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Molecularly imprinted polymer based quartz crystal microbalance sensor system for sensitive and label-free detection of synthetic cannabinoids in urine

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ABSTRACT

Herein, we prepared a novel quartz crystal microbalance (QCM) sensor for synthetic cannabinoids (JWH-073, JWH-073 butanoic acid, JWH-018 and JWH-018 pentanoic acid,) detection. Firstly, the synthetic cannabinoid (SCs) imprinted (MIP) and non-imprinted (NIP) nanoparticles were synthesized by mini-emulsion polymerization system. The SCs-imprinted nanoparticles were first characterized by SEM, TEM, zeta-size and FTIR-ATR analysis and then were dropped onto the gold QCM surface. The SCs-imprinted QCM sensor was characterized by an ellipsometer, contact angle, and AFM. The limit of detection was found as 0.3, 0.45, 0.4, 0.2 pg/mL JWH-018, JWH-073, JWH-018 pentanoic acid and JWH-073 butanoic acid, respectively. The selectivity of the SCs-imprinted QCM sensor was shown by using JWH-018, JWH-018 pentanoic acid, JWH-073 and JWH-073 butanoic acid. According to the results, the SCs-imprinted QCM sensors show highly selective and sensitive in a broad range of synthetic cannabinoid concentrations (0.0005–1.0 ng/mL) in both aqueous and synthetic urine solutions.

1. Introduction

The recent European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) annual report summarized that the most commonly used drug is cannabis (87.7 million). Additionally, among all the new psychoactive substance reported to UNODC by the end of 2016, synthetic cannabinoids constitute the biggest class in terms of the number of different substances informed (UNODC Report, 2016; EMCDDA Report, 2016).

Synthetic cannabinoids (SCs) are chemical compounds that mimic the effect of Δ–9-tetrahydrocannabinol (Δ9-THC), the main active component of cannabis. They bind to cannabinoid receptors (CB1 and CB2) in the brain, such as Δ9-THC, and were originally developed as therapeutic agents in the treatment of pain. SCs don't include cannabis, but when smoked produce marijuana-like effects. It is known that some SCs are 4–100 times forceful in effect to marijuana. Naphtalen-1-yl-(1-butylindol-3-yl)-methanone (JWH-073) and naphtalen-1-yl-(1-pentylindol-3-yl)-methanone (JWH-018) are the best known amnioalkylindole of synthetic cannabinoids. There are different structures of SCs. These numbers are increasing as new legal drugs and also sold under brand names such as K2 and SPICE (Tai and Fantegrossi, 2017). The analysis of SCs in human matrices is of especial significance in the clinical toxicology and forensic areas. Negative effects such as rapid heart rate, dizziness, confusion, agitation, and nausea of SCs have been reported.

The variety of the structures shows a challenge in SCs detection in biological samples. Conventional methods for SCs detection are mainly chromatography and immunoassay methods which include the enzyme-linked assay, colorimetric technique, chemiluminescence, and fluorescence assays as well as electrochemical sensing systems (Barnes et al., 2014; Concheiro et al., 2015; Znaleziona et al., 2015; Namera et al., 2015; Balbino et al., 2016; Smith et al., 2016; Shaw and Dennany, 2017; Smolianitski-Fabian et al., 2017) However, most of these devices need complex and heavy tools with a time-consuming process, which is not suitable for most portable or examine on-site. In particular, SCs detection requires fast, selective, sensitive response devices for rapid screening (Yang et al., 2015; Znaleziona et al., 2015; Cannaert et al., 2016, 2017; Dronova et al., 2016).

QCM sensors, which are mass-based chemical sensors, have significant features such as highly selective, cost-effective, ease of use,

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simplicity, portability, and stability. Many methods can be applied to produce QCM sensor surface, but the most promising approach is the molecularly imprinted technique. Molecularly imprinted polymers (MIPs) are materials adapted from materials that can mimic recognition of biological receptors (Cieplak and Kutner, 2016). The technique principally relies on the molecular identification, is a kind of polymerization that takes place around the targets called as a template and produces specific cavities in the extremely polymeric matrix. The three-dimensional specific cavities are generated after the template removal. The common applications of molecularly imprinted polymers in QCM sensor have been reported for different molecules detection (Stanley et al., 2003; Tai et al., 2005; Wu and Syu, 2006; Saylan et al., 2017) but there is no any combination of MIP and QCM study to detect SCs in biological matrices.

The objective of this study design stable, field able, sensitive, and selective molecularly imprinted QCM sensors for SCs; JWH-073, JWH-018 and their major metabolites (JWH-073 butanoic acid, JWH-018 pentanoic acid) for real-time detection in both aqueous solutions and synthetic urine sample.

2. Experimental

2.1. Chemicals and materials

Naphtalen-1-yl-(1-butylindol-3-yl)-methanone (JWH-073), JWH-073 N-butanoic acid, naphtalen-1-yl-(1-pentylindol-3-yl)-methanone (JWH-018), JWH-018 N-pentanoic acid were obtain Lipomed services to health (Arlesheim, Switzerland). 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EDMA), sodium dodecyl sulfate (SDS), poly vinyl alcohol (PVA), ammonium persulfate (APS), sodium bisulfite and sodium bicarbonate was obtained from Sigma Chemical Co. (St. Louis, USA). Synthetic urine was obtained from Sigma-Aldrich Co. (TX, USA). The other chemicals were analytical grade purity and obtained from Merck A.G. (Darmstadt, Germany). 0.1 M phosphate buffered saline (0.1 M PBS, 0.01 M Na_2HPO_4, 0.002 M KH_2PO_4, 0.137 M NaCl, 0.0027 M KCl, pH 7.4) was used as equilibrium buffer for the QCM system.

2.2. Synthesis of N-methacryloyl-L-phenylalanine (MAPA) monomer

The 2-methacryloyl-(L)-phenylalanine (MAPA) monomer was used to interact with synthetic cannabinoids (Akgönüllü et al., 2016). Briefly, an aqueous potassium carbonate solution (30 mL; w/v, 5%) was dissolved L-phenylalanine methyl ester and sodium nitrite and the prepared mixture was cooled down to 0 °C. In the nitrogen atmosphere, methacryloyl chloride was easily poured into this solution and the mixture was stirred magnetic stirrer at for 2 h. pH was fixed to 7.0, and the final solution was extracted. In a rotary evaporator was evaporated the aqueous phase. The MAPA residue was crystallized from cyclohexane and ether (Fig. S1).

2.3. Preparation of synthetic cannabinoids imprinted nanoparticles

SCs-imprinted nanoparticles were synthesized by using two phases mini-emulsion polymerization system. The first aqueous phase was prepared by dissolving PVA (93 mg), SDS (14 mg) and sodium bicarbonate (12.5 mg) in 5 mL of deionized water. The second phase was prepared by dissolving PVA (50 mg) and SDS (50 mg) in 100 mL of deionized water. The organic phase was prepared by using EDMA (1.0 mL) and HEMA (0.5 mL). The organic phase was slowly added to the first aqueous phase. In order to obtain mini-emulsion, the mixture was homogenized at 6000 rpm by a homogenizer (T10, Ika Labortechnik, Germany). After homogenization, the SCs template molecules (0.01 mmol) and MAPA (0.01 mmol) as functional monomer was added to mini-emulsion solution and the mixture was stirred to obtain effectively interacted monomer-template pre-polymerization complex for 2 h. Then, the mixture was slowly added to the second aqueous phase while the final phase has been stirring in a sealed-cylindrical reactor (250 mL). System was stirred at 500 rpm (Heidolph, Germany). After passing nitrogen gas through the solution, $NaHSO_3$ (100 mg) and $(NH_4)_2S_2O_8$ (50 mg) was added as initiators. Temperature was set at 40 °C and the polymerization was continued for 24 h. SCs-imprinted NPs were cleaned with H_2O and H_2O/EtOH mixture to remove surfactant, initiator and unreacted monomers. The mixture was then centrifuged at 40,000 rpm for 45 min (Allegra-64R Beckman Coulter, USA).

The non-imprinted nanoparticles (NIP) were prepared in the same way without using SCs template molecules.

2.4. Characterization of synthetic cannabinoids imprinted nanoparticles

2.4.1. Zeta-size measurement

Firstly, 3 mL deionized water was used to dilute SCs-imprinted nanoparticles sample was placed into the holder of the zeta-size device (NanoS, Malvern Inst., London, UK). The incidence angle (90° and 25°) was used for light scattering. The nanoparticle number per second was calculated with light scattering signal. The concentration of nanoparticles in the sample was enough for measurement.

2.4.2. Transmission electron microscope (TEM)

The SCs-imprinted nanoparticles were suspended in tubes (2 mL) with deionized water and dissolved in water for 2 min with vortex for homogenous distribution. Each SCs-imprinted nanoparticles sample was dropped onto 300 mesh carbon-coated copper grid and then dried. In the TEM microscope was taken TEM images (JEOL, JEM-101, USA).

2.4.3. Scanning electron microscope (SEM)

The surface morphologies of SCs-imprinted nanoparticles were examined by using SEM (Zeiss, Supra55, Germany) at the accelerating voltage of 15.0 kV. Firstly, the chip is fixed on the stub. The stubs were placed on the sample holder. Then, the platinum coating was applied to the sample for providing conductivity (Quorum, Q150R, UK). SEM images of the chips on the sample holder were taken under the appropriate magnification and voltage.

2.4.4. FTIR-ATR spectrophotometry

The FTIR-ATR characterization of SCs-imprinted nanoparticles was performed by FTIR-ATR spectrophotometry (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA). Samples were placed in a sample holder and measured in the range of 400–4000 cm^{-1}.

2.5. Preparation of synthetic cannabinoids imprinted QCM sensor

Firstly, the gold QCM surface was cleaned with acidic piranha solution (3:1 H_2SO_4:H_2O_2, v/v) and then SCs-imprinted nanoparticles were attached onto the gold surface. Acidic piranha solution was dropped onto the gold QCM surface for 30 s. Afterwards; QCM sensor was washed with pure EtOH and dried at 40 °C for 3 h. The MAXTEK 5 MHZ Cr/Au polished quartz crystal chip was provided from USA by INFICON. The AT-cut quartz is chosen for its superior mechanical and piezoelectric properties. The SCs-imprinted nanoparticles (5 µL) were dropped onto the gold QCM surface (Scheme 1). Next, QCM sensor was dried using UV light (100 W, 365 nm) (37 °C, 30 min). Lastly, the SCs-imprinted QCM sensor was cleaned four times with H_2O and EtOH and dried.

2.6. Template removal

The hydrophobic interactions were occurred between MAPA monomer and template molecules because of interactions originated from secondary forces. A template molecule is removed from the breakdown of these interactions. For this purpose, mixtures of ethylene