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Surface molecularly imprinted silica for selective solid-phase extraction of biochanin A, daidzein and genistein from urine samples☆

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ABSTRACT

Selective molecularly imprinted silica polymer (SiO_2MIP) for extraction of biochanin A, daidzein and genistein was synthesized using the surface molecular imprinting technique with the silica gel as a support. Biochanin A (BCA) was used as a template, 3-aminopropyltriethoxysilane (APTES) as a functional monomer, and tetraethoxysilicane (TEOS) as a cross-linker. Non-imprinted polymer with the sol–gel process (SiO_2NIP) was also prepared for comparison. The synthesized polymers were characterized by Fourier transform infrared spectrometry (FTIR), scanning electron microscopy (SEM) and a standard Brunauer-Emett-Teller (BET) and Barret–Joyner–Halenda (BJH) analysis. The obtained results indicated the structural differences between imprinted and non-imprinted polymers. Finally, the SiO_2MIP and SiO_2NIP were adopted as the adsorbents of solid phase extraction for isolation and preconcentration of biochanin A and its structural analogues–daidzein and genistein from aqueous and urine samples. The performance analysis revealed that SiO_2MIP displayed better affinity to the three investigated isoflavones compared with SiO_2NIP. The recoveries of spiked samples for studied analytes ranged from 65.7% to 102.6% for molecularly imprinted silica polymer and 8.9–16.0% for non-imprinted sorbents.

1. Introduction

Isoflavonoid phytoestrogens (IFS) are the group of naturally occurring non-steroidal, secondary plant metabolites. They can be widely found in more than 300 kinds of plants, mostly in fibre-rich flaxseeds, soybeans, lentils and berries or alfalfa sprouts. In plants, isoflavones are present in biologically inactive form as glucoside conjugates or their malonyl and acetyl derivatives. In the intestine (by the action of intestinal bacteria) they are hydrolyzed to active forms named aglycones. Taking into account their structural similarity to the natural endogenous hormone 17β-estradiol, isoflavones are called estrogen-like molecules or non-steroidal estrogens. Moreover, they demonstrate the ability of binding to estrogens' receptors and consequently the weak estrogenic activity [1,2]. Among isoflavonoid phytoestrogens, biochanin, daidzein and genistein are considered to be important constituents of animal and human food, as they have a serious influence on human health [3]. These compounds, with antioxidant activity, have positive effects in prevention of osteoporosis, cardiovascular diseases and attenuation menopause symptoms [4–7]. Unfortunately, isoflavones, which interact with estrogen receptors, can evoke also some undesirable effects like disruptions in endogenous hormone levels, development of female reproductive track and problem in brain sexual differentiation [1,8,9]. Because of their wide use and biological activity, monitoring of isoflavonoid phytoestrogens has been giving rise to international concern. Many food products and dietary supplements are based on isoflavones what results in the fact that they are commonly present in human biological fluids, such as blood or urine. Generally, samples containing isoflavones are of complicated matrices, and IFS exist in very low concentration, so their determination often requires effective sample preparation prior to instrumental analysis. Heretofore, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most used technique for the extraction of isoflavonoid phytoestrogens from body fluids samples [10–21]. However, the biggest disadvantage of LLE method is the usage of an enormous amount of solvents, so nowadays it is rather replaced with other, more environmentally-friendly methods like SPE. Whereas, traditional solid-phase extraction with the application of commercially available sorbents lack selectivity and specificity, which makes the subsequent analysis of isoflavones difficult. Depending on the procedural complexity, sometimes it is coupled with other sample pre-treatment techniques [22–24]. The problem of poor selectivity of SPE sorbents can be addressed by using packing materials based on molecularly imprinted polymers

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(MIPs). These materials are synthetic polymers that possess specific recognition sites complementary in shape, size and functional groups to the compounds that were used as template molecules during polymerization process [25,26]. For example, the molecularly imprinting materials have been investigated as stationary phase for analysis of stilbene-type estrogenic compounds using high performance liquid chromatography [27]. Recently, molecularly imprinted polymers (MIPs) for IFS have been used as SPE sorbents for phytoestrogens' extraction, but they were mostly prepared by bulky or precipitation polymerization [28–30]. The main disadvantages of these kinds of MIPs are poor accessibility to the target molecules (because of the large material thickness), slow mass transfer and problems with template removal. Furthermore, the grinding and sieving processes of MIPs prepared by bulk polymerization are rather time consuming and the imprinted sites can be broken. In order to overcome these problems, the surface molecular imprinting technique has been developed using silica gel supporter [31–35]. Silica gel particles, because of their high stability, chemical inertness and non-swelling properties, have been considered to be a proper material for surface molecular imprinting. In this kind of polymer, the specific recognition sites are situated at the surface of polymer what provides high specificity and selectivity for a target molecules, a large number of easily accessible binding sites and well-defined shape of the materials. Therefore, the disadvantages of traditional MIPs have been avoided to some extent.

In this paper, a novel molecularly imprinted silica polymer was synthesized by the surface molecularly imprinted technique, using biochanin A as template (SiO_2MIP). The APTES and TEOS were selected as a functional monomer and a cross-linking agent, respectively. Non-imprinted polymer with the sol–gel process (SiO_2NIP) was also prepared for selectivity comparison. Then, the silica gel sorbents were characterized by SEM, FTIR, BET and BJH method. Finally, the efficiency of synthesized BCA-imprinted silica nanoparticles was evaluated during solid-phase extraction experiments. An off-line SiO_2MIP-SPE method was established for the determination three investigated isoflavones (biochanin A, daidzein and genistein) from human urine samples using high-performance liquid chromatography system with photo-diode array detection. To our knowledge, this is the first work describing the preparation of SiO_2MIP for biochanin A and a real biological application of the SiO_2MIP as a sorbent for solid-phase extraction.

2. Experimental

2.1. Materials

Silica gel (63–200 mesh, pore volume ~ 0.8 cm^3/g; pore size 60 Å; surface area 550 m^2/g; J.T. Baker, Netherlands) was used as a support to prepare the BCA-imprinted sol–gel polymer. Biochanin A, daidzein and genistein were from Sigma-Aldrich (Poznań, Poland). The chemical structures of investigated analytes are shown in Fig. 1. 3-Aminopropyltriethoxysilane (APTES) and tetraethoxysilicane (TEOS) (both from Sigma-Aldrich) were used as the functional monomer and cross-linker in synthesis of imprinted and non-imprinted polymers. Analytical grade acetonitrile, methanol (MeOH), ortho-phosphoric acid and THF were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland). HPLC grade acetonitrile (MeCN) was supplied by Merck (Darmstadt, Germany). The enzymes β-glucuronidase from *Helix Pomatia* (type H1, ≥3000 units/g solid) and sulfatase from *Helix Pomatia* (type H1, ≥10.000 units/g solid), used to hydrolyze the urine samples were purchased from Sigma-Aldrich. Double deionized water was purified in our laboratory using a Milli-Q system (Millipore, Bedford, MA, USA). Individual stock solutions of BCA, Da and Gen (1 mg ml^{-1}) were prepared in MeOH and stored at 4 °C in the dark and was stable for at least a month. Working mixed standard solutions were prepared daily by the appropriate dilution of the stock solutions.

2.2. Instruments

HPLC separations were performed on a Dionex UltiMate 3000 HPLC System (Sunnyvale, CA, USA) equipped with a quaternary pump, on-line degasser, autosampler, automatic injector, column thermostat and diode array detector (PDA). Data acquisition and integration were performed using Chromeleon series software from Dionex. Investigated analytes were separated on Gemini-NX C18 (150 mm × 2 mm, I.D. 3 μm, 110 Å) protected by a column Gemini NX (4 × 3 mm), under isocratic conditions at a flow rate of 200 μl min^{-1}. The mobile phase was a mixture of MeCN/water (containing 0.5% H_3PO_4, 40:60$_{v/v}$). The temperature of the column oven was 30 °C. The detection wavelength was set at 254 nm. The solid-phase extraction experiments were performed using GX-271 ASPEC system with TRILUTION LH Liquid Handling Software (Gilson Inc., Middleton, USA).

Fourier transform infrared (FTIR) spectra in KBr were recorded on a Philips PU9800 spectrometer (Philips Analytical, Cambridge, UK). The imprinted and non-imprinted silica polymers were observed using a Hitachi TM3000 scanning electron microscopy (Hitachi, Japan). The specific surface areas and porosity of the developed materials were measured by nitrogen sorption porosimetry using an Accelerated Surface Area and Porosimetry Analyzer ASAP 2020 (Micromeritics Instrument Corporation, Norcross. GA, USA).

2.3. Preparation of BCA-imprinted and non-imprinted silica polymers

The surface imprinted polymers were prepared by TEOS hydrolysis with ammonium hydroxide in according to methods previously described in literature [36–38] with some modifications. The procedure of synthesis the BCA-imprinted and non-imprinted silica polymers consisted of four steps: (1) activation of silica, (2) ionic interaction between the functional monomers and template molecules in the pre-polymerization solution, (3) modification of silica with TEOS and (4) grafting of activated silica gel surfaces with the molecularly imprinted polymer. Briefly, in order to increase the number of surface silanol, the silica gel was activated by dispersing 5 g of silica gel (SiO_2) in 40 ml of methanesulfonic acid (33%, CH_3SO_3H) with stirring and heating for 8 h). The solid product of activated silica gel was filtered and washed with deionized pure water to neutrality, and dried at 75 °C for

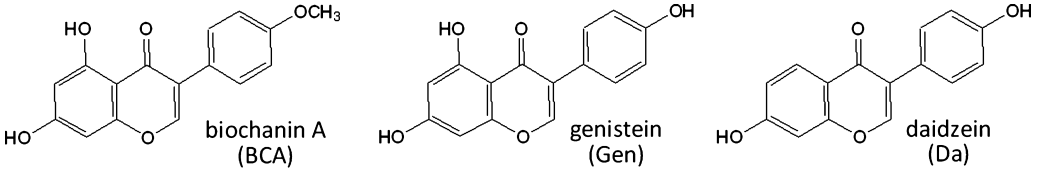

Fig. 1. Chemical structures of biochanin A (BCA), genistein (Gen) and daidzein (Da).