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A novel molecular imprinted nanosensor based quartz crystal microbalance for determination of kaempferol

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ABSTRACT

The molecular imprinting technique relies on the molecular recognition. It is a kind of polymerization which is formed around the target molecule. Hence this technique forms specific cavities in the cross-linked polymeric matrices. In this report, we developed quartz crystal microbalance (QCM) nanosensor for the real-time detection of kaempferol (KAE). Firstly, the modification of gold surface of QCM chip was carried out by self-assembling monolayer formation of allyl mercaptane to introduce polymerizable double bonds on the chip surface. Then, KAE imprinted poly(2-hydroxyethyl methacrylate–methacryloylamidoaspartic acid) [p(HEMA–MAAsp)] film was generated on the gold surface. The non-modified and KAE-imprinted p(HEMA–MAAsp) surfaces were characterized by using atomic force microscopy (AFM), Fourier transform infrared (FTIR) spectroscopy and ellipsometry. The linearity range and the detection limit were obtained as 2.0×10^{-10} to 1.5×10^{-9} M and 6.0×10^{-11} M, respectively. The developed method was applied to real samples such as orange and apple juices for the determination of KAE in the presence of quercetin (QR), myricetin (MYR) and apigenin (API). In addition, isotherm models were applied to data to explain adsorption process.

1. Introduction

Flavonoids are polyphenolic compounds commonly found in vegetables and fruits and constitute a significant part of the human diet [1]. The antioxidant and anti-inflammatory capacities of these compounds are well reported [2] and many show cancer fighting potential. Flavonoids were reported to inhibit cancer cell proliferation and angiogenesis [3].

KAE (Scheme 1) is found in tea, broccoli, apples, strawberries and beans [4]. It has been demonstrated to invoke several different mechanisms in the regulation of cancer cells. Not only is KAE a potent promoter of apoptosis [5], but it also modifies a host of cellular signaling pathways. In addition, KAE is much less toxic to normal cells in comparison to standard chemotherapy drugs [6]. Various analytical methods have been developed for determination of KAE such as high-performance liquid chromatography (HPLC) [7], liquid chromatography–tandem mass spectrometry (LC–MS/MS) and differential pulse voltammetry (DPV) [8]. But these methods have some disadvantages such as large material consumption, personal skill, and expensive equipment. In addition, several literatures were reported for selective determination of KAE via molecular imprinting technique. For example, KAE imprinted polymer (MIP) microspheres were prepared. The linearity range was obtained as 4.5–200 mg L^{-1} and the limit of detection was obtained as 8.0 μg g^{-1} [9]. The new molecularly imprinted polymer (KAE–MPS/SiO_2) with high performance for recognizing KAE was prepared by adopting the surface molecular imprinting technique with silica nanoparticles modified with 3-methacryloxypropyltrimethoxysilane (MPS) as a carrier material [10]. The selectivity coefficients (k) of KAE–MPS/SiO_2 for KAE in relation to competition species MYR and chlorogenic acid were 2.51 and 4.24, respectively. In this report, the detection limit was obtained as 6.0×10^{-11} M with high selectivity (k = 18.80 for QR, k = 12.70 for API and k = 20.14 for MYR).

Recently, the various nanosensors have been developed for sensitive determination of biomolecules or drugs [11–24]. QCM is sensor method which calculates the changes of mass on quartz crystal surface via measuring its difference of frequency in real time [25]. The QCM technique is selective, simple and sensitive method [26], in which has been utilized for the determination of clinical targets [27], environmental pollutants [26,28], oxidative stress [29,30], some proteins [31] and investigation of bimolecular interactions [32].

Molecular imprinting is a popular approach to create artificial counter parts having affinity constants as high as natural ones of

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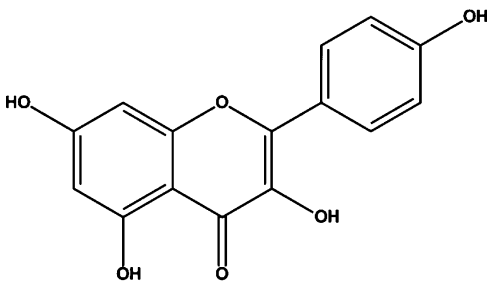

Kaempferol

Scheme 1. The chemical structure of KAE.

the interested molecules. In this approach, interested molecules also called as template are complexed with functional monomers; then, high degree of crosslinking converts these complexes into solid matrix that have ability to recognize the template molecule. Although various methods are used to generate the sensitive QCM sensor, the most effective method is molecular imprinting technique [33]. Molecular imprinted polymers (MIPs) have various applications such as artificial enzymes [33], solid-phase extraction [34], bioseparation [35–38], affinity detoxification [39,40] and sensor devices [41–43].

In this study, we prepared KAE-imprinted p(HEMA–MAAsp) film on gold surface of QCM chip. In literature, there is no report about determination of KAE which is performed by KAE-imprinted p(HEMA–MAAsp) film on gold surface of QCM chip. In addition, the developed QCM method shows high sensitivity and high selectivity for determination of KAE in real samples. The developed non-modified and modified surfaces were characterized by using AFM, FTIR and ellipsometry. After that, the developed nanosensor was applied to some real samples for the determination of KAE.

2. Experimental

2.1. Materials

KAE, QR, API and MYR were purchased from Fargem Company (Düzce, Turkey) and used as received. The stock solution of KAE (1.0 mM) was prepared by dissolving it in ultra pure quality water and methanol (1:1) then diluting it with ultra pure quality water to 50 mL. The working solutions were prepared by diluting the stock solution with 0.10 M phosphate buffer (pH 6.5). Allyl mercaptane (CH_2CHCH_2SH) (Sigma–Aldrich, USA), HEMA (Sigma–Aldrich, USA), ethylene glycol dimethacrylate (EGDMA) (Sigma–Aldrich, USA), N,N′-azobisisobutyronitrile (AIBN) (Sigma–Aldrich, USA) and sodium chloride (NaCl) (Sigma–Aldrich, USA) were used as received. MAASsp was obtained Nanoreg Ltd. Şti., Ankara, Turkey.

2.2. Surface modification of the QCM chips

2.2.1. Allyl mercaptane modification of QCM chip

To modify gold surface of the QCM chip with CH_2CHCH_2SH, the chip was washed with alkaline piranha solution (3:1 NH_4OH:H_2O_2, v/v). After the QCM chip was dipped in 10 mL of cleaning solution for 5 min, it was washed with ethyl alcohol and dried in vacuum oven (200 mm Hg, 35 °C) for 2 h. To let vinyl groups into the gold surface, the chip was dipped in an ethanol/water (4:1, v/v) solution containing 3.0 M CH_2CHCH_2SH allowed to form self-assembled monolayer for 24 h. The gold and sulfur formed covalent bond on the sensor surface. Then, it was cleaned with ethyl alcohol and dried with nitrogen gas.

2.2.2. Polymer preparation on QCM chip surface

KAE-imprinted p(HEMA–MAAsp) film on CH_2CHCH_2SH modified QCM chip was prepared according to this protocol. Firstly, 1.0×10^{-6} mol of KAE and 2.0×10^{-6} mol of MAAsp monomer were mixed with 500 μL of phosphate buffer (pH 6.5) at room temperature for 1 h. MAAsp–KAE molar ratio was 2:1. After that, 5.0 mg of AIBN as initiator was dissolved in 1250 μL of HEMA and 500 μL of EGDMA and 200 μL of MAAsp–KAE complex was added into this solution to prepare stock monomer solution. The solutions were passed with nitrogen gas for 15 min to sweep away the dissolved oxygen before the experiments and make a blanket to block the oxygen. Then, 20 μL of aliquot was taken from the stock monomer solution and dropped onto the QCM chip surface by using *spin coating method*. The method is used to deposit uniform thin films to QCM surface. After 10 s, the QCM chip was removed from spin coater and polymerization was started by UV light (100 W, 365 nm). After 60 min, polymer coated QCM chip was washed with ethanol three times, then and dried in vacuum oven. After that the developed QCM chips were stored in closed box without fluctuations of temperature and pressure.

2.3. KAE removal from QCM chip surface

There are the electrostatic interactions and hydrogen bonding between the carboxylic acid groups of MAAsp monomer and polar groups of KAE molecules. In order to break the interactions, we used 1.0 M NaCl solution in water as a desorption agent. Firstly, the removal study of KAE was performed via batch system. KAE-imprinted p(HEMA–MAAsp) surface was dipped into 25 mL of desorption agent. The QCM chip was swinged in bath (200 rpm) at room temperature. After KAE removal, the QCM chip was washed with ultra pure quality water and dried with nitrogen gas under vacuum (200 mmHg, 25 °C).

2.4. Characterization methods

In order to characterize the surfaces, tapping mode AFM was used (Nano Magnetics Instruments, Oxford, UK). QCM chip was installed on sample holder. 2 μm × 2 μm sample area was showed with a 128 × 128 pixels resolution. The scan rate was 2 μm s^{-1}. The studies were performed in air atmosphere.

Ellipsometer measurements were also performed by using an auto-nulling imaging ellipsometer (Nanofilm EP3, Germany) to characterize the surface of QCM chips. The measurements have been carried out at a wavelength of 532 nm with an angle of incidence of 72°. In the layer thickness analysis, a four-zone auto-nulling procedure integrating over a sample area of ~50 × 50 μm^2 followed by a fitting algorithm has been performed. The measurements were performed at six different points of QCM chip and the results were obtained as mean value.

For FTIR measurements, KAE-imprinted p(HEMA–MAAsp) nanosensor was put into sample holder of FTIR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA). The spectra were obtained in the wave number range of 650–4000 cm^{-1} with 2 cm^{-1} resolution.

2.5. Sample preparation

The orange and apple juices were bought from a supermarket in Ankara/Turkey. The samples were filtered with a 0.50 μm filter. The values of pH for both samples were adjusted using 0.10 M phosphate buffer. Additionally the samples were (1:1) diluted with 0.10 M phosphate buffer (pH 6.5). Then, the results were evaluated. The measurements were repeated for six times. The concentrations of KAE in the samples were evaluated using calibration curves.