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CURCUMIN NANO ENCAPSULATION AND OXIDATIVE STRESS IN NEURODEGENERATIVE DISEASES

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ABSTRACT

Back ground*: Curcumin as a chemical found in turmeric has attracted significant interest from researchers today, due to its medicinal uses consequently is regarded as a potent antioxidant. It may have significant in neurodegenerative diseases in improvement of working memory, reduced fatigue and stress reactivity in older peoples, Curcumin protects against mitochondrial dysfunction by lowering ROS levels and increasing glutathione production. However, limitations in curcumin such as its exceedingly low oral bioavailability limit its use as a medicinal agent, Curcumin bioavailability will be improved with the latest Calcium phosphate Nanoparticles encapsulation due to the valuable properties of calcium phosphate nanoparticles, including biocompatibility, biodegradability, and high affinity for binding to nucleic acids, make these particles suitable materials for the administration of drugs. The size-controllable, stable, pH-responsive, and targeted CaP nanocarriers have been made using an array of methodologies. Cap nano curcumin might break through blood-brain barrier crucially to treat neurodegenerative diseases.* ***Methods:*** *Four variety of Turmeric were dried and powdered. 5 gm of turmeric was weighed and Soxhlet extracted in 250 ml of ethanol. Extract was evaporated and sample was weighed to calculate. 50 ml of ethanol was added to get a specific concentration, Fractions with highest concentration of curcumin were selected for HPLC analysis. 1mg/ml Standards of curcumin was prepared by dissolving in methanol, The amorphous calcium phosphate (ACP) nanoparticles were prepared through the co precipitation method.* ***Aims****; The goal of this research is to present a current Nano biotechnological approach of curcumin encapsulation with CaP nanoparticles that will be use for in vitro study in treatment of age-related neurodegenerative diseases using SH-SY-5Y neuroblastoma cells induce with Alzheimer's and Parkinson's diseases,* ***Conclusion*** *Curcumin is a naturally occurring functional polyphenol whose inadequate solubility and low bioavailability limit its applications, thus It is difficult to achieve the necessary therapeutic efficiency of neurodegenerative diseases drugs due to the presence of physiological barriers, This research aims to encapsulate curcumin with calcium phosphate nanoparticles which will be use for in vitro studies targeting triggered neurodegeneration using SH-SY-5Y neuroblastoma cells.*

Key words: Oxidative Stress, Neurodegenerative Diseases *and Calcium phosphate Nanoparticles.*

1.0 Introduction

Although its natural fluorescence characteristics are frequently ignored, curcumin is a naturally functional polyphenol that its effectiveness is limited by inadequate solubility and low absorption. . It has been found that oxidative stress affects aging and a number of neurological disorders; however it makes it difficult to achieve curcumin desired therapeutic efficiency of treatments due to the presence of physiological barriers, (13,14,15) Antioxidants are utilized as food additives that prevent the oxidative decomposition of lipids and oils in processed food. When there are too many oxidants without adequate antioxidants, there is an imbalance between oxidation and reduction in organisms. Thus, oxidative stress is caused by a surplus of reactive species (oxygen, hydroxyl free radicals, etc.) and can be brought upon by an absence in the antioxidant system. Oxidative stress is a consequence of an imbalance among prooxidants (free radicals) and antioxidants,(12,13,14,15,16).Oxidative stress is caused upon by both oxidants and free radicals. As a result, they trigger the body's oxidation process. It plays a role in the development of diseases because of the various impacts it causes. As well as Proteins, lipids, and DNA are all damaged. Important functions of mitochondria include the production of essential biological components and the supply of ATP to cells through oxidative phosphorylation.. Low levels of ROS are essential for healthy cellular signaling. Oxidative stress serves a significant role in the etiology of chronic diseases such cancer, diabetes, neurodegenerative diseases, and cardiovascular diseases,(13,15,16) Excessive levels of pro-oxidant exposure gradually might end up in functional changes in a number of enzymes and cellular components, problems in mitochondrial DNA, and defects in gene expression. The amount of polyunsaturated fatty acids in neuronal membranes has been found to make them particularly prone to reactive oxygen species (ROS). Numerous neurological diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), may be put on by a change in metabolism (induced by oxidative stress) in bimolecular components,(9). That neuro degeneration may occur as a result of an imbalanced antioxidant defense system, excessive production, or integration of free radicals from the environment into the living system. Neurodegenerative diseases affect brain cells and result in functional or sensory loss. Antioxidant-rich medicinal plants, however, have been shown to have the ability to treat or prevent an array of human diseases where oxidative stress looks to be a contributing component, (2). That Curcumin, a hydrophobic polyphenol derived from the dried rhizomes of Curcuma longa L., displays significant promise for treating neurological diseases and brain tumors,(5,6). The blood-brain barrier (BBB) is the primary obstacle to curcumin transport to the brain. This is why curcumin has several limitations that hinder its use as a medication, including its extremely poor oral bioavailability. Recently developed drug encapsulation of amorphous Calcium Phosphate (ACP) Nanoparticles will boost the bioavailability of curcumin. Consequently Calcium phosphate nanoparticles are effective drug delivery materials because of their beneficial qualities, such as biocompatibility, biodegradability, and a strong affinity for attaching to nucleic acids, (1,3,4,6).Several methods have been used to generate size-controllable, stable, pH-

responsive, and targeted ACP nanocarriers. ACP nano curcumin might effectively penetrate the blood-brain barrier for the treatment of neurological disorders, (3, 4, 5, 6, 7, 9, 10, 11, 21, and 23).

2.0 Sample preparation:

Four variety of Turmeric were dried and powdered. 5 gm of turmeric was weighed and Soxhlet extracted in 250 ml of ethanol. 5-6 cycle for each sample. Extract was evaporated and sample was weighed to calculate. 50 ml of ethanol was added to get a specific concentration.

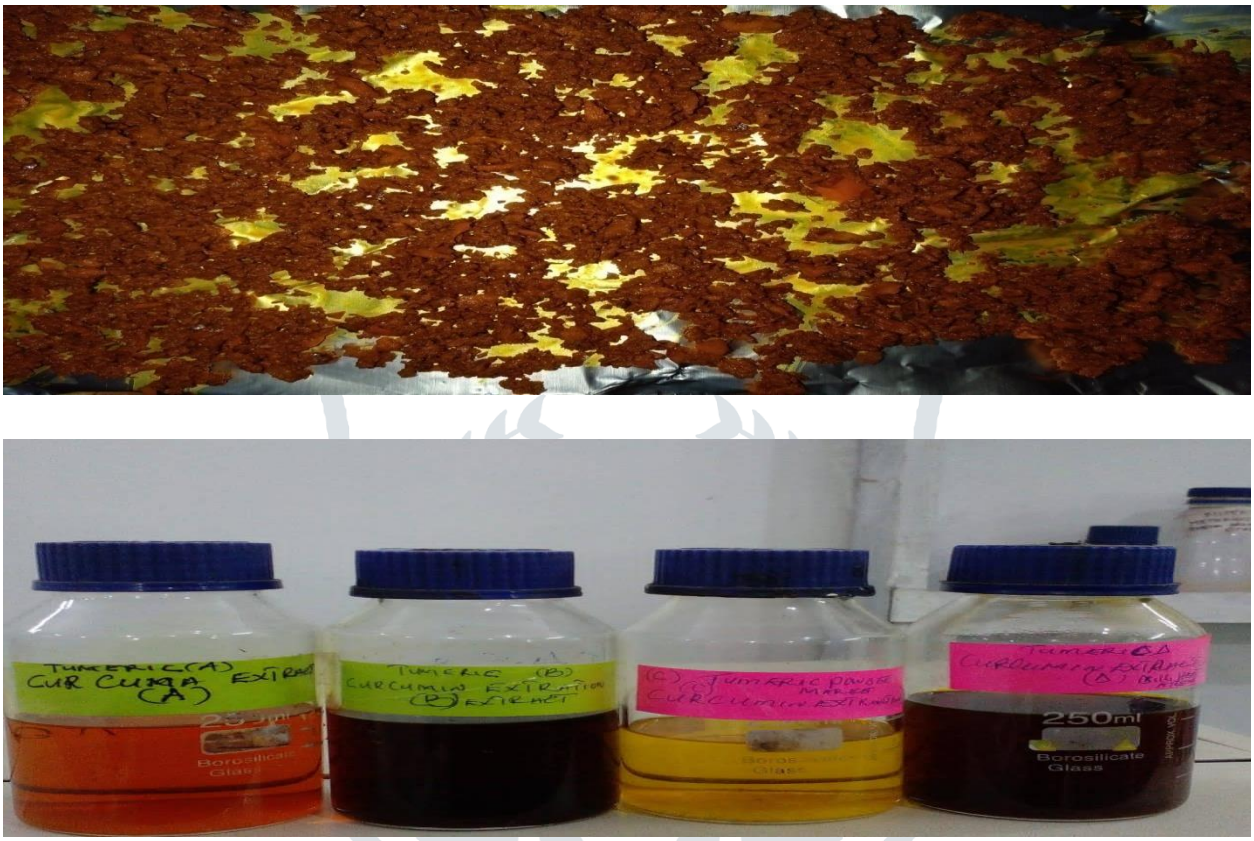


Fig. 1

3.0 Antioxidant assay of extracts.

ABTS assay:

Ascorbic acid standard: 1mg/ml in water

Ascorbic acid was diluted to 10, 20, 30, 40, 50, 60 µg/ml in water.

ABTS reagent:

7mM of ABTS: 0.180 g ABTS in 50 ml of water.

2.45 mM potassium persulphate: 0.033 gm of potassium persulphate in 50 ml of water.

Both the reagent was added in equal quantity and freshly diluted. Incubate for 12 to 16 hrs. to develop colour.

Absorbance of 0.7 was obtained by diluting the ABTS reagent with methanol. Five µl of standards and extracts were added in separate test tube followed by addition of 3.395 ml of diluted ABTs reagent and incubated at room temperature in dark for 4 min. Absorbance was taken at 734 nm. % ABTS inhibition was calculated as:

% ABTS inhibition: ((Ao-At)/Ao)*100

Where Ao: absorbance of control reagent

At: absorbance of test

CEAC(Vitamin C equivalent antioxidant capacity was calculated by linear curve equation from plotting a graph between Absorbance and concentration of ascorbic acid.

4.0 Column purification of extracts.:

Extracts was purified by column chromatography.

Briefly silica 60-120 mesh size was added to acetone to make slurry. Column was wet packed. Length of packed column was 20 cm. 5 ml of sample was loaded to top of column and eluted with gradient ratio of acetone and water. Fractions were collected for 5-5 min. Solvent system was:

20 ml of acetone, 16:4 (Acetone: water), 12:8 (Acetone: water), 8:12 (Acetone: water), 4:16(Acetone: water)

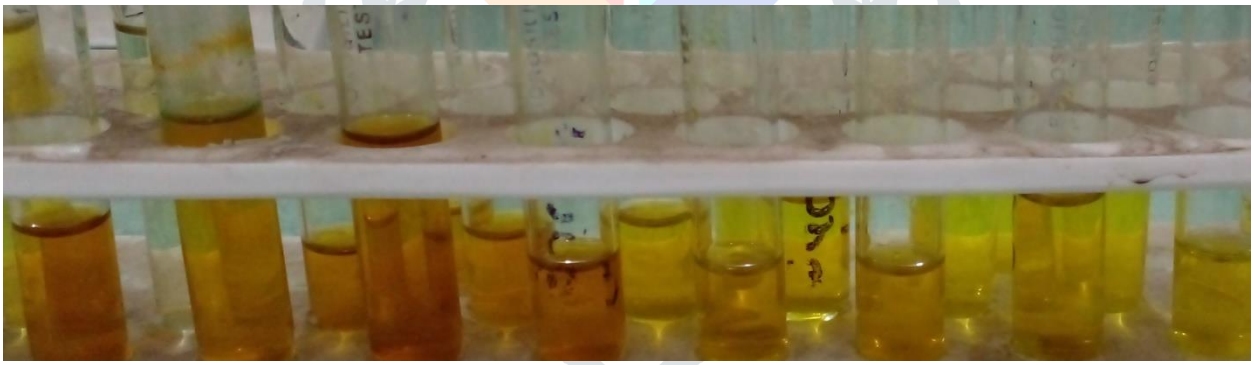


Fig.2

5.0 Curcumin concentration of purified fraction in spectrophotometer.

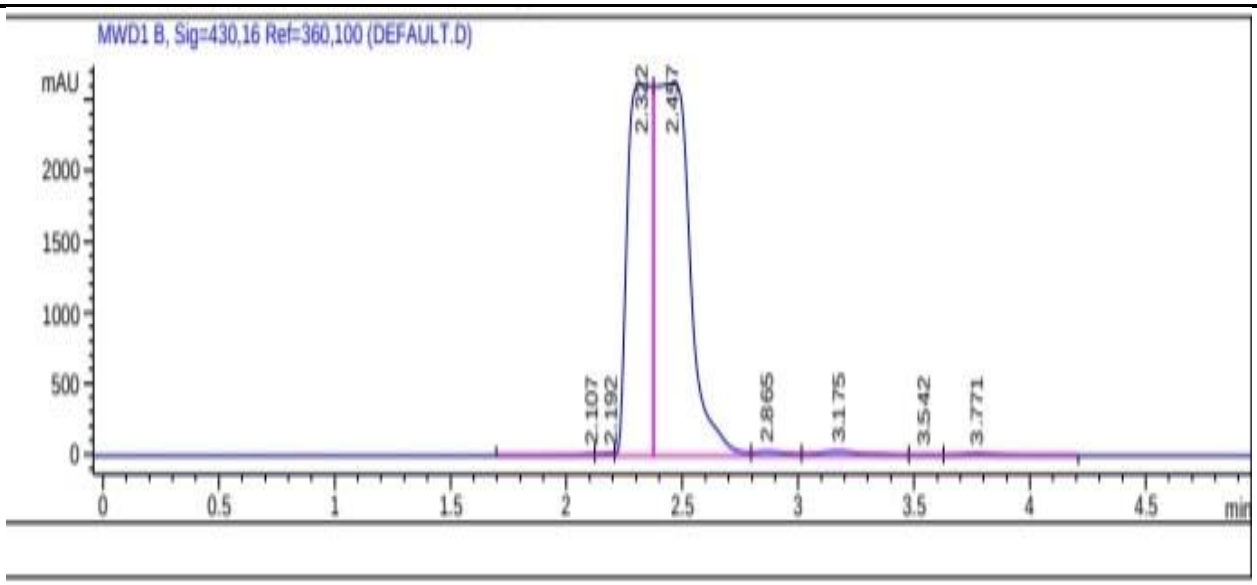
Standard curcumin of 1 mg/ml was prepared and diluted in different concentrations. Λmax of curcumin was estimated by scanning the standard curcumin from 400 to 700nm (visible range). The Λmax was found to be 430nm. Absorbance of standards and curcumin fraction was taken at 430 nm. Graph was plotted against absorbance and curcumin concentration. Concentration of curcumin in fractions was estimated by linear curve equations.

Result and discussion

6.0 HPLC analysis of fractions

Fractions with highest concentration of curcumin were selected for HPLC analysis. 1mg/ml Standards of curcumin was prepared by dissolving in methanol. Working solutions (0.5–75.0 µg mL^{-1}) were made by diluting aliquots of the standard solution in methanol. Standards and fractions were syringe filtered through 0.45 µm. HPLC was used to evaluate curcumin, which was run on a C18 column with UV detection at 425 nm at a flow rate of 1.2 mL min^{-1}, the mobile phase was acetonitrile and water (50:50 v/v) acidified with 2 percent acetic acid Fractions having peaks with retention time similar to retention time of standard of curcumin were selected All the selected fractions were dried and weighed for encapsulation with synthesize calcium phosphate nanoparticles. Due to its ability to enhance bioavailability, reduce the dosage of harmful and expensive bioactive substances, and boost product performance, (Xia, atal,. 2018).

Furthermore, amorphous calcium phosphate (ACP) nanoparticles possess a size in the nanometer range, and their high surface to volume ratio determines their high reactivity, solubility, biocompatibility, and bioactivity. As thus, ACP offers new opportunities in nanobiotechnology, particularly for improving the solubility, biocompatibility, and bioactivity of antioxidant plants including curcumin,(19). The co-precipitation method at 8pH values was successful in synthesizing amorphous calcium phosphate nanoparticles. IR spectroscopy, DLS, while SEM shows the encapsulation of curcumin and calcium phosphates nanoparticles, thus images of synthetic ACP have been shown in Figures 4a–4c and 3. Exhibit ACP nanoparticles in their dry form.

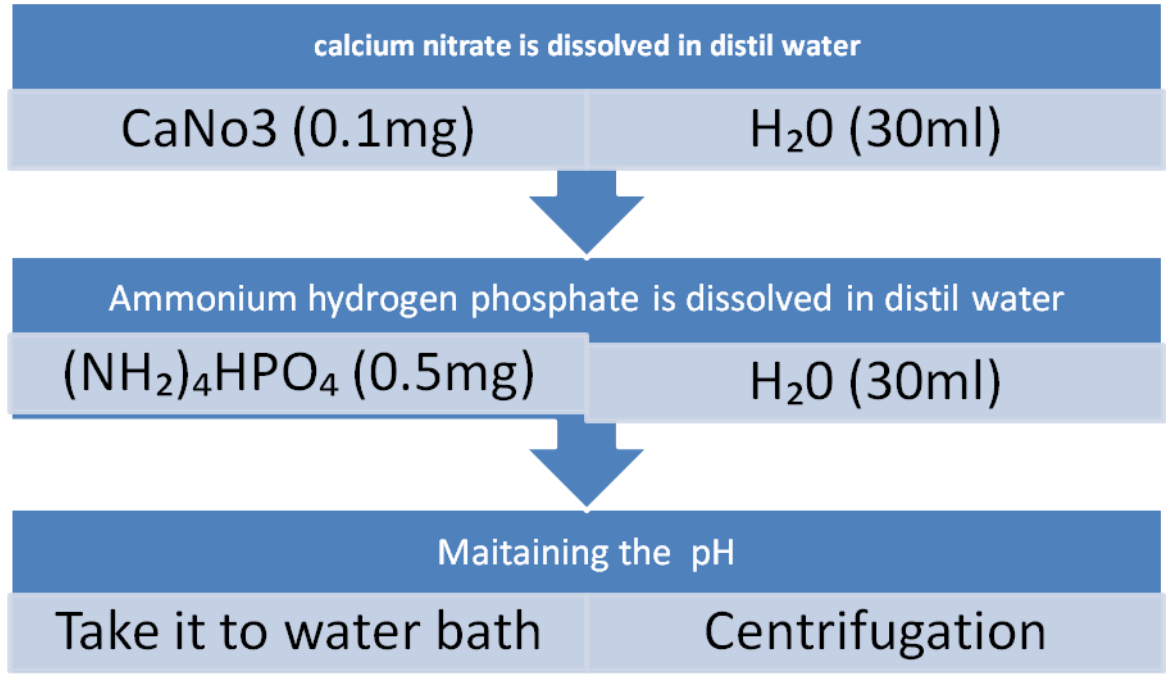


6a.Fractions with highest concentration of curcumin were selected for HPLC analysis. 1mg/ml Standards of curcumin was prepared by dissolving in methanol. Working solutions (0.5–75.0 µg mL^{-1}) were made by diluting aliquots of the standard solution in methanol.

Table.1

Co-Precipitate Method of Calcium Phosphate Nanoparticle Synthesis

Using the co precipitation method, ACP nanoparticles were synthesized in accordance with (Xia et al. 2018) with a few minor modifications. That Ca (NO3)2 4H2O was turned into an aqueous solution by dissolving it in deionized water. The aqueous solution immediately turned white as the (NH4)2HPO4 phosphate solution was added drop by drop to the previously mentioned solution. With adding NaOH solution, the pH value remained between 8 and 9. The resulting mixture was stirred for 10 minutes at 30 °C. For the purpose to remove any residual ions, the produced nanoparticles were washed three times with deionized water. The sample was then collected by centrifugation and freeze-dried.



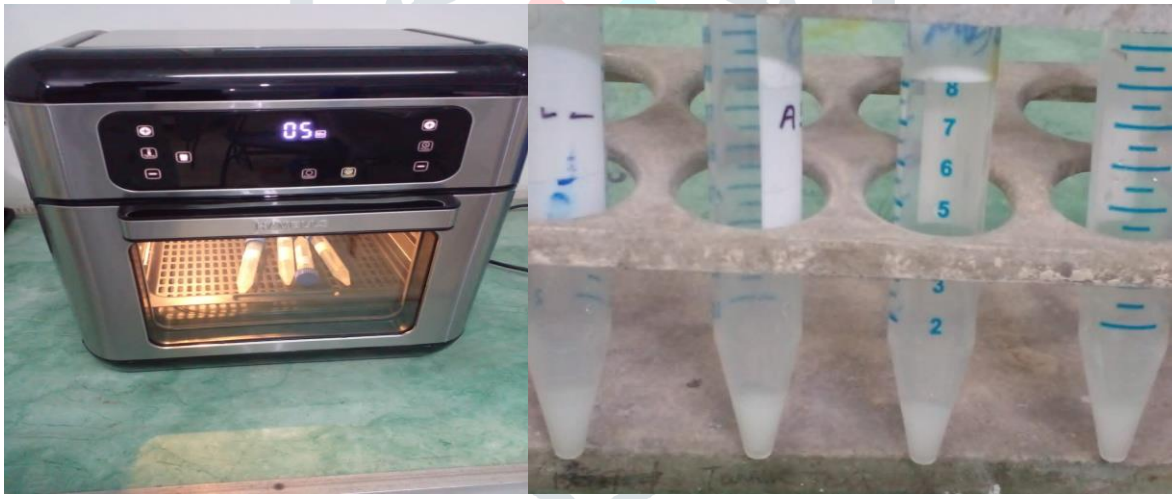


Figure.3 Synthesized calcium phosphate nanoparticles

Drug loading: Cur (0.5 mg/mL) was added to the solution of Ca $(NO3)_2$. The mixture was stirred for 1 h, An aqueous solution of Na2HPO4 was then gradually added, and the mixture was gently agitated at 30 °C for an additional 15 minutes. After the suspension was immediately centrifuged to separate the products.

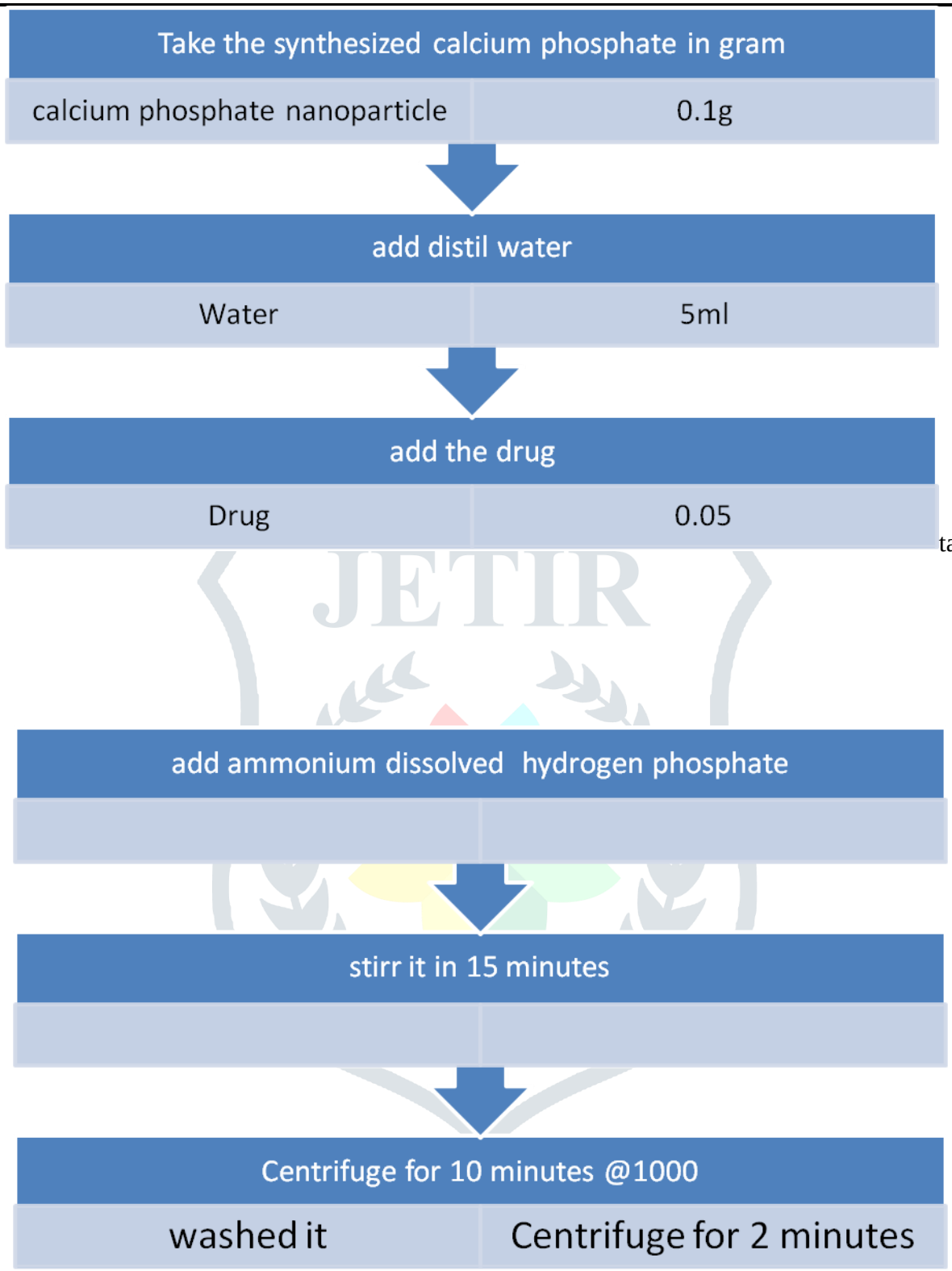


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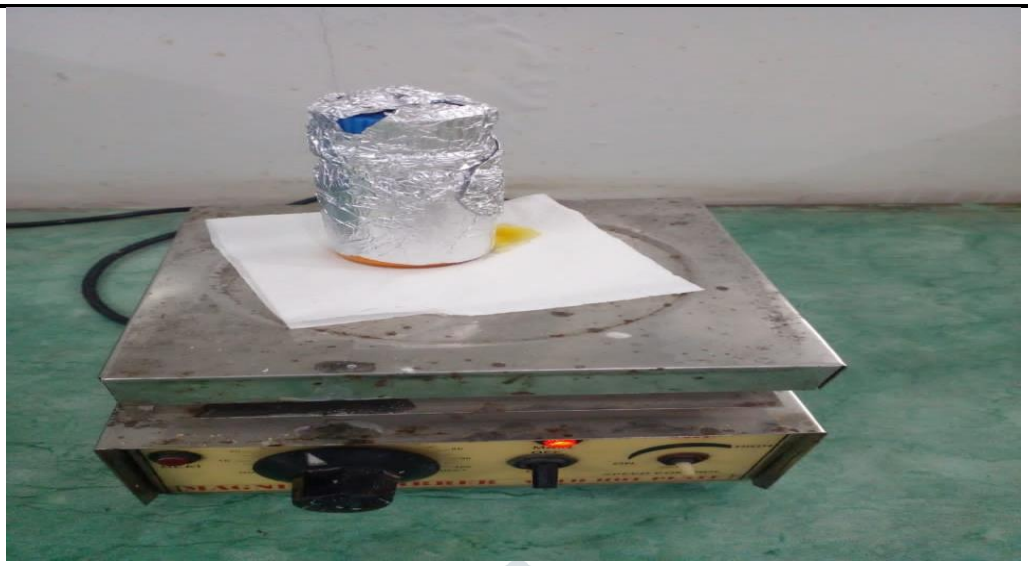
Table.2 Present curcumin calcium phosphate nanoparticles encapsulation the calcium phosphate (Xia, atal. 2018).



Characterization of calcium phosphate nanoparticles encapsulated with curcumin nanoparticles.

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	70.45	Peak 1:	113.2	83.6	40.93
PdI:	0.663	Peak 2:	13.01	11.8	2.838
Intercept:	0.952	Peak 3:	4871	4.7	686.8
Result quality	Good				

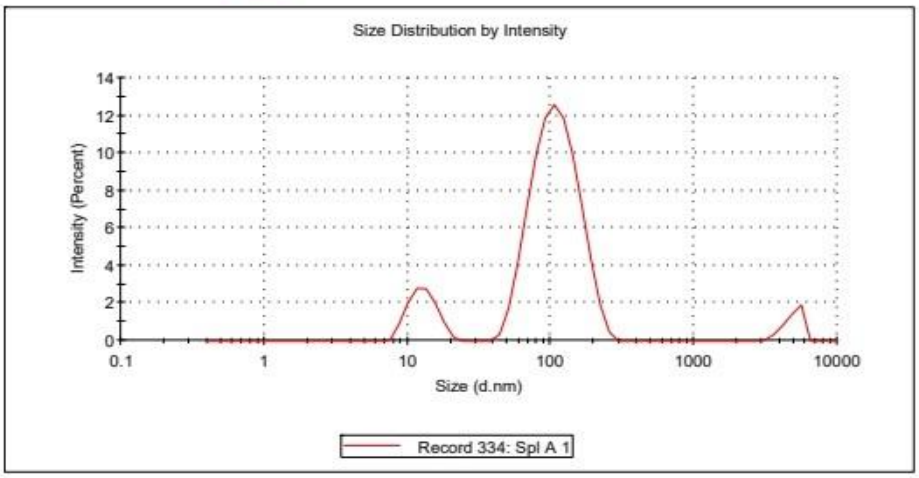


Fig. 4a present good quality of the nanoparticles having Pdi of 0.663, Z-average (d.nm) 70.45 as indicated by the DLS machine.

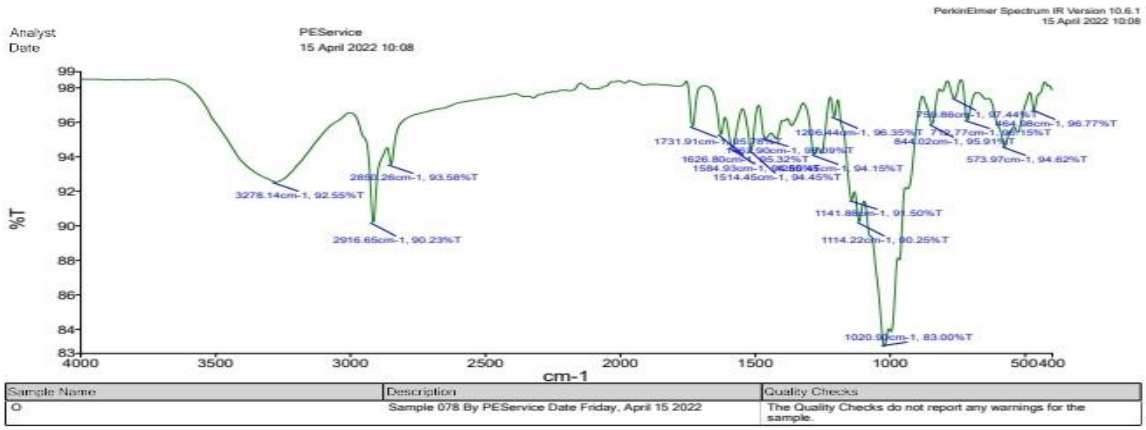


Fig.4b. point out curcumin's infrared spectroscopy. Arrows point to the molecule's characteristic bands.

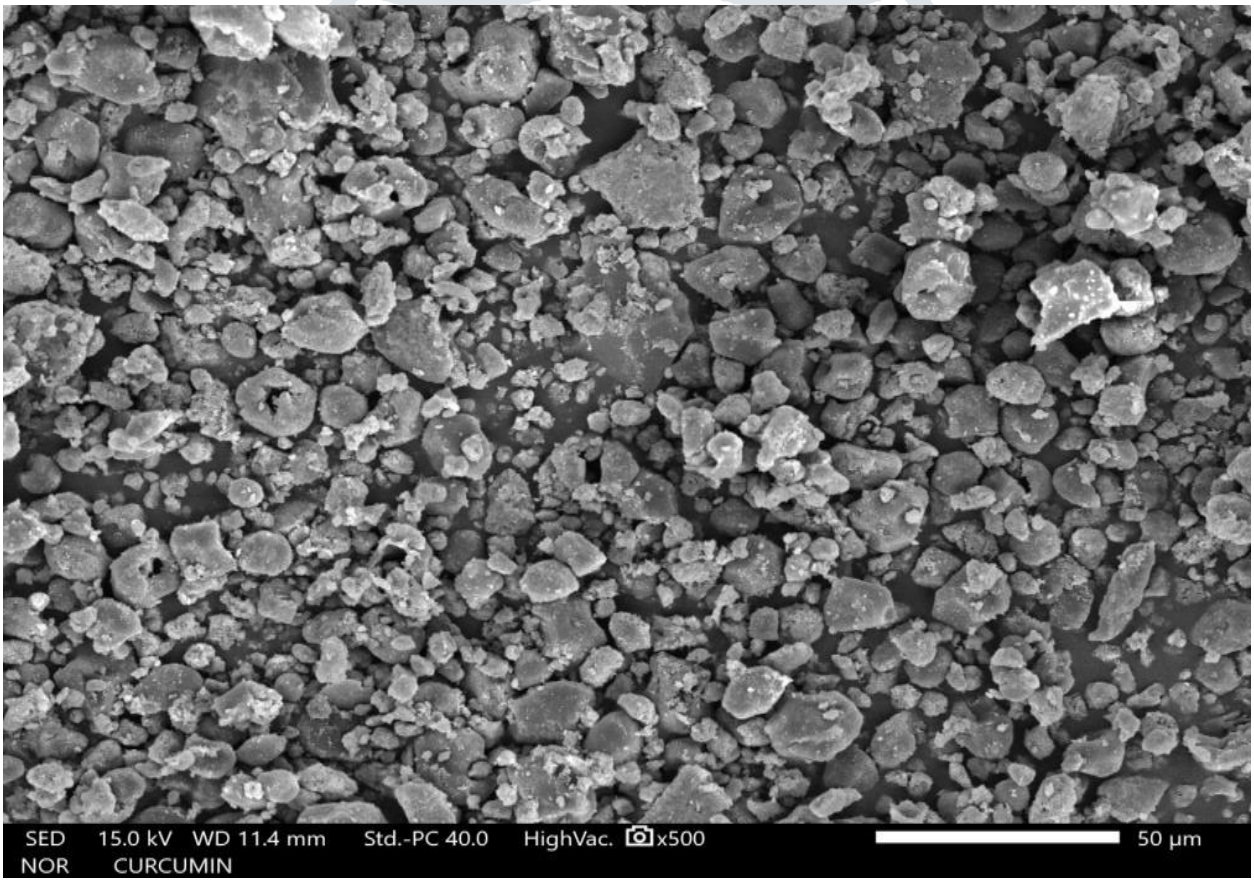


Fig. 4c. demonstrate the encapsulated curcumin AMP with 50µm Std. pc 40.0

Conclusion

The synthesis of calcium phosphate nanoparticles was carried out utilizing a simple, quick and easy surfactant-free, and environmentally friendly appropriate procedure, in which an aqueous solution containing Ca(NO3)2 was employed as a calcium source and (NH4)2HPO4 as a phosphorus source. The temperature, time frame, pH, and (NH4)2HPO4 concentrations of the reaction have a major impact on the product's crystal phase. calcium phosphate nanoparticles improved pH-responsive drug release property and a high Curcumin drug loading capacity,

consequently, the calcium phosphate nanoparticles that have been loaded with curcumin will exhibit enhanced radical scavenging abilities and a strong resistance to neurological diseases .

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