Food Chemistry 235 (2017) 14–20

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Graphene oxide-sensitized molecularly imprinted opto-polymers for charge-transfer fluorescent sensing of cyanoguanidine

Huilin Liu [a], Kaiwen Zhou [a], Xiaomo Chen [a], Jing Wang [a,*], Shuo Wang [a,b], Baoguo Sun [a]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University (BTBU), 11 Fucheng Road, Beijing 100048, China
[b] Key Laboratory of Food Nutrition and Safety, Ministry of Education of China, Tianjin Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, 29 The Thirteenth Road, Tianjin Economy and Technology Development Area, Tianjin 300457, China

ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form 2 May 2017
Accepted 6 May 2017
Available online 8 May 2017

Keywords:
Molecularly imprinted opto-polymers
Graphene
Quantum dots
Cyanoguanidine
Infant formula

ABSTRACT

The hierarchical structuring of materials offers exciting opportunities to construct functional sensors. Multiple processes were combined to create complex materials for the selective detection of cyanoguanidine (CYA) using graphene oxide-sensitized molecularly imprinted opto-polymers (MIOP). Molecular imprinting was used to construct molecular-scale analyte-selective cavities, graphene oxide was introduced to provide a platform for the polymerization, and increase the stability and binding kinetic properties, and 3-methacryloxy propyl trimethoxy silane-modified quantum dots were combined with a functional monomer to increase the fluorescence quantum yield. Polymer cross-linking and fluorescence intensity were optimized for molecular recognition and opto-sensing detection. Selective and sensitive, fluorescence sensing of CYA was possible at concentrations as low as to 1.6 μM. It could be applied to the rapid and cost-effective monitoring of CYA in infant formula. The approach is generic and applicable to many molecules and conventional opto-sensors, based on molecularly imprinted polymer formulations, individually or in multiplexed arrays.

1. Introduction

Cyanoguanidine (CYA) belongs to a group of nitrogen-rich chemical compounds based on cyanamide. Owing to it high nitrogen content and low price, CYA is used as a nitrification inhibitor to prevent nitrogen loss, but it can enter the food chain via cattle. In New Zealand in 2014, CYA-contaminated milk powder was an issue that caused much concern globally. In mouse studies, the acute toxicological properties of CYA were similar to those of melamine, where the CYA LD_{50} mortality dosage was 5000 mg/kg. Many analytical methods have been used to detect CYA residues, such as liquid chromatography–tandem mass spectrometry (LC–MS) (Abernethy & Kerianne, 2013; Inoue, Sakamoto, Min, Todoroki, & Toyo'oka, 2014), ultra-fast LC–MS (Zhang, Gong, Zhao, & Zhou, 2014), macro-scale Raman chemical imaging (Qin, Chao, & Kim, 2013), microwave-assisted extraction coupled with LC–MS (Shen et al., 2013), and micro-solid-phase extraction coupled with ultra-fast LC–MS (Chen, Zhou, Zhao, Pan, & Jin, 2014). These used large-scale instrumentation to improve accuracy, but costly, time-consuming procedures and complex pretreatment steps restricted practical and widespread use. Therefore, developing an effective and reliable method for the detection of CYA residues is important.

Graphene is a promising sorption material with a high loading capacity due to an ultrahigh specific surface area (theoretical value 2630 $m^2 g^{-1}$) (Stoller, Park, Zhu, An, & Ruoff, 2008). Graphene has many advantages, such as remarkable thermal and chemical stability, ultra-high mechanical strength, and low production cost (Chen, Muller, Gilmore, Wallace, & Li, 2008; Matthew, Vincent, & Richard, 2010). Graphene oxide contains polar moieties, such as hydroxy, epoxy, and carboxy groups (Compton & Nguyen, 2010), which create good solubility in water and offer a range of sites to interact with other molecules. Simialrly, graphene oxide is expected to have excellent performance for potential applications in electronics, energy research, catalysis, and biomedical research (Pyun, 2011; Robinson et al., 2008; Wang et al., 2010; Wilson et al., 2009; Zhu et al., 2010). There has been explosive growth in studies using graphene oxide as an enhancing material or a platform for probes and recognition elements in the development of high-performance sensing devices (Azimzadeh, Rahaiea, Nasirizadeh, Ashtari, & Naderi-Manesh, 2016; Zhang, Yin, Tan, & Ye, 2011; Zhang et al., 2016).

Molecularly imprinted technology has received considerable attention since Wulff et al. first reported it in 1973. Analytes can be recognized and separated successfully through preparation of

* Corresponding author.
E-mail address: wangjing@th.btbu.edu.cn (J. Wang).

http://dx.doi.org/10.1016/j.foodchem.2017.05.036
0308-8146/

molecularly imprinted polymers (MIPs) (Baggiani et al., 2012; Ge & Turner, 2009). The stability, ease of preparation, and low cost make MIPs particularly attractive. As reported previously, highly selective MIPs combined with organic fluorescent dyes can be used to rapidly analyze trace substances in samples of kinds.

Semiconductor nano-materials quantum dots (QDs) have many advantages compared with other organic fluorescent dyes, such as high quantum yield, controllable size and emission wavelength, and good chemical stability. Thus, they have been used in a wide range of fluorescence sensors (Bhargava, Gallaghar, Hong, & Nurmikko, 1994; Bruchez, Moronne, Gin, Weiss, & Alivisatos, 1998; Chan & Nie, 1998; Lan, Lin, Huang, & Chang, 2007). QDs-based MIP sensing materials are constructed by incorporating QDs into MIPs using a bioconjugation procedure. Li, Li, and Cheng (2010) reported a MIP fluorescence nanosensor that was developed by anchoring the MIP layer on the surface of silica nanospheres embedded with CdSe QDs via a surface molecular imprinting process. Zhang, He, Li, and Zhang (2012) developed a thermo-sensitive imprinted polymer coating of CdTe QDs to prepare fluorescent thermo-sensitive protein affinity materials, which exhibited a specific recognition capacity for target proteins. Wang, He, Ji, and Yan (2009) developed a room-temperature phosphorescence MIP opto-sensor by anchoring MIPs on the surface of Mn-doped ZnS QDs, which was used to detect trace pentachlorophenol in water samples without interference from auto-fluorescence and light scattering of matrixes (Wang et al., 2009). As an alternative method to first preparing QDs-MIP and attempting to bond QDs on the surface of MIP, we prepared double-bond-modified QDs combined with a functional MIP monomer, which could increase the QDs fluorescence intensity.

In this study, a simple and rapid opto-sensing method was developed to prepare graphene oxide-sensitized molecularly imprinted opto-polymers (MIOP) based on 3-methacryloxy propyl trimethoxy silane (MPTS)-modified CdSe/ZnS QDs through a one-pot polymerization. MPTS-modified QDs were combined with the functional monomer methacrylic acid (MAA) to increase the fluorescence quantum yield of MIOP. Graphene oxide was introduced to provide a platform for the polymerization and increase stability and the binding kinetic properties of MIOP. To illustrate the uses of this new protocol, cyanoguanidine (CYA) was chosen as the target molecule in infant formulae. The novel method was compared with traditional high-performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Materials and reagents

Carboxylated graphene oxide was purchased from Xfnano (Nanjing, China). MPTS used for the modification of QDs was purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). CdSe/ZnS QDs were purchased from Jiayuan (Wuhan, China). The diameter of CdSe/ZnS QDs was about 5 nm and the peak width of half height was less than 30 nm. CYA, cyanamide (CM), melamine (MM), and cyanuric acid (CA) were purchased from J&K Scientific Ltd. (Beijing, China). The functional monomer methacrylic acid (MAA, 99%) and the crosslinker ethylene glycol dimethacrylate (EGDMA, 98%) were purchased from J&K Scientific Ltd. 2,2-Azobisisobutyronitrile (AIBN, 99%) was purchased from Beijing Solarbio Science and Technology Co., Ltd. Methanol and acetonitrile were analytical reagent grade from Beijing Chemicals (Beijing, China). Double deionized water (DDW, 18.2 MΩ cm^{-1}) was obtained from a Water Pro water purification system (Labconco, Kansas city, MO, USA). Five different kinds of infant formula samples were obtained from a local supermarket.

2.2. Characterization

A scanning electron microscope (SEM, S-4800, Hitachi, Japan) was employed to observe the surface morphology and microstructure of synthetic material. Transmission electron images were obtained on a 2010 FEF microscope (JEOL, Tokyo, Japan). The BET surface areas of QDs and the opto-sensing material were measured on a Quantachrome Autosorb-1 (Boynton Beach, FL, USA). Fourier transform infrared (FT-IR) spectra (650–4000 cm^{-1}) were recorded using KBr pellets in a Nicolet iS10 FT-IR spectrophotometer (Thermo Scientific, Waltham, USA).

2.3. Preparation of graphene oxide-sensitized MIOP

Hydrothermal polymerization was used to synthesize graphene oxide-sensitized MIOP. The MPTS was used to modify CdSe/ZnS QDs as described by Stöber, Finker, and Bohn (1968). In a 25-mL round-bottomed flask, 1 mmol of template molecule (CYA) and 1 mg of carboxyl graphene oxide were mixed in 1 mL of acetonitrile and stirred for 30 min in the dark. 4.0 mmol MAA and 4.0 mmol MPTS-functionalized QDs were added and, after the mixture was stirred for 1 h in a water bath at 60 °C, 5.0 mmol of EGDMA and 45 mg of AIBN were added, and the mixture stirred overnight. The template molecule of CYA was removed by Soxhlet extraction with at least 200 mL of methanol until CYA was not detected by UV–vis, and the extract placed in a vacuum dryer at 40 °C for 10 h.

2.4. Preparation of graphene oxide-sensitized non-MIOP

Graphene oxide-sensitized non-molecularly imprinted opto-polymers (Non-MIOP) were used as a control to evaluate the molecular recognition properties of fluorescence-imprinted materials, and synthesized using the same procedure as the graphene oxide-sensitized MIOP. To obtain a reliable comparison with graphene oxide-sensitized MIOP, equal amounts of carboxyl GO, MPTS-functionalized QDs, functional monomer and cross-linker were used in the synthesis of the graphene oxide-sensitized non-MIOP.

2.5. Fluorescence measurements

Fluorescence measurements were performed on a multifunctional microplate (Biotek, Winooski, VT, USA). The excitation wavelength was 430 nm with a recording emission range of 500–700 nm. During dynamic adsorption, the graphene oxide-sensitized MIOPs were placed in a Labsystems 96-well plate and 0.5 mM of CYA standard solution was added to each well. Readings were recorded to obtain the optimum adsorption time.

During the static study, also in a Labsystems 96-well plate, 1 mg of the graphene oxide-sensitized MIOP was dispersed in 200 μL phosphate-buffered (PB) buffer (pH = 7.5) before various concentrations of CYA standard solution were added, and the absorption recorded. The detection protocol for the non-MIOP was the same as for MIOP. The ratio of the Stern–Volmer constant of K_{SV} values for the MIOP and the non-MIOP was defined as the imprinting factor to evaluate selectivity of the opto-sensing materials.

During the selectivity study, 1 mg of the graphene oxide-sensitized MIOP or non-MIOP was dispersed in 200 μL PB buffer, before various concentrations of CYA, CM, MM, CA, CYA/CM, CYA/MM, or CYA/CA standard solution were added. The ratio of K_{SV} values was compared to evaluate the performance of the opto-sensing material.

2.6. Analysis of food samples

CYA-contaminated milk powder has been a concern in the past. Therefore, five brands of infant formulae were used in the study,