FORMULATION, CHARACTERIZATION AND IN-VITRO PHARMACOLOGICAL SCREENING OF CURCUMIN-LOADED QUANTUM DOTS

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ABSTRACT

Curcumin's poor water solubility and limited oral bioavailability limit its therapeutic usefulness. Due to a lack of malignancy selectivity, conventional approaches have failed to progress clinically. To overcome these obstacles, a new curcumin nano formulation that can enhance tumor localization and cancer-specific therapy was created by mechanically shaking chitosan polymer. Our research demonstrated the successful production of curcumin-loaded quantum dots that were smooth and spherical and had an encapsulation efficiency of 94.75 0.62%. The range of the particle size distribution was from 87.68 nm to 88.25 nm. These curcumin-loaded quantum dots were examined in an anti-inflammatory and anti-bacterial investigation. The anti-inflammatory and antibacterial study revealed that the curcumin-loaded quantum dots were able to produce a more dramatic effect. Our investigations were effective in creating curcumin-loaded quantum dots, demonstrating the high potential of curcumin nanoparticle-based formulation as an adjuvant medicine for use in clinical antibacterial and anti-inflammatory research. In the future study we aimed to target the cancer cell by the conjugation of quantum dots & produce the anticancer effect in effectively.

Keywords: Curcumin, Quantum dots

1. INTRODUCTION

Nanotechnology has significant applications in our daily lives and is transforming society as a whole. The 'nano' prefix, which means 'dwarf' or very small in Greek, represents one billionth of a meter (10-9 m). It is important to differentiate between nanoscience and nanotechnology. Nanoscience refers to the examination of structures and molecules on nanometer scales ranging from 1 to 100 nm, whereas nanotechnology is the technology that employs it in practical applications, such as devices, among other things. [1,2] The nanotechnology processes primarily include Separation, Consolidation, and Deformation, which are performed on a single atom or a single molecule. The technological innovation being discussed herein presents a wide range of potential applications due to the alteration it creates in material properties. Specifically, materials that are traditionally considered non-conductive or insulating can exhibit properties akin to semiconductors at the nanoscale. [3,4]

1.1. QUANTUM DOTS:

Quantum dots (QDs) are luminescent semiconductor nanocrystals of nanometer dimensions. Their optical characteristics, including high luminosity, enduring stability, multiplexed signal detection, and adjustable emission spectra, render them a promising diagnostic and therapeutic modality for oncological applications.[5]

Quantum dots, which are small particles of nano crystalline material, possess distinctive optical and transport properties.[6] Typically measuring between 1-10 nm, quantum dots are extensively employed for their ability to emit light of specific wavelengths when energy is applied. The precise tuning of these wavelengths is achievable through modifications of particle shape, material composition, and size.[7] Example: CdSe, CdTe, PbSe, InP, etc…

Turmeric, renowned for its distinctive taste and hue, is a commonplace spice in the culinary preparations of India and other Asian nations. [8,9] The compound has demonstrated the ability to target multiple cancer cell lines. However, the applications of curcumin are limited due to its low water solubility, resulting in poor oral bioavailability and chemical stability.[10] The curcumin molecule tends to penetrate the cell membrane and bind to the fatty acyl chains of membrane lipids through hydrogen binding and hydrophobic interactions, resulting in low availability of curcumin within the cytoplasm. In order to surmount the obstacles and augment the overarching anticancer efficacy of curcumin, numerous structural alterations have been proposed to amplify selective toxicity towards particular cancer cells, heighten bioavailability, or fortify stability. [11]

These curcumin derivatives are compounds that retain the fundamental structural characteristics of curcumin, such as the two dioxy-substituted benzene rings, the -- C=C----CO----CH2--CO--C=C-linker, and the oxy substituents on the benzene rings. Essentially, curcumin derivatives are synthesized through derivatization, starting from curcumin. For instance, the phenolic hydroxy group may be modified through processes such as acylation, alkylation, Glycolation, and demethylation. [12]

Originally derived from marine polysaccharides found in shrimps and crabs, chitosan was initially considered an easily obtainable substance from food industry waste.[13]

The utilization of chitosan in conjunction with carcinoma cell lines resulted in a decrease in the level of MMP9 protein activity, with a corresponding increase in antimetastatic behavior observed with increased chitosan concentration. Chitosan, a bio-based polysaccharide, exhibits substantial biological and antitumor properties and holds great promise in this regard. [14,15]

2. MATERIALS AND METHODS:

2.1. Determination of λ max for curcumin:

A quantity of 10 milligrams of curcumin was dispersed in a solution containing 100 milliliters of 0.1 normal hydrochloric acid having a pH value of 1.2. The resulting solution had a stock concentration of 0.1 milligrams per ml. Using this stock solution, a diluted solution, with a concentration of 10 micrograms per ml, was prepared. Subsequently, this diluted solution was subjected to UV spectrophotometric scanning, within the wavelength range of 200-400 nanometers, to determine the maximum absorption wavelength of curcumin in this medium. [16]

2.2. Formulation of curcumin quantum dots:

2.2.1. Preparation of zinc oxide (ZnO) quantum dots:

In accordance with the prescribed methodology, zinc chloride was dissolved in nitric acid (HNO3), followed by the addition of methanol and subsequent drying at an appropriate temperature. The resultant dried sample was further dissolved in methanol. Subsequently, sodium hydroxide, which was dissolved in methanol, was added to the zinc nitrate solution with vigorous stirring after a certain period of time. The temperature was sustained for a specific duration, and as a result, the solution turned translucent. Thereafter, the quantum dots began to precipitate, leading to a turbid solution. The stirring and heating procedures were halted, and the precipitation of these nano particles continued for an additional period of time. The resultant precipitate was obtained, and the mother liquor was separated, following which the precipitate was washed with methanol. [17]

2.2.2. Drug loading on ZnO quantum dots:

Initially, ods (QDs) were dispersed in methanol. Subsequently, curcumin was introduced into the mixture, whereby it dissolved owing to its solubility in methanol. Cold water was then gradually added to the QDs-drug mixture. The purpose of this step was to enable the coating of curcumin onto the QDs. Notably, curcumin was released and loaded onto the uncoated QDs. Ultimately, the drug-loaded QDs were subject to drying in an oven at 70 °C.

2.2.3. Polymer coating on drug loaded quantum dots:

To commence the experiment, the chitosan polymer was dissolved in chloroform. Subsequently, the drug loaded QDs were quantified and dispersed in the aforementioned polymer solution. Following this step, the resultant mixture was left undisturbed for a duration of 15 minutes, after which it was agitated. Following this step, the mixture was subjected to filtration using a Whatman filter paper. Finally, the polymer-coated QDs were left to air dry overnight for a period of 24 hours. [18]

S.No	Formulation Code	Drug(gm)	Polymer (gm)
1.	F1	1	1
2.	F2	1	1.5
3.	F3	1	2
4	F4	1	2.5
5	F5	1	3

Table 1: Composition of drug loaded using different ratio of polymer.

3. EVALUATION OF CURCUMIN QUANTUMN DOTS:

3.1. Particle Size analysis:

The evaluation of the dimensions of curcumin quantum dots was conducted utilizing the microtac blue wave particle size analyzer. The outcomes acquired for the distributions of particle dimensions were implemented to validate the establishment of particles at the nanoscale. [19,20]

3.2. Zeta Potential Analysis:

The zeta potential, which serves as a measure of particle charge in colloidal systems, was determined by analyzing the electrophoretic mobility of said particles under the influence of an electrical field. The zeta potential analysis of the formulation containing curcumin quantum dots was conducted utilizing a Malvern zeta seizer, an instrument provided by Malvern. Prior to measurement, the samples underwent dilution with de-ionized water, and conductivity was adjusted through the addition of sodium chloride. [21,22]

3.3. Drug Content Determination:

The drug content of the curcumin quantum dot was examined utilizing a UV spectroscopic approach. The curcumin quantum dot that was prepared was precisely weighed to constitute 10 mg of curcumin and was dissolved in 10 ml methanol. Subsequently, the stock solutions were suitably diluted with distilled water and subject to UV spectroscopy at 425 nm. [23,24]

$$\text{Drug content} = \frac{\text{Abs. of sample X Wt. of standard X Total weight}}{\text{Abs. of standard X Wt of sample.}} \times 100$$

3.4. Determination of Entrapment Efficiency (EE) of curcumin quantum dot:

A total of 10 milliliters of curcumin quantum dot underwent centrifugation at a speed of 5,000 revolutions per minute for a duration of 20 minutes. Following this process, the supernatant solution was subjected to filtration and separation. A fraction of 1 milliliter of this filtrate was diluted with water and the absorbance was examined at maximum λ max by utilizing a UV spectrophotometer at a wavelength of 425 nanometers, with water serving as a reference. [25,26] The quantity of free drug in the formulations was measured, and the entrapment efficiency is then calculated from Eq.1

$$E.E\ \% = \frac{\text{Total drug in formula} - \text{Free drug}}{\text{Total drug in formula}} \times 100$$

3.5. Saturation Solubility Studies:

The investigation on saturation solubility was conducted for both curcumin and curcumin quantum dots. 10 mg of unprocessed curcumin, as well as curcumin formulation that is equivalent to 10 mg of curcumin, were accurately weighed and introduced separately into 25 ml stopper conical flasks containing 10 ml of distilled water. The flasks were firmly sealed and put into a rotary shaker for 24 hours at 37 °C followed by 2 days. After the specified time interval, the samples were collected, filtered, and analyzed via UV spectrophotometer at 425 nm. [27,28]

3.6. In-Vitro Drug Release Studies:

The in-vitro release of pure curcumin and its formulation were conducted using the USP dissolution test apparatus with the paddle method at a rotation speed of 50 RPM. The dissolution profile was assessed in freshly prepared acidic buffer (pH 1.2) and phosphate buffer (pH 7.4). A quantity of 10 mg of curcumin and curcumin formulation that contained 10 mg of curcumin equivalent were placed in the dissolution medium. The volume and temperature of the dissolution medium were 900 ml and 37.0 ± 0.2 °C, respectively. The samples were withdrawn at predetermined time intervals and subsequently filtered. The filtered samples were analyzed at 425 nm using a UV spectrophotometer. All determinations were performed in triplicate. The results obtained from the formulation were then compared with the dissolution profile of the unprocessed drug. [29,30]

3.7. Scanning Electron Microscopy:

Microphotographs were captured at various levels of magnification to observe surface morphology. The utilization of scanning electron microscopy facilitated the analysis of curcumin quantum dot, ultimately confirming the nanosized formulation.[37] The process involved the gentle application of the sample onto a double-sided adhesive tape, adhered to an aluminum stub. The substrate was then coated with platinum, having a thickness of approximately 10 A, under an argon atmosphere, utilizing a gold sputter module within a high vacuum evaporator. Following these steps, the stubs containing the coated sample were placed within the scanning electron microscopy chamber to analyze surface morphology.[31]

3.8. Thermal analysis:

The thermal properties of curcumin quantum dot were examined through differential scanning calorimetry analysis utilizing the Toledo-DSC II instrument. In order to identify the modifications in the internal structure, DSC analysis was executed for pure curcumin, polymer, and the curcumin quantum dot.[32] A total of 5 mg of the sample were placed in an aluminum vial and inserted into the instrument. Under a stream of nitrogen at a flow rate of 50 ml/min, the sample was heated from 20°C to 200°C at a heating rate of 10°C/min. Enthalpy changes (ΔH) were assessed peak to investigate the polymeric changes in the formulations. [33,34]

3.9. Powder X-Ray Diffraction Pattern:

The deployment of X-ray powder diffractometry facilitated the recording of the crystalline structure of curcumin dispersed in quantum dot. [35,36] To further investigate, X-ray diffraction pattern (XRD) studies of curcumin quantum dot will be conducted utilizing X-ray diffractometer with Cu as target filter, operating at a voltage/current of 40 KV/40 Ma and a scan speed of 1°/min45,46. Analysis of the samples will be executed. [37,38]

4. ANTIMICROBIAL ACTIVITY:

4.1. Determination of Zone Inhibition:

The present study employed the well diffusion method for the purpose of assaying the antibacterial activity against test strains on agar plates. In order to achieve this, a total of 100µL of diluted inoculum from organism suspensions was carefully spread on the surface under aseptic conditions on the agar medium. The plates were subsequently filled with solutions of nanocurcumin and curcumin, both at a concentration of 400µg, while DMSO was utilized as the control. Following an incubation period of 24h at 37℃, the antimicrobial activity was evaluated by measuring the diameter zone of transparent inhibition against the test microorganism. [39,40]

4.2. Invitro Anti Inflammatory Activity:

In accordance with previously reported protocol, the reaction mixture was composed of 0.2ml of egg albumin derived from fresh hen's eggs, 2.8ml of phosphate buffered saline with a pH of 6.4, and 2mi of varying concentrations of the test extract. An equivalent volume of double-distilled water was implemented as a control. The mixture was then subjected to incubation at 37 ℃± 2℃ in an incubator for 15 minutes, followed by heating at 70 for 5 minutes. The absorbance was measured at 660 nm after cooling, with vehicle as the blank. Diclofenac sodium was utilized as the reference.[41] The percentage inhibition of protein denaturation was determined using the subsequent formula. **% inhibition = {(Abs of control - Abs of test)/Abs of control} x 100**

5. RESULTS AND DISCUSSION:

5.1. Zeta potential analysis:

It is generally recommended to maintain a zeta potential of at least -30 mV for electrostatically stabilized systems or -20 mV for sterically stabilized systems to achieve physical stability in nano formulations. In the present investigation, it was observed that the zeta potential of curcumin nano formulation exhibited negative values due to the presence of negatively charged soya lecithin. The measured zeta potential values were found to be low, ranging from -1.67 to -26.3 mV. Furthermore, a reduction in surface diffusion of drug nanoparticles was observed, resulting in an increase in the concentration gradient and ultimately leading to an enhanced dissolution rate. This makes it a suitable option for both active and passive targeting in cancer therapy. Additionally, research has indicated that negatively charged nanoparticles coated with hydrophilic polymers can effectively evade the opsonization process, regardless of their particle size, promoting improved drug delivery into cancer cells.

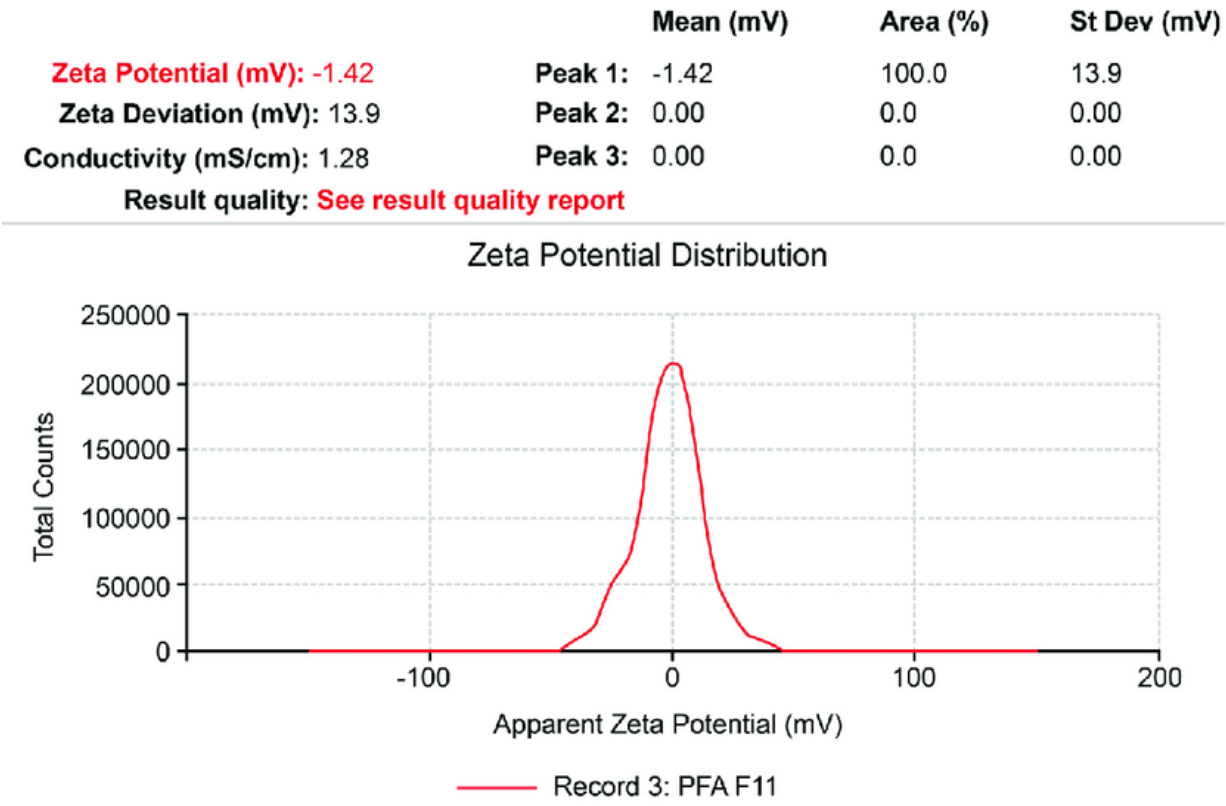


Figure1: Zeta potential of nano formulation

5.2. Drug content determination:

Notably, the F1 nano formulation exhibited a particularly high percentage of drug loading, thereby making it a clinically viable option for drug delivery.

5.3. Drug entrapment efficiency:

The entrapment efficiency of curcumin in F1 (95.49±0.63%) was significantly higher when compared to other nano formulations, potentially attributed to the presence of optimal polymer concentrations relative to the remaining formulations.

S.No	Formulations	Drug content	Drug entrapment efficiency (%)
1.	F1	95.49±0.63	70.0 ± 0.23
2.	F2	96.61±0.32	82.75±0.62
3.	F3	90.73±0.12	78.26±0.86
4	F4	92.00±0.61	76.12±0.48
5	F5	91.34±0.56	80.45±0.01

Table 2: Percentage of drug content & drug entrapment efficiency of curcumin nano formulation

Each value represents the mean ± SD(n=3).

5.4. Saturation solubility studies:

S.No	FormulationCode	Absorbanceat425 nm(mg/L)	
		0.1N HCL BUFFER	PHOSPHATEBUFFER
1.	Pure	19.67 ± 0.04	25.45 ± 0.23
2.	F1	53.73±0.56	95.21±0.61
3.	F2	61.34 ± 0.17	74.71 ± 0.53
4.	F3	55.45±0.16	72.10±0.61
5	F4	58.10±0.91	64.17±0.81
6	F5	48.28±0.61	61.61±0.51

Table 3: Saturation solubility studies of pure drug and nano formulation.

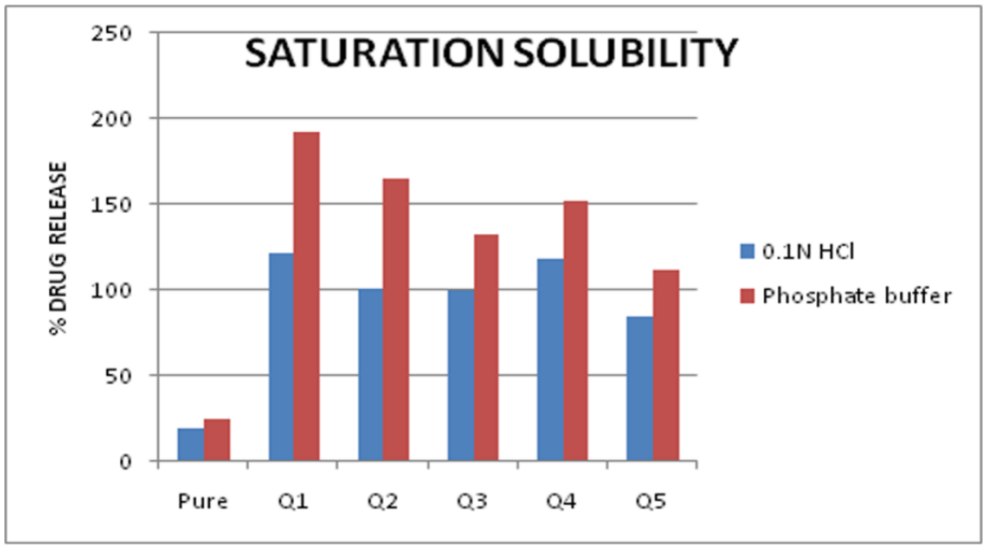


Figure2: Saturation solubility studies of curcumin and curcumin nano formulation

Table 4: *In-vitro* dissolution study for curcumin QD formulation

S.NO	TIME (min)	PURE	F1	F2	F3	F4	F5
1	0	0	0	0	0	0	0
2	30	1.94143	23.6207	14.2371	10.3543	6.795	4.53
3	60	3.88286	29.7686	20.385	15.5314	10.6779	7.44214
4	90	5.50071	33.6514	25.5621	19.4143	12.9429	9.06
5	120	6.14786	37.5343	27.8271	23.2971	15.5314	10.3543
6	180	7.11857	42.7114	34.2986	28.4743	19.0907	12.6193
7	240	9.38357	47.2414	39.1521	33.6514	22.0029	18.7671
8	360	11.6486	66.0086	52.4186	42.3879	33.6514	25.2386
9	480	24.2679	73.7743	68.5971	56.9486	46.2707	39.4757
10	600	30.4157	86.7171	77.01	67.95	57.5957	46.5943
11	720	38.1814	91.2471	80.2457	70.5386	62.1257	49.83

5.5. *INVITRO* DRUG RELEASE:

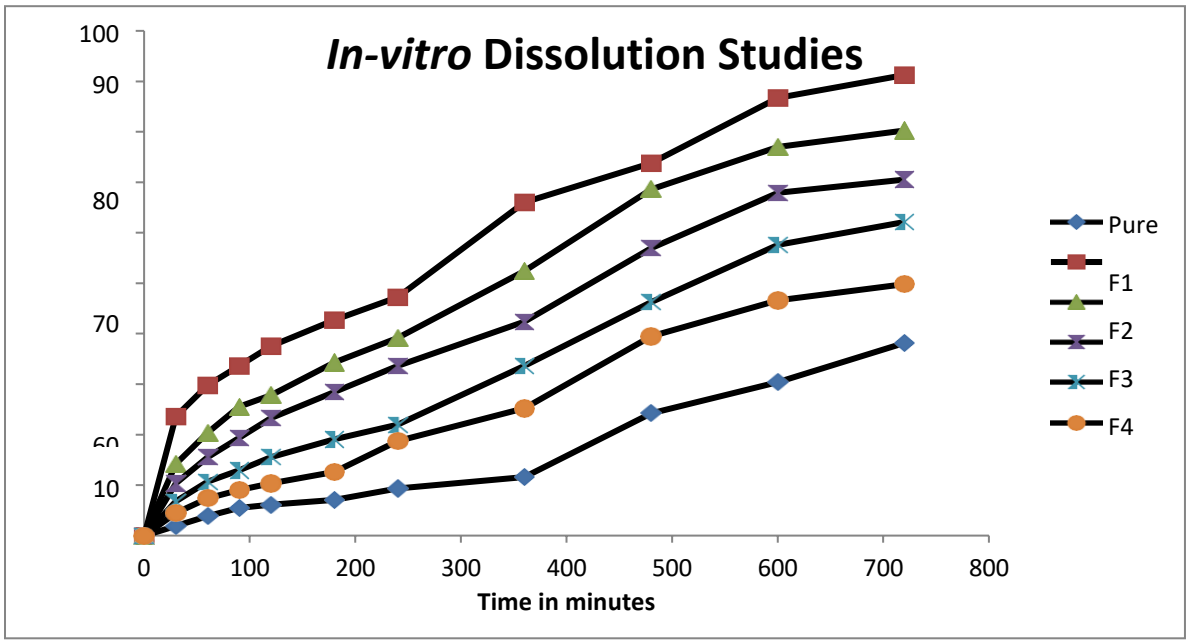
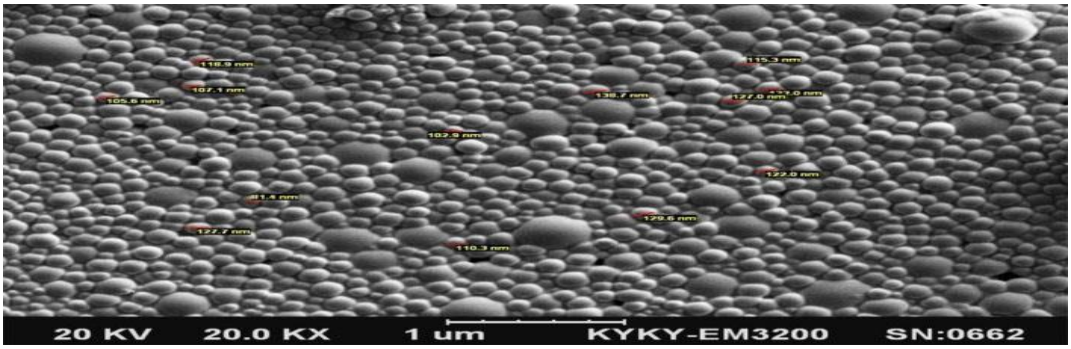


Figure3: Comparative *in-vitro* dissolution profile of pure drug and their formulation

5.6. Scanning Electron Microscopy

The surface morphology of particles was examined through the application of scanning electron microscopy. Notably, the SEM image of pure curcumin displayed a greater abundance of larger particles when compared to the nano formulation that utilized chitosan, soya lecithin, and cholesterol, and thus exhibited superior surface characteristics. Specifically, the curcumin nano formulation featured particles that were smaller in size, with most exhibiting similar dimensions, and furthermore demonstrated a smooth texture devoid of polymeric aggregates. This outcome can be attributed to the hydrodynamic process that occurs during particle preparation, which facilitates higher loading efficiency of curcumin in the surrounding aqueous phase.



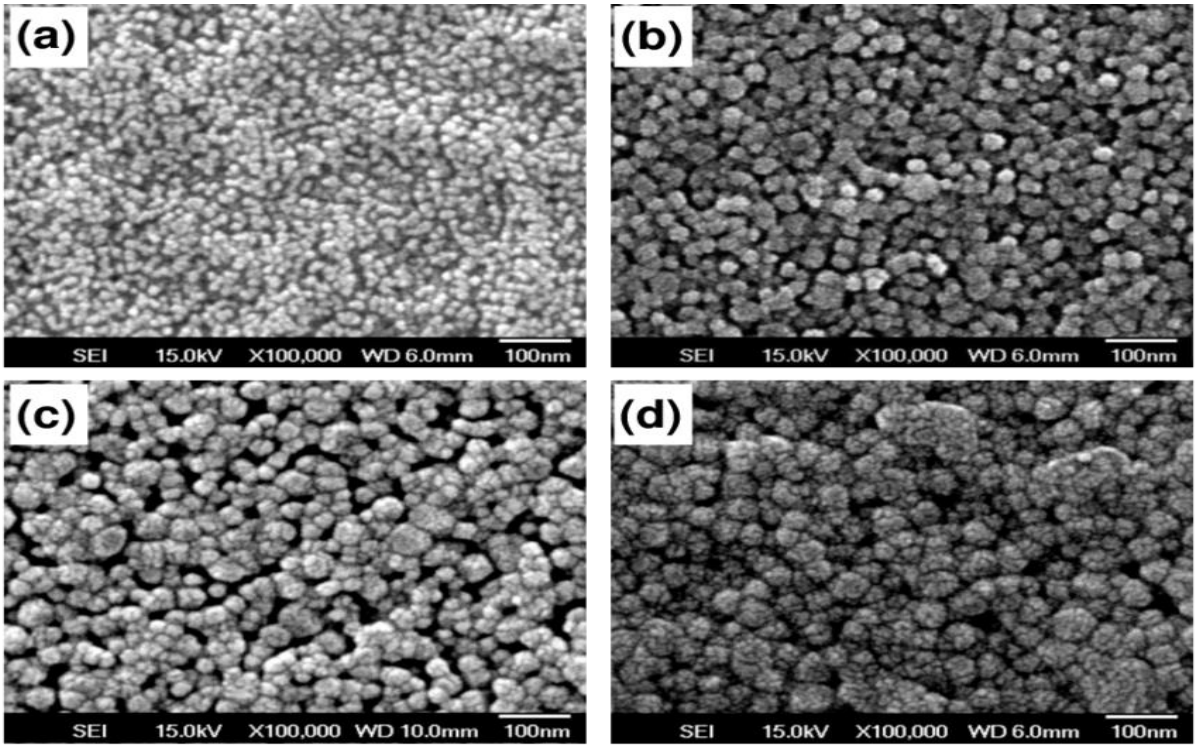


Figure4: SEM image of quantum dots.

5.7. Powder X-ray diffraction analysis (PXRD):

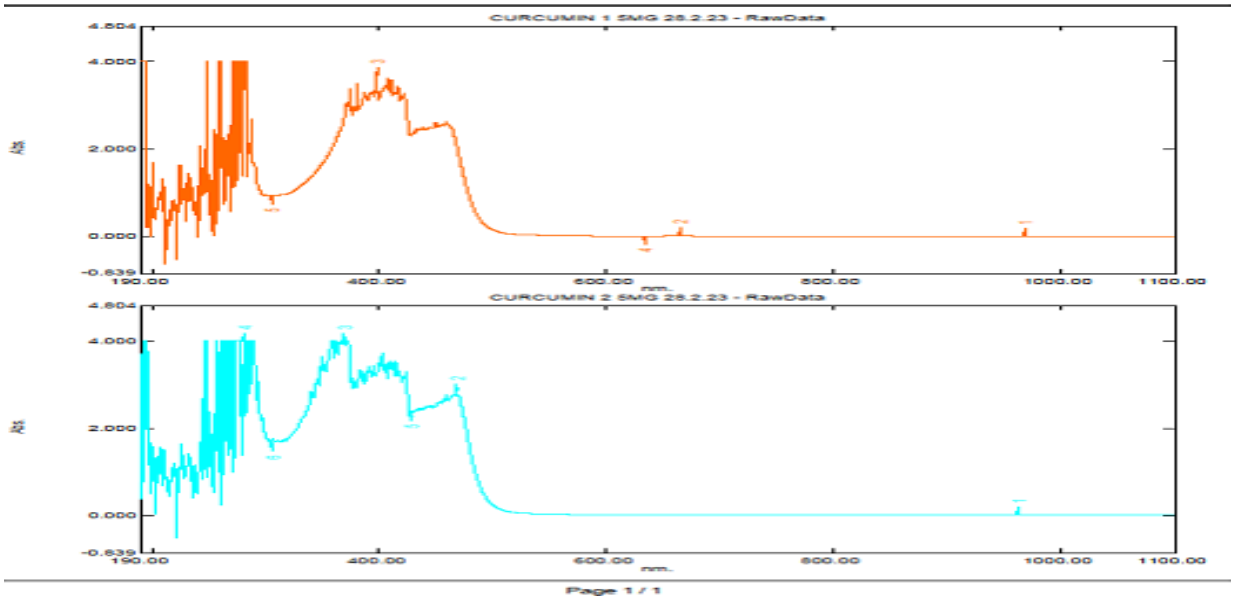


Figure5: P-XRD of curcumin pure drug and nano formulation

The utilization of powder X-ray diffraction methodology is a valuable instrument in ascertaining the physical properties of particles. In the provided figure, the patterns of powder X-ray diffraction for free curcumin, polymer, and the curcumin nano formulation are depicted. The characteristic crystalline peaks of 2θ of 10.83◦, 11.49◦, 15.75◦, 17.27◦, 18.07◦, 20.35◦, 23.73◦, 25.37◦, and 27.71◦ were displayed by free curcumin. However, curcumin nano formulation did not exhibit any such crystalline peaks. The absence of detectable crystalline domains of curcumin in the nano formulation clearly indicates that it is in an amorphous or disordered crystalline phase, or in the solid solution state

5.8. Thermal analysis

The DSC thermograms of free curcumin and curcumin nano formulation are shown in the figure. The melting endothermic peak observed at 170 to 183 ◦C in free curcumin indicates its crystalline nature.

5.9. Antimicrobial study:

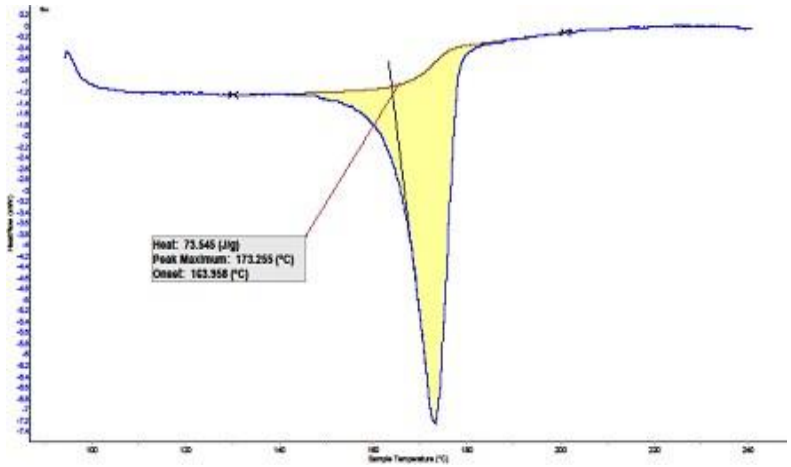


Figure6: DSC of curcumin nano formulation

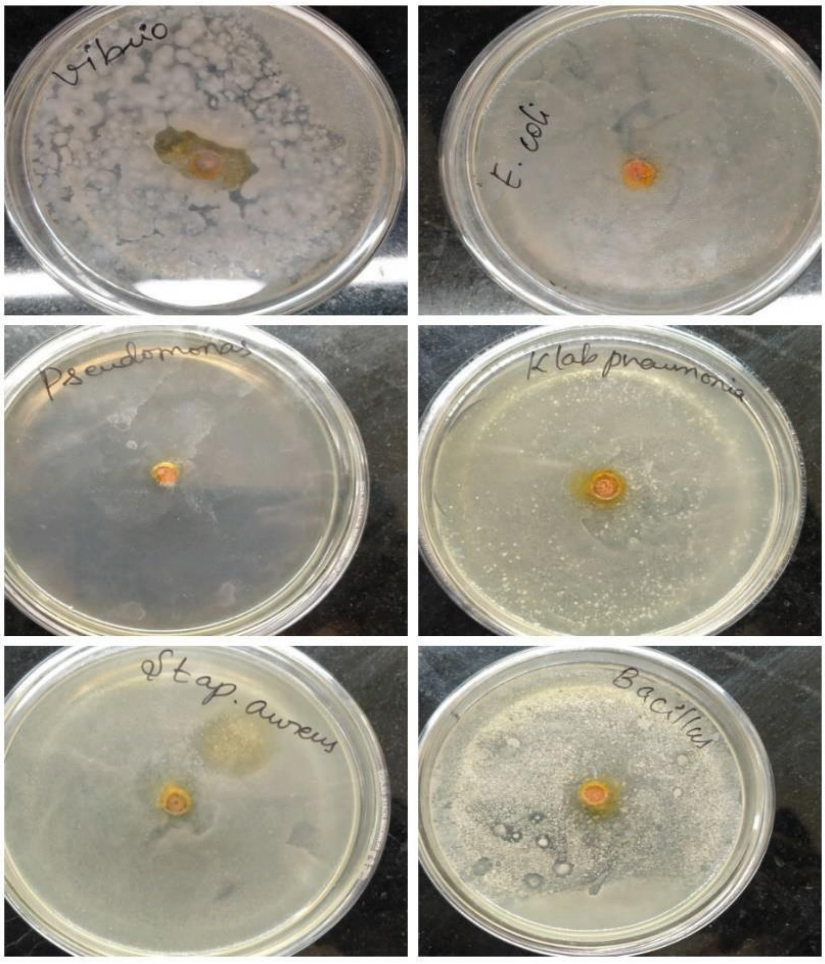


Figure7: Antimicrobial study for curcumin QD in various microorganism.

Organism	Zone of Inhibition in mm				
	PureCurcumin 10µg/ml	Curcumin of Qd in µg/ml			
		0.5	2.5	5	10
E.coli	15.65±2.65	6.78±1.12	15±1.2	21±3.2	25.45±1.34
S.aureus	17.46±3.13	5.85±0.50	12.02±2.1	20±0.54	24.67±0.98
Pseudomonas	18.20±2.32	6.35±1.43	10±1.21	19±1.89	22.56±1.23
Bacillus	19±2.94	6.23±0.49	11±2.34	18.22±2.76	20±2.78
Vibrio	15.78±2.10	5.58±2.10	8±1.56	19.23±3.45	21.67±2.59
Pneumonia	10.66±1.19	5.12±1.19	7.95±4.2	12.34±1.2	18.32±2.9

Table 5: Zone of inhibition of curcumin QD

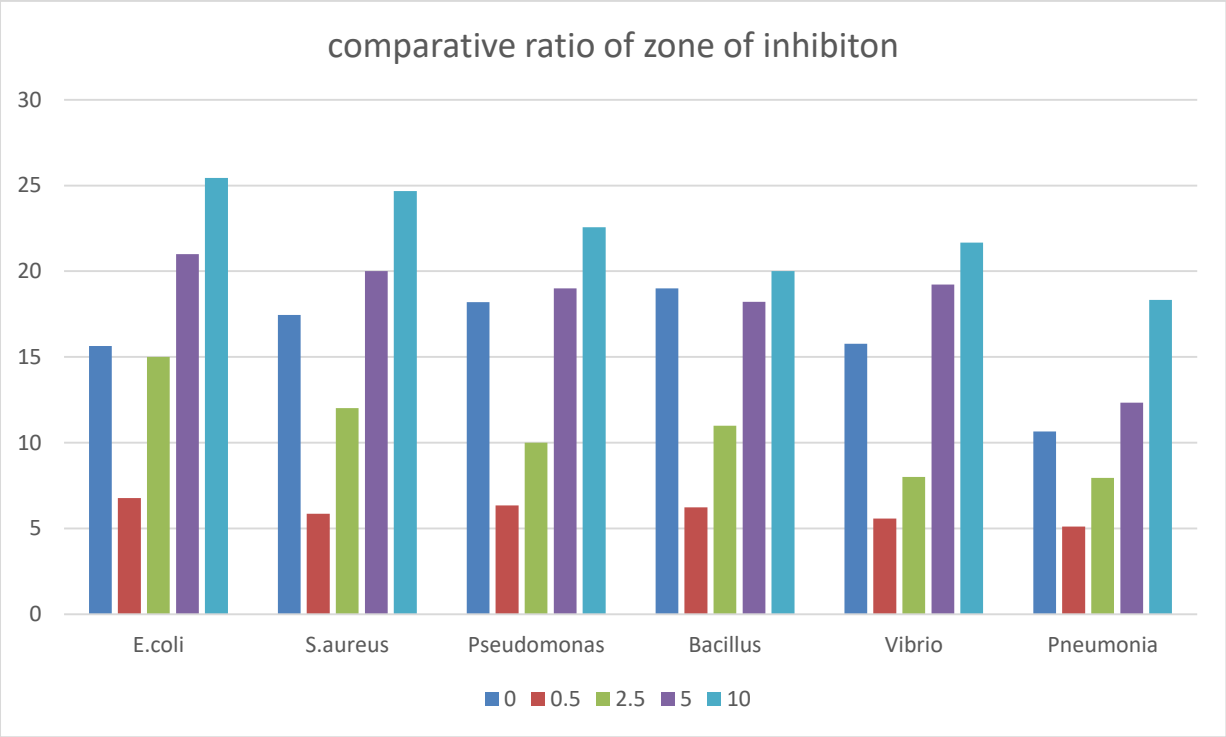


Figure 8: Antimicrobial activity of curcumin QD

5.10. Anti-inflammatory study

S.no	Concentration	Pure curcumin	Curcumin QD
1	0	0	0
2	50	31.42	30.65
3	100	43.22	42.45
4	200	58.45	56.87
5	400	70.34	68.98

Table6: Anti-inflammatory activity of Curcumin QD

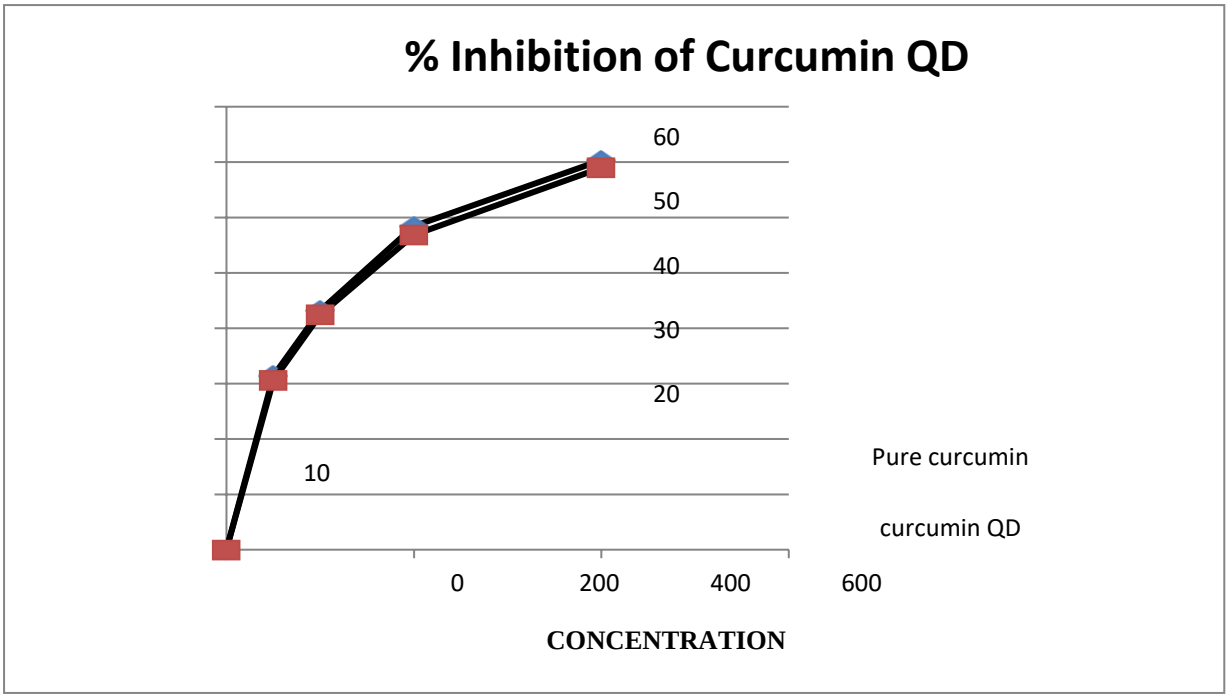


Figure 9: Percentage inhibition of curcumin QD

6. SUMMARY AND CONCLUSION:

The clinical efficacy of curcumin is constrained by its poor aqueous solubility and low oral bioavailability. Conventional methods have proven unsuccessful in clinical development due to a lack of cancer specificity. To surmount these challenges, a novel curcumin nano formulation was successfully developed through the mechanical shaking of chitosan polymer, which can improve tumor localization and cancer-specific therapy. These innovative formulations have the potential to enhance curcumin dissolution and BBB penetration, making them a promising means of administering cancer treatment.

The utilization of a nano formulation has been found to enhance the anti-cancer and antimicrobial effects. The study's findings provide compelling evidence for the potential use of curcumin-loaded nano formulations as an agent for brain targeting, chemotherapeutic and cytotoxic activities in brain tumor cancer cells. This novel formulation holds great promise for improving the efficacy of future cancer therapies while remaining cost-effective. The *in-vivo* anticancer study has identified numerous future prospects for further research in this area. Specifically, the nano formulation should be developed as a targeting method for brain tumor cells, with the ability to target images and kill tumor cells without negatively affecting normal cells through the use of active targeting methods. Modifications to the surface of quantum dots and the conjugation of ligands are recommended for future prospects studies.

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