Trends in Biomaterials and Artificial Organs
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Synthesis of Nanocurcumin and Evaluation of its Properties for Biomedical Applications

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Received: *1 October 2021*
Accepted: *25 November 2021*
Published online: *23 February 2022*

Keywords: *curcumin, nanocurcumin, ball milling, antioxidant*

Curcumin is a highly non-toxic, bioactive agent found in natural turmeric herb (Curcuma longa) and has been known for centuries as a household remedy to many ailments. However, it shows low solubility nature. The study aimed to develop a method to prepare curcumin nanoparticles to improve their aqueous-phase solubility and examine the effect of its anti-oxidant properties. Nano-curcumin was prepared by a process based on a wet-ball milling technique and was found to have a narrow particle size distribution in the range of below 150nm. Unlike curcumin, nanocurcumin was found to be readily dispersible in water in the absence of any surfactants. The chemical structure of nano-curcumin was obtained as same as that of natural curcumin, and there was no modification during nanoparticle preparation. The prepared yellow-colored nano-curcumin was analyzed by using various physio-chemical techniques. A low inhibitory concentration of nanocurcumin was determined for anti-oxidant strain which was compared with curcumin. It was decided that the aqueous dispersion of nanocurcumin was much more effective than curcumin is radical scavenging assays. The results demonstrated that the anti-oxidant activity and water solubility of curcumin markedly improved by particle size reduction up to the nano range. These results conclude that the ball-milled nano-curcumin is efficient for anti-oxidant application.

Introduction

Curcumin is a biologically active hydrophobic and polyphenolic organic compound extracted from turmeric root [1], the powdered rhizome of Curcuma longa, along with bisdimethoxy curcumin and dimethoxy curcumin. A lot of previous reports have demonstrated that curcumin exhibits huge biological and pharmacological properties such as anti-inflammatory, antimicrobial, antifungal, anticancer, arthritics, and anticarcinogenic activities [2]. Curcumin, that has significant *in vitro* and *in vivo* chemopreventive and neuroprotective characteristics potential drug, for numerous forms of cancers, including leukemia, melanoma, lymphoma, breast cancer, ovarian cancer, lung cancer, and bone cancer [3]. The curcumin prevents the infection of layers within the soft tissues such as dermis, subcutaneous tissue, or muscle linked with necrotizing changes [4]. However, the application of curcumin in clinical studies has been slowed down due to its extremely low aqueous solubility combined with its chemical instability in an aqueous medium [5] and also, measurements of blood plasma levels and biliary excretion showed that the curcumin was poorly absorbed from the gut and the quantity of curcumin that reached tissues outside the gut was pharmacologically insignificant [6]. Various delivery vehicles, including nano/microparticles, micelles, and emulsion capsules for enhancing the water solubility in which curcumin is encapsulated in liposomes, solid lipid microparticles, such as bovine serum albumin and chitosan are complexed with phospholipids have been reported [7].

Generally, various techniques used to manufacture the nano-sized curcumin nanoparticles are solvent-based processes, which include emulsification-solvent diffusion and precipitation methods. However, the problem with these methods is that they require the addition of surfactants to prevent coalescence during particle formation [8]. This report mainly focused on the reduction of particle size of curcumin by planetary ball milling using agate jar and balls for the preparation of drug nanocolloids with diameters of less than 150nm range. The milled nano-sized curcumin was characterized by physio-chemical techniques. Further, nano-sized curcumin was evaluated by anticancer scavenging activity.

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Materials and Methods

The soxhlet method was plainly attempted for the extraction of curcumin then planetary ball milling method was adopted to produce nanoscale curcumin particles. For this design, precursors such as curcumin dried powder from *C.longa rhizome* were commercially purchased from Sigma Aldrich Chemical Company USA. The ethanol, hexane, and isopropyl alcohol of analytical grade were obtained from Himedia India.

Curcumin from *C.longa rhizome* 10g was embedded in a thimble and place in the soxhlet equipment. The extraction solvent of ethanol was gradually added to the soxhlet apparatus. The reaction was maintained at 60°C for 10hrs to accomplish the extraction in the form of colored compound. After the extraction of the compound, using the rotary evaporator then removed for ethanol. 10ml of hexane was used as a solvent for extracted crude sample and continuous magnetic strings condition for 8hrs. The solution mixture was centrifuged and then the crude curcuminoid sample was dissociated at 30°C in the hot air oven for 3hrs. 5ml mixture of 1:1.5 molar ratio of isopropyl alcohol and hexane was used as a hot solvent for 5g of the crude curcuminoid powder. The prepared solution was cooled at room temperature to acquire pure crystalline curcumin, and the extracted curcumin was separated by filtration.

The prepared powder was processed by using the milling method. This milling method was conducted in a planetary ball mill (Retsch PM 100) with a 150ml agate jar and 2mm alumina milling balls. The program was set the for 350 rpm rotation, and the mill prevented overheating by a pause of 5min for every 5min of milling. The procedure was carried out for the more diminutive size of curcumin nanoparticles. Nanocurcumin's radical-scavenging activity was evaluated by using the DPPH and Phosphomolybdenum assay method. The strategy used to dissolve nanocurcumin was similar to that seemed to dissolve DPPH. Ethanol was mixed with DPPH solution to make the control solution (0.1 mM). The solutions were stored in the dark at room temperature for 60 minutes to allow the nanocurcumin to complete the interaction with the reactive oxygen species. Using a UV spectrophotometer, the absorbance of all the samples were analyzed at 760 nm (max) after incubation. The anti-oxidant ability of the produced nanocurcumin was measured using a phosphomolybdenum assay. Ammonium molybdate, Sulphuric acid, and sodium phosphate were combined to make a phosphomolybdenum solution. The test sample was combined with various diluted concentrations of phosphomolybdenum reagent and incubated in a water bath at 80°C for 90 minutes. The mixture's absorbance was measured at 996nm in comparison to a blank reagent.

Results and Discussions

X-ray diffraction analysis of curcumin and nanocurcumin

The phase formation of extracted curcumin nanoparticles has been investigated by the Rigaku Ultima IV X-ray diffractometer (XRD) with Kα radiation (λ=1.54187 Å) at 40kV and 40mV. Curcumin solution is introduced to an aqueous solution; it tends to form crystals since it is a highly hydrophobic substance. If nano-sized crystal form inside the matrix of the NPs, drug elution from the NPs will be hampered, and the release profile will be uneven.XRD patterns of curcumin are shown figure 1. A number of peaks in the 2θ = 12.26°, 14.58°, 17.32°, 18.20°, 24.68°, 25.68° and 27.46° observed in the nano formulation of curcumin. NPs, on the other hand, did not show a peak in this area. This confirms that curcumin in the NPs is amorphous or disordered-crystalline.

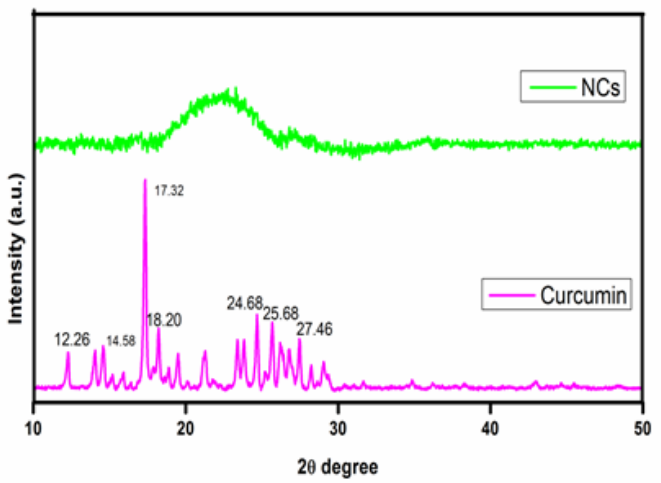

Figure 1: XRD pattern for curcumin and nanocurcumin

FT-IR spectral analysis of nanocurcumin

Chemical bonding analysis of curcumin nanoparticles was recorded with a fourier transform infrared spectroscopy (FTIR) in the range of about 40-4000cm^{-1} using Bruker Tensur 27. The nanocurcumin FT-IR spectroscopy spectra were scanned in the mid-infrared region (3500-500cm^{-1}), as shown in figure 2. The stretching vibration of hydrogen-bonded O-H found in nanocurcumin correlates to the broad, strong band at wave number 3315cm^{-1} in nanocurcumin. Sp^2 has asymmetric stretching vibrations at 2939cm^{-1}. The aromatic overtone's C-H frequency is found to be 1624 cm^{-1}. The stretching vibration of the conjugated carbonyl (C=O) is shown by the strong characteristic band centered at 1508cm^{-1}. Double-bonded carbon, sp^2, and sp^3 bonds have stretching vibrations of 1600, 1268, and 1116cm^{-1}, respectively.

Particle size analysis

Dynamic light scattering (DLS) was used on the Malvern Zetasizer 7.3 series to determine the mean particle diameter of curcumin nanoparticles. 1mg lyophilized nanocurcumin powder was dispersed in 10mL distilled water to make the sample. The production of nanoparticles with an average hydrodynamic diameter of 140nm was demonstrated by DLS of an aqueous dispersion of nanocurcumin (figure 3a). The microstructure of curcumin nanoparticles powder was analyzed by field emission scanning

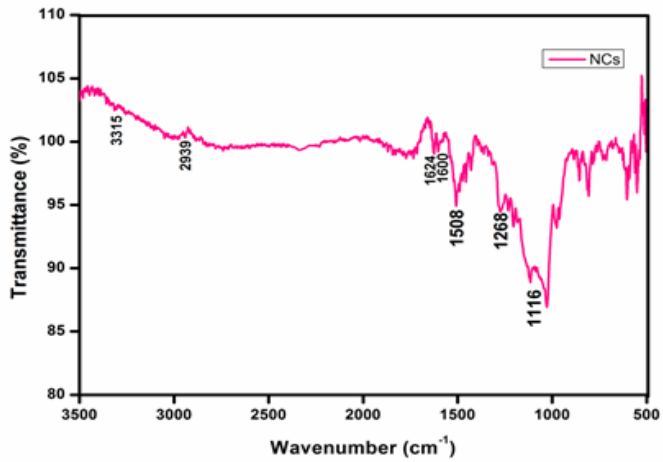

Figure 2: FTIR Spectrums for Nanocurcumin

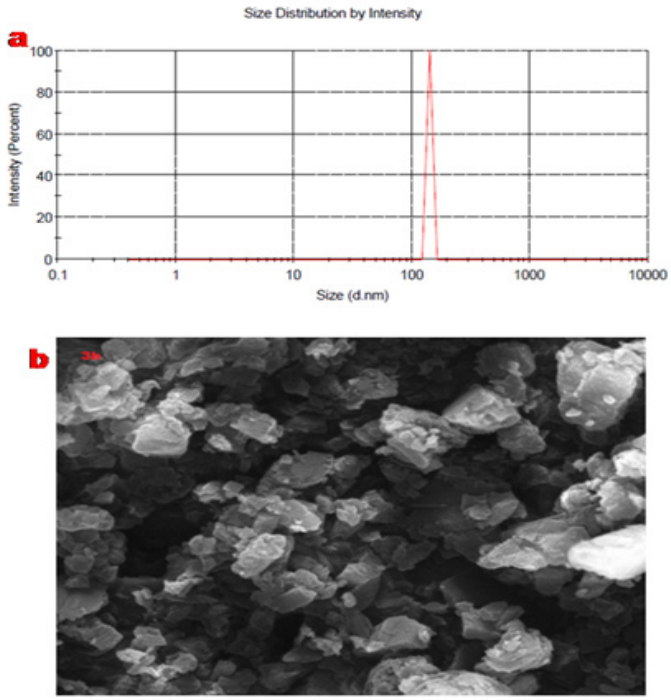

Figure 3: (a) DLS analysis for particle size and (b) FESEM showing the morphology of the nanocurcumin

electron microscopy (FESEM) Quanta-250 FEG at 15kV of acceleration voltage. The particles in the powdered sample were around 5µm in diameter, according to FESEM (figure 3b). Nanocurcumin powder, dry and lyophilized, was high in physical and chemical stability; was readily dispersible in water, and could be kept at room temperature for over 12weeks without breakdown or aggregation. The greater surface area of nano-sized curcumin particles, which facilitates dissolution, may account for their increased water solubility. Similar findings have been reported in earlier research, where reducing the particle size of active substances to nanoparticle size improved their effectiveness, solubility, and bioavailability.

Nanocurcumin's free radical scavenging activity

DPPH Assay

The DPPH is a persistent organic nitrogen radical that is generally soluble in water and other solvents. It has a purple hue and significant absorption at 760nm; however, it reacts and loses its color in the presence of anti-oxidants. The radical-scavenging capacity of nanocurcumin is tested using the DPPH scavenging technique. The prepared test was diluted with methanol to the appropriate concentration and equilibrated at 27°C, yielding a distinctive absorbance color that exhibits the maximam at 844nm (figure 4). All concentrations (20-100 uL) of nanocurcumin were mixed in methanol with a diluted DPPH solution, and absorbance was measured 20 minutes later at 27°C. Using UV spectroscopy, the proportion of scavenging activity was determined against the blank absorbance at 844nm. The sample's total anti-oxidant activity was determined to be 56.86 percent. The radical scavenging activity of nanocurcumin was determined using the formula below.

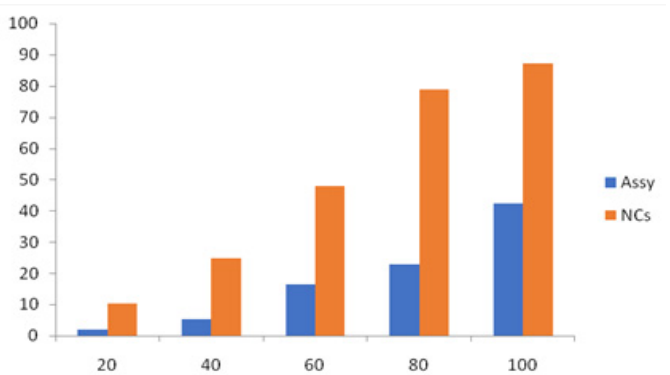

Figure 4: DPPH absorption of different concentration nanocurcumin

Phosphomolybdenum assay

The total anti-oxidant capacity was expressed as milligrams of ascorbic acid equivalent (AAE) per gram of sample after the absorbance readings were collected. The average total anti-oxidant capacity value indicated by phosphomolybdenum is 4.763.

Conclusions

The nanoscale curcumin particles were synthesised from curcumin longa rhizome on a scale ranging from 100-140nm successfully. The resultant curcumin nanoparticles were analysed for their properties and the experimental results confirm the presence curcumin. The synthesised curcumin nanoparticles were subjected to antioxidant studies and the results reveal that the curcumin with reduced particle size showed free radical capturing ablity.

Acknowledgment

The authors gratefully acknowledge the RUSA 2.0 – BEICH *(Bharathiar Entrepreneurship, Innovation and Career Hub),* Government of India. Infrastructure facilities provided by DST-FIST and UGC-SAP are also acknowledged.

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