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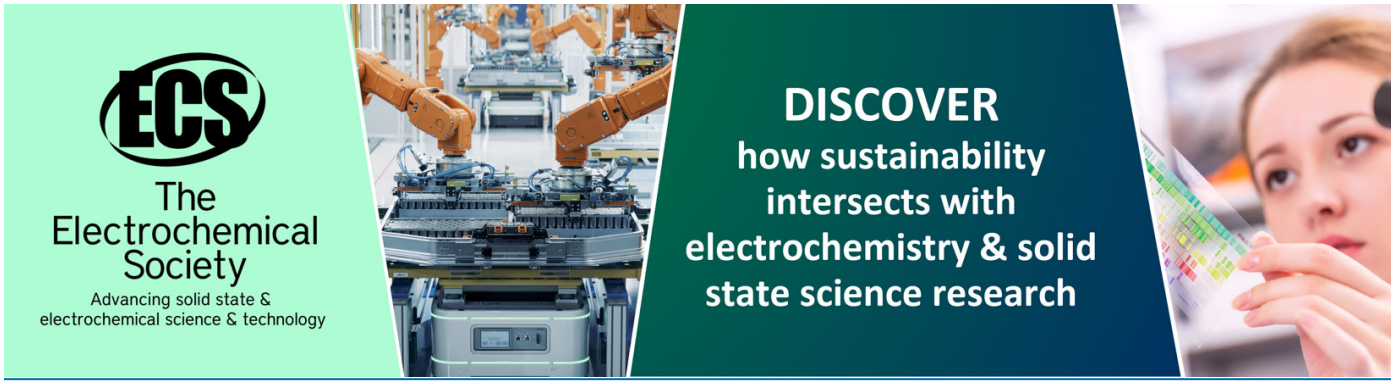

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Synthesis of biocompatible carbon dots from yogurt and gas vapor sensing

S Moonrinta[1], S Jamnongsong[2], S Sampattavanich[2], S Kladsomboon[3], W Sajomsang[4] and P Paoprasert[1*]
[1]Department of Chemistry, Faculty of Science and Technology, Thammasat University, Pathumthani, 12120 Thailand
[2]Department of Pharmacology, Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkoknoi, Bangkok, 10700 Thailand
[3]Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Nakhon Pathom, 73170 Thailand
[4]National Nanotechnology Center, Pathumthani, 12120 Thailand
*Corresponding author, email: peerasak@tu.ac.th

Abstract. Carbon dots are fascinating nanomaterials due to their low cost, straightforward preparation, unique optical properties, and biocompatibility. In this work, carbon dots were prepared from yogurt using pyrolysis method. The carbon dots were characterized using UV-visible spectroscopy, fluorescence spectroscopy, Fourier transform infrared spectroscopy, and transmission electron microscopy. The carbon dots were applied for detecting formic acid vapor using electronic nose system. Furthermore, the biocompatibility of carbon dots were investigated to show their potentials for biomedical applications. Based on these research, carbon dots from yogurt were multifunctional fluorescent nanomaterials for various applications.

1. Introduction

In 2004, Xu and coworkers isolated a novel spherical carbon-based nanomaterial, from an eletrophoretic method for the purification of single-walled carbon nanotubes derived from arc-discharge soot.[1] This new material is now known as “carbon dots”. Carbon dots are luminescent nanoparticles that have attracted broad attention in recent years. Carbon dots can fluoresce when they are excited by ultraviolet or visible light. In addition, they have low toxicity,[2] excellent photostability,[3] high water solubility[4] and can be prepared by several methods such as, laser ablation,[5] pyrolysis,[6] hydrothermal,[7] and microwave irradiation.[8] Because of these unique and attractive properties, carbon dots have been used in various applications such as sensing,[9] electronics,[10] and biomedical applications[7]. Carbon-dots have been synthesized from various precursors, such as wastes, edibles, and chemicals such as watermelon peel,[11] garlic[12] and ethanol.[13]

In this work, a method for synthesizing carbon-dots form yogurt was developed. As one of the oldest and most popular fermented foods, yogurt is a known delicacy around the world. It contains vitamins and minerals. It is also rich in protein, a source of nitrogen atoms known to enhance quantum efficiency of carbon dots. The carbon dots were synthesized from fermented milk via pyrolysis method. They were then characterized using UV-visible spectroscopy, fluorescence spectroscopy, Fourier transform infrared spectroscopy, and transmission electron microscopy. They were used in

many sensing applications such as gas vapor detection using electronic nose system. In addition, they were also tested cytotoxicity with human bile duct cancer (KKU213) cell line.

2. Experimental section

2.1. Materials

"Dutchie" original flavor yogurt was purchased from a local supermarket. All chemicals were purchased from Sigma-Aldrich. Cellulose dialysis membrane (1000 Da MWCO) was bought from Spectrum Labs. Deionized (DI) water was used throughout the experiment.

2.2. Methods and preparations

2.2.1. Synthesis of carbon dots

Carbon dots were synthesized by pyrolysis method using yogurt as a precursor. Yogurt (50 g) and hydrochloric acid (0.5 M, 25 mL) were mixed in a 200 mL evaporating dish. The reaction mixture was heated at 180 ℃ for 2.5 h. Then remaining black solid was rinsed with DI water and adjusted pH 7. Next, the brown solution was purified using dialysis membrane for 3 days. The solution was then centrifuged for 15 minutes at 10000 rpm. After that the dot solution was dried by a freeze dryer overnight to give a brown solid and then kept in the bottle.

2.2.2. Quantum yield

Quantum yield (Φ) of carbon dots was measured using fluorescence spectrometry. Quinine sulfate was used as a reference. The carbon dots were dissolved in DI water (n = 1.33). Quinine sulfate was dissolved in 0.1 M H_2SO_4 solution (quantum yield of 54%, n = 1.33). Quantum yield was calculated using equation (1):

$$\Phi = \Phi_R \times \frac{I}{I_R} \times \frac{A_R}{A} \times \frac{n^2}{n_R^2} \quad (1)$$

where I is the integrated emission intensity, A refers to the absorbance, and n is refractive index of the solvent. The subscript R denotes the reference sample.

2.2.3. Electronic nose measurement

The optical electronic nose system consists of light sources, sensing materials, and a photo detector. Light-emitting diodes (LEDs) and a commercial photo detector (ET-TCS230) were used to detect light intensity. The eight gas sensor arrays were generated based on eight colors of LEDs. The transmitting light intensity through sensing thin film was observed in the form of photon frequency (Hz), captured by a photo-detector.

2.2.4. Cytotoxicity test

Human bile duct cancer (KKU213) cells, containing the nuclear marker NLS (nuclear localization signal)-mCherry, were chosen for the cell viability and imaging experiments. First, cells were placed in the wells of a microtiter plate at a ratio of 8,000 cells per well and incubated for 24 h at 37 ℃ under 5% CO_2. The media (150 μL per well) for cell culture was then replaced. Carbon dots (2.0 mg) were dissolved in a sterile 1X PBS solution (1 mL). The carbon dot solution was passed through a syringe filter (0.45 μm) and sonicated for 30 min at room temperature. The carbon dot solution (2 g L^{-1}, 100 μL) was then added to the first row of wells, and then diluted two-fold at each of the sequence of eight rows, to obtain carbon dots concentrations of 2.0000, 1.0000, 0.5000, 0.2500, 0.1250, 0.0625, 0.0313, 0.0156, and 0.0078 g L^{-1}. The last row of wells contained only cells and culture media with no carbon dot solution and was used as a control. The cells were then incubated for 24 h at 37 ℃ under 5% CO_2, which the culture media and carbon dot solution were removed. The incubated cells were washed three times with 1X PBS (200 μL). The incubated cells were then imaged (Operetta, 60X high NA objective

lens) using a 475 nm excitation wavelength and a 525 nm emission wavelength. The graph of relative viability against carbon dots concentration (0-2 g L^{-1}) was plotted from cell counting.

3. Result and discussion

3.1. Preparation and characterization of the carbon dots

The carbon dots were prepared from yogurt by pyrolysis reaction. The functional groups of carbon dots were identified using FTIR. The FT-IR spectra indicated the characteristic absorption bands of O–H at 3278 cm^{-1} and N–H stretching vibrations of amine groups at 2966 cm^{-1} and C=O stretching vibrations located at around 1640 cm^{-1} (Figure 1a). This supports that carbon dots are highly hydrophilic and water soluble. Size and morphology of carbon dots were determined by TEM. The TEM image showed spherical carbon dots with a diameter of about 3.5 nm (Figure 1b). The optical properties of carbon dots were characterized using UV-visible and fluorescence spectroscopy. The UV–visible spectrum exhibited a broad absorption band from 250 to 500 nm (Figure 1c). The absorption peak centered at 282 and 359 nm, corresponding to π-π* transition of the aromatic sp^2 domains and n-π* transition of C=O bonds due to the presence of carbonyl-based groups on the surface.[14] The emission was found to be excitation wavelength dependence, showing the highest emission intensity at 320 nm excitation wavelength (Figure 1d). The carbon dots showed blue emission under UV illumination with a quantum yield of 2.4%.

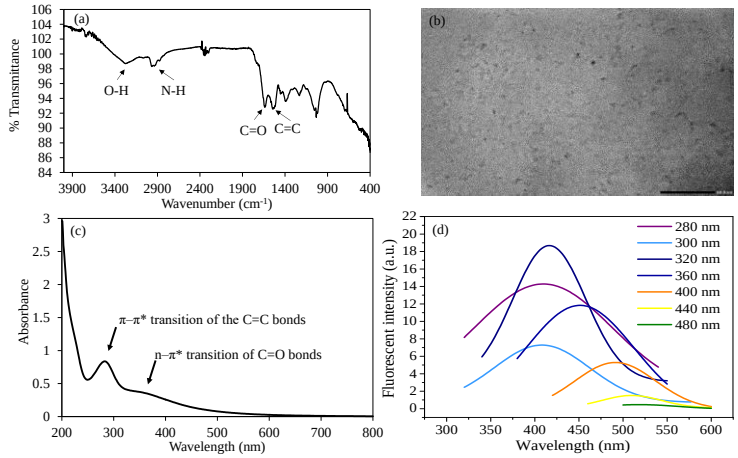

Figure 1. (a) FT-IR spectrum, (b) TEM image, (c) absorption, and (d) excitation-dependent emission spectra of carbon dots.

3.2. Sensing applications of the carbon dots

3.2.1. Formic acid vapor sensing

Carbon dots were used to detect chemical vapor, in this case, formic acid. The dynamic optical responses of the film (Figure 2a and b) corresponded to a change in the light intensity. When the nitrogen reference gas flowed through the film, the light transmission in the frequency unit increased rapidly. The light transmission then dropped dramatically when the flow was switched to reference gas. The carbon dots showed different responses when 10% and 20% formic acid solutions were used. This suggested that the carbon dots were successfully tested as a formic acid vapor sensor.

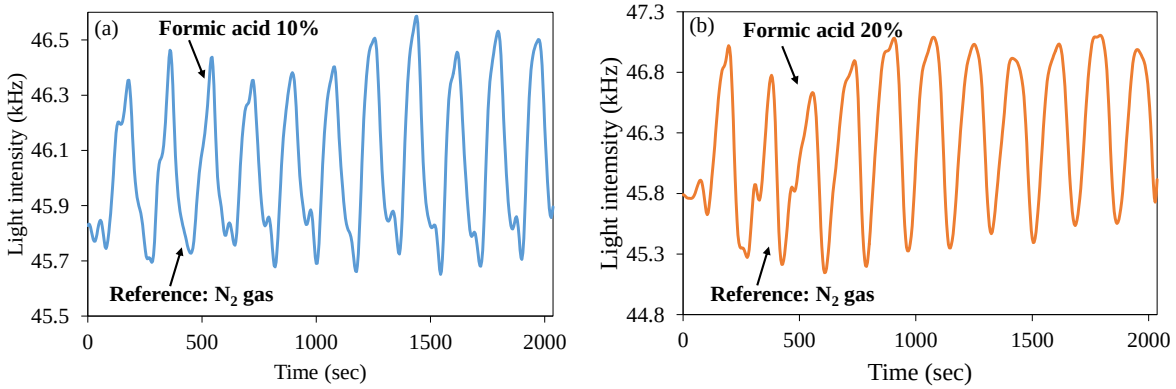

Figure 2. Gas sensing responses with carbon dots film for (a) formic acid 10%, and formic acid 20%.

3.2.2. Cytotoxicity

In this work, KKU213 cell line, a human bile duct cancer, was used to test the cytotoxicity of carbon dots. Cells were treated with different concentrations of the carbon dots for 24 h. The relative cell viability showed more than 90% up on 2 g L^{-1} concentration of carbon dots (Figure 3). The result suggested that carbon dots showed excellent biocompatibility.

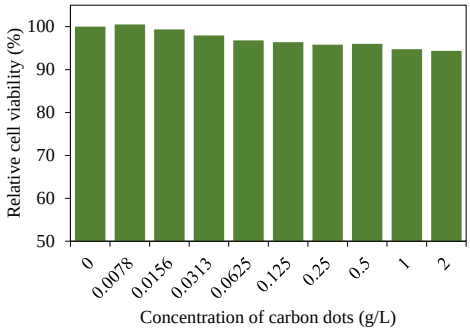

Figure 3. Relative cell viability human bile duct cancer cells treated with carbon dots for 24 h**.**

4. Conclusion

From this study, carbon dots were prepared from yogurt using a simple pyrolysis method. The carbon dots have been successfully applied for formic acid vapor. In addition, they exhibited excellent biocompatibility in living cell. The multifunctional carbon dots from yogurt developed in this work will be suitable for sensing and biomedical applications. Future work includes cell imaging and chemical sensing using our carbon dots.

5. Acknowledgement

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