Preparation, Characterization and Antiviral Evaluation of Curcumin-PLGA and Curcumin-metal oxide nanocomposite against SARS-CoV-2

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Abstract

Our usual control strategies for viral infections are still not as effective as demand, and the need for different ways of treatment is still of high priority. Viruses undergo genetic mutations that prevent specific drugs from affecting new strains. This research is concerned with the usage of nanotechnology in the preparation of natural drug classes that can be more effective when dealing with emerging pathogens. Curcumin is a natural extract of Curcuma longa that is known for its wide therapeutically effect due to its binding with different enzymes and proteins in the body. The curcumin can bind to viral proteins and block their activity; however it has poor water solubility and bioavailability. Likely, curcumin nanoparticles have much better solubility and bioavailability. This can improve our traditional treatment or prophylactic methods for viral infections and pandemics. This study is concerned with curcumin nanoparticle formulations and their anti-viral activity. Curcumin has been loaded onto PLGA to form Cur-PLGA nanoparticles. In parallel, curcumin was added to ZnO to form a Cur-ZnO composite. The compositions of PLGA and ZnO preparations with different concentrations of curcumin were confirmed with a panel of physiochemical assays. In vitro, the Cur-ZnO composite demonstrated significantly higher anti-SARS-CoV-2 activity than Cur-PLGA with significantly higher host cell safety. The study emphasizes the preference of curcumin composite nanoparticles over curcumin loaded nanoparticles. This study highlights the robust antiviral activity of different curcumin-based ZnO nanoparticles. This preliminary study demands further preclinical and clinical studies to confirm the obtained results in vivo.

1. Introduction

Respiratory viral infections, especially influenza viruses, have attracted researchers due to the deficiency of effective cures or vaccines as a result of viral continuously RNA alterations, which form a huge challenge while facing epidemic or pandemic times [1]. Our current situation in relation to the current pandemic Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has made us deeply appreciate the role of nutraceuticals. As of March 2022, more than 6 million deaths worldwide have been caused by SARS-CoV-2. This has had a catastrophic effect on the world’s demographics, making it the most significant global health crisis since the 1918 influenza pandemic [2]. Initially, early in the pandemic, the understanding of COVID-19 and its therapeutic management was limited, creating an urgency to mitigate this new viral illness with experimental therapies and drug repurposing. Due to the intense efforts of clinical researchers globally, significant progress has been made, which has led to a better understanding of not only COVID-19 and its management but also has resulted in the development of novel therapeutics and vaccines at an unprecedented speed [3]. Currently, a variety of therapeutic options are available that include antiviral drugs (e.g., molnupiravir, paxlovid, remdesivir), anti-SARS-CoV-2 monoclonal antibodies (e.g., bamlanivimab/etesevimab, casirivimab/imdevimab), anti-inflammatory drugs (e.g., dexamethasone), immunomodulators (e.g., baricitinib, tocilizumab) are available under FDA issued Emergency Use Authorization (EUA) or are being evaluated in the management of COVID-19 [4]. However, many herbal supplements, vitamins, probiotics, and micronutrients have demonstrated significant efficacy in the treatment and prevention of viral infection [5].
Although the American Foundation for Innovation in Medicine defined nutritional supplements (nutraceuticals) in 1989 as any product made from edible parts of an animal or plant that can affect health or improve disease conditions [6], the use of many herbal products in medicine dates back much further. This research is focused on curcumin as a nutritional supplement derived from the plant turmeric (\textit{Curcuma longa Linn}). Turmeric is one of the traditional medicines used over 2000 years ago in Asia, mainly China and India, and then it became known to the West by the 14\textsuperscript{th} century.

The first isolation of curcumin was in 1824 by Vogel, then characterized later in 1910 by Lampe and Milobedeska [7]. Ancient use of turmeric was primarily for wound healing and as an anti-inflammatory, but now, curcumin possesses many approved effects on different biological pathways. In \textit{vitro} trials indicating its inhibitory action on human immunodeficiency virus through inhibiting viral protease were firstly documented in 1990. The antiviral activities of curcumin on diverse viruses have been discussed in several studies.

Unfortunately, most of the promising \textit{in vitro} activities of curcumin are inapplicable \textit{in vivo} owing to curcumin's low solubility in water and poor bioavailability [8]. Nanotechnology offers ways for preparations that can overcome these limitations. Manipulations of material nearer to the atomic scale can produce different physical and chemical properties, and now much research indicates that nano-formulations of curcumin show improved solubility and bioavailability, resulting in better \textit{in vivo} activities. Encapsulated curcumin in poly (Lactic-co-Glycolic) acid (PLGA) nanoparticle is one of the curcumin delivery systems that showed perfect relief of previously explained limitations in addition to excellent biodegradability [9]. In this study, we also tried another improved bioavailability of curcumin nano-formulation as Cur-ZnO. The Cur-ZnO nano-composite has higher anti-bacterial activity than pure single counterparts [10].

This study aims to discuss the anti-viral activity of both curcumin nano-formulations, Cur-PLGA and Cur-ZnO, on the normal Vero E6 cell line that was infected by SARS-CoV-2 (COVID-19) and to demonstrate their effects on Vero E6 and SARS-CoV-2 in order to determine the toxic and inhibitory dose on the normal cells and viruses, respectively. Considering Cur-PLGA, two preparations of Cur-PLGA were synthesized, one of them is a fully encapsulated curcumin core in a PLGA shell in which excess curcumin was added during preparation, we referred to it here as non-stoichiometric Cur-PLGA. The other is exactly one mole of curcumin was added to one mole of PLGA where just a covalent bond reaction between curcumin and PLGA polymer had formed as a result of dehydration reaction without encapsulation, so curcumin here is nearer to a kind of termination of the PLGA molecules and we refer to this product (1:1) as stoichiometric Cur-PLGA. On the other hand, three preparations of Cur-ZnO were prepared with curcumin percentages of 5\%, 10\% and 20\%. We refer to them as X = 5, X = 10 and X = 20, respectively.

2. Materials And Methods

2.1. Materials
The PLGA polymer with M.wt (7000–17000 Dalton) 50:50 RESOMER® RG502H was purchased from Sigma-Aldrich. The curcumin 95% was purchased from LOBA CHEMIE in India. The polyvinyl alcohol (PVA), dichloromethane, methanol, zinc acetate, formaldehyde, Dulbecco's Modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), Bovine Serum Albumin (BSA), and potassium hydroxide were purchased from Merck Millipolids Co., Ltd.

2.2. Methods

2.2.1. Preparation of stoichiometric and non-stoichiometric Cur-PLGA

Cur-PLGA was prepared using the solid-in-oil-in-water (s/o/w) solvent evaporation technique as previously described [11]. In a beaker, one gram of PLGA was dissolved in dichloromethane until it formed a uniform PLGA solution. Then the beaker was filled with 0.023 gm of curcumin. Because this is the exact stoichiometric amount, we referred to this product as stoichiometric Cur-PLGA. A second beaker was prepared in the same manner as the previous one, but this time 0.039 grams of curcumin was added to the PLGA solution resulting in a light yellow fluorescence solution. In the second beaker, an excess of curcumin was evenly distributed throughout the solution for few seconds concurrently with the light yellow fluorescence color formation and then was encapsulated by PLGA self-assembly behavior, forming a dark yellow solution with no fluorescence, which we named this product as a non-stoichiometric Cur-PLGA. Both the stoichiometric and non-stoichiometric Cur-PLGA were sonicated for 2 min in a probe sonicator (VCX-1500, Sonics) in order to generate the s/o primary emulsion. Then the stoichiometric and non-stoichiometric Cur-PLGA s/o primary emulsion were added in-separate drops through filter paper into two separate flasks containing 160 mL of PVA solution (1% w/v) and sonicated for 3 min to generate the final s/o/w emulsion. The organic solvent was removed through rotary vacuum evaporation at 50 °C, while the free PLGA/PVA polymers were eliminated by centrifugation at 3000 rpm using a Hermle Z32 (German centrifuge) for 10 min. Finally, solutions were lyophilized to be stored as solids at 4 °C for further use.

2.2.2. Preparation of Cur-ZnO nano composite with different percentages of curcumin

The ZnO nanoparticles were first prepared following Thanasi et al.[12]. In brief, a solution of potassium hydroxide in methanol (2% W/V) was added to a solution of zinc acetate dehydrate (2% W/V) as a dropwise addition on a magnetic hot plate stirrer at 60°C for 2 h, forming a precipitate, which was separated, washed twice with methanol and dried at 60°C. Loading of curcumin on ZnO-Nps was done according to Al-Ajmi et al. [13] with some modifications. Three beakers were prepared in which the previously prepared ZnO-Nps were added to 50 ml of ethanol. In each beaker, 0.95, 0.9, and 0.80 gm .Then curcumin powder were added to them with a percent of 0.05, 0.1, and 0.2, respectively. All beakers were sonicated for 30 minutes to trap curcumin on ZnO, and then the solutions were centrifuged at 10,000 rpm for 10 minutes to remove solvent and unreacted materials before drying at 60°C overnight. In final, three preparations of Cur-ZnO were obtained with curcumin percentages of 5%, 10% and 20%. We refer to them as X = 5, X = 10 and X = 20, respectively.
2.3 Characterizations techniques

2.3.1. X-Ray Diffraction (XRD)

Empyrean Malverpanalytical and Netherland X-ray diffraction were used to document the XRD data. The device worked with a 30 mA current, a functioning voltage of 40 kV (power of 1200 W), and a scanning speed of 2/min (step size = 0.04 degree and step time = 1.0s) in the 10-80 scanning range (2 Theta scale) at room temperature. The crystallite size was calculated from the XRD peaks using Scherrer's equation.

\[
D = \frac{0.94\lambda}{\beta \cos \theta}\quad (Eq. 1).
\]

Where D is the average crystallite size, \( \lambda \) is the radiation wavelength, \( \beta \) is the corrected full width at half the maximum of the diffraction peak, and \( \theta \) is the Bragg's diffraction angle.

2.3.2. Fourier-transform infrared (FTIR)

The functional groups were determined by FTIR spectra that were obtained using a Bruker (Vertex 70 RAM II IR (Bruker, Germany) spectrometer. Samples were ground and mixed with potassium bromide (KBr) to make pellets. It was compressed under high pressure to prepare pellets of 10.0 mm and 1–2 mm thick. The pellets were scanned over a range of 4000 to 500 cm\(^{-1}\). The spectral of free Curcumin and free PLGA and free ZnO in addition to the five prepared formulas were recorded in the transmission mode. The resolution used in the scans was 1 cm\(^{-1}\), and the spectra were averaged over three scans.

2.3. 3. Morphological and size analysis

The size determination of all five prepared curcumin nanoparticles was determined using a JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 KV. All samples were diluted by water, and some samples were treated with phosphotungstic acid (PTA) to improve the resolution.

2.4. Antiviral Study

2.4.1. Vero-E6 cells with SARS-CoV-2 infection

To culture Vero-E6 cells, Dulbecco's Modified Eagle's medium (DMEM) was used. The cells were supplemented with 10% Fetal Bovine Serum (FBS) (Invitrogen) and 1% Penicillin/Streptomycin mixture (pen/strep) and then incubated at 37 °C in a humidified incubator with 5% CO\(_2\). The hCoV-19/Egypt/NRC-3/2020 SARS-CoV-2 "NRC-03-nhCoV" virus was proliferated in Vero-E6 cells. Simply, confluent VERO-E6 cell monolayers were infected with NRC-03-nhCoV in infection medium (DMEM with 1% pen/strep, 0.2% Bovine Serum Albumin (BSA), and 1 µg/ml TPCK-treated trypsin) at a multiplicity of infection (MOI) of 0.1 for 2 h. Following virus adsorption, the infection medium containing the inoculum of the virus was discarded and replaced with new infection media. The infected VERO-E6 monolayers were incubated for 4 days post-infection at 37°C in a humidified incubator with 5% CO\(_2\). The cell supernatants were collected.
and centrifuged at 2500 rpm for at least 5 min to remove cellular remains. The clear supernatant containing propagated viruses was then liquated and preserved at -80°C in a freezer. An aliquot of the saved NRC-03-nhCoV virus was subjected to virus titration using a plaque titration assay.

2.4.2. Cytotoxicity assay

2.4.2.1 Cytotoxic Concentration CC\textsubscript{50}

To assess the cytotoxic concentration CC\textsubscript{50} of the prepared products as described before (citations ), stock solutions were prepared by dissolving the mentioned formulae in 10% DMSO "in 1X DMEM" and serially diluting them with 1X DMEM to prepare the working concentrations. The CC\textsubscript{50} of each formula was examined in Vero-E6 cells by using a crystal violet assay in which 100 µl of the VERO-E6 cell suspension was dispersed into 96-well plates (3*10\textsuperscript{5} cells/mL). Incubation for the seeded plates then occurred at 37°C in a 5% humidified CO\textsubscript{2} incubator for 24 h. Then cell monolayers were co-incubated with a combination of different concentrations of each drug in triplicates at 37°C in a 5% humidified CO\textsubscript{2} incubator. After three days, the media supernatants were thrown away and the cell monolayers were washed once with 1X PBS and fixed with 10% formaldehyde for 1 h at room temperature (RT). The plates were later dried and stained at RT with 0.1% crystal violet for 20 min on a bench rocker. Again, the monolayers were then washed, dried, and the crystal violet dye in each well was then treated with 200 µL methanol for 20 min on a bench rocker at RT. Eventually, the absorbance was measured at \lambda\textsubscript{max} 570 nm using the Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The cytotoxicity concentrations of the mentioned formulae to control were compared using nonlinear regression analysis. The cytotoxicity concentrations of the mentioned formulae to control were compared using nonlinear regression analysis.

2.4.2.2 Inhibitory concentration 50 (IC\textsubscript{50})

The IC\textsubscript{50} values were determined as previously described by crystal violet assay (citations ). Briefly, after washing the 24 h cultured VERO-E6 monolayers in the 96-well tissue culture plates once with 1x PBS: The NRC-03-nhCoV "virus was co-incubated with serial diluted concentrations of the mentioned formulae for 1 h at 37 °C. The Vero-E6 cells were classified as follows: the uninfected cells were represented as control, the infected cells which remain untreated represent the virus control, and the infected cells were treated with mentioned formulae. All cells were kept in a 5% CO\textsubscript{2} incubator for 72 h at 37 °C. The fixation of the cell monolayers took place by the addition of 100 µL of 10% formaldehyde for 20 min, and the staining process occurred by the addition of 0.1% crystal violet "in distilled water" for 15 min at RT. Then 100 µL of absolute methanol was added per well to dissolve the crystal violet dye. The optical density of the color was measured at \lambda\textsubscript{max} 570 nm using the Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC\textsubscript{50} values were calculated using nonlinear regression analysis through plotting log inhibitor versus normalized response.
3. Results And Discussion

3.1 Preparation and characterization

Figure 1 (I) exhibited the XRD of ZnO, Cur, and Cur-ZnO nano 5%, Cur-ZnO nano 10%, and Cur-ZnO nano 20%. The XRD spectrum of the prepared ZnO nanoparticles was well matched with the ICDD card No [01-080-0075]. No characteristic peaks were observed other than ZnO. The XRD pattern of ZnO was in accordance with Reza et al and reflected a pure phase, wurtzite phase. The crystallographic parameters revealed a hexagonal crystal system or crystal lattice structure, the space group p63mc, and space group number 186. The lattice parameters were (a) 3.2539 Å, (b) 3.2539, Å and (c) 5.2098 Å, (α) 90 °, (β) 90 °, and (γ) 120 °. The figure showed strong and distinct diffraction peaks at 2θ [31.72 °, 34.40 °, 36.21 °, 56.51 °, 62.80 °, 66.28 °, 68.99 °, and 72.51 °] with corresponding diffraction plans (100), (002), (101), (110), (103), (200), (201), and (004). The diffraction peaks reflected the high crystallinity of the synthesized ZnO nanoparticles. The basal diffraction peaks of the plan (100) and (101) are distinct peaks of the ZnO nanoparticles. The diffraction peak of (101) was the highest intensity in agreement with [14]. A definite line broadening of the diffraction peaks is an indication that the synthesized materials are in the nanometer range. According to equation 1, the calculated crystallite size of the pure ZnO nanoparticles was 2.47861 Å.

The same figure paraded the crystal form of a neat Cur drug. The spectrum was well-matched with ICDD reference code No. (00-063-0943). The crystallographic parameters revealed a monoclinic crystal system, in agreement with [15]. The lattice parameters were (a) 12.56 Å, (b) 7.04 Å and (c) 19.95 Å, (α) 90 °, (β) 94.9 °, and (γ) 90 °. The figure covers an abundance of diffracted peaks reflecting numerous structural plans and abundant electron densities of its chemical formula C21H20O6. The high signal intensity with a narrow width of the diffracted peaks denotes a good crystallinity state. The crystallographic detected planes were (110), (004), (-301), (114), (-303), (023) at diffraction angles [14.42 °, 18.01 °, 21.07 °, 23.22 °, 24.39 °, and]. These results agree with another study which observed that the characteristic peaks in the spectrum of CUR originate from its crystalline structure [16]. The calculated crystallite size of the pure drug was 5.13976 Å. The obtained XRD spectrum of Cur was similar to a previous study [17].

These two parent nanoparticles were taken as reference for their three products, where X = 5, 10, and 20. The identification process illustrated indexing of all ZnO peaks, which resemble the high percent in all product composites, while for Cur peaks, only the highest peaks were roughly indexed, and it is clear that as the percent of Cur (X) increased, the intensity of Cur peaks increased. The appearance of two parent peaks indicates the successful preparation of composites.

Figure 2 displays the FTIR spectrum of the Cur, PLGA, stoichiometric and non-stoichiometric Cur-PLGA, in addition to nano ZnO, nano Cur-ZnO where X= 5, 10 and 20. According the chemical structure of Curcumin (scheme), it showed its signature peaks at 3450 cm⁻¹ (phenolic O-H stretching vibration), 2850 cm⁻¹ C-H stretch vibration, 1630 cm⁻¹ (aromatic moiety C=C stretching), 1590 cm⁻¹ (benzene ring stretching vibrations), 1510 cm⁻¹ (C=O and C=C vibrations), 1420 cm⁻¹ (olefinic C-H bending vibrations),
1280 cm\(^{-1}\) (aromatic C–O stretching vibrations), and 970 cm\(^{-1}\) (C–O–C stretching vibrations). The obtained Cur spectrum agreed well with that of Xing et al [18]. The stretch of -C-H- was measured in the PLGA (scheme) sample bands at 2890 cm\(^{-1}\). A band at 1750 cm\(^{-1}\) was assigned to the stretching vibration of C=O of the ester bond (strong and narrow), and a band at 1163-1020 cm\(^{-1}\) was assigned to C-O stretching, which is one of the PLGA molecule’s characteristic peaks[19]. The stoichiometric Cur-PLGA (scheme) revealed specific peaks of curcumin and PLGA with slight shifting to the right of the PLGA vibration peaks and evanescence of the vibration peak of the phenolic O-H group of Cur, indicating the formation of a dehydration reaction on conjugation of Cur with PLGA. Figure 3 represents the chemical equation for this dehydration reaction [20].

It is worth noting that all PLGA bands showed more domination rely on the fact that it is the major partner and due to similarities in functional groups of both PLGA and Cur. By increasing Curcumin over the stoichiometric (non-stoichiometric), the phenolic O-H group re-appeared again because the excess Curcumin encapsulated physically inside the PLGA spheres without covalent bond of dehydration reaction. The shift and broadness of the re-appeared band may be due to hydrogen bonds between Curcumin molecules with each other’s In the FTIR spectrum of ZnO, in the fingerprint region, a strong and significant vibration band ranging from 400 cm\(^{-1}\) to 500 cm\(^{-1}\) is assigned to the characteristic stretching mode of the Zn-O bond [21].

After Cur loading, the ZnO bands showed a slight shift to a lower wave number. This result indicates that Cur is hydrogen-bonded to the surface of ZnO. When pure Cur was loaded on ZnO, the intensity of the stretching band decreased in the case of X = 5. This could be due to hydrogen bonds between the oxygen of ZnO and the hydrogen of Cur’s hydroxyl group. The O-H band for Cur cannot be identified in samples with higher percentages of cur, which could be due to hydrogen bond formation between curcumin molecules. Further, one can see a non-significant shift to the right of the Cur carbonyl group, which appears at 1630 cm\(^{-1}\). This shift may be due to resonance between ketone groups and methoxy groups attached to the same aromatic integrity [22]. The bending vibration peak mainly persists and appears in the range of 1479 and 970 cm\(^{-1}\). Many other bands for C-C, C-H, C-O and benzene rings can be identified in the fingerprint region within 1290 cm\(^{-1}\)[23].

Figure 4 represents the HR-TEM images of (a) pure Cur, (b) stoichiometric Cur-PlGA, and (c) non-stoichiometric Cur-PLGA. The images indicated successful conversion of bulk pure curcumin into spherical nanoparticles in the size range of 200 nm, which is the self-assembly behaviour of PLGA molecules. When treated with phosphotungstic acid, Formula (b) showed a majority of hollow spheres appeared as rings. This may be due to the binding of most Cur with PLGA covalently without encapsulation, while formula (c) illustrates opaque spheres, ensuring cur encapsulation inside the spheres physically. (d) Pure ZnO and Cur-ZnO preparations are represented as (e), (f), and (g) for X = 5, 10, and 20, respectively. All formulas showed nearly the same size range below 50nm with an obvious hexagonal shape that well-agreed with what was mentioned in XRD analysis.

### 3.2 Anti-viral activity
The table (1) reported the value of CC50 (µg/ml), IC50 (µg/ml), and selectivity index (CC50/IC50). For the composite, there is a clear and directly proportional relationship between the percent of Cur in the sample and the viral inhibition capability. On comparing results of CC50 and IC50, we found that the Cur-ZnO where X=20 composite showed best results over other lower percentages with an IC50 value of 10.24 micrograms/ml with a significant improvement in CC50 (421 micrograms) over raw Cur (150 micrograms). Generally, ZnO improved the cell toxicity of pure cur. In Cur-ZnO, where X=5 samples, CC50 is nearly similar to pure ZnO. When the percent of Cur became 10% for the composite Cur-ZnO where X=10, CC50 decreased around half its previous value, then nearly was conserved to the same value for Cur-ZnO where X=20. On the other hand, the combination of PLGA with Cur was not a good choice for our application. Cur-PIGA products showed much lower antiviral activity than pure PLGA and pure cur. It seems that the combination between them partially hides the active groups that affect the viral activity. This hypothesis is supported also by the slightly improved antiviral activity by increasing Cur concentration over the stoichiometric weight where some Cur is freely encapsulated without direct reaction with PLGA, but the results are still not promising compared with those of Cur-ZnO where X=20 and even Cur-ZnO where X=10 , figure (5) shows these results.

4. Conclusion

Cur attaches to ZnO physically while there is a chemical covalent bond formed during combination with PLGA that may hide active sites for viral inhibition in both PLGA and Cur. Although pure PLGA owns the best selectivity index owing to its super safe dose, the most potent anti-virus here was proven to be the Cur-ZnO composite with X=20. The promising results of Cur-ZnO where X=20 samples with its economic price compared with Cur-PLGA samples give it the preference over all other samples. It was also clear that the combination of Cur and PLGA for antiviral purposes did not work well as this combination decreases the activity of both pure members or roughly stays the same for the nonstoichiometric formula that has a better effect than the stoichiometric formula, which only relies on chemical bonding. Physical attachments do better for Cur, so it is recommended to investigate Cur-ZnO where X=20 composite activity in many other applications where Cur-PLGA sounds well, as the physically combined composite may sound better.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication Not applicable.

Competing interests: The authors have no competing interests of any type

Availability of data and materials data will be available upon request

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23. Textmap W. Organic chemistry.

**Tables**

**Table (1).** A comparison between CC50, IC50 and selectivity index of all raw and prepared products in this study
<table>
<thead>
<tr>
<th>Sample</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt; µg/ml</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; µg/ml</th>
<th>Selectivity index CC&lt;sub&gt;50&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt;</th>
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<tr>
<td>Curcumin</td>
<td>150</td>
<td>14.24</td>
<td>10.53</td>
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<tr>
<td>PLGA</td>
<td>2975</td>
<td>29.28</td>
<td>101.61</td>
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<tr>
<td>Cur-PLGA (stoichiometric)</td>
<td>935.1</td>
<td>50.55</td>
<td>18.50</td>
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<tr>
<td>Cur-PLGA (Non-stoichiometric)</td>
<td>844.3</td>
<td>26.33</td>
<td>32.07</td>
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<td>48.38</td>
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<td>Cur-ZnO 10%</td>
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<td>18.49</td>
<td>22.59</td>
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<td>Cur-ZnO 20%</td>
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</table>

**Figures**
Figure 1 shows XRD results of Cur, ZnO, Cur-ZnO with X=5, 10 and 20
Figure 2 illustrates FTIR results for Cur, PLGA, stoichiometric and non-stoichiometric Cur-PLGA, ZnO, Cur-ZnO with X=5, 10 and 20.
Figure 3

illustrates the chemical equation of dehydration reaction on binding curcumin with PLGA
Figure 4
shows the pictures obtained from HRTEM for (a) pure Curcumin, (b) stoichiometric Cur-PLGA, (c) non-stoichiometric Cur-PLGA, (d) pure ZnO, (e) Cur-ZnO where X=5, (f) X=10, (g) X=20

Figure 5
shows of the relation between product concentrations and CC$_{50}$ plus IC$_{50}$. (a) shows the results for PLGA, (b) stoichiometric Cur-PLGA, (c) non-stoichiometric Cur-PLGA, (d) Curcumin, (e) Cur-ZnO where X=10, (f)
Cur-ZnO where $X=20$, (g) ZnO, while (h) represent the results of Cur-ZnO where $X=5$

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