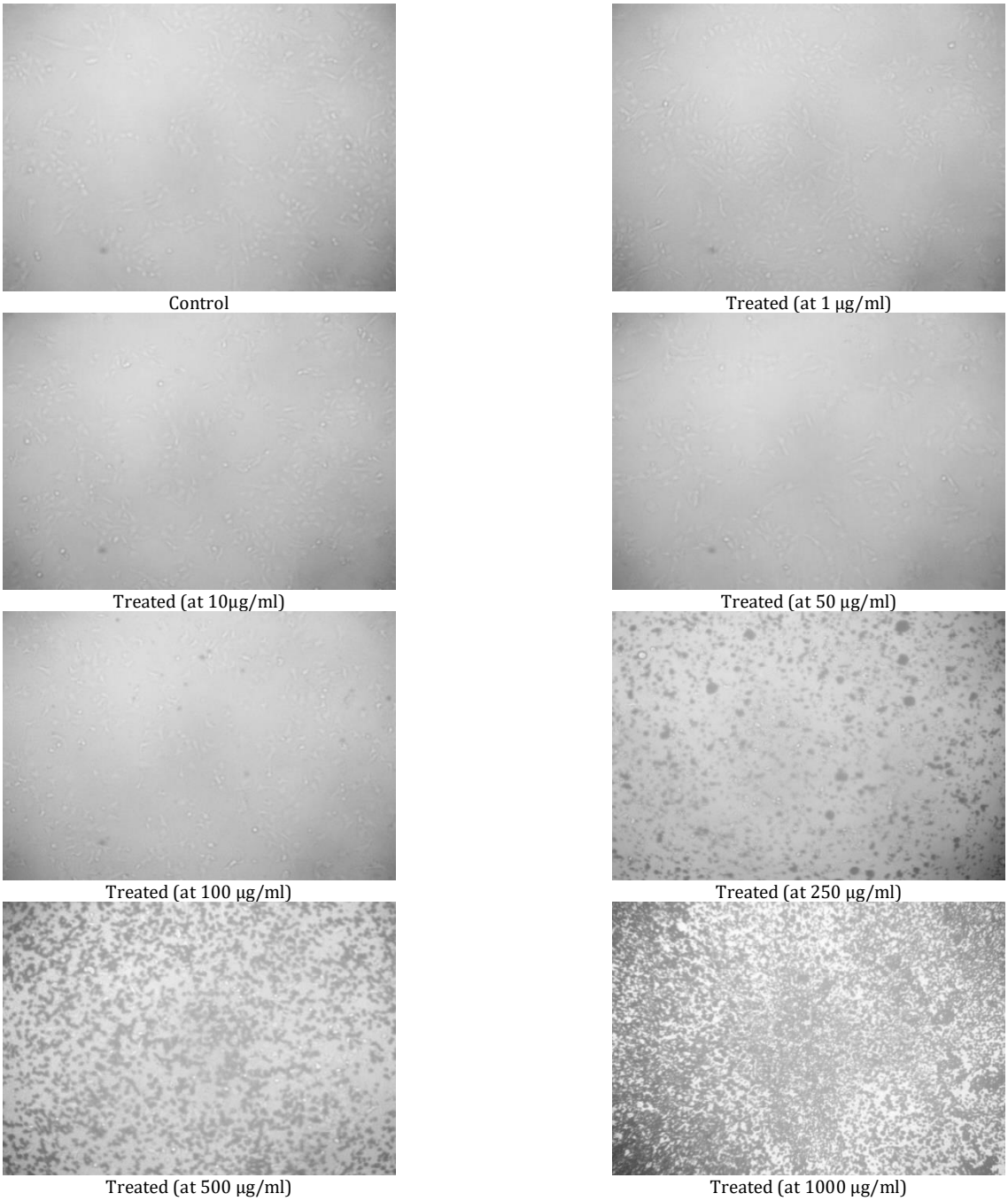


Fig. 14: MTT assay of curcumin nanoparticles

Table 11: Results of MTT assay

Sample conc. (µg/ml)	**Mean**	**SD**		**SEM**	**N**
0	100	5.749789		2.874895	4
1	95.75812	0.616543		0.308272	4
10	91.33574	0.625289		0.312645	4
50	63.71841	1.932898		0.966449	4
100	55.77617	0.779873		0.389936	4
250	44.31408	2.982439		1.491219	4
500	34.47653	2.020801		1.0104	4
1000	24.00722	3.027616		1.513808	4
IC_{50} value: 165.6±0.084 (µg/ml)					
ANOVA Summary					
Source	Degrees of Freedom (DF)	Sum of squares (SS)	Mean square (MS)	F-stat	P-value
Between groups	7	17931.81	2561.687	339.7445	0
Within groups	16	120.6406	7.54		
Total	23	18052.45			

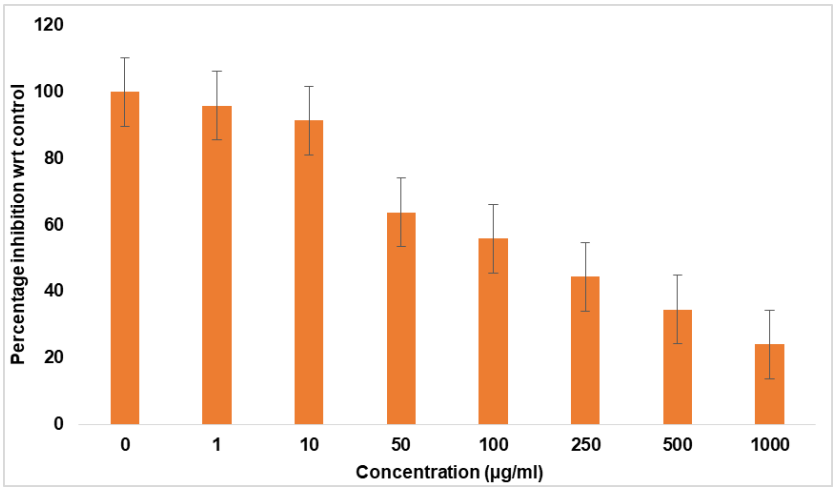


Fig. 15: MTT assay of curcumin nanoparticles, data are expressed as mean±SD, n=3

Table 12: Stability studies

Months	Temperature/Relative humidity			Drug content (%)	
1	1 Mo, 25±2 °C, 60±5% RH			99.67±0.12	
	1 Mo, 37±2 °C, 65±5% RH			99.46±0.32	
	1 Mo, 45±2 °C, 75±5% RH			99.34±0.25	
3	3 Mo, 25±2 °C, 60±5% RH			99.45±0.15	
	3 Mo, 37±2 °C, 65±5% RH			99.25±0.24	
	3 Mo, 45±2 °C, 75±5% RH			99.13±0.23	
6	6 Mo, 25±2 °C, 60±5% RH			99.32±0.43	
	6 Mo, 37±2 °C, 65±5% RH			99.04±0.54	
	6 Mo, 45±2 °C, 75±5% RH			98.01±0.13	
Data are expressed as mean±SD, n=3ANOVA Summary					
Source	Degrees of freedom	Sum of squares	Mean square	F-stat	P-value
	DF	SS	MS		
Between Groups	8	5.4967	0.6871	7.675	0.0002
Within Groups	18	1.6114	0.0895		
Total:	26	7.1081			

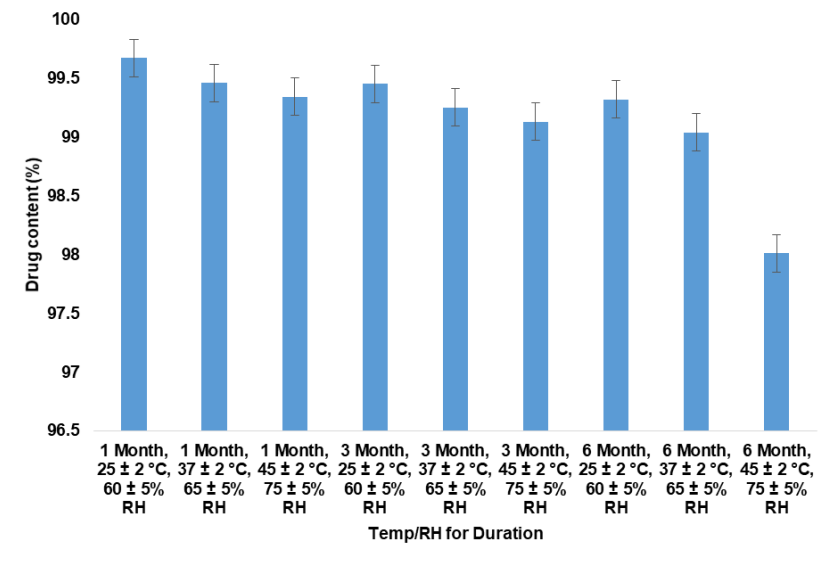


Fig. 16: Stability study of curcumin nanoparticles, data are expressed as mean±SD, n=3

CONCLUSION

The comparative FTIR of curcumin and curcumin nanoparticles showed no changes in bonds and groups that indicate the compatibility of curcumin (drug) with menthol (excipient). The Scanning electron microscopy of curcumin nanoparticles showed the particle size ranged between 10-400 nm. The mean particle size was 2 nm obtained using SmileView software. The differential scanning

calorimetry showed no interaction between curcumin and menthol. Both curcumin and curcumin nanoparticles had the same endothermic peak. The solubility profile and dissolution profile of nanoparticles improved rather to pure curcumin. The curcumin nanoparticles showed an IC_{50} value than pure curcumin indicating an improved antioxidant activity of curcumin nanoparticles than pure curcumin. The *in vitro* cytotoxicity of curcumin nanoparticles against MCF-7anticancer cell lines using MTT assay was 165.6±0.084 (µg/ml). The curcumin nanoparticles were stable. It concluded that the sublimation technique can used to prepare the nanoparticles of the drug or might be for thermo-labile drugs.

CONSENT FOR PUBLICATION

Not applicable

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AUTHORS CONTRIBUTIONS

Procure of materials, idea generation, design, lab experiment, writing was done by author and Indian Patent was granted on 18/09/2024 on this research.

CONFLICT OF INTERESTS

The author declares no conflicts of interest.

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