

# A Metal Oxide Quantum Dot and Method of Forming Thereof

## Technical Field

The present invention generally relates to a metal  
5 oxide quantum dot. The present invention also relates to a  
method for forming a metal oxide quantum dot.

## Background

Conventional quantum dots such as CdSe and CdTe have  
10 been used in a number of applications such as in  
biomedical imaging. These quantum dots possess excellent  
optical properties such as (i) narrow and symmetric  
emission peaks, (ii) particle size dependent emission  
across the whole visible spectrum and (iii) different  
15 emission colors with a single excitation source. These  
remarkable properties and advances in synthesis of  
different composite nanostructures have allowed these  
quantum dots to continue to drive this research field.  
However, the toxicity of CdSe and CdTe based quantum dots  
20 is an issue. Cadmium is basically carcinogenic and  
therefore, cadmium-based quantum dots pose a threat to the  
environment, especially in optical and biological  
applications. Hence, due to the toxic effect of such  
quantum dots, they cannot be utilized properly in  
25 biological applications and have only been used  
restrictively.

In addition, the process used to form such quantum  
dots such as colloidal synthesis may be complicated and  
irreproducible. Here, colloidal semiconductor nanocrystals  
30 are synthesized from precursor compounds and organic  
surfactants dissolved in solution. Upon heating the  
solution, the precursors decompose to form monomers, which  
then nucleate and generate nanocrystals. The temperature  
during the synthetic process and the concentration of the

monomers must be appropriately chosen as they are critical factors which must be stringently controlled. The choice of temperature affects the nanocrystal growth, which involves rearrangement and annealing of atoms during the synthesis process and the growth of the crystal. The choice of the monomer concentration affects the growth process of the nanocrystals. Hence, this process is a sophisticated process that requires control of a number of factors to achieve quantum dots of the desired size and distribution. In addition, due to the unpredictability associated with the crystal growth stage, this method may result in quantum dots that vary between batches, affecting the quality of the quantum dots.

There is a need to provide a quantum dot that overcomes, or at least ameliorates, one or more of the disadvantages described above.

There is a need to provide a method for forming a quantum dot that overcomes, or at least ameliorates, one or more of the disadvantages described above.

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### Summary

According to a first aspect, there is provided a metal oxide quantum dot having a plurality of pores disposed on the surface of the metal oxide quantum dot.

Advantageously, the metal oxide quantum dot itself is porous. Hence, the metal oxide quantum dot may not need to be encapsulated or integrated with another porous compound to impart the porous property to the quantum dot. Advantageously, the pores present on the metal oxide quantum dot may be used for incorporating therapeutic agents or small molecules for delivery to a desired site.

Advantageously, the metal oxide quantum dot may be biocompatible and may not be carcinogenic.

According to a second aspect, there is provided a method for forming a porous metal oxide quantum dot comprising the step of decomposing a metal salt in the presence of a pore-forming agent to form said porous metal  
5 oxide quantum dot.

Advantageously, the method may form the pores *in situ* during formation of the metal oxide quantum dot.

Advantageously, the method may form a quantum dot having a desired fluorescence emission. This may be due to  
10 the selection of a decomposition temperature of the metal salt that then determines the particle size of the formed metal oxide, which in turn affects the color emitted by the quantum dot.

Advantageously, the method may be easy to scale up  
15 and may be less complicated than conventional methods. The disclosed method may avoid the nucleation and growth stages associated with conventional methods, leading to a more consistent product that is reproducible and does not suffer from batch-to-batch variation. According to a  
20 third aspect, there is provided a method for forming a water soluble porous metal oxide quantum dot comprising the steps of (a) decomposing a metal salt in the presence of a pore-forming agent to form a porous metal oxide quantum dot; and (b) coating the porous metal oxide  
25 quantum dot with a hydrophilic compound to form the water soluble porous metal oxide quantum dot.

Advantageously, the water soluble quantum dot may be used in biomedical applications.

According to a fourth aspect, there is provided a  
30 water soluble porous metal oxide quantum dot for use as a drug delivery agent.

Advantageously, the drug may be incorporated in the pores of the metal oxide quantum dot and delivered to a desired site *in vivo* or *in vitro*.

According to a fifth aspect, there is provided a water soluble porous metal oxide quantum dot for use as a chemotherapeutic agent.

According to a sixth aspect, there is provided use of  
5 a water soluble porous metal oxide quantum dot in the manufacture of a medicament for the treatment of cancer.

### Definitions

The following words and terms used herein shall have  
10 the meaning indicated:

The term "quantum dot" is to be interpreted broadly to refer to any semiconductive or metallic nanoparticle that is capable of emitting a light signal. The particle size of the nanoparticle may be about 1 nm to about 1000  
15 nm, about 2 nm to about 100 nm, about 10 nm to about 60 nm, or about 30 nm to about 60 nm. The shape of the quantum dot is not limited and may be in the shape of a sphere, a rod, a wire, a pyramid, a cube, or other geometric or non-geometric shapes. The colour of the  
20 light emitted by the quantum dot depends on a number of factors that include the size and shape of the quantum dot. For example, a quantum dot with a larger particle size emits light with a lower energy as compared to a quantum dot made of the same material but with a smaller  
25 particle size.

The term "mesopore" is to be interpreted broadly to refer to a pore having a size in the range of about 2 nm to about 50 nm. The size of the particle may refer to the diameter, and hence a mesopore may refer to a pore that  
30 has a diameter in the range of about 2 nm to about 50 nm, or about 2 nm to about 15 nm, or about 2 nm to about 12 nm.

The term "hydrophobic" is to be interpreted broadly to refer to a substance, such as a quantum dot, that

exhibit a low intermolecular attraction for aqueous solvents such as water. Alternatively, the term "hydrophilic" is to be interpreted broadly to refer to a substance, such as a surface modified quantum dot, that  
5 exhibits a high intermolecular attraction for aqueous solvents such as water.

The term "peptide" is to be interpreted broadly to refer to a molecule containing at least two amino acid residues which are linked to each other by peptide bonds.  
10 The term "amino acid residue" as used herein refers to an amino acid either in the D or L form or to an amino acid mimetic that can be incorporated into a polypeptide by an amide bond. The amino acid residues may be naturally occurring amino acids or synthetic amino acids as well as  
15 modified amino acids and derivatives of naturally occurring amino acids.

The terms "decomposition", "decomposing" or grammatical variants thereof are to be interpreted broadly to refer to a chemical breakdown of a chemical compound  
20 into its elements or a simpler compound. Decomposition may include thermal decomposition in which the chemical compound breaks down when heat is applied such that the temperature of the chemical compound is at or above its decomposition temperature.

25 The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

30 Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

#### **Detailed Disclosure of Embodiments**

Exemplary, non-limiting embodiments of a metal oxide quantum dot will now be disclosed.

The metal oxide quantum dot has a plurality of pores disposed on the surface of the metal oxide quantum dot.

The pores may form areas of depression on the surface.

5       The pores may have a dimension in the range of about 2 nm to about 50 nm, about 2 nm to about 40 nm, about 2 nm to about 30 nm, about 2 nm to about 20 nm, about 2 nm to about 10 nm, about 5 nm to about 50 nm, about 10 nm to about 50 nm, about 20 nm to about 50 nm, about 30 nm to  
10   about 50 nm, about 40 nm to about 50 nm, about 5 nm to about 15 nm, about 5 nm to about 6 nm or about 10 nm to about 11 nm. The above dimension may refer to the diameter of the pores.

      Due to the size of the pore, the pore may be termed  
15   as a "mesopore" and the quantum dot may be termed as a mesoporous (metal oxide) quantum dot. The pore size may be favourable for the incorporation of a therapeutic agent or a small molecule for delivery to a desired site.

      The metal of the metal oxide may be selected from  
20   Groups 8 to 14 of the Periodic Table of Elements, particularly Groups 8, 10, 11, 12 or 14 of the Periodic Table. The metal may be selected from the group consisting of zinc, iron, copper, tin and nickel. Hence, the metal oxide may be zinc oxide, iron oxide, copper oxide, tin  
25   oxide or nickel oxide. The metal oxide may be biocompatible and may be non-carcinogenic.

      The metal oxide quantum dot may have a dimension in the nano-size range. The dimension of the metal oxide quantum dot may be at least 30 nm, about 30 nm to about  
30   1000 nm, about 30 nm to about 100 nm, about 30 nm to about 60 nm, about 30 nm to about 40 nm, about 30 nm to about 50 nm, about 40 nm to about 50 nm, about 40 nm to about 45 nm, or about 45 nm to about 50 nm. The dimension may refer to the diameter of the quantum dot. The dimension may

refer to the average diameter of the quantum dot. Where the quantum dot is not exactly spherical in shape, the diameter (or average diameter) may refer to the equivalent diameter (or average equivalent diameter).

5           The quantum dot may be substantially hydrophobic. The metal oxide quantum dot may emit a fluorescence having a wavelength in the range of about 350 nm to about 650 nm. The color emitted by the quantum dot may be tuned from green to yellow spanning the visible wavelength spectrum.

10           The metal oxide quantum dot may have a specific surface area in the range of about 30 to about 60 m<sup>2</sup>g<sup>-1</sup>, about 30 to about 40 m<sup>2</sup>g<sup>-1</sup>, about 30 to about 50 m<sup>2</sup>g<sup>-1</sup>, about 40 to about 60 m<sup>2</sup>g<sup>-1</sup>, about 50 to about 60 m<sup>2</sup>g<sup>-1</sup>, about 30 to about 35 m<sup>2</sup>g<sup>-1</sup>, or about 50 to about 55 m<sup>2</sup>g<sup>-1</sup>.

15           In order to tailor the solubility of the quantum dot, a hydrophilic coating may be provided on the metal oxide quantum dot. The hydrophilic coating may make the coated quantum dot water soluble. The hydrophilic coating may comprise a water soluble compound. The water soluble  
20 compound may be a water soluble polymer. The water soluble polymer may be selected from the group consisting of a polysaccharide, a protein, a peptide and a synthetic polymer. The water soluble polymer may be selected from the group consisting of xanthan, pectin, chitin, chitosan,  
25 dextran, carrageenan, guar gum, hyaluronic acid, albumin, glutathione, starch, starch derivative, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, sodium carboxy methyl cellulose, poly(ethylene glycol), polyvinyl pyrrolidone, polyvinyl  
30 alcohol, polyacrylic acid, polyacrylamides, N-(2-hydroxypropyl) methacrylamide, divinyl ether-maleic anhydride, polyoxazoline, polyphosphate and polyphosphazene. Exemplary, non-limiting embodiments of a method for forming a porous metal oxide quantum dot will

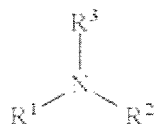
now be disclosed. The method comprises the step of decomposing a metal salt in the presence of a pore-forming agent to form the porous metal oxide quantum dot.

The metal of the metal salt (and hence, that of the corresponding metal oxide) may be selected from Groups 8 to 14 of the Periodic Table of Elements, particularly Groups 8, 10, 11, 12 or 14 of the Periodic Table. The metal may be selected from the group consisting of zinc, iron, copper, tin and nickel. The metal salt may form the metal oxide upon thermal decomposition. Hence, the metal salt may be a metal acetylacetonate, metal chloride, metal nitrate or a metal acetate.

The temperature used during the decomposing step may depend on the type of metal salt to be decomposed. Where the metal salt is zinc acetylacetonate, the decomposition temperature is at least 200°C. By increasing the decomposition temperature, the particle size of the formed quantum dot increases. As the colour emitted by a quantum dot depends in part on its particle size, by altering the particle size through control of the decomposition temperature, the colour emitted by the quantum dot can be tailored as desired. Hence, the method is a one-step method to form porous metal oxide quantum dots having tunable fluorescence (or photoluminescence).

The pore-forming agent may contribute to the formation of the pores and may be a coordinating agent. The coordinating agent may be a templating surfactant which may induce the formation of pores such as mesopores. The coordinating agent may be selected from the group consisting of amines, fatty acids, alkyl thiols, alkyl phosphine oxides, alkyl phosphonic acids, alkyl phosphines, nitrogen-containing aromatics, and mixtures thereof.

The coordinating agent may be an amine. The coordinating agent may be an amine of Formula (I):



wherein:

- 5  $R^1$  is an optionally substituted  $C_{1-35}$  alkyl,  $C_{1-35}$  alkenyl or  $C_{1-35}$  alkynyl; and  
 $R^2$  and  $R^3$  are hydrogen, optionally substituted  $C_{1-35}$  alkyl,  $C_{1-35}$  alkenyl or  $C_{1-35}$  alkynyl.

The amine coordinating agent may be derived from its  
10 acid precursor.

The coordinating agent may be dodecylamine, tetradecylamine, hexadecylamine, octadecylamine, oleylamine, oleic acid, or mixtures thereof.

The solvent may be a solvent with a high boiling  
15 point to increase the temperature of the reaction such that the decomposition temperature (or higher temperatures) can be reached. The solvent may aid in the formation of the metal oxides. The boiling point of the solvent may be above the decomposition temperature of the  
20 metal salt. The solvent may be selected from the group consisting of benzyl ether, alkyl benzoate (such as methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, pentyl benzoate or hexyl benzoate), ethylene glycol and benzyl alcohol.

25 The combination of the coordinating agent and the solvent may aid in the formation of the metal oxide with pores thereon.

The method may be undertaken in an inert atmosphere. The inert atmosphere may be a nitrogen or an argon  
30 atmosphere. By having an inert atmosphere, the nucleation and growth of the quantum dots may be controlled, leading

to quantum dots with a uniform particle size and distribution. The inert atmosphere may also aid in the creation of the uniform pores on the metal oxide quantum dots. After the metal oxide quantum dots are formed, the  
5 quantum dots may be purified and isolated.

As the quantum dots obtained are substantially hydrophobic, they may be coated with a hydrophilic coating to form water soluble quantum dots. Hence, a method for forming a water soluble porous metal oxide quantum dot  
10 comprises the steps of (a) decomposing a metal salt in the presence of a pore-forming agent to form a porous metal oxide quantum dot; and (b) coating the porous metal oxide quantum dot with a hydrophilic compound to form the water soluble porous metal oxide quantum dot.

15 The hydrophilic compound may comprise a water soluble compound. The water soluble compound may be a water soluble polymer. The water soluble polymer may be selected from the group consisting of a polysaccharide, a protein, a peptide and a synthetic polymer. The water soluble  
20 polymer may be selected from the group consisting of xanthan, pectin, chitin, chitosan, dextran, carrageenan, guar gum, hyaluronic acid, albumin, glutathione, starch, starch derivative, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, sodium carboxy methyl cellulose, poly(ethylene glycol), polyvinyl  
25 pyrrolidone, polyvinyl alcohol, polyacrylic acid, polyacrylamides, N-(2-hydroxypropyl) methacrylamide, divinyl ether-maleic anhydride, polyoxazoline, polyphosphate and polyphosphazene.

30 The porous metal oxide quantum dot may be coated with the hydrophilic compound using a reverse microemulsion method. Here, reverse micelles may first be formed using a suitable surfactant in an organic solvent. The surfactant may be an anionic surfactant, a cationic surfactant or a

non-ionic surfactant. The surfactant may be of the Igepal® brand (from Sigma-Aldrich) such as CA-210, CA-420, CA-520, CA-620, CA-630, CA-720, CA-887, CA-890, CA-897, CO-520, CO-630 or CO-897. The surfactant may be selected from  
5 sodium lauryl sulphate, polysorbate or poloxamer. Once the reverse micelles are formed, the water soluble compound may be added, followed by the porous metal oxide quantum dots. The coated metal oxide quantum dots may then be subjected to purification and isolation.

10        Upon coating, the size of the quantum dot may increase while the pore size may decrease. The pore size after coating may be in the range of about 2 nm to about 10 nm. The fluorescence emitted by the quantum dot may remain the same after coating.

15        The coated quantum dots may be multifunctional in the sense that it is photoluminescent, porous and has a chemotherapeutic effect.

      The water soluble porous metal oxide quantum dot may be well dispersed in water with good colloidal stability  
20 for a prolonged period of time. The water soluble porous metal oxide quantum dot may be stable for close to 2 months, or close to 3 months.

      The water soluble porous metal oxide quantum dot may be used to label a cell in vivo or in vitro. The water  
25 soluble porous metal oxide quantum dot may be used for cell imaging.

      The water soluble porous metal oxide quantum dot may be used as a drug delivery agent. The drug may be incorporated into the pores and delivered to a desired  
30 site.

      The water soluble porous metal oxide quantum dot may be cytotoxic and hence may be used as a chemotherapeutic agent. There is also provided the use of a water soluble porous metal oxide quantum dot in the manufacture of a

medicament for the treatment of cancer. The dosage of the quantum dots may be in the micromolar concentration range for killing cancer cells.

5

### Brief Description Of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1(a) is a digital photograph ZnO(200) under a UV lamp at a wavelength of 365 nm. Fig. 1(b) is a digital photograph ZnO(300) under a UV lamp at a wavelength of 365 nm. Both ZnO(200) and ZnO(300) were obtained according to the process of Example 1 below.

Fig. 2 is a graph showing the UV-Vis absorption spectra of ZnO(200) and ZnO(300) quantum dots.

Fig. 3 is a graph showing the photoluminescence spectra of bluish-green emitting ZnO(200) quantum dots samples excited at different wavelengths (300 nm, 310 nm, 320 nm, 330 nm, 340 nm and 350 nm).

Fig. 4 is a graph showing the photoluminescence spectra of yellow emitting ZnO(300) quantum dots samples excited at different wavelengths (300 nm, 310 nm, 340 nm, 350 nm and 360 nm).

Fig. 5(a) and Fig. 5(c) are transmission electron microscopy (TEM) images of ZnO(200) and ZnO(300) quantum dots respectively. The scale on these images is 100 nm. Fig. 5(b) and Fig. 5(d) are high-resolution transmission electron microscopy (HRTEM) images of ZnO(200) and

ZnO(300) quantum dots respectively. The scale on these images is 10 nm.

Fig. 6 is a graph showing the X-ray diffraction (XRD) patterns of ZnO(200) (depicted by (a)) and ZnO(300) (depicted by (b)).

Fig. 7(a) is a graph showing the energy dispersive X-ray (EDX) analysis of ZnO(200) while Fig. 7(b) shows the same for ZnO(300).

Fig. 8(a) is a graph showing the nitrogen adsorption/desorption isotherms of ZnO(200) while Fig. 8(b) shows the same for ZnO(300).

Fig. 9 is a graph showing the UV-vis absorption spectra of uncoated ZnO(300) (depicted by (a)) and coated ZnO(300)@GSH (depicted by (b)).

Fig. 10 is a graph showing the nitrogen adsorption/desorption isotherms of ZnO(300)@GSH-5x.

Fig. 11 shows a series of fluorescence microscopy images of labeled HeLa cells incubated with ZnO(300)@GSH(3x) at concentrations of 0.25 mg/ml (depicted by (a)), 0.35 mg/ml (depicted by (b)), 0.45 mg/ml (depicted by (c)), 0.55 mg/ml (depicted by (d)), 0.65 mg/ml (depicted by (e)) and 0.75 mg/ml (depicted by (f)). The scale bar on these images is 20  $\mu$ m.

Fig. 12(a) is a graph showing the cell viability of CHO cells when incubated with 0.25 mg/ml of ZnO(300)@GSH(1x). Fig. 12(b) is a graph showing the cell viability of HeLa cells when incubated with 0.25 mg/ml of ZnO(300)@GSH(1x).

Fig. 13(a) is a graph showing the cell viability of CHO cells when incubated with 0.75 mg/ml of ZnO(300)@GSH(3x). Fig. 13(b) is a graph showing the cell

viability of HeLa cells when incubated with 0.75 mg/ml of ZnO(300)@GSH(3x).

### Examples

5 Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

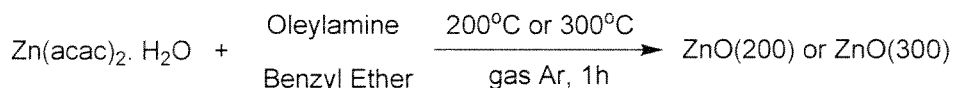
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### Example 1

#### Synthesis of Mesoporous Zinc Oxide Quantum Dots

Zn(acac)<sub>2</sub>·H<sub>2</sub>O (0.844 g, 3.0 mmol), oleylamine (15 ml) and benzyl ether (15 ml) were added into a 100 ml three-necked round-bottom flask connected to a Schlenk line. The mixture was pumped under vacuum and purged with Argon for 15 to 30 minutes. The mixture was heated to 200°C or 300°C under Argon flow and magnetically stirred at 650 rpm. The reactions were kept at that temperature for 1 hour to form the zinc oxide particles, as shown in Scheme 1 below.

20



### Scheme 1

The heating mantle was removed and the white-milky solution was cooled down. 15 ml of CHCl<sub>3</sub> was added to the mixture. The particles were purified with CHCl<sub>3</sub>/EtOH (volume 1:3) by centrifugation/re-dispersion cycles (3 times at 3500 rpm for 15 minutes). The precipitate was dissolved in 10 ml of chloroform and centrifuged once again to separate bigger quantum dots (6000 rpm for 10 minutes). The final precipitate was dissolved in 5 ml of CHCl<sub>3</sub>. Most of the chemicals used were purchased from

Sigma-Aldrich (of Missouri of the United States of America).

The zinc oxide (ZnO) quantum dots formed at 200°C and 300°C, were denoted as ZnO(200) and ZnO(300) respectively.

5 Under the UV trans-illuminator, ZnO(200) and ZnO(300) gave green and yellow fluorescence, respectively, as shown in Fig. 1.

In Fig. 2, the UV-Vis absorption spectra of ZnO(200) and ZnO(300) quantum dots showed the absorption bands at  
10 371 and 375 nm, respectively. The photoluminescence spectra of ZnO(200) and ZnO(300) quantum dots are shown in Fig. 3 and Fig. 4, respectively. As seen in Fig. 3, the emission peaks are centered at 385 nm and 468 nm for ZnO(200) quantum dots while as seen in Fig. 4, the  
15 ZnO(300) quantum dots exhibited a broad emission spanning the wavelengths 450 to 550 nm with a small peak at 475 nm. The corresponding digital images of the quantum dots samples are shown in the insets of Fig. 3 and Fig. 4.

The morphology of the ZnO quantum dots was visualized  
20 by TEM (refer to Fig. 5(a) for ZnO(200) and Fig. 5(c) for ZnO(300)) and HRTEM (refer to Fig. 5(b) for ZnO(200) and Fig. 5(d) for ZnO(300)). The HRTEM images (Fig. 5(b) and Fig. 5(d)) of ZnO(200) and ZnO(300) quantum dots showed an average diameter of 41 and 47 nm, respectively. Both  
25 quantum dots synthesized at these temperatures displayed mesoporosity.

The XRD patterns (Fig. 6) of ZnO(200) (depicted in Fig. 6 by (a)) and ZnO(300) (depicted in Fig. 6 by (b)) quantum dots showed an intense [101] peak, confirming the  
30 Wurtzite-type single-crystalline ZnO. In order to determine the composition of ZnO quantum dots, an elemental mapping using EDX was performed. The elemental mapping of Zn and O for ZnO(200) and ZnO(300) quantum dots are shown in Fig. 7(a) and Fig. 7(b) respectively.

The nitrogen adsorption/desorption isotherms of the as-synthesized mesoporous ZnO(200) and ZnO(300) quantum dots are shown in Fig. 8(a) and Fig. 8(b) respectively. The shape of the isotherms corresponds to a type IV isotherm according to the Brunauer-Deming-Derning-Teller (BDDT) classification. This indicated the presence of mesoporosity. The BET analysis revealed that the specific surface area of ZnO(200) quantum dots was  $54.40 \text{ m}^2\text{g}^{-1}$  with a pore diameter of 6.74 nm while the specific surface area of ZnO(300) quantum dots was  $32.06 \text{ m}^2\text{g}^{-1}$  with a pore diameter of 10.71 nm.

## Example 2

### Coating of ZnO with L-Glutathione Peptide

The ZnO quantum dots from Example 1 were purified according to conventional purification procedures in which the quantum dots were purified three times with 1:3 volume ratio of cyclohexane/ethanol and centrifuged at 3500 rpm for 10 minutes, and finally dispersed in deionized water. The purified ZnO quantum dots were coated with L-glutathione (GSH) peptides using a reverse microemulsion method such that initially hydrophobic ZnO quantum dots became water soluble ZnO quantum dots.

Reverse micelles were prepared by adding 1 mL of Igepal CO-520 and 9 mL of cyclohexane. To 2 mL of reverse micelles, 100  $\mu\text{L}$  of GSH (5mg/mL deionized water) was added. The solution was stirred until it became clear. Then, 100  $\mu\text{L}$  of tetramethyl ammonium hydroxide (TMAH) was added, followed by loading specific amounts of ZnO quantum dots (1 mg, 3 mg and 5 mg). The solution was stirred at room temperature (of about  $25^\circ\text{C}$ ) for 18 hours.

The coated ZnO quantum dots were purified by re-dispersion in ethanol and centrifugation cycles. The coated particles were dissolved in 4 mL deionized water.

The various samples with 1 mg ZnO(300), 3 mg ZnO(300) and 5 mg ZnO(300) were denoted as ZnO(300)@GHS(1x), ZnO(300)@GHS(3x) and ZnO(300)@GHS(5x), respectively.

Fig. 9 shows the UV-vis absorption spectra of uncoated (depicted in Fig. 9 by (a)) and coated ZnO(300)@GHS(1x) (depicted in Fig. 9 by (b)). As shown in Fig. 9, the absorption band at 374 nm, which is similar to the absorption band for the uncoated quantum dots at 375nm. The nitrogen adsorption/desorption isotherms of the coated ZnO(300)@GHS(5x) quantum dots are shown in Fig. 10. The shape of the isotherms corresponds to a type IV isotherm according to the Brunauer-Deming-Derning-Teller (BDDT) classification. This indicated the presence of mesoporosity. The BET analysis revealed that the specific surface area of the coated ZnO(300)@GHS(5x) quantum dots was  $61.09 \text{ m}^2\text{g}^{-1}$  with a pore diameter of 2.37 nm. Hence, coating of the quantum dots resulted in an increase of the specific surface area and a decrease in the pore diameter, which can be attributable to the presence of the glutathione peptides on the surface of the quantum dots. This pore diameter allows for the incorporation of drug molecules.

In order to determine the fluorescence ability of the coated quantum dots in a cell, the coated quantum dots were incubated with HeLa cells for 2 hours. Here, the HELA cells were grown on 6 well-plates with cover slips in respective media. The samples of ZnO were added to the cover slips having cells with media in the ratio of 1:10 and incubated for 2 h at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$ . The cover slips containing ZnO treated cells were thoroughly washed three times with PBS and fixed by incubating in 4% paraformaldehyde for 15 minutes followed by PBS washing three times.

The fluorescence microscopy images of labeled HeLa cells incubated with GSH-coated ZnO QDs (ZnO(300)@GHS(3x)) at different concentrations (0.25-0.75 mg/mL) are shown in Figure 11. It is obvious that these QDs will be suitable for understanding the endogenous activity of cells.

### Example 3

#### Cytotoxicity of Coated ZnO Quantum Dots

The cytotoxicity effects of quantum dots are tested on the viability of two different cell lines - non-cancerous CHO and cancerous HeLa cells.

The HeLa and CHO-K1 cells were obtained from ATCC (Manassas, Virginia of the United States of America) and cultured in a T75 flask with complete growth media (DMEM high glucose and F-12 Kaighn's modification media were used for HeLa and CHO-K1 cells respectively, containing 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin) at 37°C and 5% CO<sub>2</sub>. For cell cytotoxicity, 1x10<sup>4</sup> HeLa cells per well were seeded in a 96-well plate. Following incubation overnight, cell culture medium was removed and 100 µL of ZnO samples diluted in phenol-free DMEM were added to the cells. The ZnO samples were then removed after 24 hours of incubation at 37°C, and the treated cells were washed with PBS. Thereafter, 100 µL of DMEM and 10 µL of cell counting kit (CCK-8, Sigma-Aldrich) solution were added to the cells. After incubation at 37°C for 3 hours, the absorbance was measured at 450 nm using a Tecan Infinite 200 microplate reader. A similar assay was setup for the 48hr time point except that the ZnO samples were incubated for 48 hours. All experiments were performed in triplicates.

Fig. 12 compares the viability of these two cells incubated with a lower concentration of quantum dots or sample ZnO(300)@GHS(1x) at 0.25 mg/ml. It is evident that

this concentration could be used for cellular studies as it is completely non-toxic for CHO cells, whereas for HeLa cells it is more toxic, indicating that the quantum dots have a chemotherapeutic effect. Furthermore, Fig. 12 shows that a shorter incubation time can be used for anti-cancer studies rather than longer incubation time.

Fig. 13 compares the viability of these two cells incubated with a higher concentration of quantum dots or sample ZnO(300)@GHS(3x) at 0.75 mg/ml. Fig. 13 shows that even at higher concentrations, the quantum dots are non-toxic in normal cells (CHO) compared with the cancer cell line (HeLa), making the quantum dots more suitable for anti-cancer studies.

### Applications

The disclosed quantum dots can be used in biomedical applications for cell labelling, cell imaging or cell targeting.

The disclosed quantum dot may be used for drug delivery or as a chemotherapeutic agent.

The disclosed quantum dot may be biocompatible and may not be toxic to non-cancerous cells.

The disclosed method may be a one-step method to form a quantum dot that is porous and which the fluorescence (or photoluminescent) property can be tuned by adjusting the temperature used in the method.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

## Claims

1. A metal oxide quantum dot having a plurality of pores disposed on the surface of said metal oxide quantum  
5 dot.

2. The metal oxide quantum dot according to claim 1, wherein said pores have a dimension in the range of 2 nm to 50 nm.

10 3. The metal oxide quantum dot according to claim 1 or 2, wherein said metal is selected from Groups 8 to 14 of the Periodic Table of Elements.

15 4. The metal oxide quantum dot according to claim 3, wherein said metal is selected from the group consisting of zinc, iron, copper, tin and nickel.

20 5. The metal oxide quantum dot according to any one of the preceding claims, wherein said metal oxide quantum dot has a dimension of at least 30 nm.

25 6. The metal oxide quantum dot according to any one of the preceding claims, wherein said metal oxide quantum dot emits a fluorescence having a wavelength in the range of 350 nm to 650 nm.

30 7. The metal oxide quantum dot according to any one of the preceding claims, wherein said metal oxide quantum dot has a specific surface area in the range of 30 to 60 m<sup>2</sup>g<sup>-1</sup>.

35 8. The metal oxide quantum dot according to any one of the preceding claims, further comprising a hydrophilic coating on said metal oxide quantum dot.

9. The metal oxide quantum dot according to claim 8, wherein said hydrophilic coating comprises a water soluble compound.

5 10. The metal oxide quantum dot according to claim 9, wherein said water soluble compound is a water soluble polymer.

10 11. The metal oxide quantum dot according to claim 10, wherein said water soluble polymer is selected from the group consisting of a polysaccharide, a protein, a peptide and a synthetic polymer.

15 12. The metal oxide quantum dot according to claim 11, wherein said water soluble polymer is selected from the group consisting of xanthan, pectin, chitin, chitosan, dextran, carrageenan, guar gum, hyaluronic acid, albumin, glutathione, starch, starch derivative, hydroxypropylmethyl cellulose, hydroxypropyl cellulose,  
20 hydroxyethyl cellulose, sodium carboxy methyl cellulose, poly(ethylene glycol), polyvinyl pyrrolidone, polyvinyl alcohol, polyacrylic acid, polyacrylamides, N-(2-hydroxypropyl) methacrylamide, divinyl ether-maleic anhydride, polyoxazoline, polyphosphate and  
25 polyphosphazene.

13. A method for forming a porous metal oxide quantum dot comprising the step of decomposing a metal salt in the presence of a pore-forming agent to form said  
30 porous metal oxide quantum dot.

14. The method according to claim 13, wherein said metal is selected from Groups 8 to 14 of the Periodic Table of Elements.

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15. The method according to claim 13 or 14, wherein said metal is selected from the group consisting of zinc, iron, copper, tin and nickel.

5        16. The method according to any one of claims 13 to 15, wherein said metal salt is a metal acetylacetonate, metal chloride, metal nitrate or metal acetate.

10       17. The method according to any one of claims 13 to 16, comprising the step of selecting said pore-forming agent from the group consisting of a coordinating agent, a solvent and combination thereof.

15       18. The method according to any one of claims 13 to 17, wherein said decomposing step is undertaken in an inert atmosphere.

19. A method for forming a water soluble porous metal oxide quantum dot comprising the steps of:

20       (a) decomposing a metal salt in the presence of a pore-forming agent to form a porous metal oxide quantum dot; and

      (b) coating said porous metal oxide quantum dot with a hydrophilic compound to form said water  
25       soluble porous metal oxide quantum dot.

20. The method according to claim 19, wherein said hydrophilic compound comprises a water soluble polymer selected from the group consisting of xanthan, pectin,  
30       chitin, chitosan, dextran, carrageenan, guar gum, hyaluronic acid, albumin, glutathione, starch, starch derivative, hydroxypropylmethyl cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, sodium carboxy methyl cellulose, poly(ethylene glycol), polyvinyl pyrrolidone,  
35       polyvinyl alcohol, polyacrylic acid, polyacrylamides, N-

(2-hydroxypropyl) methacrylamide, divinyl ether-maleic anhydride, polyoxazoline, polyphosphate and polyphosphazene.

5        21. A water soluble porous metal oxide quantum dot for use as a drug delivery agent.

22. A water soluble porous metal oxide quantum dot for use as a chemotherapeutic agent.

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23. Use of a water soluble porous metal oxide quantum dot in the manufacture of a medicament for the treatment of cancer.