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# Microwave-assisted synthesis and formation mechanism of fluorescent carbon dots from starch



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ARTICLE INFO	A B S T R A C T		
<i>Keywords:</i> Multicolor fluorescence Carbon nanodots Green chemistry Food	We report the synthesis of fluorescent carbon dots (CDs) <i>via</i> the microwave (MW)-assisted hydrothermal treat- ment of starch in an aqueous phosphoric acid solution. CDs emitting blue, orange, and green fluorescence in different solvents were obtained. The fluorescence quantum yield of the CDs emitting blue fluorescence is 30%, which is comparable to those of semiconductor quantum dots. The formation of the CDs under MW irradiation was monitored using a spectroscopic approach. The formation of blue-fluorescent CDs began at an early stage of the reaction. Subsequently, the CDs underwent aggregation. The aggregations were effectively extracted to the toluene layer by liquid—liquid extraction, and they emitted orange fluorescence in toluene and green fluores- cence in water. The formation of the aggregates was followed by a steep increase in the yield of the CDs that emit blue fluorescence. MW heating for a certain duration was required to increase the yield of the blue-fluorescent CDs		

# 1. Introduction

Fluorescent carbon dots (CDs), a new family of carbon materials, have attracted significant interest because of their potential applications in fluorescence bioimaging, energy devices, and sensing devices (Georgakilas et al., 2015; Li et al., 2019; Li et al., 2012; Lim et al., 2015; Wang & Hu, 2014; Wang et al., 2019; Wang et al., 2015). They are typically composed of carbon and a few ubiquitous elements, such as oxygen and hydrogen. Therefore, they are free from toxic elements, such as Cd and Se that are components of a typical fluorescent quantum dot. The application of natural products to produce CDs is considered a low-cost and eco-friendly synthetic strategy characterized by low emission of toxic materials, because natural products are biodegradable and sustainable resources (Bag et al., 2021; Zhu et al., 2020). Many natural products have been employed as carbon sources of CDs, such as fruits (Wang et al., 2016), fruit juices (Sahu et al., 2012), vegetables (Alam et al., 2015; Feng et al., 2015; Shen et al., 2017; Xu et al., 2015), food waste (Vandarkuzhali et al., 2017), and carbohydrates (Ansi & Renuka, 2018; Yang et al., 2011).

Plants-based polysaccharides have engaged attention for the application to a variety of industrial products because they are nontoxic, and biodegradable (Ahmad, 2021). Starch belongs to a family of polysaccharides composed of D-(+)-glucose units. Carbohydrates, including sucrose, and starch, are made from plants, and easily available as food in a moderately purified form. The application of carbohydrates allows the eco-friendly synthesis of fluorescent CDs using easily available and moderately pure chemicals. Many methods have been employed to synthesize fluorescent CDs from starch, including pyrolysis using sulfuric acid (Chin et al., 2012; Yan et al., 2015), hydrothermal reaction with NaOH or HCl (He et al., 2011), pyrolysis in water (Chen et al., 2018), pyrolysis in a mixture of polyethylene glycol and water (Chandra et al., 2012), hydrothermal synthesis using polyethylene imine (Liu et al., 2015), and microwave (MW)-assisted hydrothermal synthesis in water (Zheng et al., 2018).

Herein, we report the MW-assisted hydrothermal synthesis of fluorescent CDs from starch using an aqueous phosphoric acid solution. The fluorescence properties and the separation of the CDs from the aqueous phosphoric acid solution are reported. Notably, a large variety of methods and carbon sources have been employed to prepare CDs (Rigodanza et al., 2021). MW-assisted hydrothermal synthesis is a facile method for preparing fluorescent CDs under milder conditions and with low energy consumption in comparison with the conditions of the conventional hydrothermal method (Wang & Hu, 2014; Zhai et al., 2012). However, this method is limited by the poor control of certain properties, such as the size of CDs (Wang & Hu, 2014). In addition, the formation mechanism of the CDs under MW irradiation is complex and

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poorly understood. Understanding the formation mechanism of fluorescent CDs is important for improving the yield of CDs and optimizing their fluorescence properties. Therefore, the dependence of the fluorescence properties of CDs on the experimental conditions was investigated to obtain insights into their formation mechanism. The findings of this study will promote the rational optimization of the process conditions for the facile MW-assisted hydrothermal synthesis of fluorescent CDs from starch.

## 2. Materials and methods

## 2.1. Materials

Starch, which was commercially available and made from potatoes harvested in the Hokkaido area of Japan (Kawamitsu Bussan Co., Ltd.), was used. Phosphoric acid (85%, guaranteed reagent grade), toluene (guaranteed reagent grade), quinine sulfate (guaranteed reagent grade), and  $H_2SO_4$  (guaranteed reagent grade) were obtained from Junsei Chemical Co. Ltd. 1-butanol (guaranteed reagent grade) was obtained from Kanto Chemical Co., Inc.

#### 2.2. Methods

A schematic of the synthetic method is shown in Fig. 1. Starch (60 mg) was mixed with distilled water in a glass flask using a magnetic stirring bar for 15 min. Thereafter, phosphoric acid was added to the aqueous starch solution. The volume of phosphoric acid was varied between 2 and 20 mL, and the total volume of the mixture was adjusted to 30 mL by adding distilled water. The concentration of phosphoric acid in the mixture is shown in Table S1 in the Supplementary Materials. The mixture was stirred for 5 min and subsequently heated in a microwave oven (Panasonic, 250 W, 2.45 GHz) for 2 - 14 min. The mouth of the flask was covered with a Kapton® film during the heating process. The water steam produced during heating was released through holes created on the film. The solution was cooled to room temperature, after which distilled water (25 mL) was added, followed by stirring for 10 min. The obtained solution was filtered using sanitary cotton, a filter paper (Advantec), and polytetrafluoroethylene membrane filters with pore sizes of 1.0 µm (Merck) and 0.2 µm (Advantec). The absorption spectra of the filtrate were recorded. When the absorbance at 390 nm

was larger than 0.1, the filtrate was diluted with distilled water to adjust the absorbance to 0.1. The diluted solution was contained in a quartz cell with an optical path length of 1 cm to measure the absorption and fluorescence spectra. The absorption and fluorescence spectra were measured using a spectrophotometer (Hitachi, U4100) and a spectrofluorometer (Hitachi, F4500), respectively. A transmission electron microscope (TEM) (JEOL, JEM-2100F) equipped with a field emission gun at an acceleration voltage of 200 kV was used to observe the morphology of the CDs. The specimen for the TEM experiment was prepared by placing a drop of the solution containing the CDs on a Cu grid with a carbon support film, followed by drying in an ambient atmosphere. Fourier-transform infrared (FTIR) spectroscopy (JASCO, FT/IR-4100) was conducted in attenuated total reflection (ATR) mode using an ATR prism of ZnSe. The spectral bandwidth was 4 cm<sup>-1</sup>. X-ray diffraction (XRD) measurements were carried out with an X-ray diffractometer (MultiFlex, Rigaku) using a Cu Ka X-ray source (1.54058 Å) operated at 40 kV and 20 mA. The fluorescence quantum yield (QY) was measured relative to the fluorescence standard (Lakowicz, 2006). Quinine sulfate dissolved in 0.1 mol/L of an aqueous H<sub>2</sub>SO<sub>4</sub> solution was used as the standard solution. The excitation wavelengths of both the standard solution and the CDs dispersed in the aqueous solution were 350 nm. In the calculation of the fluorescence QY, we employed a fluorescence QY of 0.577 for the standard solution (Lakowicz, 2006).

For the liquid—liquid extraction, distilled water was added to the filtrate solution containing the CDs. The volume of the solution was adjusted to 30 mL, and half of this solution was mixed with 15 mL of toluene or butanol in a separation funnel. Both organic and water layers were used for the spectroscopic measurements.

## 3. Results and discussion

#### 3.1. Dependence on the phosphoric acid concentration

The absorption and fluorescence characteristics of the CDs prepared using different phosphoric acid concentrations were compared. MW irradiation was performed for 12 min. The absorption spectra are shown in Fig. 2a. A sharp absorption band with a maximum at 240–250 nm was observed, and a broad absorption band in the range of 300–400 nm was also observed. These absorption bands were attributed to the  $\pi$ – $\pi$ \* transition of the sp<sup>2</sup>-conjugated system, and the n– $\pi$ \* transition from



Fig. 1. Schematic of the synthetic method of CDs from starch, and photographs of the CDs in an aqueous phosphoric acid solution under (left) ambient light and (right) excitation at 365 nm.



**Fig. 2.** (a–c) Absorption, fluorescence divided by the absorbance at the excitation wavelength, and fluorescence excitation spectra of the CDs prepared using different concentrations of phosphoric acid. W and P represent the volumes of water and phosphoric acid in the reactant solution. The MW irradiation time was 12 min. (d-f) Absorption, fluorescence, and fluorescence excitation spectra. The CDs were prepared by MW irradiation time for 2–14 min. The fluorescence intensities of the CDs prepared by MW irradiation for 2–10 min were multiplied by 25.

the non-bonding orbital on heteroatoms or functional groups (e.g., C=O) to the  $\pi^*$  orbital of the carbon double bonds (Ding et al., 2016; Gómez et al., 2021; Rigodanza et al., 2021).

The CDs emitted blue fluorescence upon ultraviolet photoexcitation (Fig. 1). The fluorescence and fluorescence excitation spectra are shown in Fig. 2b and 2c, respectively. The maximum wavelength in the fluorescence excitation spectrum was used to measure the fluorescence spectrum. A relative measure of the fluorescence QY of the CDs prepared using different concentrations of phosphoric acid was obtained by dividing the fluorescence intensity by the absorbance at the excitation wavelength ( $\lambda_{exc}$ ). The fluorescence spectra divided by the absorbance are shown in Fig. 2b. A constant fluorescence maximum of 436 nm was observed, irrespective of the concentration of phosphoric acid.

The maximum wavelength of the fluorescence excitation spectrum varied slightly (Fig. 2c). The fluorescence was weak upon photoexcitation at  $\lambda_{exc}$  which was shorter than ~300 nm. The absorbance at  $\lambda_{exc}$  =

240–260 nm was larger than 0.8 for all the samples (Fig. 2a). A solution with an absorbance of 0.18 at 241 nm was prepared to determine if the weak fluorescence was an artifact because of the inner filter effect (Lakowicz, 2006). The fluorescence excitation spectrum was measured by observing the fluorescence at 429 nm (Fig. S1 in Supplementary Material). Although the inner filter effect was considerably smaller than that of the samples in Fig. 2a, a weaker fluorescence was observed with the excitation at  $\lambda_{\rm exc} < 300$  nm compared to that at  $\lambda_{\rm exc} = 320-400$  nm. This result shows that the weak fluorescence at  $\lambda_{\rm exc} < 300$  nm is not an artifact.

The mechanism in the excited state that explains the fluorescence in the CDs is generally controversial and has not been adequately understood (Cadranel et al., 2019; Gómez et al., 2021). Surface states originating from functional groups containing oxygen and phosphor atoms are formed in CDs (Gómez et al., 2021). The observed shift of the excitation band with changes in the concentration of phosphoric acid is probably due to the change in the composition of the CDs, such as the density of the defect sites, and the functional groups that produce the surface states. It was proposed that the fluorescence of nitrogen-doped CDs occurs from the defect levels originating from vacancies and functional groups containing oxygen and nitrogen atoms together with the band-to-band transition in the sp<sup>2</sup>-conjugated structure (Gómez et al., 2021). A few studies on the fluorescence mechanism have indicated that the surface states play an important role in the fluorescence of CDs (Bao et al., 2015; Cadranel et al., 2019). Contrarily, the constant fluorescence maximum of the CDs prepared using phosphoric acid of different concentrations is explained by the energy level, which is unchanged for the different compositions of the CDs. The fluorescence occurs from the lowest energy level in the excited states as a result of relaxation processes including exciton migrations in a CD particle. There is probably negligible change in the lowest energy level of the surface state for the CDs having different compositions.

The fluorescent and nonfluorescent CDs probably coexist in the solution. The fluorescence excitation spectrum showed a negligible intensity at  $\lambda_{\rm exc} < 300$  nm, indicating that the absorption band with the peak at 240–260 nm is ascribed to the nonfluorescent CDs. The absorption spectrum of the CDs prepared using 10 mL of water and 20 mL of phosphoric acid showed the tail of the absorption band in the region between 260 and 500 nm. This absorption band is probably also ascribed to the nonfluorescent CDs. The absorption band of fluorescent CDs was observed in the region of  $\lambda_{\rm exc} > 300$  nm (Fig. 2c and f).

The fluorescence intensity divided by the absorbance as a function of the volume fraction of phosphoric acid is shown in Fig. S2 in Supplementary Material. The relative magnitude of the fluorescence QY of the CDs prepared using different phosphoric-acid concentrations can be evaluated from Fig. S2. The fluorescence QY of the CDs prepared with 4 mL of phosphoric acid and 26 mL of water was measured using the fluorescence standard. The fluorescence QY was approximately 30% at the excitation wavelength of 350 nm. This value is comparable to that of CdTe quantum dots, which are extensively used as fluorescence emitters in various applications (Grabolle et al., 2009). The CDs prepared using a relatively large volume of phosphoric acid exhibited small fluorescence QY values. The mechanism of fluorescence QY change involves the increase in the amount of nonfluorescent CDs relative to that of fluorescent CDs with the phosphoric acid concentration in the solution. The fluorescence QY must decrease with an increase in the absorption originating from the nonfluorescent CDs if the absorption originating from the fluorescent CDs is constant or decreased. The change in the composition of CDs might also contribute to the change in fluorescence QY.

The fluorescence of CDs prepared from citric acid has recently been explained by the presence of small organic fluorophores (Schneider et al., 2017). It was reported that mixtures of carbon particles and organic fluorophores are synthesized from citric acid and amines, which are frequently used combination of precursors for the synthesis of CDs. The organic fluorophores were identified as derivatives of pyridine, and the nitrogen atom in pyridine ring is derived from amines. The fluorophores are likely attached to the surface of CDs, which emit quite intense fluorescence. It was also demonstrated that CDs containing no fluorophores of pyridine derivatives emit fluorescence. In our study, amines were not used to synthesize CDs. Therefore, it is likely that the origin of fluorescence of CDs synthesized in our study is not ascribed to pyridine derivatives.

#### 3.2. Dependence on the MW irradiation time

We studied the dependence of the fluorescent characteristics on the MW irradiation time. Although the CDs prepared using 2 mL of phosphoric acid showed the highest fluorescence QY (Fig. S2), the absorbance in the range of 350-390 nm, which is a measure of the amount of the CDs, was lower than 0.1. We could increase the amount of the CDs by increasing the concentration of phosphoric acid. We employed a mixture

of 4 mL of phosphoric acid and 26 mL of water to study the change in fluorescent CDs upon varying the MW irradiation time.

The absorption, fluorescence excitation, and fluorescence spectra of the solution prepared using MW irradiation times in the range of 2–14 min are shown in Fig. 2d–f. The absorbance of the solution prepared by MW irradiation for 2–8 min was considerably lower than 0.1 at 390 nm. The preparation of a small amount of CDs that emit blue fluorescence was indicated by the fluorescence excitation spectra (Fig. 2f).

The CDs prepared by MW irradiation for 9 and 10 min showed the fluorescence maximum at approximately 520 and 440 nm (Fig. 2e). In addition to the main excitation band at 357 nm (Fig. 2f), other excitation band at 490 nm was observed when the monitoring wavelength was changed (Fig. 3). At wavelengths longer than 400 nm, an increase in the absorbance was also observed in the CDs prepared by MW irradiation for 9 and 10 min (Fig. 2d). The absorption and fluorescence excitation bands at wavelengths longer than 400 nm were not discernible for the CDs prepared by MW irradiation for 12 min. This result indicates that some of the CDs prepared by MW irradiation for 9–10 min exhibited absorption and fluorescence bands at longer wavelengths than those for the CDs prepared by MW irradiation for over 11 min. Thus, we successfully prepared CDs with different fluorescence characteristics by varying the MW irradiation time.

The intensity of the blue fluorescence also changed with the MW irradiation time. The fluorescence intensity divided by the absorbance as a function of the MW irradiation time is shown in Fig. S3 in Supplementary Material. The fluorescence QY of the blue fluorescence steeply increased between 10 and 11 min. The mechanism behind this steep change in the fluorescence QY of the CDs showing blue fluorescence will be discussed later.

### 3.3. Separation of the CDs using the liquid-liquid extraction technique

The results of the fluorescence spectra suggested that the CDs with different fluorescence characteristics were obtained by MW irradiation for 9,10 min. The separation of these CDs is important for obtaining multicolor CDs from starch. The liquid-liquid extraction technique was employed to separate these CDs. The fluorescence and fluorescence excitation spectra of the original solution containing the CDs before the liquid-liquid extraction are shown in Fig. 4a and 4b, respectively. The CDs were prepared by MW irradiation for 10 min, and blue fluorescence was observed (Fig. 5). While the water layer exhibited blue fluorescence, the toluene layer exhibited orange fluorescence after the liquid-liquid extraction. The fluorescence and fluorescence excitation spectra of the toluene and water layers are shown in Fig. 4c-f. The CDs that exhibited the orange fluorescence were extracted to the toluene layer. The orange fluorescence was not observed in the toluene layer obtained from the solution prepared by MW irradiation for 11 min (Fig. S4, Supplementary Materials). These results demonstrate that the CDs that exhibited orange fluorescence were prepared by MW irradiation for 9,10 min. Further, we succeeded in separating the CDs showing blue and orange fluorescence by liquid-liquid extraction.

The solvatochromism of CDs extracted to the toluene layer was investigated. The toluene dissolving the CDs was removed under a vacuum, after which the CDs were dispersed in water and ethanol (Fig. S5, Supplementary Materials). Green fluorescence was observed in water, and a blue shift of the fluorescence maximum was observed upon the change in the solvent.

The CDs that showed blue fluorescence in the original solution were separated using butanol as the organic layer. Although phosphoric acid was also extracted to the butanol layer, it was removed by washing the butanol layer two or three times with water or saltwater. Blue fluorescence was observed for the CDs extracted to the butanol layer (Fig. S6, Supplementary Materials). The liquid–liquid extraction using toluene and butanol was useful for separating the CDs with different fluorescence properties.

We compared the sizes of the CDs that exhibited blue and orange



Fig. 3. The fluorescence excitation spectra of the CDs prepared by MW irradiation for 9 min.  $\lambda_{obs}$  represents the observed wavelength.

fluorescence using TEM. The TEM image of the CDs prepared by liquid–liquid extraction using toluene is shown in Fig. 6a. The aggregated structure of the CDs with sizes smaller than 10 nm was observed. The lattice fringe in the TEM image corresponds to a d-spacing of 0.22 nm. The observed spacing is close to the interplanar distance of 0.213 nm between the (100) planes of graphite (National Institute for Materials Science (NIMS) AtomWork, 2022). Therefore, the nanoparticles observed in the TEM images can be ascribed to the CDs. The sizes of the CDs extracted using butanol were approximately 2–4 nm (Fig. 6b). The aggregated structure of the CDs was not observed. The lattice fringe observed in the TEM image corresponds to the d-spacing of 0.22 nm, which is ascribed to the diffraction of the graphite.

# 3.4. Coagulation of the CDs forming sediment

Although the CDs prepared in the phosphoric acid aqueous solution were successfully extracted using toluene and butanol, the amount of the extracted CDs was considerably small to perform analyses such as powder XRD. Therefore, the preparation of solid materials containing the CDs was investigated. We found that sediment was formed spontaneously due to coagulation. CDs are probably dispersed in aqueous solution as colloidal dispersions. Although the colloidal dispersion system is very stable, coagulation of colloids may occur. The sediment was collected by a filtration, and a white powder was obtained after drying (Fig. 7). The powder showed blue fluorescence originating from the CDs, and it was barely soluble in water. The FT-IR spectrum of the powder is shown in Fig. 8. The IR absorption band observed at 1635  $\text{cm}^{-1}$  was assigned to the C=O group (Gong et al., 2016). The broad absorption peak in the range of approximately 2500-3700 cm<sup>-1</sup> includes the contribution of the hydroxyl group (Gong et al., 2016). There is a broad absorption band in the range of  $850-1300 \text{ cm}^{-1}$ , with the absorption maximum at 998 cm<sup>-1</sup>, which is generally assigned to C–O stretching (Xu et al., 2014). The vibrations of the P–O–C (1063  $\text{cm}^{-1}$ ) and P–O–H (966  $\text{cm}^{-1}$ ) groups (Gong et al., 2016) may also contribute to this broadband. These results suggest that the solid in the sediment consists of O-containing and P-containing functional groups. The XRD pattern of the powder is shown in Fig. 9. A broad peak centered at approximately 23° was observed, corresponding to a d-spacing of 3.9 Å. The XRD pattern of the CDs typically shows a peak at 3.4 Å, which is assigned to the diffraction due to the (002) plane of graphite (Qu et al., 2012). The difference in the d-spacings of the sediment and graphite is not negligible; therefore, it is unlikely that the sediment is solely composed of CDs. We speculate that the sediment might be an amorphous solid composed of phosphate ions and that the CDs are dispersed in the matrix of the amorphous solid.

#### 3.5. Formation mechanism of CDs

Starch, which is the source of the carbon employed in the current work to produce CDs, is a polysaccharide composed of glucose units. Many studies on the synthesis of CDs using carbohydrates as the carbon source have been conducted (Chandra et al., 2012; Chen et al., 2018; Chin et al., 2012; He et al., 2011; Liu et al., 2015; Tang et al., 2012; Xu et al., 2014; Yan et al., 2015; Zheng et al., 2018). Fluorescence properties of CDs and graphene quantum dots prepared from carbohydrates are summarized in Table 1.

Although synthesis of CDs from carbohydrates has been extensively studied, synthetic pathway of CDs remains unclear, because there exist diverse intermediates and it is practically difficult to identify all of them (Xu et al., 2014). One plausible mechanism is that the synthetic pathway of CDs begins by the hydrolysis of starch in aqueous phosphoric acid solution. Phosphoric acid was proposed to act as a chemical agent of polysaccharide (Xu et al., 2014). In fact, starch is converted to glucose by hydrolysis in the presence of H<sup>+</sup> under hydrothermal condition (Nagamori & Funazukuri, 2004). Although the major product of hydrolysis is glucose, a variety of saccharides including maltose and fructose are also produced (Nagamori & Funazukuri, 2004). Glucose and other saccharides undergo degradation reaction by dehydration, forming furfural intermediates (Titirici & Antonietti, 2010). It is possible that the furfural intermediates are polymerized, and changed to aromatic polymers, which finally condenses to a carbon-like material (Titirici & Antonietti, 2010; Xu et al., 2014). Moreover, hydrogen atoms in glucose may react with hydroxyl groups of other glucose, and formyl groups may react with hydroxyl groups (Bayat & Saievar-Iranizad, 2017). This dehydration of glucose molecules has been proposed as another possible route to condensed carbon-like materials (Bayat & Saievar-Iranizad, 2017; Sun & Li, 2004). Carbon atoms in condensed substances may undergo aromatization to form polymers with aromatic rings under



**Fig. 4.** (a, c, e) Fluorescence, and (b, d, f) fluorescence excitation spectra of the CDs in (a, b) the original solution, (c, d) toluene layer, and (e, f) water layer. The original solution was obtained as the filtrate of the solution prepared by MW irradiation for 10 min.  $\lambda_{exc}$  and  $\lambda_{obs}$  represent the excitation and observed wavelengths, respectively. The same color codes for  $\lambda_{exc}$  were used in (a, c, e).

hydrothermal condition (Sun & Li, 2004). Nucleation and growth of aromatic polymers are expected to subsequently occur via cross-linking and cycloaddition reactions of polymers (De & Karak, 2013; Sun & Li, 2004). The resulting carbonized nuclei which are soluble to aqueous solvent become CDs. These CDs emit blue fluorescence with the maximum at approximately 435 nm (Fig. 2e). It has been proposed that fluorescence is emitted from the surface state originating from the functional groups containing oxygen atoms, such as carboxyl groups, on the surface of the CDs (Cadranel et al., 2019; Gómez et al., 2021).

The aggregated CDs were prepared by MW irradiation for 9–10 min. This observation suggests that some of the CDs start aggregating. The aggregated CDs were effectively extracted to the organic layer. The solubility to organic and aqueous phases depends on the surface structure of CDs. It was reported that the formation of ammonium salts from amines in amine-terminated CDs upon acid-base reaction resulted in the phase transfer of CDs from organic phase to aqueous phase (Pei et al., 2018). Moreover, water contact angle measurements of CDs indicated that hydrophilicity of CDs increased upon the formation of ammonium salts. Therefore, it is probable that aggregates of CDs, which are extracted to toluene layer, have hydrophobic surface structures. CDs dispersed in aqueous phase without aggregation may have hydrophilic surface structures. The aggregation of the CDs is related to the change in the fluorescence color from blue to orange or green. An increase in the broad absorption band intensity at approximately 400 nm was also observed for the CDs produced by MW irradiation for 9–10 min. Contrarily, the fluorescence QY obtained by dividing the fluorescence intensity by the absorbance was very low (Fig. S3). This result indicates that the QY of the aggregated CDs is low, and the nonfluorescent CDs exhibit broad absorption band at approximately 400 nm. The increase in the absorption band observed for the MW irradiation times of 9,10 min

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Fig. 5. Photograph of CDs under ambient light (left) and excitation at 365 nm (right). The photoexcitation was performed from the bottom side of the glass vials. The original solution of CDs was prepared by MW irradiation for 10 min. The CDs in the toluene and water layers were obtained using liquid–liquid extraction of the original solution.



Fig. 6. TEM images of the nanoparticles prepared by the liquid–liquid extraction using (a) toluene and (b) butanol. (Insets) Magnified images of the nanoparticles. The yellow lines represent the lattice fringe of the nanoparticles.



# Excitation at 365 nm



Fig. 7. Photographs of the dried sediments that were formed spontaneously in the aqueous phosphoric acid solution containing the CDs. The sediment is placed on a filter paper.

is mainly caused by the increase in the amount of aggregated and nonfluorescent CDs.

The proposed model for the formation process of CDs is shown in Fig. 10. Phosphoric acid can activate the formation of polar functional groups containing oxygen atoms on the surface of CDs (Xu et al., 2014). It is likely that the degree of the coverage by functional groups on the surface of CDs is low at MW irradiation time shorter than 10 min. The rate of the aggregation of CDs may increase as the concentration of CDs

increases with MW irradiation time. The aggregation may efficiently occur at MW irradiation time of 9,10 min. The coverage of the surface functional groups might decrease with the degree of the aggregation of CDs, and accordingly the hydrophobicity of CDs may be increased. This mechanism is consistent with the result of the liquid–liquid extraction and the low fluorescence QY of the aggregated CDs.

The steep increase in the fluorescence QY at MW irradiation times longer than 10 min (Fig. S3) is explained by some mechanisms. One



Fig. 8. The FT-IR spectrum of the dried sediment in powder form.



Fig. 9. The XRD pattern of the dried sediment in powder form.

plausible mechanism is the removal of nonfluorescent CDs by the formation of debris. The results of the liquid-liquid extraction suggest that the aggregation of CDs occurs at MW irradiation time of 9,10 min. The concentration of the aggregated CDs may increase with MW irradiation time. The rate of the coalescence of the aggregations may become faster with increasing the concentration of the aggregates in solution. The coalescence of the aggregation of CDs may also make the size of the aggregation larger. It is assumed that nonfluorescent CDs are prepared by the growth of the aggregations. The further increase in the amount and size of nonfluorescent CDs under MW irradiation probably leads to the formation of carbon-like black debris in the reaction vessel.

A second mechanism is that the formation of CDs of good solubility is facilitated by phosphoric acid. When MW irradiation was performed for longer than 10 min, a large part of the water in the solution evaporated. Accordingly, the solution was composed of hot and concentrated phosphoric acid. In fact, the temperature of the solution prepared by MW irradiation for 12 min was approximately 270 °C. The formation of the oxygen-containing functional groups on the surface of CDs could be facilitated in concentrated phosphoric acid. The hydrophilicity of CDs increases with the increase in the number of the functional groups, resulting in the increase in the solubility of CDs to solvent (Pei et al., 2018). The rate of the formation of aggregation of CDs may decrease because of the repulsion due to the surface charge of CDs. This results in the increase in the number of fluorescent CDs. In addition, the increase in the number of surface functional groups may lead to the enhancement of fluorescence QY of CDs. Therefore, it is speculated that blue-fluorescent CDs of good solubility are effectively synthesized in such a concentrated phosphoric acid solution. Aggregated CDs, or nonfluorescent CDs of good solubility.

We can assume that the formation step of CDs involves the nucleation and growth of carbon substances (Xu et al., 2014). Therefore, a third plausible mechanism is that the increase in the amount of blue-fluorescent CDs is ascribed to a burst of the nucleation at MW irradiation of  $\sim$ 11 min.

The critical role of phosphoric acid in the synthetic pathway of CDs was verified by trying the synthesis of CDs without adding phosphoric acid to solution. The intensity of absorption and fluorescence spectra of solution prepared without adding phosphoric acid was very small (Fig. S7 in the Supplementary Materials). It is suggested that the rate of the formation of CDs is slow unless phosphoric acid is added to solution. This result demonstrates that phosphoric acid plays a critical role in the synthetic pathway of CDs.

The synthesis of N-doped graphene quantum dots (N-GQDs) using glucose and aqueous ammonia with MW-assisted hydrothermal treatment has been reported (Tang et al., 2014). The proposed role of ammonia in the growth of N-GQDs was twofold; it acts as catalyst for the intra-molecular and inter-molecular dehydration of glucose, and for N-doping. In our work, it is assumed that starch is hydrolyzed in aqueous phosphoric acid solution into saccharides including glucose (Nagamori & Funazukuri, 2004). Glucose molecules may subsequently undergo hydrothermal reaction. Glucose may be the common carbohydrate precursors both for CDs in our work and for N-GQDs in the other study. We infer that the facilitation of the formation of CDs could be ascribed to differences in volatility of ammonia and phosphoric acid, and concentration of carbohydrates, rather than the property of starch. While ammonia plays the two roles as shown above, a part of ammonia is

#### Table 1

Fluorescence properties of CDs and graphene quantum dots prepared from carbohydrates.

Carbon sources	Methodology	Fluorescence properties	Refs.			
Glucose, sucrose, starch	Hydrothermal, aqueous NaOH, HCl	Visible and near-infrared	He et al., 2011.			
Starch	Carbonization, H <sub>2</sub> SO <sub>4</sub>	430 nm	Chin et al., 2012.			
Glucose, sucrose, fructose	MW <sup>a</sup> , water	Deep ultraviolet, $\Phi_F=7-11\%$ $^{\rm b}$	Tang et al., 2012.			
Polysaccharides and polyethylene glycol	MW, aqueous acetic acid	Blue and green	Chandra et al., 2012.			
Sucrose	Acidic oxidation, H <sub>3</sub> PO <sub>4</sub>	Green and yellow, $\Phi_F=3-4\%$	Xu et al., 2014.			
Glucose	MW, aqueous ammonia	300–1000 nm,	Tang et al., 2014.			
Starch and polyethyleneimine	Hydrothermal, water	470–550 nm, $\Phi_F~=9.8\%$	Liu et al., 2015.			
Starch	Chemical oxidation, aqueous acetic acid, H <sub>2</sub> O <sub>2</sub>	Blue, $\Phi_F = 11.4\%$	Yan et al., 2015			
Starch	Hydrothermal, water	Visible and near-infrared, $\Phi_F = 21.7\%$	Chen et al., 2018.			
Starch	MW, water	Blue, $\Phi_F = 2.46\%$	Zheng et al., 2018.			
Glucose	Hydrothermal, water	540 nm	Bayat and Saievar-Iranizad, 2017			
Starch	MW, aqueous H <sub>3</sub> PO <sub>4</sub>	Blue, green and orange, $\Phi_F=30\%$	This work.			

<sup>a</sup> MW-assisted hydrothermal method

<sup>b</sup> Fluorescence quantum yield.



Fig. 10. The proposed formation pathway of the CDs from starch dispersed in an aqueous phosphoric acid solution by MW irradiation. The aggregation of the CDs may efficiently occur at MW irradiation time of 9–10 min. At MW irradiation time equal to or longer than 11 min, the blue-fluorescent CDs of good solubility may be synthesized without forming aggregates. The size variation between CDs at these MW irradiation times in the figure represents this change in CDs. The photographs of the reaction vessel are taken at the MW irradiation times of 8, 10, and  $\geq$  11 min.

expected to evaporate, and the amount of ammonia in solution may be decreasing during the MW-assisted hydrothermal treatment. Phosphoric acid hardly evaporates even at high temperatures. One plausible explanation of the facilitation of the formation of CDs is that phosphoric acid can stay in the solution as concentrated acid at high temperatures due to low volatility.

# 4. Conclusions

Fluorescent CDs were synthesized from starch using MW-assisted hydrothermal treatment in an aqueous phosphoric acid solution. The full characterization of fluorescence and optical property, and new insights into the formation mechanism of CDs are valuable from technological point of view of carbohydrate polymer. We succeeded in increasing the fluorescence QY from a few% reported in a previous study to approximately 30%, which is comparable to that of semiconductor quantum dots. The small amount of blue-fluorescent CDs was prepared at the initial stage of the reaction. Thereafter, the CDs began to aggregate, affording CDs with a hydrophobic surface. The liquid–liquid extraction was employed to separate CDs that show different fluorescence colors. The orange fluorescence of the aggregating CDs was observed in the toluene layer. The amount of nonfluorescent CDs also increased with the MW irradiation time, and carbon-like debris was finally formed in the solution. Blue fluorescent CDs were also prepared, together with nonfluorescent CDs. The information on the formation mechanism is technically important for the efficient synthesis of fluorescent materials showing specific fluorescence colors.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpta.2022.100218.

#### References

- Ahmad, M. M. (2021). Recent trends in chemical modification and antioxidant activities of plants-based polysaccharides: A review. Carbohydrate Polymer Technologies and Applications. 2, Article 100045.
- Alam, A. M., Park, B. Y., Ghouri, Z. K., Park, M., & Kim, H. Y. (2015). Synthesis of carbon quantum dots from cabbage with down- and up-conversion photoluminescence properties: Excellent imaging agent for biomedical applications. *Green Chemistry : An International Journal and Green Chemistry Resource : GC, 17*(7), 3791–3797.
- Ansi, V. A., & Renuka, N. K. (2018). Table sugar derived carbon dot a naked eye sensor for toxic Pb<sup>2+</sup> ions. Sensors and Actuators B: Chemical, 264, 67–75.
- Bag, P., Maurya, R. K., Dadwal, A., Sarkar, M., Chawla, P. A., Narang, R. K., & Kumar, B. (2021). Recent development in synthesis of carbon dots from natural resources and their applications in biomedicine and multi-sensing platform. *ChemistrySelect*, 6(11), 2774–2789.
- Bao, L., Liu, C., Zhang, Z. L., & Pang, D. W. (2015). Photoluminescence-tunable carbon nanodots: Surface-state energy-gap tuning. Advanced Materials, 27(10), 1663–1667.
- Bayat, A., & Saievar-Iranizad, E. (2017). Synthesis of green-photoluminescent single layer graphene quantum dots: Determination of HOMO and LUMO energy states. *Journal of Luminescence*, 192, 180–183.
- Cadranel, A., Margraf, J. T., Strauss, V., Clark, T., & Guldi, D. M. (2019). Carbon nanodots for charge-transfer processes. Accounts of chemical research, 52(4), 955–963.
- Chandra, S., Pathan, S. H., Mitra, S., Modha, B. H., Goswami, A., & Pramanik, P. (2012). Tuning of photoluminescence on different surface functionalized carbon quantum dots. RSC Advances, 2(9), 3602–3606.
- Chen, W., Li, D., Tian, L., Xiang, W., Wang, T., Hu, W., Hu, Y., Chen, S., Chen, J., & Dai, Z. (2018). Synthesis of graphene quantum dots from natural polymer starch for cell imaging. *Green Chemistry : An International Journal and Green Chemistry Resource : GC*, 20(19), 4438–4442.
- Chin, S. F., Yazid, S. N. A. M., Pang, S. C., & Ng, S. M. (2012). Facile synthesis of fluorescent carbon nanodots from starch nanoparticles. *Materials Letters*, 85, 50–52.
- De, B., & Karak, N. (2013). A green and facile approach for the synthesis of water soluble fluorescent carbon dots from banana juice. *RSC Advances*, *3*(22), 8286–8290.
- Ding, H., Yu, S. B., Wei, J. S., & Xiong, H. M. (2016). Full-color light-emitting carbon dots with a surface-state-controlled luminescence mechanism. ACSs Nano, 10(1), 484–491.

Feng, X., Jiang, Y., Zhao, J., Miao, M., Cao, S., Fang, J., & Shi, L. (2015). Easy synthesis of photoluminescent N-doped carbon dots from winter melon for bio-imaging. *RSC Advances*, 5(40), 31250–31254.

Georgakilas, V., Perman, J. A., Tucek, J., & Zboril, R. (2015). Broad family of carbon nanoallotropes: Classification, chemistry, and applications of fullerenes, carbon dots,

nanotubes, graphene, nanodiamonds, and combined superstructures. *Chemical Reviews*, 115(11), 4744–4822.

- Gómez, I. J., Vázquez Sulleiro, M., Dolečková, A., Pizúrová, N., Medalová, J., Roy, R., Nečas, D., & Zajíčková, L. (2021). Exploring the emission pathways in nitrogendoped graphene quantum dots for bioimaging. *Journal of Physical Chemistry C*, 125 (38), 21044–21054.
- Gong, X., Zhang, Q., Gao, Y., Shuang, S., Choi, M. M. F., & Dong, C. (2016). Phosphorus and nitrogen dual-doped hollow carbon dot as a nanocarrier for doxorubicin delivery and biological imaging. ACS Applied Materials & Interfaces, 8(18), 11288–11297.
- Grabolle, M., Spieles, M., Lesnyak, V., Gaponik, N., Eychmüller, A., & Resch-Genger, U. (2009). Determination of the fluorescence quantum yield of quantum dots: Suitable procedures and achievable uncertainties. *Analytical Chemistry*, 81(15), 6285–6294.
- He, X., Li, H., Liu, Y., Huang, H., Kang, Z., & Lee, S. T. (2011). Water soluble carbon nanoparticles: Hydrothermal synthesis and excellent photoluminescence properties. *Colloids and Surfaces B: Biointerfaces*, 87(2), 326–332.
- Lakowicz, J. R. (2006). Principles of fluorescence spectroscopy. Principles of fluorescence spectroscopy (3rd ed.). New York: Springer.
- Li, H., Kang, Z., Liu, Y., & Lee, S. T. (2012). Carbon nanodots: Synthesis, properties and applications. Journal of Materials Chemistry, 22(46), 24230–24253.
- Li, M., Chen, T., Gooding, J. J., & Liu, J. (2019). Review of carbon and graphene quantum dots for sensing. ACS Sensors, 4(7), 1732–1748.
- Lim, S. Y., Shen, W., & Gao, Z. (2015). Carbon quantum dots and their applications. Chemical Society Reviews, 44(1), 362–381.
- Liu, M., Zhang, X., Yang, B., Li, Z., Deng, F., Yang, Y., Zhang, X., & Wei, Y. (2015). Fluorescent nanoparticles from starch: Facile preparation, tunable luminescence and bioimaging. *Carbohydrate Polymers*, 121(0), 49–55.
- Nagamori, M., & Funazukuri, T. (2004). Glucose production by hydrolysis of starch under hydrothermal conditions. *Journal of Chemical Technology and Biotechnology*, 79 (3), 229–233.
- National Institute for Materials Science (NIMS) AtomWork. (2022). http://crystdb.nims. go.jp/.
- Pei, X., Xiong, D., Wang, H., Gao, S., Zhang, X., Zhang, S., & Wang, J. (2018). Reversible phase transfer of carbon dots between an organic phase and aqueous solution triggered by CO<sub>2</sub>. Angewandte Chemie International Edition, 57(14), 3687–3691.
- Qu, S., Wang, X., Lu, Q., Liu, X., & Wang, L. (2012). A biocompatible fluorescent ink based on water-soluble luminescent carbon nanodots. *Angewandte Chemie International Edition*, 51(49), 12215–12218.
- Rigodanza, F., Burian, M., Arcudi, F., Dorđević, L., Amenitsch, H., & Prato, M. (2021). Snapshots into carbon dots formation through a combined spectroscopic approach. *Nature Communications*, 12(1), 2640.
- Sahu, S., Behera, B., Maiti, T. K., & Mohapatra, S. (2012). Simple one-step synthesis of highly luminescent carbon dots from orange juice: Application as excellent bioimaging agents. *Chemical Communications*, 48(70), 8835–8837.
- Schneider, J., Reckmeier, C. J., Xiong, Y., von Seckendorff, M., Susha, A. S., Kasák, P., & Rogach, A. L. (2017). Molecular fluorescence in citric acid-based carbon dots. *Journal of Physical Chemistry C*, 121(3), 2014–2022.
- Shen, J., Shang, S., Chen, X., Wang, D., & Cai, Y. (2017). Facile synthesis of fluorescence carbon dots from sweet potato for Fe<sup>3+</sup> sensing and cell imaging. *Materials Science* and Engineering: C, 76, 856–864.
- Sun, X., & Li, Y. (2004). Colloidal carbon spheres and their core/shell structures with noble-metal nanoparticles. Angewandte Chemie International Edition, 43(5), 597–601.
- Tang, L., Ji, R., Cao, X., Lin, J., Jiang, H., Li, X., Teng, K., Luk, C., Zeng, S., Hao, J., & Lau, S. P. (2012). Deep ultraviolet photoluminescence of water-soluble selfpassivated graphene quantum dots. ACS Nano, 6(6), 5102–5110.
- Tang, L., Ji, R., Li, X., Bai, G., Liu, C. P., Hao, J., Lin, J., Jiang, H., Teng, K. S., Yang, Z., & Lau, S. P. (2014). Deep ultraviolet to near-infrared emission and photoresponse in layered N-doped graphene quantum dots. ACS Nano, 8(6), 6312–6320.
- Titirici, M. M., & Antonietti, M. (2010). Chemistry and materials options of sustainable carbon materials made by hydrothermal carbonization. *Chemical Society Reviews*, 39 (1), 103–116.
- Vandarkuzhali, S. A. A., Jeyalakshmi, V., Sivaraman, G., Singaravadivel, S., Krishnamurthy, K. R., & Viswanathan, B. (2017). Highly fluorescent carbon dots from Pseudo-stem of banana plant: Applications as nanosensor and bio-imaging agents. Sensors and Actuators B: Chemical, 252, 894–900.
- Wang, C., Strauss, V., & Kaner, R. B. (2019). Carbon nanodots for capacitor electrodes. Trends in Chemistry, 1(9), 858–868.
- Wang, N., Wang, Y., Guo, T., Yang, T., Chen, M., & Wang, J. (2016). Green preparation of carbon dots with papaya as carbon source for effective fluorescent sensing of Iron (III) and Escherichia coli. *Biosensors & Bioelectronics*, 85, 68–75.
- Wang, Y., & Hu, A. (2014). Carbon quantum dots: Synthesis, properties and applications. Journal of Materials Chemistry C, 2(34), 6921–6939.
- Wang, Z., Zeng, H., & Sun, L. (2015). Graphene quantum dots: Versatile photoluminescence for energy, biomedical, and environmental applications. *Journal*
- of Materials Chemistry C, 3(6), 1157–1165. Xu, H., Yang, X., Li, G., Zhao, C., & Liao, X. (2015). Green synthesis of fluorescent carbon dots for selective detection of tartrazine in food samples. *Journal of Agricultural and Food Chemistry*, 63(30), 6707–6714.
- Xu, Z. Q., Yang, L. Y., Fan, X. Y., Jin, J. C., Mei, J., Peng, W., Jiang, F., Xiao, Q., & Liu, Y. (2014). Low temperature synthesis of highly stable phosphate functionalized two color carbon nanodots and their application in cell imaging. *Carbon*, 66, 351–360.
- Yan, Z., Shu, J., Yu, Y., Zhang, Z., Liu, Z., & Chen, J. (2015). Preparation of carbon quantum dots based on starch and their spectral properties. *Luminescence : The Journal of Biological and Chemical Luminescence*, 30(4), 388–392.
- Yang, Z. C., Wang, M., Yong, A. M., Wong, S. Y., Zhang, X. H., Tan, H., Chang, A. Y., Li, X., & Wang, J. (2011). Intrinsically fluorescent carbon dots with tunable emission

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derived from hydrothermal treatment of glucose in the presence of monopotassium

- phosphate. Chemical Communications, 47(42), 11615–11617.
  Zhai, X., Zhang, P., Liu, C., Bai, T., Li, W., Dai, L., & Liu, W. (2012). Highly luminescent carbon nanodots by microwave-assisted pyrolysis. Chemical Communications, 48(64), 7955–7957.
- Zheng, J. X., Liu, X. H., Yang, Y. Z., Liu, X. G, & Xu, B. S. (2018). Rapid and green synthesis of fluorescent carbon dots from starch for white light-emitting diodes. New Carbon Materials, 33(3), 276-288.
- Zhu, L., Shen, D., Wu, C., & Gu, S. (2020). State-of-the-art on the preparation, modification, and application of biomass-derived carbon quantum dots. *Industrial & Engineering Chemistry Research*, 59(51), 22017–22039.