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Transformation of crystalline starch nanoparticles into highly luminescent carbon nanodots: Toxicity studies and their applications

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ABSTRACT

Being abundant in many tropical part of the world, *Dioscorea sp.* as food is limited due to its toxicity. However polysaccharides derive from these tubers could be important for other applications. Here we developed a Highly Luminescent Carbon Nanodots (C-dots) via acid hydrolysis of Gadong starch (GS). The hydrolysis rate of GS increased from 49% to 86% within 7 days while the X-ray diffraction showed the native GS particle is a C-crystalline type. The GS particles were either round or oval with diameters ranging from 50–90 nm. Further acid dehydration and surface oxidation reduced the size of GS nanoparticles to 6–25 nm. The C-dots produced a fluorescent emission at wavelength 441 nm. Toxicity tests demonstrate that zebrafish embryo were able to tolerate the C-dots for 48 h after exposure. This study has successfully demonstrated a novel approach of converting GS into excellent fluorescent C-dot.

1. Introduction

Quantum dots (QDs) were known as a substitution candidate for the conventional phosphor materials (Hong et al., 2012; Li et al., 2011a; Li et al., 2010a; Lightcap & Kamat, 2012; Song & Yang, 2012; Zhao et al., 2012; Zhuo, Shao, & Lee, 2012) and widely used in various filed such as biomedical and bioimaging due to their unique optical, electronic and fluorescent properties (Zhai et al., 2012). However interest in QDs was reduced for being costly, ineffective, complicated and impractical in real applications (Li, Kang, Liu, & Lee, 2012; Uu, Li, Zhan, Liu, & Huang, 2011). The usage of QDs is slowly declining due to the introduction of carbon based quantum dots. The explosion of nanotechnology in 2004 proposed new types of carbon nanodots via the electrophoresis of carbon nanotube thereby attracting researchers to better study carbon nanodots to replace QDs (Xu et al., 2004). Recently, carbon based quantum dots known as carbon nanodots (C-dots) have drawn considerable interest due to their stable photoluminescence property, less toxicity, high biocompatibility, cost effectiveness, good fluorescent properties, easy-functionalisation, excellent water solubility, high sensitivity, excellent selectivity to target analyte, tunable fluorescence emission and excitation and high quantum yield (Baker & Baker, 2010; Li et al., 2012; Saxena & Sarkar, 2012;). However, understanding the origin of fluorescence in C-dots is far from sufficient and information regarding the nanostructure and surface of C-dots remain unclear.

Based on the impressive advances of C-dots, various methods have been established to synthesis non-toxic fluorescing C-dots which is categorised into top-down and bottom-up methods (Cao et al., 2007; Chin, Yazi, Pang, & Ng, 2012; Jia, Li, & Wang, 2012; Li et al., 2010b; Li et al., 2011a; Li et al., 2011b; Liu, Ye, & Mao, 2007; Liu, Tian, Wang, Luo, & Sun, 2012; Liang, Ma, Shi, Li, & Yang, 2013a; Long et al., 2012; Ma, Ming, Huang, Liu, & Kang, 2012; Ming et al., 2012; Qin, Lu, Asiri, Al-Youbi, & Sun, 2013; Sahu, Behera, Maiti, & Mohapatra, 2012; Wang, Wang, & Chen, 2012; Wu, Li, Tan, Wu, & Liu, 2015). Different starting materials have been used in the preparation of C-dots (Das, Liu, Yeom, Kim, & Richards, 2014; Dong et al., 2012a; Hsu, Shih, Lee, & Chang, 2012; Lai, Hsiao, Peng, & Chou, 2012; Liang, Ma, Shi, Li, & Yang, 2013b; Liu et al., 2012; Shi et al., 2015a; Wang et al., 2011; Wu et al., 2015; Zhu, Zhai, & Dong, 2012). Nevertheless, the majority of the synthesis methods require complicated equipment, catalyst, non-environmental friendly chemicals or harsh experimental condition which affect the production cost (Tan, Romainor, Chin, & Ng, 2014). In advance, C-dots synthesised from different methods and various sources were applicable in numerous biological and chemical fields (Ahmed, Laino, Calzon, & Garcia, 2015; Guo, Wang, Shao, & Jiang, 2013; Liu, Zhao, & Zhang, 2014; Qin et al., 2013; Qu, Chen, Zheng, Cao, & Liu, 2013; Quyang, 2013; Shi et al., 2015a; Shi et al., 2015b; Wang et al., 2015; Xu, Lai, Feng, Weng, & Huang, 2014; Yazid, Chin,

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Pang, & Ng, 2013). For example, C-dots based on carbon powder with diameter size 4–5 nm was synthesised using laser radiation while C-dots with size 1 nm in size were synthesised from candle soot by combustion and refluxed with nitric acid for 12 h (Ray, Saha, Jana, & Sarkar, 2009). These synthesised fluorescence particles had been used in fluorescent-based cell imaging applications. Starch based fluorescent C-dots, however, are poorly studied compared to other carbon-based materials such as carbon nanotube, citric acid, and glycerol (Chin et al., 2012).

Nanocrystals from polymeric material can be utilised to synthesise C-dots. Polymeric nanocrystals can be extracted from naturally occurring polysaccharides such as starch, cellulose, and chitin due to their semi-crystalline nature. In recent years, the demand for starch nanocrystals (SNCs) has seen an increase in various sectors. For example, these non-toxic nanoparticles have been approved as a suitable candidate for a renewable nano-filler (Chin, Azman, & Peng, 2014a; LeCorre, Bras, & Dufresne, 2010). Moreover, SNCs have also been proposed as an alternative material to replace carbon black used to reinforce natural rubber. SNCs are also known as an excellent material to be used for drug delivery systems and have been employed as a slow-release drug-carrier (Han, Borjihan, Bai, Chen, & Jing, 2008; Rodrigues & Emeja, 2012; Simi & Abraham, 2007; Yu, Xiao, Tong, Chen, & Liu, 2007). SNCs are also used in biotechnology as a nano-encapsulation agent or as a stabilising agent for nano-emulsions (Chin, Yazid, & Pang, 2014b; Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011;). In biomedical applications, SNCs have been introduced into polymeric matrices in order to improve their mechanical strength and biodegradability (Kristo & Biliaderis, 2007).

Dioscorea hispida Dennst. is categorised as a *Lasiophyton* and taxonomically ascribed to the Dioscoreacea family and Dioscorea genus (Baah, 2009). There are 1137 species of Dioscorea around the world, but only 600 species are reported to be harmless (Agbar-Egbe & Treche, 1995; Nashriyah, Yusoff, Tajuddin, Ngah, & Rejab, 2010; Poornima & Rai, 2009). The Gadong tuber is recognised as a largely abandoned species of *Dioscorea* due to containing poisonous alkaloids known as dioscorine (Ashri et al., 2014; Azman et al., 2015; Hudzari, 2012). To obtain C-dots, various starting materials were used and passivated with various compound such as BPEI and PEG_{1500N} or combining several chemicals to enhance fluorescent properties (Cao et al., 2007; Dong et al., 2012b; Sun et al., 2006; Sun et al., 2008; Zhang et al., 2013). On the other hand, no literature reported on the preparation of C-dots based on the exotic wild yam, *D. hispida.* Apart from that, none demonstrated that starch based C-dots can act as a fluorescent ink.

This research explores the synthesis of C-dots by a simple synthesis route from a low cost precursor, Gadong starch and we investigate its resistance to toxicity and fluorescent properties. The SNCs and C-dots were then characterised to determine its optimum performance. The prepared nanocrystals and synthesised C-dots have been characterised by transmission electron microscopy (TEM) to investigate the morphology, X-ray diffraction (XRD), Fourier transformed infrared FTIR, UV–vis, and fluorescence measurements to introduce this C-dots as a new type of fluorescent ink. Further investigations were carried out to determine the toxicity level of the synthesised C-dots. The C-dots were tested on 1-h post fertilization zebra fish embryos.

2. Materials and method

2.1. Materials

All chemicals were of reagent grade and were used without further purification. Gadong tuber was obtained from Terengganu, Malaysia. Native Gadong starch was extracted from Gadong tuber according to the procedure reported by Nand, Charan, Rohindra, and Khurma (2008). Sulphuric acid (95–97%) was purchased from Friendemann Schmidt (USA), Ultrapure water (~18.2 MΩ, 25 °C) was obtained from Water Purify System (Sartorius, Model arium®611DI). Sodium hydroxides were purchased from Sigma-Aldrich (USA).

Embryos were obtained from the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. E3 embryo media (5.03 m M NaCl, 0.17 m M KCl, 0.33 m M $CaCl_2{\cdot}2H_2O$, 0.33 m M $MgSO_4{\cdot}2H_2O$ and 0.1% (w/v) methylene blue) were used to wash and maintain viable fertilised eggs at room temperature at all times.

2.2. Acid hydrolysis

Acid hydrolysis was preformed according to the method reported by Kim, Lee, Kim, Lim, and Lim (2012). Native Gadong starch (15 g, dry) was dispersed in an aqueous sulphuric acid solution (100 mL, 3.16 M), and the dispersion was stirred for 7 days at 40 °C. On the seventh day, the dispersion was neutralised by washing with distilled water filtered by centrifuging the suspension at 20,000 rpm for 20 min. The wet precipitate was then washed with distilled water and stirred for 30 min before the dispersion was again centrifuged at 20,000 rpm for 20 min. This washing process was repeated several times to remove the residual sulphate. The precipitated starch hydrolysate was then freeze-dried, and the solid weight of the starch hydrolysate was measured. The degree of hydrolysis (%) was calculated as the percent mass ratio of the dissolved starch solids to the initial starch solids.

$$\text{Degree of hydrolysis}\,(\%) = \frac{\text{Dissolved starch solids}}{\text{Initial starch solids}} \times 100 \qquad (1)$$

2.3. Acid dehydration

Starch nanoparticles were prepared from Gadong tuber based on Kim's et al. (Kim et al., 2012) method. The starch nanoparticles were converted to carbon nanoparticles based on Chin's et al. (Chin et al., 2012) method by dehydration with concentrated sulphuric acid. In typical synthesis, 2.0 g of starch nanoparticles were dispersed in 5 mL of ultrapure water and 8.0 mL of concentrated sulphuric acid was added to the starch solution. The reaction was allowed to proceed for 40 min, followed by the addition of 40 mL of ultra-pure water. The black carbon nanoparticles produced was collected by centrifugation (20,000*g*, 20 min) and washed three times with ultra-pure water.

2.4. Surface oxidation

The black carbon nanoparticles were dispersed in 20 mL of nitric acid (2.0 M) and the mixture was refluxed for 1 h. The solution was neutralised by sodium hydroxide at room temperature (Chin et al., 2012).

2.5. Morphology and average particle size

Morphology and particle size of the starch nanoparticles and C-dots were observed using transmission electron microscopy (TEM) (Philips) operated at 300 kV. The precipitates (10 mg, dry basis) were dispersed in 5 mL of water and then ultrasonically homogenised for 3 min at 13,000 rpm to ensure dispersion of the starch particles. A drop of the suspension was deposited on a carbon-coated microscopy grid. The grid was negatively stained with a drop of uranyl acetate solution (2% w/v) before it was dried at room temperature (Kim et al., 2012). Besides that, Field Emission Scanning Electron Microscopy (FE-SEM) was used to confirm the shape and size of the starch nanoparticles. The liquid C-dots were diluted with ultra-pure water in ratio of 1:5 (mL). A drop of the suspension was deposited on a carbon-coated microscopy grid