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Development and application of novel clonazepam molecularly imprinted coatings for stir bar sorptive extraction

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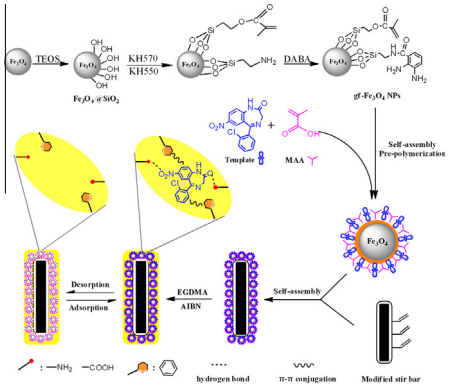
GRAPHICAL ABSTRACT



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ABSTRACT

The molecularly imprinted magnetic stir bar coatings were created based on graft-functional Fe_3O_4 nanoparticles with magnetic field-induced self-assembly. The magnetic complex including clonazepam as template, the graft-functional Fe_3O_4 nanoparticles and methacrylic acid as monomers was pre-assembled through π–π interaction and hydrogen bonding, then was directionally adsorbed on the surface of magnetic stir bar under the magnetic induction. The molecularly imprinted coating with well-ordered structure was generated by one-step copolymerization based on the cross linking of ethylene glycol dimethacrylate. The molecularly imprinted coating with multiple recognition sites could be manufactured and applied in polar solvents, and showed superior selectivity and fast binding kinetics for benzodiazepines. The analytes in herbal health foods, treated by stir bar sorptive extraction, were determined by HPLC-UV. Good linearity was observed in the range of 0.01–2 μg mL^{-1}. The content of clonazepam in the herbal health foods was found to be 44 ng g^{-1}, and the average recoveries were 89.8–103.3% with a relative standard deviation (RSD) <6.5%, demonstrating the successful application in real sample analysis.

1. Introduction

In the tense and competitive society, lack of sleep or sleep disorders are gradually becoming common social problems. The diseases coming along, such as anxiety and insomnia, can do severe damage to health without proper treatment. Benzodiazepines

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(BZ), with clonazepam (CZP) as the representative, are the most widely prescribed medicine for the treatment of these diseases [1]. Due to BZ's inhibition effect on central nervous system [2], it is often illegally added into herbal health foods (Hhfs), which has complex composition and imperfect quality standard, to gain sedative hypnotic effect more remarkably and quickly [3]. These counterfeit Hhfs, advertised as "pure Chinese medicine", would give rise to typical side effects of BZ, such as drowsiness, fatigue, ataxia and confusion. What's worse, the drug interaction between Hhfs and BZ probably potentiates certain side effects [4–6] which might be life-threatening. Thus, identification and quantification of BZ in Hhfs is important.

Several methods have been reported for the determination of BZ, such as high-performance liquid chromatography, thin layer chromatography, high performance liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, and high performance capillary electrophoresis. However, due to the low concentration of BZ and the interference of coexisting substances, sample preparation is always indispensable prior to instrumental determination, which may result in a loss of analytes [7–9]. As a result, selective sample pretreatment plays an important role before instrumental determination.

As a novel sample preparation technique, molecularly imprinted polymers stir bar sorptive extraction (MIPs-SBSE) has been widely applied to selective separation and enrichment for analytes in the sample pretreatment [10–12]. It was first reported by Zhu and coworkers. Zhu et al. prepared MIP stir bar coatings based on commercial PDMS stir bar substrate for the adsorption of monocrotophos [13] and L-glutamine [14]. Li et al. reported ractopamine [15], terbuthylazine [16], sulfamethazine [17] and metsulfuron–methyl [18] MIPs for SBSE coatings. Yang et al. [19] developed a monolithic material MIP stir bar coating. Hashemi et al. [20] summarized the recent research of MIPs-SBSE technique. Stir bar sorptive extraction (SBSE) is based on the partitioning of target analytes between a liquid sample and a stationary phase coated stir bar (SB) [21]. As reported, molecularly imprinted polymers (MIPs), which have characteristics of good selectivity, easy preparation and low cost, have provided an outstanding coating for SB. The molecularly imprinted polymers stir bar (MIPs-SB) showed not only the expected high selectivity, but also rapid equilibrium adsorption thanks to the porous nature of the imprinted polymer combined with a proper thickness of the coated polymer film [22]. More recently, MIPs-SB prepared by chemical bonding of the MIPs to the stir bar through silylation followed by multiple copolymerization reactions was proposed for the determination of various components in different samples.

Despite the advantages of MIPs-SBSE, many challenges still remain to be addressed. Certain factors can directly affect the adsorption capacity and kinetics, such as the structure and thickness of the coating, the porogen used for the preparation of the MIPs-coating, etc. The thick MIPs-coating may lead to deep embedding of the residual template molecules and the recognition sites, which may cause template leakage and slow kinetics during adsorption. Therefore, it's necessary to obtain a thin MIPs-coating with good structure formation, high adsorption amount and fast kinetics. Paradoxically, the preparation of MIPs-coating often needs a repeated polymerization process to obtain larger adsorption capacity and it may also affect the dynamic performance of the coating [23,24]. MIPs traditionally demonstrate their best performance in hydrophobic organic solvents. In non-covalent imprinting, the MIPs can be established relying on various interactions like hydrogen bond which is frequently employed. But the presence of polar solvents can disturb the formation of the pre-polymerization complex during the imprinting procedure, and disrupt the interactions between monomers and template because the hydrogen bond is unstable in polar solvents. The imprinting effect and recognition capability can be weakened or even damaged by polar solvents [25]. As many molecules are lack of solubility in non-polar solvents, the application of MIPs is strictly limited [26].

In recent years, magnetic nanoparticles (MNPs) have attracted particular interest due to their superparamagnetic nature as well as their unique physical and chemical properties such as high water dispersibility, relative large surface area and the high surface-to-volume ratio, resulting in a higher adsorption capacity [27–29]. Among MNPs, Fe_3O_4 magnetic nanoparticles (Fe_3O_4 NPs) have received considerable attention owning to their many advantages [30]. Especially, graft-functional Fe_3O_4 NPs (gf-Fe_3O_4 NPs) with new features such as well-controlled, highly branched structure and enriched functional groups were introduced into the study of MIPs, and the controllable material with special functional parts was generated [31–33].

Self-assembly of nanocomposites is one of the most promising methods for the preparation of novel materials and devices with desired morphologies and properties [34–36]. During this process, the building blocks spontaneously organize into ordered structures by thermodynamics and other constraints [37,38]. There are two broad divisions containing template-guided self-assemblies (e.g. physical template, chemical template and biological template) and field-guided self-assemblies (e.g. magnetic field, electric field, pressure gradient, light and laser) [39,40]. Magnetic field-induced self-assembly, a recently emerged external self-assembly approach, has been more introduced as a powerful strategy for controlling the structure and showed an excellent prospect for the fabrication of MIPs-coating [41,42]. In this system, magnetic field could rapidly drive and conveniently orient MNPs, synthesizing and assembling the MIPs-coating with special structures and thickness [43].

In this study, we demonstrate a satisfactory separation and enrichment performance of BZ in Hhfs with the application of the MIPs magnetic stir bar (MIPs-MSB) based on gf-Fe_3O_4 NPs. Fe_3O_4 NPs was modified by silane coupling agent and diaminobenzoic acid (DABA) to produce functional groups on the surface. Gf-Fe_3O_4 NPs which form pyknotic and homogeneous MIPs-coating could increase effective binding sites and enhance recognition for template molecules in polar solvent. The thickness and morphologies of the MIPs-coating could be easily controlled by adjusting the amount of gf-Fe_3O_4 NPs and showed superior selectivity and fast binding kinetics. Herein, a well-designed MIPs-coating was obtained through a simple synthetic method.

2. Experimental

2.1. Reagents and materials

Nitrazepam, clonazepam and midazolam were obtained from the Food and Drug Administration of Jiangsu Province., γ-Aminpropyl triethoxysilane (KH550) was obtained from Diamond Advanced Material of Chemical Inc., γ-methacryloxypropyl trimethoxysilane (KH570) was purchased from Shanghai Aladdin Reagent Co., Ltd., tetraethyl orthosilicate (TEOS) was acquired from Sinopharm Chemical Reagent Co., Ltd., 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai No.4 Reagent & H.v Chemical Co., Ltd., methacrylic acid (MAA) was acquired from Shanghai Enfujia Technology Co., Ltd., ethylene glycol dimethacrylate (EGDMA) was bought from Sigma–Aldrich Inc., diaminobenzoic acid (DABA) was bought from Shanghai Puzheng Biological Science and Technology Co., Ltd., the MSB was manufactured by Nanjing University Chemical Lab, Hhfs (for tranquilizing and allaying excitement, the fundamental composition as described by the manufacturer: semen ziziphi spinosae, ligusticum wallichii, rhi-