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Size-driven fluorescent properties in Nanophotonics

Munzir Akhtar[1], Abhilasha Mishra[2]*, Priyank Vyas[1], Harsh Pratap Singh[3], Adeeba Mirza[2] & Pallavi Joshi[4]

[1]Department of Biotechnology; & [2]Department of Chemistry, Graphic Era Deemed to be University; [3]Department of Mechanical Engineering; & [4]Department of Agriculture, Graphic Era Hill University, Dehradun-248 002, Uttarakhand, India

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Despite the diverse application of nanotechnology, nanophotonics is still behind the curtains. There's limited insight into how intrinsic and extrinsic factors affects the photonic properties of nanoparticles. In this work, an attempt is made to critique the nature of particles exploiting its micro, nano, and quantum size as intrinsic factors. This fluorescence can be regulated with the aid of extrinsic factors too *viz*- temperature, pH *etc*. *Ex-vivo* study in hepatic tissue portray bioaccumulation as well as enzymatic shifts. Fluorescence decreases with increases in temperature but tends to be constant at 26±2°C for all three sizes and it increases with increases in pH with transition between pH=2 and 4 however, this increase is maximum in Quantum size. Concentration has parabolic gradient in all three sizes. Quantum particles show greater accumulation and effect on peroxidase activity as compared to other sizes. It is concluded that the fluorescence intensity of particles increases with decrease in size. However, its value can be regulated by altering extrinsic factors. Size variations also meet with extent of bioaccumulation. As reduced peroxidase activity indicates compromised antioxidant defence and increased susceptibility to oxidative damage. These studies serve as basis for engineering application of fluorophores regulating their fluorescent properties.

Keywords: Bioaccumulation, Peroxidase activity, Stoke shift, Microwaves, Quantum size

Nanophotonics entails exploiting light for molecules in the nanoscopic range. Nanophotonics, a frontier in nanoscience where light meets the nanoworld holds the key to revolutionary advancements in technology and science[1]. The ability to control light on such a tiny scale is like having a traffic system of photons. It is on the nanoscale where light reveals its most intriguing behavior and untapped potential. Here light can be manipulated to create outer efficient LED improving solar cell performances[2] or even developing new ways to travel to transmit data in telecommunication[3]. Fluorescence also display application in biological[4] as well as pharmaceutical sectors[5]. Fluorescence appears when a molecule absorbs light of lower wavelength (higher energy) and emits light of higher wavelength (lower energy). Fluorescence is a qualitative parameter and is known by fluorescence intensity on quantitative scale. “Fluorescence Intensity” is an indirect measure of Stoke’s shift. In fluorescence spectroscopy, stoke’s shift can be defined as the difference between spectral positions of the maximum of first absorption band and the maximum of the fluorescence emission band (spectral shift) which is expressed in wavelength[6]. In spectroscopy laboratories, the stoke shift is measured as the separation between lambda max in the excitation and emission spectra of flurophore. The shift varies with molecular structure and thus crucial for fluorescence experiments[7]. The value of Fluorescence Intensity or Stoke’s Shift depends upon either the intrinsic structure of a fluorophore or its solvent properties. For a molecule, its structure remains the same unless its size is exploited.

The environmental factors include temperature[7,8], pH[7], concentration (Ionic Strength)[7], viscosity *etc*. Some researchers reported that the fluorescence intensity increases with decrease in temperature and vice versa.[8]However, in case of pH, the fluorescence is quenched at particular pH value or may be due to solvent’s properties. The process is called as fluorescence masking or fluorescence quenching[9,10]. Fluorescence also increases when the concentration of fluorophore decreases but upto a certain limit. Beyond this limit, the intensity decreases. This limit is known as Threshold Concentration of Fluorescence[11,12]. The value of viscosity also influenced the fluorescence intensity of a fluorophore. It was observed that Fluorescence intensity increases with decrease in viscosity[13]. The above-mentioned parameters can be explored for regulating the fluorescence action of the fluorophores.

*Correspondence:
E-mail: abhi1680@geu.ac.in

When size as an intrinsic factor is concerned, the fluorescence is increased while switching from micro to nanosize due to greater surface area volume ratio. Moreover, while acquiring quantum size *i.e.*- 1 to 10 nm, the fluorescence becomes everlasting. When attained this small range, the particles are said to be "Quantum Dots"[14,15].

Quantum dots are semiconducting nanocrystalline materials with diameters usually 2-10 nm meaning around 10-15 atoms. These display unique electronic properties intermediate of those between bulk semiconductor and discrete molecules[16]. It is on account of extra-large surface area volume ratio and the quantum confinements due to limited number of atoms due to limited number of atoms that leads to quantization leading to the yield of colors[17]. When the quantum yield or the stoke shift is large, the sample becomes fluorescent. Fluorescence of quantum dots can be ascertained in the same way like that of other fluorescent molecules. Rhodamine 6G is a fluorophore whose absorption ranges between 440 nm and 570 nm, with the absorption maxima recorded at 530 nm. On the other hand, its emission spectra range extends from around 510 nm to about 710 nm with the emission maxima at 550 nm[18,19]. However, there seem possibilities of increasing quantum confinements when the size of the particles decreases. Nanoparticle-based technologies present promising opportunities for advancing disease diagnosis and treatment. Leveraging the distinctive properties of nanoscale materials requires careful optimization of their size, shape, and surface chemistry to tailor their functionalities for various biomedical applications[20].

However, Extrinsic factors such as temperature[21], pH[22], concentration[23] as well as nature of solvent[23] has a vital role in displaying the extent of fluorescence. In this study, an attempt is made to undertakes on how nanoparticle size influences its fluorescent intensity and the comparative behavior of the same at varying extrinsic factors funnelling their outputs in biomedical applications and also assessing bioaccumulation in tissues, including aspects such as cellular uptake, tissue distribution, and retention over time.

Experimental Procedure

Materials and requirements

The materials and equipment used in this study included Rhodamine G60 (RhG60) (Sigma-Aldrich), de-ionized water, and a variety of laboratory instruments. RhG60 was weighed and dissolved in 30 mL of de-ionized water. A microwave reactor (Biotage India Pvt. Ltd, India) was used for microwave ablation of the prepared mixture, followed by dilution and further microwave treatment. For dynamic light scattering (DLS) and particle size analysis (PSA), a Zeta-sizer Lab (Malvern Instruments) was employed, along with a LabMan ultrasonicator (LabMan Instruments) for sonication of the samples. Fluorescence spectroscopy was conducted using a Globe Instruments spectrophotometer (Globe Instruments, India) to analyze the absorption and emission properties of the samples, along with a temperature-controlled setup involving a spirit lamp and thermometer to assess temperature effects. pH adjustment and concentration variation in solutions were done using hydrochloric acid (Sigma-Aldrich), sodium hydroxide (Sigma-Aldrich), and a pH meter (Thermo Fisher). For bioaccumulation studies, confocal microscopy (Leica Microsystems) was used to analyze nanoparticle uptake in chicken liver tissue slices, which were sourced from a common slaughterhouse. Peroxidase activity was assessed qualitatively by observing the decomposition of hydrogen peroxide on liver tissue slices.

Preparation of fluorophore

0.03 g of rhodamine G60 (RhG60) was weighed and dissolved in 30 mL de-ionized water. The solution was mixed thoroughly on the magnetic stirrer.

Microwave ablation

Size reduction was executed with same protocol as done by Hatim et al, 2013 with minor modifications in time lapse.[25]Microwave reactor used was of Biotage India Pvt. Ltd, India. Before ablation, the 8 mL of this mixture was taken in a separate vial and labelled as α-size (microparticle). The remaining solution was microwaved for 30 sec. After 30 sec, 10 mL of De-ionized water is added to it. The colloid was again microwaved for 30 sec. After 30 sec, 10 mL of de-ionized water was added to it and shaken well. 8 mL of this solution was taken in a separate vial and labelled as β-size (nanoparticle). 10 mL de-ionized water was again added to remaining 2 mL solution and microwaved for 30 sec. After 30 sec, 20 mL of de-ionized water was added to it. The colloid was microwaved for 60 sec. The resulting colloid was taken in a separate vial which was labelled as γ-size (quantum particle)[24,25].

DLS-PSA

DLS- PSA estimated the average size of particles. The polydispersity index corresponds to the measure of

dispersion. The DLS-PSA model used was Zeta-sizer lab, Malvern. The prepared colloid was diluted to 25% in De-ionized water and ultrasonicated for 20 min using LabMan ultasonicator at the room temperature (25°C). The sonicated sample was subjected to DLS in DTS0012 cuvette and using the tabulations, the plot of size *vs* percent intensity is constructed.

Fluorescence spectroscopy

Effect of size

All the three samples were subjected to fluorescence spectrophotometer of model, Globe Instruments, India. The absorption and emission plots were plotted on the same graph for a sample. The absorption and emission maxima were determined from each sample in the range of 500 nm to 650 nm.

Calculation of stoke shift

The intensity of fluorescence of any particle is the direct function of stoke shifts. Stokes shift is nothing but the difference between lambda max of the absorption spectrum and emission spectrum of the single electronic transition. Therefore, it could be calculated with the aid of formula.

Stokes shift = Absorption (wave number) – Emission (wave number)

However, the wavelength was converted to wave number by reciprocating it[26].

Fluorescence spectroscopy

Effect of temperature

The dilute solution of a sample was prepared by mixing 5 mL of the colloid in 95 mL of de-ionized water. The dilution factor was also ascertained. The apparatus was set up near the fluorescence spectrophotometer. A beaker containing the prepared dilute colloid was put on the tripod stand. The spirit lamp was used as the heat source and the thermometer was installed to record the increase of temperature. As the temperature kept on increasing, the aliquots were taken at the temperature of 10, 20, 30, 40, 50, 60°C. The spectroscopy was performed instantaneously. The readings of stoke shift were factorized based on the dilution factor. Using the final tabulations, a graph of temperature *vs* fluorescent intensity (Stoke Shift) was plotted. The protocol was repeated for two other samples as well[27].

Effect of pH

The pH solution kit was prepared by using Hydrochloric acid and sodium hydroxide solution. The pH of all the solutions was adjusted from 2-13 with the aid of pH meter. The 96 well titre plate was taken and the horizontal columns were taken for all three sizes of particles, taken in duplicates whereas the vertical row was exploited as pH gradient. The particles and samples were made to react in the wells and the titre plate was observed after 5 min. The readings were taken using the spectrophotometer. Using the tabulations, a graph of pH *vs* fluorescence intensity was plotted. The protocol was repeated for two other samples as well[28].

Effect of concentration

All the three prepared colloid were diluted to various concentration as 20%, 40%, 60%, 80% and then subjected to fluorescence spectrophotometry. A graph of concentration *vs* fluorescence intensity was plotted if there exit a significant correlation between the two parameters[29].

Bioaccumulation in liver tissues

Novel protocol was established to investigate the bioaccumulation of nanoparticles in chicken liver tissue using confocal microscopy. Thinly sliced liver tissues (~300-500 μM) were cultured in a nutrient-rich medium at 37°C and 5% CO_2. The tissues were then exposed to fluorescent nanoparticles for 4-24 h, washed with PBS, and fixed in par formaldehyde for subsequent imaging and analysis[30].

Peroxidase activity of liver slice

An assessment of liver's peroxidase activity can be made qualitatively based on the action of enzyme to decompose hydrogen peroxide into hydrogen and oxygen. To qualitatively assess liver peroxidase activity, fresh liver tissue slices are prepared and placed in a reaction vessel. A small volume of hydrogen peroxide (H_2O_2) solution is added directly onto the tissue slices. The enzymatic decomposition of H_2O_2 is indicated by the immediate formation of oxygen bubbles on the tissue surface. The rate and intensity of bubble production are observed as a qualitative measure of peroxidase activity. Controls using tissue slices pre-heated to denature enzymes or without H_2O_2 are included to ensure specificity of the reaction

Results and Discussion

Microwave ablation

The following colloidal solutions are prepared.

- α size (Microparticles)
- β size (Nanoparticles)
- γ size (Quantum Particles)

Beyond this size, the degradation of dye started as loss of fluorescence and appearance of impurities prevails. Figure 1 reveals the uprise in fluorescence.

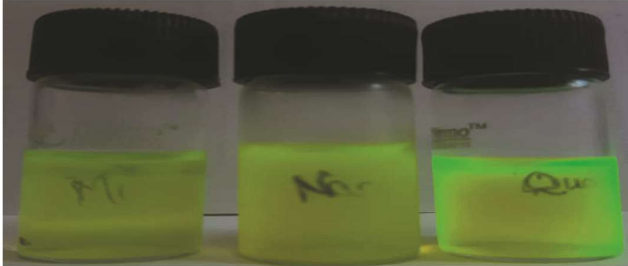

Fig. 1 — The fluorescence of particles observed in dim light

Dynamic light Scattering- Particle size analyzer

The size of micro, nano, quantum particles come out to be 1033 nm, 78 nm and 6 nm with the polydispersity index of 0.2, 0.2 and 0.6, respectively. These particles lost their properties beyond quantum size and thus, the quantum size is the most native size that expresses maximum fluorescence. From the results, it is clear that Fluorescence increases with decrease in size (Fig. 2).

Absorption and Emission and calculation of stoke shift

As the average size of particles decreases, the fluorescence intensity increases. The greater surface area volume ratio accounts for the statement. Moreover, the approach of quantum size directly

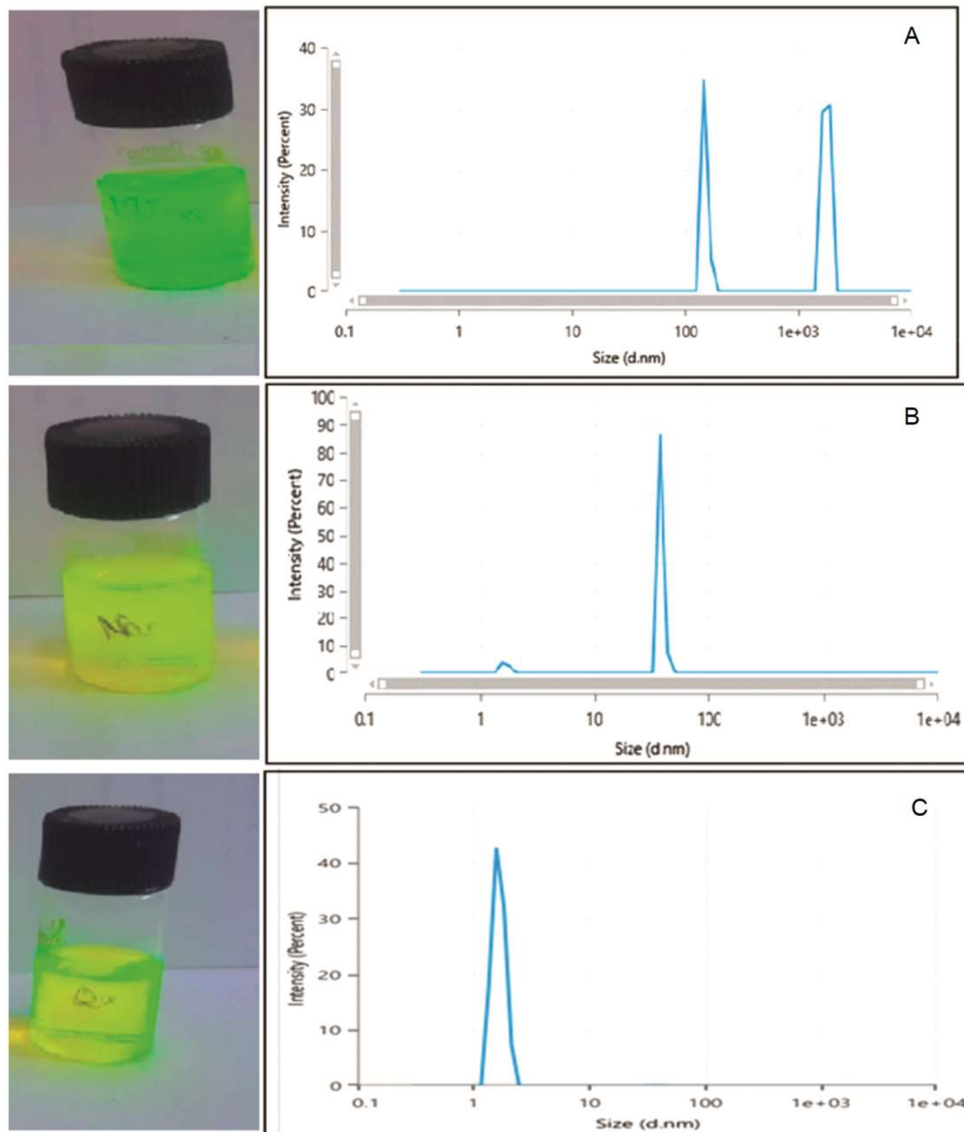

Fig. 2 — The fluorescence observation and the PSA plot of the (A) micro particles; (B) Nanoparticle; and (C) Quantum particles

established the fact that the quantum confinements hold potential for the emission of tremendous fluorescence. In Figure 3A the absorption and emission peak occur around 530 nm and 583 nm resulting in stoke shift of 53 nm. The absorbance values extent up to ~1.0, indicating a relatively strong absorption, but the fluorescence emission intensity is comparatively lower than in the nano and quantum-sized counterparts. This is principally because larger micro-sized particles have a smaller surface area-to-volume ratio, leading to limited surface reactivity and reduced quantum confinement effects. These particles do emit fluorescence, but not as intensely as smaller particles. Figure 3B shows nano-sized particles, with absorption maxima near to 526 nm and emission maxima upto 590 nm yielding stoke shift of 64 nm. Compared to the microsized particles, the emission peak is broader and more intense, though the absorbance is slightly lower (~0.55). The increase in Stokes shift and emission intensity demonstrates that as particle size reduces to the nanoscale, quantum effects begin to influence fluorescence. The enhanced fluorescence can be attributed to a higher surface area-to-volume ratio, which allows for more surface-active sites and effective electron-hole recombination, enhancing the emission. Figure 3C highlights quantum-size particles — typically in the range of quantum dots. These particles exhibit a pronounced quantum confinement effect, as reflected by the largest Stokes shift of 85 nm, with absorption around 520 nm and emission close to 605 nm. Though the absorbance peaks around 0.6, the emission is significantly more intense and red-shifted, showcasing strong fluorescence. This shift is a signature of quantum confinement, where the electronic properties are dictated by particle size. In quantum-sized particles, energy levels become discrete, leading to tuneable fluorescence properties and greater efficiency in light emission. The progression from micro to nano to quantum-size particles in Figure 3 illustrates a clear enhancement in fluorescence as particle size decreases. The increasing Stokes shift and emission intensity confirm that quantum confinement and surface area effects play vital roles in fluorescence.

Effect of temperature

From the Figure 4, it is interpretated that fluorescence decreases with increase in temperature however, after a certain limit, it tends to be constant as the statement holds true by previous researches as well[13]. In this case this limit is 26±2°C. This limit is approximately same for all the three size and thus can be considered as a constant for a particular molecule as RhG6 in this case.

Effect of pH

The fluorescence increases with increase in pH milieu. The Fluorescence is highest in Quantum size with less fluctuations after attaining the alkaline pH. The same was being reported by Han *et al*. in 2016[14]. In all three particles, the fluorescence is nearly zero at the lower pH. Therefore, the acids or salts with lower pH can be used to mask the fluorescence, the process being called as fluorescence quenching. The nanosize as well as the quantum size bears immediate transition however, in case of microsize, fluorescence increases on gradual scenario (Figs. 5 & 6).

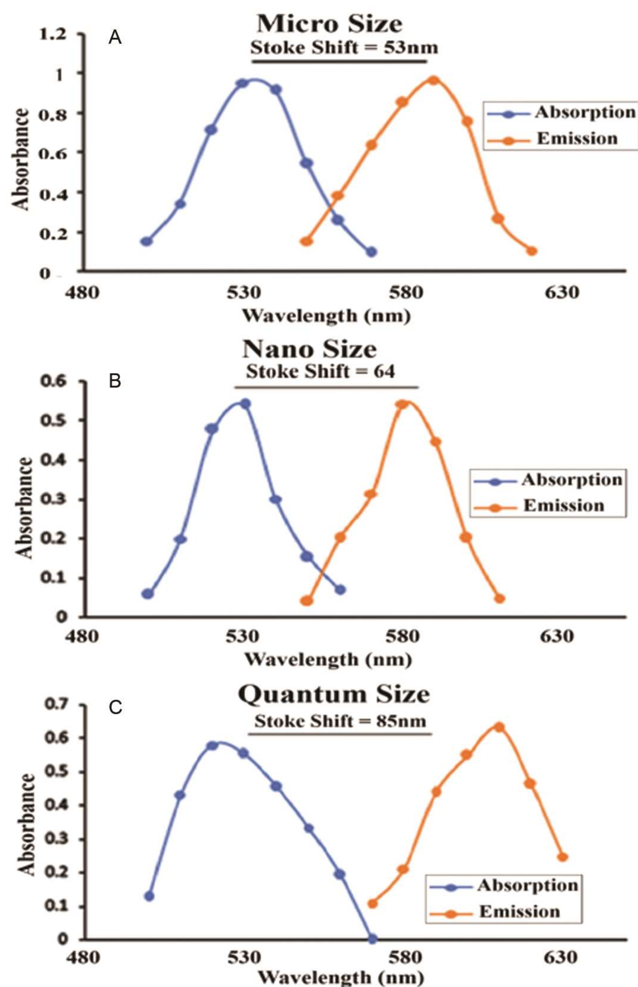

Fig. 3 — Absorption and emission spectra of RhG6 particles at varying size scales: (A) micro-size particles display a Stokes shift of 53 nm; (B) nano-size particles exhibit a Stokes shift of 64 nm; and (C) quantum-size particles display a maximum Stokes shift of 85 nm. As particle size decreases, the Stokes shift increases and fluorescence emission becomes more intense due to enhanced surface area-to-volume ratio and quantum confinement effects

Effect of Concentration

The fluorescence of fluorophore increases with increase in concentration but upto a certain limit. Exceeding this limit fluorescence decreases. This limit can be called as fluorescence maximawhich reflects the optimal concentration for maximum emission before quenching effects become significant. Some researchers point out the conclusion that at higher concentration, particles aggregate reducing the fluorescence. However, it depends on the specific solvent used[15]. The graph in Figure 7 illustrates the variation in Stoke shift with respect to concentration for three different sizes of particles: microparticles, nanoparticles, and quantum particles. Each curve demonstrates a distinctive fluorescence behavior where the Stoke shift increases with concentration up to a certain point, after which it declines. For microparticles, the maximum Stoke shift observed is 50 nm at a concentration of 10%. In the case of nanoparticles, the Stoke shift peaks at 67 nm around 12% concentration, while quantum particles show the highest Stoke shift of 83 nm at approximately 11.4% concentration.

This behaviour can be explained by the increased availability of fluorophore molecules at lower concentrations, leading to enhanced fluorescence. However, as the concentration increases beyond a certain threshold, particle aggregation becomes more likely, especially in dense solutions. Aggregation leads to energy transfer between closely spaced particles and non-radiative losses, resulting in fluorescence quenching and a subsequent decrease in the Stoke shift. The differences in the peak values among the three particle types highlight how particle size and optical properties influence fluorescence behavior. Microparticles tend to aggregate more readily, while nanoparticles and quantum particles can sustain higher concentrations before aggregation becomes significant. Moreover, the solvent enviro nment plays a crucial role in stabilizing these particles and modulating their fluorescence response, although this was held constant in the current context.

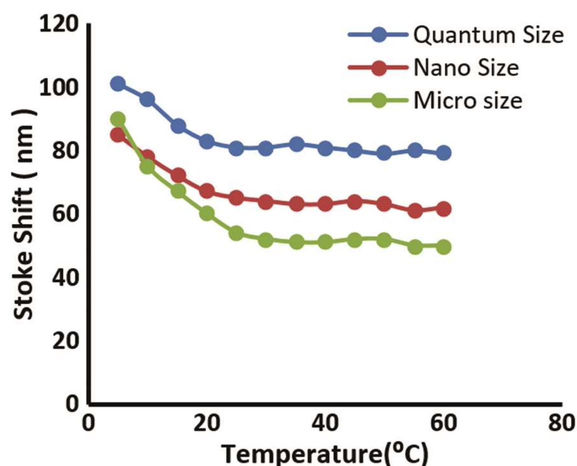

Fig. 4 — The plot of Temperature *vs* Stoke Shift in all three sized particles

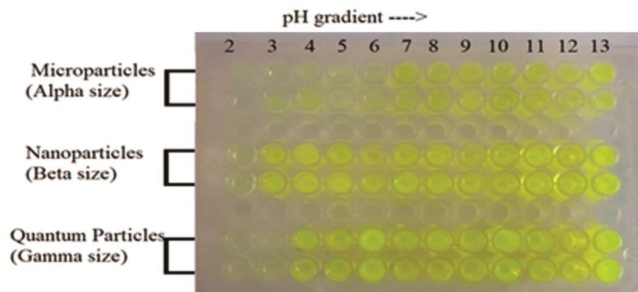

Fig. 5 — The titre-plate containing the reaction mixture: Size *vs* pH Mileau

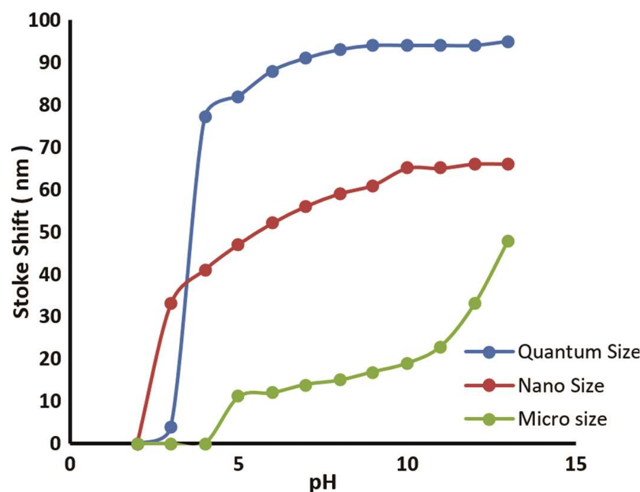

Fig. 6 — The plot of pH *vs* Stoke Shift of the three sized particles

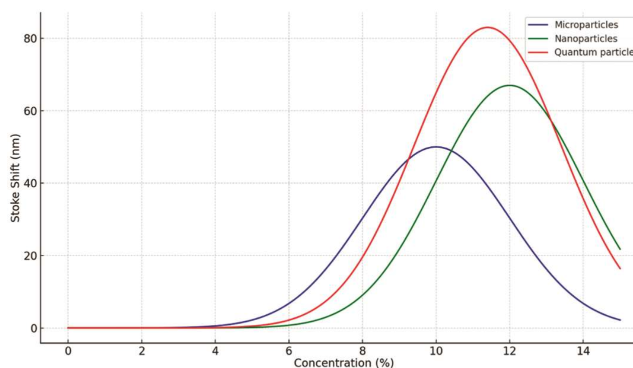

Fig. 7 — The plot of Stoke shift *vs* concentration of three sized particles portraying fluorescence maxima for being Quantum particles having highest value indicating greater fluorescence between concentration of 11 to 12%

Accumulation in liver tissue

The amount of accumulation is the direct function of visible green fluorescent area in the microscopic section. The microscopy results clearly demonstrate the higher accumulation in case of quantum nanoparticles whereas there is but not much in nanoparticles (Fig. 8). However, very less accumulation is observed in microparticles.

Peroxidase activity

The control sample exhibited the highest peroxidase activity, as evidenced by the substantial froth production observed. This indicates that the peroxidase enzyme in the control tissue slices effectively decomposed hydrogen peroxide, leading to rapid oxygen release. In contrast, the quantum particles displayed minimal froth formation, suggesting that these particles have a profound effect on peroxidase activity. The nanoparticle-treated tissues showed a slower reaction, with less froth formation compared to the control, indicating that their enzymatic activity was less pronounced but still detectable. On the other hand, the microparticle-treated tissues displayed froth production similar to that of the control, implying that the microparticles did not significantly alter the peroxidase enzyme's ability to decompose hydrogen peroxide. These observations suggest that the size of the particles influences the rate of peroxidase activity, with the control and microparticles showing the most rapid enzymatic response, while the nanoparticles and quantum particles exhibited slower or reduced activity (Fig. 9). A reduction in peroxidase activity in liver tissue is a momentous indicator of oxidative stress[31]. Peroxidases enzymes, like glutathione peroxidase and catalase, play a vital role in neutralizing hydrogen peroxide and other reactive oxygen species (ROS). When their activity is diminished, hydrogen peroxide accumulates, leading to oxidative damage of cellular components, including lipids, proteins, and nucleic acids[32-33]. This oxidative damage can compromise membrane integrity, disrupt enzyme function, and ultimately lead to hepatocellular injury. Such damage is especially concerning in the liver, a vital organ responsible for detoxification and metabolic homeostasis[34]. Furthermore, decreased peroxidase activity can impair mitochondrial function, reduce ATP synthesis and contribute to energy deficits in hepatocytes. Over time, this can promote cell death through necrosis or apoptosis, exacerbating liver inflammation and possibly contributing to chronic liver conditions such as fibrosis or cirrhosis[35]. Additionally, the liver's reduced ability to detoxify harmful compounds may increase systemic toxicity, making the organism more vulnerable to environmental or chemical insults[36]. Thus, lowered peroxidase activity not only reflects oxidative imbalance but also has cascading effects on liver function and overall physiological health.

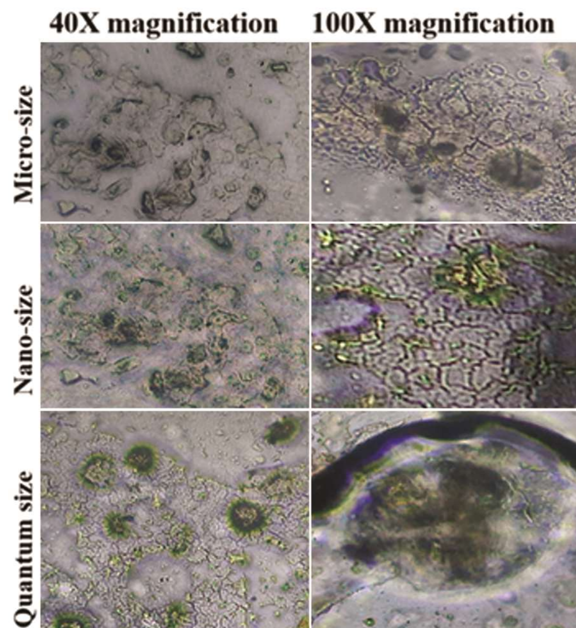

Fig. 8 — Accumulation of three size nanoparticles in liver tissue of *Gallus gallus*

Fig. 9 — Peroxidase activity of liver tissue slices as observed as froth production when exposed to (A) Quantum particle; (B) Nanoparticle; (C) Microparticle; and (D) Control

Conclusion

This study demonstrates that the size of colloidal particles plays a crucial role in determining their fluorescence properties and interactions with liver tissue. By utilizing microwave ablation, three distinct particle sizes were generated: microparticles, nanoparticles, and quantum particles. The smallest quantum particles, measuring 6 nm, exhibited the highest fluorescence intensity due to their increased surface area-to-volume ratio, which enhances their optical properties. The study revealed that fluorescence

intensity is inversely related to particle size, with quantum particles displaying the highest Stokes shift. Temperature and pH were also found to influence fluorescence, with optimal conditions observed at 26°C and alkaline pH. Additionally, concentration-dependent fluorescence showed a maximum at intermediate concentrations, beyond which aggregation reduced fluorescence. Furthermore, the study highlighted the role of particle size in modulating peroxidase activity, with microparticles exhibiting the strongest enzymatic response. In contrast, quantum particles demonstrated reduced peroxidase activity which indicates oxidative stress, leading to accumulation of reactive oxygen species (ROS) and cellular damage. This impairment compromises liver function, promotes hepatocellular injury, and may contribute to chronic liver conditions. Lowered peroxidase activity has cascading effects on liver function and overall physiological health, increasing vulnerability to toxicity. Notably, quantum particles showed the highest accumulation in liver tissue, suggesting their potential for targeted applications, such as imaging and drug delivery.

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Conflict of interest

All Authors declare no conflict of interest.

References

1 Iqbal MA, Nanophotonics: fundamentals, challenges, future prospects and applied applications. Nonlinear Optics-Nonlinear Nanophotonics and Novel Materials for Nonlinear Optics (IntechOpen), (2021).
2 Amalathas PA & Alkaisi MM, Nanostructures for light trapping in thin film solar cells. *Micromachines*, 10 (2019) 619.
3 Craiciu I, Heshami K, Holzinger S, Bourgoin J P, Sinclair N & Tittel W, Nanophotonic quantum storage at telecommunication wavelength. *Phys Rev Appl*, 12 (2019) 024062.
4 Conde J, Tian F & Baptista PV, Nanophotonics for molecular diagnostics and therapy applications. *Int J Photoenergy*, 2012 (2011) 619530.
5 Ullah F, Abbas M, Ahmad B, Ullah R, Sohail M, Liu H & Wang L, Fluorescent and phosphorescent nitrogen-containing heterocycles and crown ethers: biological and pharmaceutical applications. *Molecules*, 27 (2022) 6631.
6 Thaer AA & Sernetz M, Fluorescence techniques in cell biology. Springer Science & Business Media, (2012).
7 Zacharioudaki DE, Fitilis I & Kotti M, Review of fluorescence spectroscopy in environmental quality applications. *Molecules*, 27 (2022) 4801.
8 Värkonyi Z & Kabók K, Effect of temperature on light-absorption and fluorescence of the peroxidase. *Acta Biochim Biophys Acad Sci Hung*, 10 (1975) 129.
9 Srisantitham S, Sukwattanasinitt M & Unarunotai S, Effect of pH on fluorescence quenching of organic dyes by graphene oxide. *Colloids Surf A*, 550 (2018) 123.
10 Patil NR & Melavanki RM, Effect of fluorescence quenching on 6BAAC in different solvents. *Can J Phys*, 92 (2014) 41.
11 Dhara A, Panja S, Guha A, Basu S & Saha S, Zero-overlap fluorophores for fluorescent studies at any concentration. *J Am Chem Soc*, 142 (2020) 12167.
12 D'Souza A V, Spring BQ, Rizvi I & Hasan T, Effects of modeled optical properties on recovered fluorophore concentration during image-guided fluorescence tomography. *Opt Methods Tumor Treat Detect Mech Tech Photodyn Ther XXII (SPIE)*, (2013), 8568.
13 Campos ECG, Silva TA, Medeiros ES, Lima FMA, Oliveira HPM, Silva FB & Paterno LG, Viscosity-induced dual-emission of europium ions containing metallopolymer. *Synth Met*, 273 (2021) 116686.
14 Gidwani B, Vyas A, Sahu R, Maravi P & Shrivastava A, Quantum dots: Prospectives, toxicity, advances and applications. *J Drug Deliv Sci Technol*, 61 (2021) 102308.
15 Kwapiszewski R, Skolimowski M, Ziolkowska K, Chudy M, Brzózka Z & Kaminski T, Effect of a high surface-to-volume ratio on fluorescence-based assays. *Anal Bioanal Chem*, 403 (2012) 151.
16 Ghosh D, Ivanov SA & Tretiak S, Structural dynamics and electronic properties of semiconductor quantum dots: Computational insights. *Chem Mater*, 33 (2021) 7848.
17 Mohamed WAA, Mohamed RG, Atta AMM, Elsayed D, Elnouby M, El Rouby WMA & Shalaby RM, Quantum dots synthetization and future prospect applications. *Nanotechnol Rev*, 10 (2021) 1926.
18 Sugiarto IT & Putri KY, Analysis of dual peak emission from Rhodamine 6G organic dyes using photoluminescence. *J Phys Conf Ser*, 817 (2017) 1.
19 Fedoseeva M, Letrun R & Vauthey E, Excited-state dynamics of rhodamine 6G in aqueous solution and at the dodecane/water interface. *J Phys Chem B*, 118 (2014) 5184.
20 Wang Z, Fluorescence mechanism and biomedical applications of graphene quantum dots. *Theor Nat Sci*, 23 (2023) 259.
21 Zaman RT, Yusuf M, Luthra P, Wright B, Barton JK & Mahadevan-Jansen A, Variation of fluorescence in tissue with temperature. *Lasers Surg Med*, 43 (2011) 36.
22 Han J & Burgess K, Fluorescent indicators for intracellular pH. *Chem Rev*, 110 (2010) 2709.
23 Zehentbauer FM, Moretto C, Stephen R, Theisen A, Boddington S, Phipps M, Penzkofer A, Hegemann P, Hempstead P & Richard-Kortum R, Fluorescence spectroscopy of Rhodamine 6G: concentration and solvent effects. *Spectrochim Acta A Mol Biomol Spectrosc*, 121 (2014) 147.
24 Hatim NA & Ahmad ZM, A novel method for conversion of eggshell hydroxyapatite particles to nano-size using microwave irradiation. *Int J Enhanc Res Sci Technol Eng*, 2 (2013) 71.
25 Wojnarowicz J, Chudoba T, Lojkowski W & Sobczak K, Effect of microwave radiation power on the size of aggregates of ZnO NPs prepared using microwave solvothermal synthesis. *Nanomaterials*, 8 (2018) 343.

26 Bagga A, Chattopadhyay PK & Ghosh S, Stokes shift in quantum dots: origin of dark exciton. *Proc Int Workshop Phys Semicond Devices (IEEE)*, (2007).

27 Lewis J, Klem EJD, Piascik JR & Cunningham G B, Fluorescence based thermometry. *RTI Int*, (2011).

28 Rahman MM, Inaba H, Sato K, Otsu H & Horibe A, Effects of pH on the fluorescence fingerprint of ATP. *Trans Jpn Soc Refrig Air Cond Eng*, 33 (2016) 16.

29 Gaigalas AK, Wang L, Zhang YZ & Marti GE, Quantitating fluorescence intensity from fluorophore: assignment of MESF values. *J Res Natl Inst Stand Technol*, 110 (2005) 101.

30 Wang Y, Wang T, Tian P, Sun R, Zhang Q, Zhang H, Li Y & Ji W, Precision-cut liver slices as an ex vivo model to evaluate antifibrotic therapies for liver fibrosis and cirrhosis. *Hepatol Commun*, 8 (2024) e0558.

31 Arauz J, Ramos-Tovar E & Muriel P, Redox state and methods to evaluate oxidative stress in liver damage: From bench to bedside. *Ann Hepatol*, 15 (2016) 160.

32 Nandi A, Yan LJ, Jana CK & Das N, Role of catalase in oxidative stress and age-associated degenerative diseases. *Oxid Med Cell Longev*, 2019 (2019) 9613090.

33 Lubos E, Loscalzo J & Handy DE, Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal*, 15 (2011) 1957.

34 Allameh A, Amiri A, Fazeli H, Kazemi A, Mohammadi S & Shariati M, Oxidative stress in liver pathophysiology and disease. *Antioxidants*, 12 (2023) 1653.

35 Shi S, Li H, Yuan R, Song Y, Qin H, Xiong J, Li J, Liu J & Wang J, Mitochondrial dysfunction and oxidative stress in liver transplantation and underlying diseases: new insights and therapeutics. Transplantation, 105 (2021) 2362.

36 Goshtasbi H, Abed A, Taghipour A, Ramezani Z, Saeedi N, Fathi M, Nouri M & Shokri M, Mitigating oxidative stress toxicities of environmental pollutants by antioxidant nanoformulations. *Nano Trans Med*, (2025) 100087.