Green Synthesis and Optimization of Zinc Oxide Quantum Dots Using the Box-behnken Design, With Anticancer Activity Against the MCF-7 cell line

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Abstract

A green strategy and cost-effective approach was adapted to prepare Zinc oxide quantum dots (ZnO-QDs) for biomedical applications. The prepared ZnO-QDs may hold great promise as sensing scanners for diagnostics and therapy, as demonstrated in our current study. Zinc Sulphate, *Azadirachta indica*, and *Catharanthus roseus* leaves extract were used to synthesis a novel natural Zinc oxide bionanocomposite (ZnO-BC) and used as a precursor to prepare ZnO-QDs by microwave-assisted technique. The ZnO-BC was characterized by SEM-EDX, FTIR, XRD, Zeta potential and particle size analysis. The optical properties of QDs were investigated using UV and PL spectrophotometers. Experimental factors like the concentrations of ZnO-NPs, *C. roseus* and *A. vera* were evaluated using Box-Behnken design (BBD). MTT and haemolysis assay was performed using ZnO-BC and ZnO-QDs. Maximum absorbance observed at optimized values of 0.5% ZnO-NPs, 1g *A. vera* gel and 0.5ml *C. roseus* leaf extract of ZnO-QDs against BBD. There was decreased viability rate, ranging from 60-15% for 0.5mg/ml ZnO-BC and 45-5% for 5 mg/ml ZnO-QDs which revealed a tenfold decrease in cell viability with less concentration scale for 5mg/ml of ZnO-QDs when compared with that of 0.5 mg/ml ZnO-BC. Also, hemolysis test shows that the hemolysis ratio was below 0.5%, indicating non-haemolysis of ZnO-QDs. cellular morphology by results was supported by phase-contrast microscopy images. A good biocompatibility and high anticancer activity was noticed for ZnO-QDs when compared to ZnO-BC and provide versatile applications in the field of Nano biomedicine.

1. Introduction

Semiconductor carbon quantum dots of low-dimensional (<10 nm) has got notable consideration for possible use in medical application such as diagnosis and treatment where larger nanoparticles (>10 nm) are ineffective [1]. Quantum dots (QDs) have gained broad thought for their dominating properties like low poisonousness, small sizes, water solvency, biocompatibility and extraordinary photoluminescence properties [2]. Quantum dots (QDs) is a family of carbonaceous nanostructures and one of the emerging nanomaterial [3]. QDs are characterized as nanoparticles with molecule size more modest than its excite on Bohr radius which is around <20 nm [4]. Additionally, QDs can be simply synthesized with low-cost methods that putaside acomplex, toxic, time-consuming and high-cost synthesis methods [5]. Different expansive range of applications like solar cells, chemo-sensor, drug delivery, biosensor, cell imaging and catalysis [6–11] have been incorporated using carbon quantum dots (CQDs) because of their distinct physical and chemical properties like water solubility, brilliant fluorescence, non-toxicity and biocompatibility. Then again, the capability of utilizing CQDs in different applications expands the need to synthesis them on an industrial scale which is harmless to the ecosystem and green cycles. To avoid the issues of the chemical method of nanoparticle preparation an eco-friendly approach was adopted using plant material which is a green strategy [12]. Heaps of literature surveys have demonstrated benefits in green strategies which are natural cost-effective, environmentally friendly, and simple to increase much of the time, the shortfall of compound chemicals, poisonous foreign substances and utilizing low-valued precursors [13–15]. Other than these there are numerous possible effective molecules

in the plant interceded synthesis of nanoparticles which is appropriate for a wide scope of biomedical applications [12].

A couple of designed strategies of QDs have been accounted, for example, pyrolysis, aqueous carbonization strategy, microwave radiation, laser removal, curve release, and plasma treatment [16–21]. Among them, microwave strategy is considered as a fundamental and green engineered approach due to its possible interaction, utilization of fluid medium and arrangement of QDs with better quantum yield. Also, Microwave radiation was thought to be the best, cheapest, and quickest way to create fluorescent QDs, on the grounds that it doesn't use a lot of equipment, is not difficult to perform, and saves time. Microwave-assisted heating method does not require any firm reaction conditions and is easy to operate compared to other synthetic methods. It is a one-step method of preparation and stabilization of QDs. Therefore, making it an economically and environmental friendly method which could be used in large scale production of fluorescent QDs [22].

Metal and metal oxide nanomaterials have demonstrated critical restorative effects in scientific progress [23] because these nanoparticles have a high surface-to-volume ratio, which allows for better interaction with cancer cells and microscopic organisms [24]. Among these, ZnO is a major semiconductor material with a direct wide band gap and high directivity at 25°C, other than because of huge excitation on restricting energy of 60 meV. The way that ZnO has solid pharmacological properties which increment its utilization in medical services regions, for example, anticancer, antimicrobial, and cancer prevention agents [25] so preparation of ZnO nanomaterial using metals like Zinc can be added with the plant-based synthesis of ZnO quantum dots and would pave a good way to fight against cancer in the forthcoming generations.

Among different cancers, Breast cancer is one of the most widely recognized reasons for the cancer disease in females in the entire world (WCR, 2008). 23% of all as of late happening cancers in ladies called breast cancer have been noticed worldwide and 13.7% has been addressed as death due to cancer because of breast cancer in both genders [26]. Option and necessary medications are at present considered to assume an arising part in the prevention of cancer. Plant-based materials from nature are inside simple reach and are an amazingly encouraging technique for chemo-prevention to impede the advancement of cancer in humans [27]. An outcome, semiconductor nanoparticles/quantum dots (QDs) of low-dimensional have obtained incredible consideration in cancer treatment using nanotechnology [28].

Optimization of various factors over evaluating independent variable and responses relationship is given by Response surface methodology (RSM) [29, 30]. This technique has been successfully applied in a variety of biotechnology fields, as well as some new studies for the green synthesis of carbon quantum dots [31, 32]. RSM was used for the optimization of variables for biological synthesis of ZnO-QDs was performed.

An enormous number of late written works uncovered that thoughtful and simple fabrications of QDs for various cancer investigations and also other biological applications have taken serious efforts. H.S.

Zavareh et al., for example, reported that carbon quantum dots made of Chitosan were used as an anticancer medication to deliver 5-fluorouracil [9], x. li et al. reported that a carbon quantum dot was used for combined photodynamic-chemotherapy examination of cancer cells [33], Ganesan Muthusankar et al. formed a productive probe for the simultaneous determination of anticancer and antibiotic drugs using nitrogen-doped carbon quantum dots implanted CO_3O_4 with multiwall carbon nanotubes. [34]. E.S.M. Cutrim et al. prepared and characterized nanoconjugate based QDs and an *in vitro* anticancer performance was checked [35]. Zinc oxide QDs were prepared for the evaluation of breast cancer, were reported by Zahra Fakhroueian et al [36]. Also a chitosan-encapsulated ZnO quantum dot was designed for *in vitro* drug delivery response by Q. Yuan et al [37]. Accordingly, these research works get the impact of the green way of QDs synthesis with compound interactions of useful hetero atom doping, a gathering of an antecedent, which gives the way to solid arrangement of quantum yield. Nature reliably gets additional thought from material scientists considering its unlimited focal point for making novel materials with less biological effect. To enrich biomass firm QDs, alternate bio-waste/plant materials were used as regular forerunners to set up the quantum dot material [38–39]. Because of their exceptional fluorescence and identifying properties, biomass-derived QDs have received a lot of attention from scientists as a candidate material for semiconductor quantum dots and other fluorescent regular components.

Catharanthus roseus, is additionally notable as Madagascar, *C. roseus* is a type of blossoming plant having a place with the family Apocynaceae. *C. roseus* originated on the Indian Ocean island of Madagascar, and it is now widely distributed in Australia, China, South America, Indonesia, and North America. The plant's leaves are abundant in monomeric indole alkaloids such as vindoline and catharanthine, which have remarkable therapeutic properties such as anticancer, antibacterial, antifungal, anti-microbial, cancer prevention agent, wound healing, and antiviral activities. Likewise, plant leaf concentrates are a rich source of chemically significant terpenoid indole alkaloids, and it is currently used in the semi-union of two anticancer medications (vinorelbine and vinflunine) in pharmaceutical companies [40–41]. Thus, it is profoundly alluring to set up a proficient QD from *C.roseus* leaves without the guide of solid corrosive dissolvable, surface passivation reagent, as well as a complicated post-treatment procedure [38].

By taking into consideration of the above facts, a novel method that combines zinc oxide bionanocomposite derived ZnO-QDs was synthesized using extract of *C. Roseus* and *Aloe vera*, which was then converted to QDs and assessment of their biological activities are reported. *C. roseus* leaves were selected since it contains vinblastine and vincristine. Chemical constituents of *C. roseus* are expected to play an important role in the development of (ZnO-QDs). Interestingly, we exhibit the anticancer activity of *C. roseus* derived ZnO-QDs which are represented in scheme 1. The connection between the biological activity of cancer cells in anticancer activity and haemolytic activity of low dimensional green synthesized QDs are the major highlights of this work. The results of commercial products and control samples were compared. We have primarily engaged one pot approach to prepare QDs without any passivation agent by simple microwave-assisted technique which is a green synthetic and eco-friendly

approach applying *C. roseus* leaves as carbon origin. Critically, our outcomes uncovered that *C. roseus* based QDs have minuscule molecule size, low harmfulness, solid fluorescence and biocompatibility, which have been utilized in cell imaging of MCF 7 cell lines. *C.roseus* based (ZnO-QDs) in ongoing has great likely application for biomedical and environmental pollution.

2. Materials And Methods

2.1. Reagents and Chemicals

C. roseus (*vinca rosea*) leaves were collected from nearby local farms in Tiruvannamalai, Neem leaves and *Aloe vera* was gathered from Arunai Engineering college campus, Tiruvannamalai. Other chemicals like Pharmaceutical grade Zinc Sulphate (ZnSO_4) were purchased from Hi-tech enterprise Pvt Ltd, Chennai, Sodium hydroxide and Glacial acetic acid were purchased from Qualiens Chennai, India.

2.2. Synthesis of ZnO-NPs using neem (*Azadirachta indica*) leaf extract

Neem leaves of around 30 g were completely washed using distilled water and were dried and crushed. 1 L distilled water was added to this and bubbled for around 15 min. The extract was obtained by filtering with Whatman No.1 filter paper with pore size of 35 μm . A pale yellowish colored solution was obtained. An aqueous solution of Zinc sulphate (ZnSO_4) of 1mM and *Azadirachta indica* aqueous leaf extract was utilized for the preparation of ZnO NPs. 20ml of *Azadirachta indica* leaf extract was added drop by drop to 80ml of 1mM ZnSO_4 aqueous solution [42–43]. It was stirred in a magnetic stirrer for about 24 hours at 37°C until a pale white paste formed. This paste was transferred to a porcelain crucible pot and warmed in a heater at 350°C for 1 hour. For the synthesis of ZnO-BC, the obtained pale white colored powder of ZnO NPs was used.

2.3. Preparation of bionanocomposite (ZnO-BC) using *C. Roseusextract*

Prior to preparation, the gathered *C. roseus* leaves, which are high in carbon source, were thoroughly washed in deionized water, hacked into small pieces, and dried at room temperature. 50 mL of deionized water was mixed with 0.5 g of *C. roseus* leaves and bubbled for 15 minutes. From then on, it was permitted to reach room temperature. The pale yellowish arrangement was obtained and separated using a 0.45 μm layer channel to remove unreacted natural moieties and unwanted large particles [38]. 0.5% ZnO NPs were dispersed in 1g of *Aloe vera* gel for 2-3 h and it was sonicated for 5 min at 50 °C. After that, 0.5 ml of *C. Roseus* leaf extract was added dropwise and stirred for 24 hours to achieve a homogeneous mixture. At last, the precipitate was dried at 50 °C overnight in the oven. A brownish-black powder of ZnO-BC was formed and used as a natural carbon source for the synthesis of ZnO-QDs.

2.4. Biogenic preparation of ZnO-QDs from *C. roseus* leaves from ZnO-BC

A one-pot synthesis of microwave assisted procedure was chosen to blend the QDs from the *C. roseus* leaves extract prepared from ZnO-BC as a natural carbon source. 20 ml of 5 mg/ml ZnO-QDs was prepared using 0.5 mg/ml ZnO-BC and suspended in distilled water of 30 ml kept under magnetic stirrer for 10 min. The ZnO-BC suspension was then exposed to microwave for 15 min and then at 8000 rpm, it was centrifuged for 10 min to assist ZnO-QDs synthesis. The supernatant was collected and observed under UV light trans-illumination for fluorescence property and stored for further applications. This entire process for the synthesis of ZnO-QDs is also shown in scheme 1.

3. Characterization Studies

The UV absorption (Perkin Elmer Lambda 9) was carried out to find the excitation wavelength. Zeta potential and Particle size (HORIBA) estimation tests were carried out by using a nanocomposite. The surface morphology and elemental analysis of ZnO-BC were examined using a scanning electron microscope (SEM; JEOL JSM 6360) coupled with an energy-dispersive X-ray (Oxford Instruments, INCApentaFETx3). To investigate the effect of organic species in plant extract on ZnO-BC, FT-IR spectrum (IR prestige 21, Shimadzu) was used. X-ray diffraction (XRD) was used to examine the crystalline nature of the as-prepared ZnO-BC (Bruker D8 Advance).

3.1. Cell culture and MTT assay

MCF7 (breast cancer cell line) was plated independently in 96 well plates with 1104 cells/well concentration in EMEM media with 1X solution of Antibiotic Antimycotic and 10% foetal bovine serum (Himedia, India) with 5% CO₂ at 37 °C in CO₂ incubator. The cells were washed with 200 L of 1X PBS before being treated with different test concentrations of 5 mg/ml prepared ZnO-QDs and 0.5 mg/ml prepared ZnO-BC in serum-free media and incubated for 24 hours. Toward the end of the treatment period, the medium was suctioned from the cells. The prepared 0.5 mg/mL MTT with 1X PBS was incubated at 37 °C for about 4 hours in a CO₂ incubator. After incubation, the MTT-containing medium was removed from the cells and washed with 200 L of PBS. The crystals were disintegrated and completely blended with 100 L of Dimethyl sulfoxide (DMSO) [44, 45]. At 570 nm, there was a development of intensified colour where the formazan dye turned purple-blue. A microplate was used to calculate absorbance at 570 nm.

3.2. Morphological analysis in MCF-7 cell line

Inverted phase-contrast microscope at 20X magnification (OLYMPUS LS CKX 31) was used to see the macroscopic and microscopic changes that occurred in MCF-7 cells which were exposed to different doses of 500-25 µg/ml of ZnO-BC (0.5 mg/ml) and 3.125 - 50 µg/ml of ZnO-QDs (5 mg/ml).

3.3. Haemocompatibility assay

To determine the toxic effect of ZnO-QDs with red blood cells (RBC) Haemolytic assay was carried out using a whole blood sample. Fresh blood was collected using a disposable syringe and stored in a centrifuge tube containing EDTA to prevent the blood from clotting. The blood sample was diluted using 1x PBS buffer in equal volume [46]. To 0.5ml of blood, 5mg/ml ZnO-QDs of the prepared sample was added in different concentrations 50 to 250 μ l which an increment of 50 μ l in an Eppendrofftube, 3 hincubation time was given with agitation for a few minutes after every 1h. Then the sample was centrifuged for 10min at 1600 rpm and then removed the supernatant and the optical density was checked at 540 nm.

3.4. Statistical Analysis for anticancer activity

Analysis of Variance (ANOVA) and Tukey's test was applied to know the high range measurable domain of difference between mean values of cell viability of different cell lines treated with ZnO-QDs Vs. ZnO-BC. All tests were carried out in triplicate, and the results were reported as mean $\pm$ SD [47]. Hence, from the results, statistical significance with p values < 0.05 was contemplated.

3.5. Data analysis of Box–Behnken design for ZnO-QDs

The Box–Behnken design was used in this study to estimate the synergy of the factors for a concentration of ZnONps (X1), A.vera (X2), and C.roseus (X3) on the absorbance response of ZnO-QDs. The range and levels of the variables were observed. For the prepared design, 17 run was performed for the desired factors and levels in this study. Design Expert- 13 software was used to analyze the variables of response surfaces inside the experimental scope. As a result, three additional confirmation experiments were carried out to validate the statistical experimental statistics. Finally, UV-VIS and PL examinations were performed in derived optimum conditions after ZnO-QDs biosynthesis to demonstrate the reasonableness of the BBD.

4. Results And Discussion

4.1. Visual observation of ZnO-QDs

With the developing interest in green synthesis and to restricting the utilization of toxic chemicals and nanoparticles of heavy metals, the improvement of organic, biomimetic, and biochemical methodologies for formingnanoparticles was wanted. Green strategies for nanoparticles synthesis are more invaluable than physicochemical techniques because of their eco-friendly and non-toxic nature. Fig. 1 shows the visual observation of prepared ZnO-QDs from *C.roseus* leaf extract by microwave-assisted method. The commercial ZnO and synthesized ZnO were used as a control sample in order to prove the fluorescence property emitted by ZnO-QDs on exposure to UV light. The presence of a good fluorescence effect was noticed in ZnO-QDs (1c) prepared from ZnO-BC alone when compared to control samples a) commercial ZnO and b) synthesized ZnO. Apart from this the prepared ZnO-BC and ZnO-QDs were characterized using XRD, FT-IR, SEM -EDX, UV spectroscopy, PL spectroscopy, Particle size analyzer and Zeta potential analysis.

4.2. UV and Photoluminescence spectrum

UV spectroscopy was finished to break down the optical properties of processed ZnO-QDs (bare) including ZnO-QDs (*C.roseus*) and shown in Fig. 2 (a) [38]. The UV range revealed broad absorption peaks at 270 and 360 nm, confirming the π - π^* and n - π^* electronic changes caused by the presence of C-O and C-C functional groups in ZnO-QDs. To impress the properties of Photoluminescence of QDs exhaustively, an excitation frequency was adjusted from 270 to 350 nm with period of 10 nm. The most extreme outflow of 500 nm and 550nm was seen at excitation frequency of 330nm for ZnO-QDs (bare) and ZnO-QDs of *C.roseus* (displayed in Fig. 2b). This wavelength confirms that the ZnO-QDs prepared out of *C.roseus* leaf extract showed high intensity when compared to that with ZnO-QDs (bare). It was seen that the extract of natural material acts as penetrating and reducing agents to synthesize quantum dots. The presence of greenish-blue color was seen with the naked eye due to the presence of high intensity which confirms the presence of good optical property [48].

4.3. X-ray diffraction analysis

The calculated crystalline size of the ZnO-BC was about 26.974 nm (Bruker D8 Advance). The obtained XRD graph was given in Fig. 3 and a broad diffraction peak centered at 25.974° (2θ) was witnessed. This implies that the synthesized ZnO-BC was amorphous [42].

4.4. FT-IR spectrum

The explained peaks of the FT-IR spectra are shown in Fig. 4 as proof of the synthetic usefulness of ZnO-BC. The expansive peaks of around 3547 cm^{-1} are traced to the extending of -OH affirms alcoholic, phenolic, and carboxylic structures and the peak at $2230\text{--}2018\text{ cm}^{-1}$ was compared to the aliphatic and aromatic C-H stretches of ZnO-BC. Besides, its C=O group was identified by the peaks at 2342 cm^{-1} and 1101 cm^{-1} . Peaks at 1142 cm^{-1} confirm the C=C bond, and a peak at 1613 cm^{-1} confirms the C-N bond. Besides, the peaks situated at $984\text{--}631\text{ cm}^{-1}$ could be allocated to the C-O and C-C, which was recorded as a single band [38, 12].

4.5. Scanning electron microscopy and energy dispersive X-ray analysis

The morphological components of ZnO-BC saw under SEM showed that the particle size went between 80 nm - 139 nm with between molecule distances. The state of ZnO-BC was seen to be spherical (Fig. 5a). The EDX spectra showed that the combination of zinc and oxygen components in ZnO-BC was 12.81% and 50.67% respectively (Fig. 5b). The adsorbed constituents of the leaf extracts are represented by the carbon peak in the spectrum [49]. Carbon is the primary chemical constituent in both the *C. roseus* and *A. Vera* leaf chemical structures, and it is abundant in leaf extracts. The EDX spectrum demonstrates the formation of particles via bioreduction of plant leaf extracts, confirming the presence of ZnO in ZnO-BC.

4.6. Particle size distribution and Zeta potential measurements

The stability of ZnO-BC was measured through Particle size distribution and Zeta potential (Zetasizer NanoZS, Malvern Instruments, UK). The measurements were made by taking the average of three repeated measurements. The ZnO-BC exhibited a generally narrow particle size distribution with a mean particle size of 557nm which is illustrated in Fig. 6a [50]. Zeta potential provides the idea about surface charges of about -2.7mV indicating that the presence of ZnO-BC dispersed or blended with zinc sulphate, *A. vera* and *C. roseus* which affected the ZnO-BC size and their surface charges. The stability of the biocomposites suggestion was given and the value of zeta potential lies within the stable low range which infers that the green ZnO-BC is unstable in an aqueous medium [49]. Therefore, ZnO-BC may have accumulation due to residual surfactants and may act as more stable components (Fig. 6b).

4.7. Optimization and confirmation experiments of ZnO-QDs using RSM

Optimization of variables concentration for the synthesis of ZnO-QDs was achieved through Box–Behnken design (BBD) of experiments. BBD was particularly preferred for accurate estimates, low-cost and high-quality products development with specific desired features demanded in researches. The data of absorbance of ZnO-QDs was analysed using analysis of variance (ANOVA), regression coefficients, and regression equation using Design Expert-13 software. The Absorbance as a contemporary function of the concentration of ZnO NPs, *A. vera* and *C. roseus* is represented in polynomial equation shown as below in equation (1)

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^3 \beta_{ij} x_i x_j \rightarrow (1)$$

We can learn from the analysis that the model's coefficient of determination (R^2) is 0.9337, indicating that the model is significant. The model F-value is 10.95, indicating that the model is suitable for addressing the genuine connections among the selected factors. Because the P-values are less than 0.05, the independent factors A, B, and C, as well as the quadratic term of AB, AC, BC, and A^2 , B^2 , C^2 , have a large effect on the absorbance of the obtained ZnO-QDs. The 2D form plots and the 3D response surfaces of the reactions using Eq. (1) for the QDs absorbance are displayed in Fig. 7. To demonstrate the intuitive effects of autonomous factors on responses, one variable was kept constant while the other two shifted within a specific range. The shapes of response surfaces and contour plots revealed the extent of interaction between various factors and its nature [29]. Less conspicuous or negligible connections are shown by circular contour plots, whereas relatively notable interactions are generally shown by elliptical shape plots (Fig. 8) illustrating that the concentration of ZnO NPs, *A. vera* and *C. roseus* significantly influences the absorbance of the as-prepared ZnO-QDs, while the circular nature in Fig. 8b demonstrates that the absorbance is not affected by the concentration value of precursor solution significantly.

Graphical optimizations by Design-Expert software provide optimum conditions for the microwave method. Thinking about the two expenses and effectiveness, the optimum operating parameters were observed to be A=1ml, B = 1 g and C = 0.5 ml. That is, the concentration of ZnO-NPs, *A. vera* and *C. roseus* values was fixed at 1ml, 1g and 0.5 ml, separately. Affirmation tests were conducted in three replicates under these conditions. The observed mean absorbance was found to be generally consistent with the expected qualities. The QDs obtained had a high absorbance up to 330nm, which corresponds to the PL properties of the synthesised ZnO-QDs.

4.8. MTT assay

MTT assays were used to compare the cytotoxicity of ZnO-QDs to ZnO-BC. A control (untreated cells) has been established for the comparison of treated cells. In our test, the control shows 100 percent feasibility with the cell lines that were developed to evaluate anticancer activity [50]. The breast cancer cell line was treated against 5 mg/ml ZnO-QDs and 0.5 mg/ml ZnO-BC of varying concentrations ranging from 3.125-50 µg/ml and 25-500 µg/ml respectively. It was inspected from the test that there was decreased viability rate, ranging from 60-15% for 0.5mg/ml ZnO-BC and 45-5% for 5mg/ml ZnO-QDs which revealed a tenfold decrease in cell viability with less concentration scale for 5mg/ml of ZnO-QDs when compared with that of 0.5mg/ml ZnO-BC. Phase-contrast microscopy images were shown in figure 9a & 9b which also distinctly supports the percentage viability of cells for ZnO-BC and ZnO-QDs. Therefore, the prepared ZnO-QDs exhibit more biocompatibility when compared to ZnO-BC [44]. The ZnO-QDs treated cells showed more strain with that of ZnO-BC due to the reduced particle size and presence of green materials (*C.roseus* & *A.vera*) act as a rich carbon sources for the synthesis of ZnO-QDs which in turn are responsible for the anticancer activity and due to its penetrating property against breast cancer cells it can be used as an efficient anticancer drug.

4.9. Hemocompatibility

Haemolytic assay of ZnO-QDs was checked to test its blood compatibility. From the quantitative assessment of Hgb and furthermore, from the actual perception the test was directed to assess the toxicity of synthesized 5mg/ml ZnO-QDs at various concentrations, i.e., 50, 100, 150, 200, and 250 µg/mL (Fig. 10), it is prominent that the OD value for haemolysis was significantly higher with 250µg/ml, while just slight haemolysis was seen with 50µg/ml rather than the negative control. The positive control caused the most hemolysis. According to ASTM (American Society for Testing and Materials Designation), which is standard practise for assessing the hemolytic properties of materials. Which indicates that hemolysis of less than 2% is considered non-hemolytic, 2–5% is considered somewhat hemolytic, and >5% is considered hemolytic [42, 50]. As a result, the results of 5mg/ml ZnO-QDs met the material standard of Hemolysis.

5. Conclusion

We, for the first time, have prepared and reported ZnO-QDs derived from bionanocomposite ZnO-BC by adapting microwave-assisted method without using any toxic chemicals. Also, ZnO-QDs were optimized using BBD of RSM to obtain the optimum reaction conditions of concentration value of ZnO-NPs, *A. vera* and *C. roseus*. Under the optimal conditions, that is, the concentration value of ZnO-NPs, *A. vera*, and *C. roseus* values was fixed at 0.5 %, 1 g and 0.5 ml, respectively. The obtained ZnO-QDs have a high absorbance at 330 nm which was in contrast to that of characterization results obtained. Low concentration range of ZnO-QDs revealed a favourable cytotoxicity when compared to ZnO-BC. From these results, it can be concluded that due to the surface penetration property of ZnO-QDs (*C. roseus* & *A. vera*) it could act as a new type of fluorescence probe that may act as a nano-carrier and can be known to the nano-medical worldwide market after finishing its clinical preliminary and provide promising potential in the field of breast cancer treatment research.

Declarations

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Conflicts of interest

The authors hereby declare no conflicts of interest.

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Scheme

Please see the Supplementary Files for the Scheme 1.

Figures

Figure 1

UV trans illuminator observation a) Commercial ZnO b) Synthesized ZnO c) Synthesized ZnO-QDs

Figure 2

(a) UV–Vis spectra of ZnO-QDs from *Catharanthus roseus* leaves and bare ZnO (b) PL excitation spectrum for excitation wavelength of 330 nm. Inset shows the digital pictures under normal light (left) and UV (right) radiation.

Figure 3

X-ray diffraction pattern of ZnO-BC

Figure 4

Fourier transform infrared spectra showing the functional group of ZnO-BC

Figure 5

(a) Scanning electron micrograph (SEM) of ZnO-BC (b) Energy dispersive X-ray (EDX) analysis of ZnO-BC

Figure 6

(a) Particle size distribution of ZnO-BC (b) Zeta Potential measurements of ZnO-BC

Figure 7

3D graphics for optimization of response surface by plotting absorbance versus concentration of (a) ZnO-NP (ml) and A. vera (g), (b) ZnO-NP (ml) and C. roseus (ml), (c) A. vera (g) and C. roseus (ml) obtained from ZnO-QDs using BBD of RSM

Figure 8

contour showing graphics by plotting absorbance versus concentration of (a) ZnO NPS (ml) and A. vera (g), (b) ZnO-NP (ml) and C. roseus (ml), (c) A. vera (g) and C. roseus (ml) using BBD of RSM for investigation of interacted parameters belonging to the synthesis of ZnO-QDs.

Figure 9

(a & b). Cell viability and MTT assay images using phase contrast microscopy of MCF-7 cells treated against ZnO-BC and ZnO-QDs of concentration ranges :9a -ZnO-BC (a) control (b) 500 $\mu\text{g/ml}$ (c) 250 $\mu\text{g/ml}$ (d)100 $\mu\text{g/ml}$ (e) 50 $\mu\text{g/ml}$ (f) 25 $\mu\text{g/ml}$; 9b -ZnO-QDs (a) control (b) 50 $\mu\text{g/ml}$ (c) 25 $\mu\text{g/ml}$ (d)12.5 $\mu\text{g/ml}$ (e) 6.25 $\mu\text{g/ml}$ (f) 3.125 $\mu\text{g/ml}$ respectively.

Figure 10

(a) Haemolytic analysis image showing plasma from human blood sample incubated with ZnO-QDs (b) Haemolysis caused by different ZnO-QDs concentrations

Supplementary Files

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