

Materials

The deionized water was prepared in the laboratory. The starch used in the synthesis of GQDs was bought from Aladdin Reagent Co.,Ltd, Shanghai, China, and its information and physical properties are listed in Table S1.

Table S1 The information and physical properties of the starch

| | |
|--------------------------|------------------------------|
| Material | Starch from potato (amylose) |
| CAS No. | 9005-25-8 |
| EC No. | 232-679-6 |
| Appearance | White powder |
| Molecular formula | $(C_6H_{10}O_5)_n$ |
| Degree of polymerization | 1100 |
| Density | 1.5 |
| Melting point | 256-258 °C |
| PH value (20g/L, 25°C) | 6.0-7.5 |
| Burning residue | ≤0.5% |
| Weight loss on drying | ≤13.0% |

Synthetic process of the GQDs

The starch of 0.3g was firstly dispersed in 25ml deionized water and stirred at 60°C for 15 min. After it was dissolved, the solution was immediately poured into a 100 ml Teflon lined stainless autoclave, which was then heated in an oven at 190 °C for 120 min. Then, the autoclave was taken out to be cooled freely. The final brown product was transferred into centrifugal tubes, and centrifuged at 15000 for 20 min to separate out the precipitate. The pale yellow liquid which was obtained finally was the solution of graphene quantum dots (GQDs).

Cell viability assay

Human cervical carcinoma cells Ca Ski (China Center for Type Culture Collection,

Wuhan) (1×10^4 /well) were seeded into 96-well microplates containing 100 μ L supplemented RPMI-1640 culture medium (Gibco Life Technologies, USA) with 10% fetal bovine serum (FBS) and incubated for 12 h in 5% CO₂ at 37°C. Then, 100 μ L of medium of various GQDs concentrations (ranging from 0.078 mg/ml to 1.25 mg/ml) were added to each well and were further incubated for 24 h. Afterwards, the cell viabilities were evaluated by MTT assays. The absorbance of formazan formed was measured at 490 nm by a microplate reader. Each experiment was repeated at least 3 times.

Cell imaging

Ca Ski cells (1×10^5 /well) were seeded into 35mm cell culture dishes (Corning, American) containing 1 ml of supplemented RPMI-1640 culture medium with 10% FBS and incubated for 24 h in 5% CO₂ at 37°C. Then, 1 mL of medium containing GQDs (1.25 mg/ml) were added and were further incubated for 6 h. Finally, cells were observed under a laser confocal fluorescence microscopy (Olympus FV1200, Japan).

Procedure of inference and supporting literatures for the reaction mechanism

As we know, there are two hydrolysis routes by which glucose can be formed from starch, i.e., acidolysis in the presence of acid and enzymolysis in the presence of enzyme. For the former, neutralization of the hydrolyzate is required after acidic hydrolysis. Meanwhile, the cost of enzymatic hydrolysis is too expensive for the latter. Accordingly, water has been the focus of attention as a hydrolytic medium because of the relatively high values of the ionic product of H⁺ and OH⁻ under hydrothermal conditions.^[1] In fact, various natural polymers such as cellulose,^[2-4] polygalacturonic acid^[5] and biomass samples^[6-7] have been hydrolyzed with water in the absence of acid catalyst or other reagents under hydrothermal conditions.

Starch is considered to be hydrolyzed more easily than cellulose, and its hydrolysis process and mechanism were researched by Nagamori et al.^[8] Their work

demonstrated that starch was converted into glucose by hydrolysis with water under hydrothermal conditions at 453-513K in a small batch reactor, without the addition of any additives. The research indicated that the major product of the hydrolysis was glucose, while small amounts of maltose and fructose, aldehydes were also produced. The maximum yield of glucose obtained was 630 g kg⁻¹ on the carbon basis at 473K and 30 min, where the quantity of carbon unrecovered was about 50 g kg⁻¹, and the production of char and gaseous products was negligible.

Additionally, synthesis of graphene or GQDs from the glucose, which is the hydrolysis product of starch, have also been reported.^[9-11] Bayat et al.^[11] offered a low-cost, high-yield, one-step, and facile process to prepare single layer GQDs from only glucose powder as precursor in DI water. In the typical synthesis, glucose powder was dissolved in the deionized water, and the mixture was treated by a one-step hydrothermal method in a Teflon-lined stainless-steel autoclave. The mechanism is presented by them that under the hydrothermal process, glucose molecules are dehydrated to form C=C, which is the elementary unit of the graphene structure. During the formation of GQDs, hydrogen atoms of a glucose molecule interact with hydroxyl groups of an adjacent glucose molecule leading to formation of water molecules; consequently, carbon atoms covalently interact with each other and finally graphene quantum dots are formed.

Based on the above literatures, the reaction mechanism of synthesis of GQDs from starch can be inferred as follows: the synthetic process contains the first hydrolyzation of starch to produce glucose, and the following ring-closure condensation of glucose to generate GQDs. At first, water is used as a hydrolytic medium because of the relatively high values of the ionic product of H⁺ and OH⁻ under hydrothermal conditions. Then, the starch continuously hydrolyze with water without any chemical reagent in the presence of H⁺, and turn into various components including glucose, fructose, maltose, and aldehydes. At the same time, traces of gaseous products are produced but relatively insignificant, according to the carbon balances. As the hydrolysis reaction goes on, the yield of glucose is increased while those of other carbohydrates are decreased sharply. Thus, the starch is converted

mainly into glucose, with small amounts of other carbohydrates being remained. Followly, hydrogen atoms of a glucose molecule interact with hydroxyl groups of an adjacent glucose one, and formyl groups react with hydroxyl groups, leading to dehydration under the hydrothermal conditions. Consequently, carbon atoms covalently interact with each other and there form the aromatic rings, the elementary unit of the graphene structure. In this way, GQDs are generated with the ring-closure condensation of glucose molecules. On the other hand, the other remained carbohydrates are changed into carbide precipitates at high temperatures and pressures due to the carbonization. Fanally, the GQDs solution are obtained by separating the precipitates out via centrifugation.

The supporting literatures for the reaction mechnism is listed as follows:

- 1 J. C. Tanger, K. S. Pitzer, Calculation of the ionization constant of H₂O to 2273 K and 500 MPa, *AIChE J.*, 1989, 35, 1631-1638.
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- 7 S. E. Jacobsen, C. E. Wyman, Xylose monomer and oligomer yields for uncatalyzed hydrolysis of sugarcane bagasse hemicellulose at varying solids concentration, *Ind. Eng. Chem. Res.*, 2002, 41, 1454-1461.

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9 X. H. Li, S. Kurasch, U. Kaiser, M. Antonietti, Synthesis of Monolayer-Patched Graphene from Glucose, *Angew. Chem. Int. Ed.*, 2012, 51, 9689-9692.

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Mesurement of quantum yields

Fluorescein in 0.1 M NaOH (QY = 92 %) was chosen as a reference.^{1, 2} The quantum yield was calculated according to the following equation:

$$\Phi_X = \Phi_{ST} \left(\frac{A_{ST}}{A_X} \right) \left(\frac{I_X}{I_{ST}} \right) \left(\frac{\eta_X^2}{\eta_{ST}^2} \right)$$

Where the subscripts ST and X denote standard and sample, respectively. Φ is the quantum yield. I represents the measured integrated fluorescence intensity. A is the absorbance at the excitation wavelength, and η is the refractive index of the solutions. The quantum yield of GQDs was calculated to be 21.7 % with being excited at 360 nm.

References

1 D. Magde, R. Wong, P. G. Seybold, Fluorescence quantum yields and their relation to lifetime of rhodamine 6G and fluorescein in nine solvents: Improved absolute standards for quantum yields, *Photochem. Photobiol.*, 2002, 75, 327-334.

2 J. Shen, R. D. Snook, Thermal lens measurement of absolute quantum yields using quenched fluorescent samples as references, *Chem. Phys. Lett.*, 1989, 155, 583-586.