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GREEN SYNTHESIS OF CARBON QUANTUM DOTS DERIVED FROM ORANGE PEEL FOR HIGH-SENSITIVITY PH BIOSENSING

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Carbon quantum dots (CQDs) are nanoscale, fluorescent carbon-based substances known for their outstanding biocompatibility, solubility in water and flexible optical characteristics. The environmentally-friendly creation of such nanoparticles through the use of non-toxic, renewable resources and energy-efficient techniques is known as green synthesis. Utilizing orange peel as an affordable, sustainable precursor, the ecologically-friendly synthesis of CQDs and nitrogen-doped CQDs (NCQDs) using both hydrothermal and microwave-assisted methods are presented. Small, homogeneous CQDs (2–3 nm) with a modest quantum yield (~11%) were created by the hydrothermal method, but the microwave-assisted method instantly produced larger CQDs (4–8 nm) with improved fluorescence intensity and enhanced quantum yield (~54%). The optical properties of both CQDs and NCQDs at different pH levels (4, 7 and 10) were studied using UV-Vis spectroscopy. With a significant redshift in absorbance peak wavelengths from 429 nm at pH 4 to 445 nm at pH 10, CQDs exhibited robust pH-responsive activity and high pH sensitivity. The slightly blue-shifted but more stable optical response with NCQDs, on the other hand, makes them suitable for applications requiring signal reliability. A pH biosensor is an analytical tool used in biomedical, industrial and environmental monitoring to analyze changes in hydrogen ion concentration, providing a rapid, visual or digital input about the acidity or alkalinity of a given environment. These findings demonstrate that CQDs and NCQDs generated from orange peel are viable, non-toxic and promising nanomaterials for colorimetric pH biosensing. Each has unique advantages tailored to particular sensing conditions that can be applied to intracellular pH imaging, water and soil quality evaluation, smart packaging, food freshness detection, biological diagnostics (such as wound and saliva monitoring) as well as medicine stability tracking.

Keywords: carbon quantum dots, orange peel, green synthesis, pH biosensor, microwave-assisted synthesis

1. Introduction

Due to their remarkable characteristics, carbon quantum dots (CQDs) are considered to be one of the most recognized and interesting varieties of nanomaterials composed of carbon. These features include outstanding physicochemical capabilities, minimal toxicity, vivid multicolor visual properties, exceptional chemical stability, high water solubility and remarkable biocompatibility [1],[2]. Carbon dots, often referred to as CDs, are semi-spherical nanoscale materials smaller than 10 nm in diameter composed of hydrogen, oxygen and carbon [3]. The optical properties of CQDs, most specifically their characteristic fluorescence emissions, are vital for potential applications among their countless other attributes. In spite of the benefits of large-scale production and straightforward surface modification, CQDs are still notable due to their bright fluorescence, enhanced fluid solubility, chemical stability and resistance to photobleaching. Photoluminescent carbon-based quantum dots are fascinating with regard to their technological applications because they contain more stable, controlled fluorescence emission levels, are inert to chemical reactions and dissolve better in aqueous solutions than conventional semiconductor quantum dots [4].

A variety of natural materials, including orange juice and peels as well as amino acids, have been used to create fluorescent CQDs. Investigating novel and eco-friendly precursors is essential to produce fluorescent CQDs at a reasonable cost [5]. Among some of their unique physicochemical characteristics are stable

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photoluminescence (PL), remarkable hydrophilicity, compatibility with biological systems, low toxicity, emission that changes according to the excitation wavelength and environmental friendliness. Numerous fields, including sensing, solar cells, bio-imaging, bio-electrochemistry, nanomedicine and photocatalysis, may benefit from the use of CQDs [6].

The most widespread species of orange, *Citrus sinensis*, produces a large quantity of peel that is thrown away once its juice is extracted. This peel could be an outstanding source of carbon for the synthesis of CQDs due to its abundance in proteins and fibers but lack of oils and antioxidants [7]. Hydrothermal processes, microwave techniques, electrochemical oxidation, chemical oxidation, ultrasonic techniques and laser ablation treatments are some of the methods used to synthesize and develop CQDs that can be classified into either the top-down or bottom-up groups [8]. Furthermore, their unique sizes, shapes and surface chemistry facilitate effective dispersion in polar solvents like water or ethylene glycol and polymer matrices, which are vital for membrane applications. In order to improve membrane features and overall performance, CQD-modified membranes have been designed [9]. Heteroatom doping of CQDs represents an intriguing and efficient strategy to enhance their quantum yield (QY). Among the various elements available, nitrogen is similar in size to carbon, thereby facilitating effective doping, which could result in significant modifications to the intrinsic properties of CQDs. These changes might affect their optical qualities, electrical features and surface chemical reactivity, which could ultimately enhance the QY [10]. One fascinating method to improve the fluorescence performance of quantum dots composed of carbon and increase their use in sensing systems is to incorporate nitrogen into them. The recently reported rise in fluorescence is probably due to the protonation caused by these nitrogen atoms on quantum dots along with the creation of various additional polyaromatic compounds brought about by the integration of nitrogen atoms into them. Research indicates that nitrogen-doped carbon quantum dots (NCQDs) can function as a multifunctional platform for fluorescence-based sensing, facilitating the measurement of pH values as well as the detection of Ag(I) and Fe(III) in aqueous solutions [11].

As the pH rose from acidic to basic values (pH 3–12), the fluorescence intensity decreased, suggesting that the CQDs are sensitive to variations in pH [12]. The concentration of free hydrogen ions in food products can be directly determined through its pH value. Bacteria, mold and yeast are just some of the microorganisms that are dependent on the pH levels of food products. Extreme pH values, whether low or high, inhibit the growth of these microorganisms [13]. Maintaining a pH balance in the body is essential for enzymatic function, nutrient absorption, cellular processes and immune response. pH measurements are typically conducted using synthetic chemical sensors which have drawbacks, including toxicity, that can pose risks to human health. This highlights the need for quick, simple, inexpensive, user-friendly and non-toxic pH sensors. Biosensors have emerged as innovative devices that are selective, affordable, portable and easy to use, making them valuable across various industries for detecting contaminants such as heavy metals, harmful microorganisms and pesticides (*Figure 1*). To efficiently detect chemical pollutants, certain biosensors use advanced colorimetric approaches. These sensors use color-sensitive receptors that react with target analytes to cause visibly distinct changes in color, resulting in rapid, cost-effective and instrument-free detection. Additionally, natural plant resources serve as an excellent source of colorants and bioactive compounds that enhance the specificity and sensitivity of these biosensors [14]. The distinct changes in color of each CQD in response to different pH levels driven by changes in surface functional groups highlight their strong potential for accurate and real-time pH detection in biosensor applications.

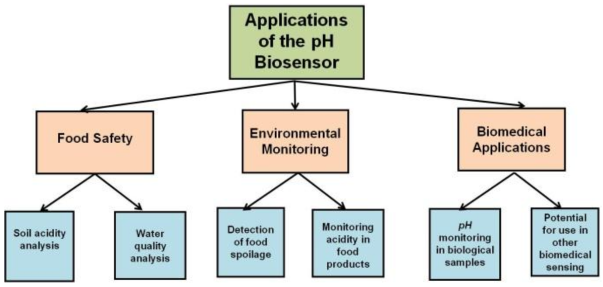

Figure 1: Applications of *pH* biosensor-derived CQDs from orange peel

The two types of synthetic methodologies for CQDs are top-down and bottom-up. The bottom-up method produces CQDs from small molecules using techniques like microwave irradiation, hydrothermal treatment or pyrolysis [10]. In order to create CQD-based nanoprobes that are especially designed for use in pH biosensors, a novel microwave-assisted technique that employs orange peel as a sustainable and natural source of carbon has been devised. Because of its amazing simplicity and time efficiency, this method is attracting a lot of interest from the scientific community. It facilitates the quick manufacture of CQDs without requiring intricate chemical reactions [15]. Orange peel, a by-product of processing oranges in one serving, is a great carbon precursor since it is rich in carbohydrates as well as contains a variety of useful components. These properties enhance the fluorescence, water solubility and biocompatibility of CQDs [16]. The present study investigates two green synthesis techniques, namely hydrothermal and microwave-assisted, for creating CQDs and NCQDs from orange peel. The absorbance peak shifts at pH 4, 7 and 10 are then compared using UV-Vis spectroscopy, revealing remarkable pH-responsive behaviors that underscore their potential as non-toxic, environmental-friendly pH biosensors.

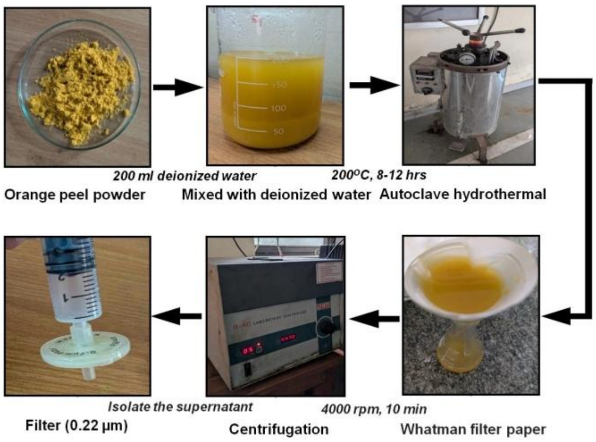

Figure 2: Schematic diagram of the hydrothermal production of CQDs from orange peel

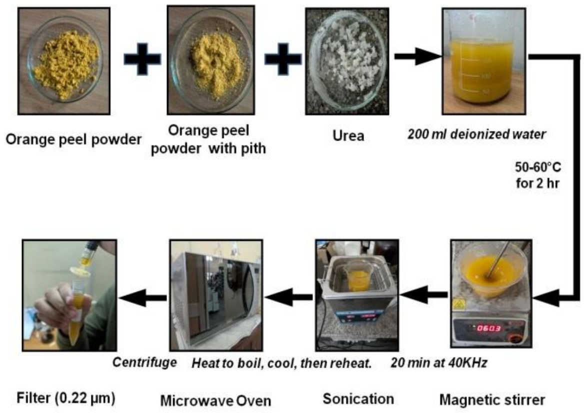

Figure 3: Microwave irradiation synthesis route for orange peel-derived CQDs

2. Experimental

2.1. Materials

In the second week of June 2025, fresh oranges were purchased from a small local market in Nagpur, India. Two kinds of peel from *Citrus sinensis* were used in the study: one with and one without the white pith layer. In Nagpur, the average diameter of oranges was 7 cm. Double distilled water, combined with either urea or ethylenediamine, was employed for the purpose of nitrogen doping. A standard laboratory pH buffer kit (alpha 01, India) typically consists of solutions calibrated at pH levels of 4, 7 and 10. All the required solutions were prepared fresh and each experiment conducted utilized distilled water.

2.2. Methods

2.2.1. Preparation of orange pericarp

To remove surface contaminants, the orange pericarp was thoroughly washed with distilled water. It was then sliced into small pieces and sun-dried for 24 hours to ensure complete dehydration. The dried material was subsequently oven-dried in a hot air oven (Bio Technics India) at 60 °C for 5 hours until a firm, crisp texture was achieved. Two types of pericarp samples were prepared: one containing the white fibrous pith layer and one without. The dried orange peel samples were then ground into a fine powder using a mechanical grinder to obtain a uniform consistency suitable for the subsequent synthesis of CQDs.

2.2.2. Hydrothermal methods

200 mL of deionized water was mixed with 5 grams of crushed orange peel that had been dried for five hours at 60 °C and ground to a fine consistency. To promote carbonization and the creation of CQDs, the suspension was moved to a stainless steel autoclave lined with Teflon and heated to 200 °C for 8 to 12 hours. Large particles were extracted from the mixture by filtering it through Whatman filter paper after it had naturally cooled down to room temperature. A Remi R-4C centrifuge was used to centrifuge the resultant filtrate for 10 minutes at 4000 rpm before the supernatant was finally collected. The solution was dialyzed against deionized water for 24 hours after passing through a 0.22 µm PVDF syringe filter to ensure subsequent purification [8]. A schematic diagram of this procedure is depicted in *Figure 2*.

2.2.3. Microwave irradiation technique

To prepare the solution, 5 grams of orange peel powder was mixed with 200 ml of double distilled water. In a separate mixture, 2.5 grams of orange peel powder (including the pith) were combined with 100 ml of double distilled water and 1 ml of urea to facilitate nitrogen doping. The latter mixture was stirred continuously at between 50 and 60 °C for 2 hours to ensure uniform dispersion as well as the partial pre-reaction of the dopant. Subsequently, the mixture was subjected to sonication (Labman, India) for 20 minutes at a frequency of 40 kHz to break down agglomerates and enhance homogeneity at the molecular level. An IFB 25SC4 convection microwave oven was then used to heat both samples at 700–900 W until the mixtures began to boil. After initially boiling, the samples were allowed to cool for 30 seconds and then reheated repeatedly until a visible color change to brown or dark brown was observed, typically within 10 mins, indicating the formation of carbonized nanostructures. Following cooling, the mixtures were centrifuged at 4,000 rpm for 15 mins and the clear supernatant carefully collected. To ensure the removal of large particles and residual impurities, the solutions were filtered using a 0.22-micrometer syringe filter, yielding purified CQD and NCQD suspensions suitable for further characterization [16]. This experimental approach is presented in *Figure 3*.

2.2.4. Microwave-assisted synthesis

5 grams of orange peel powder, which had been previously dried in a hot air oven at 60 °C for 5 hours and subsequently ground into a fine powder, was mixed with 200 ml of deionized water. The resulting mixture was

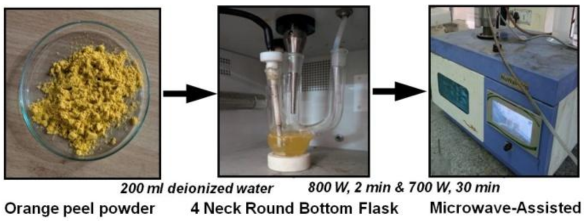

Figure 4: Stepwise green synthesis of CQDs from orange peel using microwave and sonication techniques

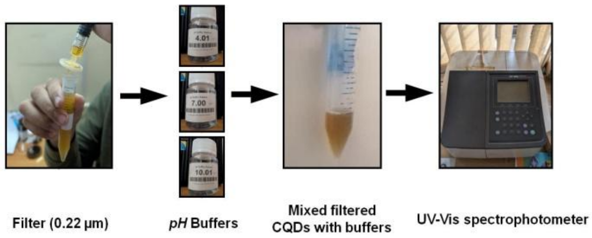

Figure 5: Workflow showing the *pH*-dependent UV-Vis spectroscopic optical examination of CQDs and NCQDs

then heated using a microwave synthesis apparatus (NuWav-Pro, NutechAnalytical, India) initially at 800 W for 2 mins followed by 700 W for an additional 30 mins. Upon completion of the heating process, the solution was allowed to cool and then filtered to obtain the carbon quantum dot suspension (*Figure 4*). It is important to note that due to the rapid boiling point of water when used as a solvent, appropriate precautions were taken throughout the procedure to avoid overheating [17].

2.2.5. Interaction of pH buffers with CQDs

The NCQDs and filtered CQDs, synthesized using the previously described methods, were utilized for pH-responsive analysis. At each pH level (4, 7 and 10), 2 ml of the CQDs solution was mixed with 2 ml of the corresponding pH buffer. The resulting mixtures were then analyzed using a UV-Vis spectrophotometer to evaluate the optical responses of both the undoped and nitrogen-doped carbon quantum dots (*Figure 5*). All prepared samples were stored in a dark environment at 4 °C to maintain their stability prior to analysis.

3. Results and discussion

The synthesis of CQDs based on precursor materials is conducted using both "top-down" and "bottom-up" approaches. However, "bottom-up" approaches using small molecules, polymers and biocompatible materials are more cost-effective and environmentally friendly as they do not require carbon-rich precursors like carbon nanotubes and graphene oxide. Generally, harsh conditions such as during hydrothermal procedures, combustion and acidic pyrolysis are used to synthesize bottom-up CQDs from tiny molecules [18].

During the hydrothermal process, precursor molecules are dissolved in water, put in an autoclave made of stainless steel lined with a Teflon coating and then heated to extremes of pressure as well as temperature over many hours inside a hydrothermal chamber. Various waste materials, organic compounds, proteins, polymers, amino acids, glucose and polyols are examples of the precursor molecules used for synthesis. The hydrothermal method has attracted a lot of interest lately worldwide because of its one-step approach, simplicity, non-toxic qualities, affordability and environmental friendliness. CQDs produced via the hydrothermal treatment exhibit a wide array of advantageous properties such as high homogeneity, water solubility, monodispersity, photostability, salt tolerance, controlled particle size and an elevated quantum yield without the necessity for surface passivation [19]. These methods often involve dissolving tiny organic raw materials or macromolecules in either a naturally occurring solvent (solvothermal) or water (hydrothermal). Next, the resultant solution is put in an autoclave for heating. The creation of carbon-based cores, which constitute the fundamental components to develop CQDs, is then initiated by subjecting the mixture to high temperatures. Various chemical compounds can be utilized during their preparation to change the functional groups on the surface of the CQDs, enabling further customization and the integration of specific properties as needed [20].

One well-known bottom-up technique for creating CQDs is microwave irradiation, which is distinguished by its straightforward, efficient approach and viability for commercial use. By activating the polar compounds in the solvent with microwave energy, this method allows for atomic-level heating despite the need for a material to be heated. The intrinsic flexibility and rapid ability to generate the high-yielding response of this method make it a time-efficient and environmentally-friendly method for producing CQDs. By using a customized version of a normal microwave device, microwave radiation creates strong interactions between polar molecules which lead to instantaneous temperature changes at the atomic level. By directly applying high-frequency electromagnetic radiation, this process significantly increases the reaction rate compared to conventional heating methods while promoting a quick and selective yield. The process is well regarded for its quick synthesis time, environmental friendliness, energy and time efficiency, low impurity incorporation as well as exact control over temperature and size parameters. All of these benefits together produce high-quality synthetic CQDs with a good level of reproducibility [21].

Microwave-assisted synthesis with the NuWav-Pro creates carbon quantum dots (CQDs) through microwave-induced dielectric heating, where polar substances (such as water, citric acid and urea) absorb microwave energy, generating heat quickly that breaks chemical bonds and initiates carbonization into nanometer-sized graphitic cores with fluorescent surface characteristics. Meanwhile, infrared (IR) sensing offers real-time, non-contact monitoring of temperature (usually with IR sensors and Pt100 probes), allowing precise regulation of the reaction to prevent overheating as well as guarantee a consistent particle size and optical features. Additionally, modalities like ultrasound for enhanced mixing or UV for surface modifications after synthesis can be integrated, while the fundamental concept is rapid employing uniform volumetric heating for the quick (within minutes) and environmentally-friendly production of CQDs which is most effective for lab-scale synthesis utilizing simple, eco-friendly precursors [22]. These green approaches have been used for various applications such as the recovery of carboxylic acids, use of natural solvents, enzymatic/catalytic reactions, etc. [23]-[26].

Hydrothermal synthesis utilizing an autoclave and microwave-assisted methods both convert orange peel into carbon quantum dots (CQDs) through bottom-up, environmentally-friendly processes; however, their mechanisms and applications are significantly different. Hydrothermal autoclaving involves the heating of biomass in sealed, pressurized vessels at temperatures typically ranging from 150 to 200 °C over several hours. This approach facilitates a slow, controlled carbonization process that yields CQDs with uniform sizes of approximately 2 to 3 nanometers in diameter, a good degree of crystallinity and moderate quantum yields of around 11%. These characteristics render them particularly suitable for applications that require precise optical properties, although this method is time-consuming and energy-intensive. In contrast, microwave-assisted synthesis, whether performed on the domestic or laboratory scale with power settings between 500 and 1000 W for durations of 2 to 10 mins, employs rapid volumetric dielectric heating to instantaneously carbonize the orange peel extract. This technique results in slightly larger CQDs, ranging from 4 to 8 nm, with a higher quantum yield of up to approximately 54%, excellent fluorescence as well as a process that is both environmentally friendly and cost-effective. However, this method is associated with less precise size control and necessitates post-process purification.

Consequently, hydrothermal autoclaving is most suitable when uniform, well-defined CQDs of controlled morphology are essential as well as when the processing time and cost are less critical, particularly in contexts such as material characterization, device fabrication or academic studies. Conversely, microwave-assisted methods are preferable when speed, simplicity, high yield and adherence to the principles of green chemistry are sought, making them suitable for applications involving rapid prototyping, sensing, bioimaging or scalable production from orange peel waste.

The optical characteristics of CQDs were investigated through the use of fluorescence and UV absorbance spectroscopy. The pH was found to be roughly 4.3 in the absence of a buffer, moreover, both CQDs and NCQDs exhibited an absorbance peak at 412 nm. Because of the interaction with orange peel, the pH was modestly acidic when the buffer was added. The UV absorbance and fluorescence spectra (Shimadzu UV-1800) of CQDs are shown in *Figure 6*. The

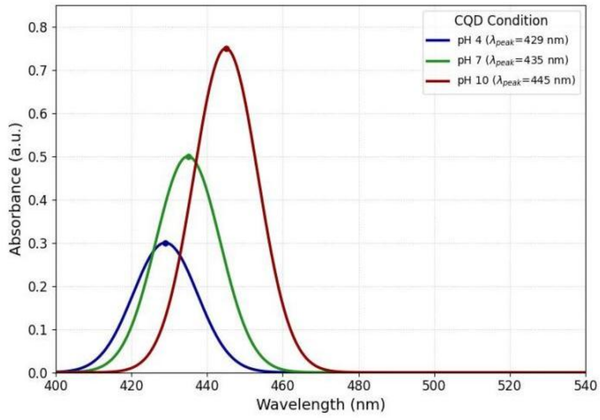

Figure 6: Absorbance spectra of CQDs at various pHs, illustrating their sensitivity to pH changes

absorbance spectra are represented on the y-axis, while the wavelength is shown on the x-axis.

The changes in UV-Vis absorption seen in this graph demonstrate a clear pH-responsive behavior of CQDs made from orange peel showing a progressive redshift and increasing absorbance as the pH rises from 4 to 10. At acidic pH, the absorption peak is approximately 429 nm and of lower intensity, whereas at alkaline pH, it shifts to around 445 nm with a significantly higher level of absorbance. This pattern is associated with the deprotonation and protonation that occur on surface functional groups, namely -OH, $-NH_2$ and -COOH, which modifies the optical transitions of the CQDs by altering the π-conjugation and electron distribution inside them. Such consistent and tunable changes in absorption suggest that orange peel-derived CQDs are well suited for use as colorimetric pH biosensors. By calibrating the peak wavelength or absorbance intensity against specific pH values, the pH of unknown samples can be accurately assessed. Potential applications include environmental monitoring (e.g. soil or water quality), wound-care sensors and food-freshness indicators, wherein visible spectral shifts provide simple, non-invasive readouts. Therefore, this experiment validates the viability of orange waste-derived CQDs as eco-friendly, cost-effective and sensitive pH probes with a broad range of applications in the biomedical, environmental and smart-packaging industries.

Both undoped CQDs and NCQDs made from orange peel exhibit distinct pH-dependent wavelength alterations as shown in *Table 1*.

CQDs exhibit a clear monotonic redshift as the pH rises from 4 to 10; the absorbance peak wavelength shifts from 429 nm at pH 4 to 435 nm at pH 7 and then 445 nm around pH 10. NCQDs, on the other hand, exhibit a subtler trend with peak wavelengths shifting from 414 nm at pH 4 to 412 nm at pH 7 and then slightly decreasing to 409 nm at pH 10. These results indicate that the protonation-deprotonation of surface amine and carboxyl groups influences their electronic structure in various ways.

Table 1: Comparison of the absorbance peak wavelengths of undoped CQDs and NCQDs at different pHs (4, 7 and 10)

pH	CQDs (nm)	NCQDs (nm)
4	429	414
7	435	412
10	445	409

The larger Δλ observed with regard to CQDs suggests a more responsive optical reaction to pH changes, making them particularly effective as pH biosensors. Calibration graphs relating wavelength to pH could thus facilitate quick, non-invasive measurements. Meanwhile, NCQDs exhibit a lower level of sensitivity but greater signal stability and enhanced quantum yield as a result of nitrogen-induced auxochromes that improve fluorescence brightness and suppress non-radiative losses. Importantly, both types of dots are synthesized via eco-friendly, water-based methods from orange peel waste, avoiding the use of heavy metals and in accordance with the principles of green chemistry. In practical applications, CQDs are well suited for tasks requiring high pH sensitivity and a wide dynamic range such as in water quality monitoring or pH-responsive indicator strips, whereas NCQDs are better suited for environments demanding consistent signal performance such as in prolonged bioassays or intracellular pH imaging.

4. Conclusions

In this study, both hydrothermal and microwave-assisted (microwave / solvothermal) methods were employed to successfully create CQDs and NCQDs from orange peel using environmentally acceptable, waste-derived resources. Characterization through UV-Vis spectroscopy at pH levels of 4, 7 and 10 showed that both CQDs and NCQDs display pH-responsive optical properties, making them suitable low-cost, non-toxic, environmentally-friendly pH biosensors. Importantly, CQDs exhibited a significant and consistent redshift in absorbance peak wavelength as the pH increased, providing a substantial and clear Δλ between pH levels that enhances sensitivity. In contrast, NCQDs displayed minimal or decreasing wavelength shifts, likely due to amine-functional surface groups balancing out their response. Both types benefit from the eco-sustainability of using orange peel as a precursor, employment of water-based production methods and the absence of hazardous chemicals. However, CQDs are particularly advantageous for applications that require a wide range of dynamics and clear spectral differences across a range of pH levels. NCQDs, with their enhanced stability and fluorescence efficiency, might be more suitable in applications where signal durability is prioritized over sensitivity. In summary, both CQDs and NCQDs show significant promise as biosensors: CQDs are superior when sensitivity and clear signal are essential, while NCQDs are preferable in scenarios that require photostability and durability.

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