Sensors and Actuators B 181 (2013) 104–113

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

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

Rapid detection assay for the molecular imprinting of gossypol using a two-layer PMAA/SiO_2 bulk structure with a piezoelectric imprinting sensor

Chen Zhao [a,b], Daocheng Wu [a,*]

[a] Key Laboratory of Biomedical Information Engineering of Education Ministry, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, PR China
[b] Xi'an Technological University, Xi'an 710032, PR China

ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form 7 January 2013
Accepted 8 January 2013
Available online 23 January 2013

Keywords:
Gossypol
Molecular imprinting polymer
Polymethacrylic acid
Silica
Piezoelectric imprinting sensor

ABSTRACT

The rapid and sensitive detection of gossypol is important in the food production and medical fields. In the current study, poly(methacrylic acid) (PMAA) was grafted onto the surface of bulk SiO_2 by solution polymerization, producing a molecular imprinting polymer (MIP) with a two-layer PMAA/SiO_2 bulk structure. A rapid detection assay for gossypol with a surface MIP having a two-layer PMAA/SiO_2 bulk structure combined with a piezoelectric imprinting sensor was developed. The morphology of the PMAA/SiO_2 bulks was characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscope (AFM). MIP functional groups were confirmed by Fourier transform infrared spectrometry (FTIR). Traditional gossypol detection methods, such as ultraviolet (UV) spectrophotometry and chromatographic analyses, were performed as controls. The material characteristics relevant to gossypol detection, such as binding rate, selectivity coefficient, and binding activity index (I), were measured by UV spectrophotometry, chromatographic analysis, and PMAA/SiO_2 bulks combined with a piezoelectric imprinting sensor. The TEM, SEM and AFM images provided clear observations of the two-layer PMAA/SiO_2 bulk structures with a 40-nm- to 50-nm-thick of PMAA shell; the temple gossypol molecule can be found on the MIP surface. Compared with traditional detection methods, such as UV spectrophotometry and high-performance liquid chromatography (HPLC)-UV, our assay has the advantages of inducing rapid, high-affinity gossypol binding with a binding activity index of 3.85, a selectivity coefficient of 2.791, and a lower detection limit of 6.0 $\mu g\,L^{-1}$, which is 500 times better than that of HPLC-UV. The detection limits (S/N = 3) of the method is ranged of 6–16 $\mu g\,L^{-1}$ and exhibit good linear relationship with the frequency (R^2 = 0.9996). Therefore, the proposed assay shows promise for the detection of gossypol and other biomolecule.

1. Introduction

Gossypol is a polyphenolic compound found naturally in members of the Malvaceae family and is mainly extracted from cotton plants, with a content of 0.3–20.0 $g\,kg^{-1}$ in cotton seeds [1]. Gossypol is soluble in some organic solvents, such as acetone, chloroform, ethanol, and ether, but not in water or petroleum ether with a low boiling point. Refined cottonseed oil has a desirable taste and contains several healthful nutrients, such as linoleic acid and vitamin E. Cotton cake, a by-product of cottonseed oil, is a high-quality yet inexpensive protein source for animal husbandry and aquaculture [2]. However, the presence of gossypol in both cottonseed oil and cotton cake restricts the use of cottonseed products in the diet because of its toxicity. People who consume food with excessive gossypol over a long time accumulate toxins, which have harmful effects on the body and can even lead to death [3–6]. Gossypol is excreted slowly from the body and can chronically accumulate. Therefore, long-term consumption of gossypol may cause its stimulation in tissues and the degeneration and necrosis of the alimentary canal and other organs.

Modern medicine has shown that gossypol has anti-fertility functions [7], anti-tumor effects [8,9], antiviral effects [10], and antibacterial action [11–13] in addition to its toxic effects. Currently, (−)-gossypol has shown promising results in a Phase III clinical trial for cancer therapy in the US. With the developments of biological and clinical investigations on gossypol and its derivatives, the demand for rapid and sensitive detection of gossypol will increase considerably. Therefore, the measurement of gossypol in food production or medicine is an important analytical challenge.

Gossypol can currently be detected in several ways, including ultraviolet spectrophotometry (UV), colorimetry, high-performance liquid chromatography HPLC-UV, and atomic absorption spectroscopy. UV is a traditional analytical method; however, its results are often unsatisfactory because of the

* Corresponding author. Tel.: +86 29 82663941.
E-mail address: wudaocheng@mail.xjtu.edu.cn (D. Wu).

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.01.025

interference of other components. In addition, UV method has lower specificity and can not effectively detect impurities in the sample without purification treatments. The poor sensitivity of UV method could not trace the samples accurately and effectively, due to separating impurities from the gossypol solution is difficult. In contrast, HPLC-UV has higher sensitivity and accuracy but requires a complex and time-consuming sample pretreatment. HPLC can be divided into HPLC-UV, HPLC-electrochemical detection (ED) and HPLC-mass spectroscopy detection (MS) with different detectors. Among them, HPLC-UV is a common method for the detection of gossypol. Cai et al. [14] reported the detection of gossypol in plants using HPLC-UV. Gossypol in plants must first be extracted by acetone and then redissolved in 1% HOAc in $CHCl_3$. Dowd et al. [15] revealed that diastereomeric gossypol was obtained from the extraction of root bark by R-9(1)-2-amino-1-propanol. The two examples above illustrate the expense and difficulty involved in sample pretreatment. Abou-Donia et al. [16] observed that gossypol is better analyzed with HPLC-UV using a methyl alcohol–water–phosphoric acid (8:1:1) mobile phase. However, gossypol samples contain some impurities that cannot be separated by HPLC-UV or any of the other methods described above. HPLC-UV not only requires pretreatment and involves high experimental cost but is also an expensive and time-consuming process, which, leads to workload and experimental difficulties caused by the derivative processes. Therefore, the traditional methods above cannot be adopted for the rapid, sensitive, and accurate determination of gossypol.

Recently, molecular imprinting (MI) has emerged as an excellent rapid detection method in the field of analysis. The principle behind the interaction between a molecular imprinting polymer (MIP) and a template molecule is similar to that of antigen–antibody reactions [17]. MI has the characteristics of rapid detection, higher specificity and can be used in organic solvents, and is stable at low/high pH, pressure, or temperature. The most important component of this method is the template polymer, which can induce the formation of specific recognition sites in a polymer, with the positioning and orientation of the polymer structural components being directed by the template. Therefore, this method can more effectively overcome the issue of a biological molecule being easily inactivated and difficult to store under normal conditions. Traditional MIP is highly cross-linked with the polymer network, resulting in difficulties in the combination and elution of the template molecules [18]. Surface MI, as an improved approach, has overcome these problems. This approach places almost all binding sites on the MIP surface, thereby facilitating the removal of the template molecule and its recombination. Silica gel, one of the most important MIP materials, is used as an MI carrier for the following reasons: (1) silica gel is inert in bulk and not highly reactive to the monomer and template molecules; (2) activated silica gel with generated hydroxyl is conducive to evenly polymerizing the monomer on its surface; (3) silica particles have a large surface area and can create several cavities on the silica gel surface, allowing better combination with the template molecules; (4) silica gel is cheap and readily available, thus reducing preparation cost. Sellergren et al. [19] and Baojiao et al. [20] studied a surface imprinting technique with the two-layer structure of surface MIP bulks and poly(methacrylic acid)/silica (PMAA/SiO_2) bulks for the detection of pirimicarb and L-phenylalanine anilide, respectively. These materials were found to possess better selectivity and an excellent eluting property for the following reasons: (1) the intermolecular forces of hydrogen bonding and the electrostatic interaction between the grafted macromolecules and template molecules, (2) the aforementioned difficulties in detecting gossypol, and (3) the characteristics of the two-layer structure of surface MIP bulks. In this study, we propose a novel strategy for the rapid and accurate detection of gossypol with a two-layer structure of PMAA/SiO_2 bulks or polyacrylamide/silica

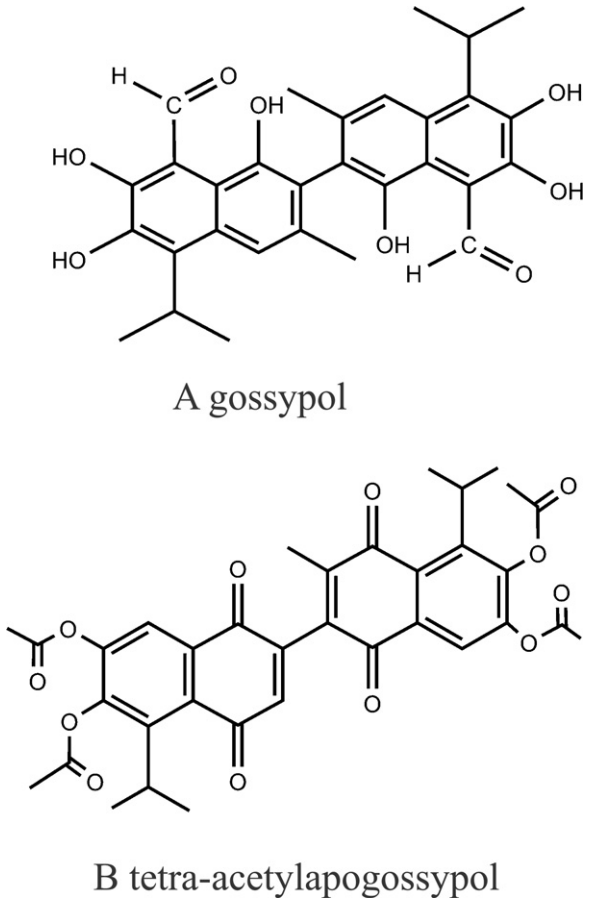

Fig. 1. Structures of gossypol (A) and tetra-acetylapogossypol (B).

(PAM/SiO_2) bulks, caused by the possible interaction of six hydroxyl groups and two aldehyde groups in the gossypol structure with MIPs containing hydrogen bonds (Fig. 1). An assay for the detection of gossypol with surface MIPs combined with a piezoelectric imprinting sensor was developed (Scheme 1). Silica gel particles were selected as the MIP core because they easily link the monomer with a multi-active hydroxyl. Next, PMAA and PAM were grafted onto the silica gel particle surface through their double bond. Their carboxyl group can bind the phenolic hydroxyl of gossypol to the hydrogen bonds. The interaction between MIP and gossypol is a noncovalent bond; thus, gossypol can be easily eluted by polarity solvents. Two-dimensional cavities are obtained on the surface of MIP, indicating that gossypol can easily combine with MIP and can be rapidly eluted. Finally, gossypol can be rapidly and accurately determined by piezoelectric quartz crystal materials in a piezoelectric imprinting sensor or MIP-quartz crystal microbalance (MIP-QCM) sensor [21]. The results of this paper are positive and consistent with our design, indicating a promising rapid and accurate method for the detection of gossypol and other biomolecule.

2. Materials and methods

2.1. Materials

Chloroform (Chinese Medical Chemicals, Shanghai, China) was distilled to remove its inhibitor. Silica gel bulks (300–400 mesh; Qingdao Ocean Chemical, Shandong, China), methanesulfonic acid (Sinopharm Chemical Reagent, Shanghai, China), methacrylic acid (MAA; Kermel Chemical Reagents Development Centre, Tianjin, China), methacrylic acid and acrylamide (AM; Kermel, Tianjin, China), triethoxyvinylsilane (TTS, coupling agent; Sigma–Aldrich,