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Magnetic molecularly imprinted polymer nanoparticles for selective solid phase extraction and pre-concentration of Tizanidine in human urine

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ABSTRACT

In this work, the magnetic molecularly imprinted polymer nanoparticles (MMIP-NPs) for the selective pre-concentration of Tizanidine have been described. The polymer nanoparticles were synthesized by the polymerization of methacrylic acid as a functional monomer, ethylene glycol dimethacrylate as a cross-linker, 2,2-azobisisobutyronitrile as an initiator and Tizanidine as a template molecule. The MMIP-NPs were characterized by scanning electron microscopy (SEM) and thermogravimeteric analysis (TGA). Imprinted Tizanidine molecules were removed from the polymeric structure using acetic acid in methanol (10:90 V/V%), as the eluent solvent. The limits of detection (L.O.D) for Tizanidine were 1.13×10^{-6} M and 1.68×10^{-6} M in ultrapure water and urine, respectively. Also, the relative standard deviations (R.S.D) in ultrapure water and urine were 2.21% and 2.58%, respectively. The method was applied to the determination of Tizanidine in the human urine samples.

1. Introduction

Magnetic nanoparticles have wide applications in the fields of cellular therapy in cell labeling, separation and purification, target-drug delivery, and hyperthermia treatment of cancers, as well as magnetic resonance imaging (MRI) [1]. Magnetic nanoparticles, especially Fe_3O_4 and maghemite (γ-Fe_2O_3), have attracted broad attention in solid phase extraction. The method of magnetic solid phase extraction (MSPE) overcomes the problem of column packing by simply using an external magnetic field [2]. It involves the addition of magnetic particles (MP_S) into the sample solution and the target analyte are then adsorbed on its surface. This is shortly followed by a separation of the adsorbed analytes from the aqueous solution using an external magnetic field, while the target analyte is desorbed by the eluent [3]. Although the term molecular imprinting was first used in 1931, but real interest in the technique was created in 1972, when organic polymers with predestined ligand selectivity were synthesized by Wulff, Sarhan, Klots and Takagishi [4]. They are synthesized from a three-dimensional polymeric matrix which is created around a template molecule [5]. Unfortunately, the manufacture of MIP nanoparticles is not an easy task; however, the most common methods of synthesis include: precipitation polymerization, mini- and micro- emulsion polymerization, core–shell (with subsequent grafting), suspension polymerization in silicon oil and living radical polymerization processes, such as atom transfer radical polymerization (ATRP) and reversible addition–fragmentation chain transfer polymerization (RAFT) [5,6].

Contrary to bulk monoliths, MIP nanoparticles possess higher surface area to volume ratios; therefore, imprinted cavities are more easily accessible by the templates [7,8].

Over the years, these nanoparticles have been widely used as enzyme substitutes [9,10], drug delivery systems [11,12], antibody substitutes [13–16], capillary electrochromatography [17–20], sensors [21–23], solid phase extraction (SPE) [24,25], solid phase micro extraction (SPME) [26,27], stir bar sorptive extraction (SBSE) [28,29], and dispersive liquid–liquid micro extraction (DLLME) [30]. For the very first time, in 1998, with the use of a magnetic iron oxide, a magnetic molecularly imprinted polymer (MMIP), having a mean diameter of 13 μm was produced from the polymerization of monomers in liquid perfloro chlorine [31]. The development of this method, in combination with the use of special functional monomers, resulted in the production of MMIP, to which some stimuli responded, such as thermal stimuli [32], optical stimuli [33]

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and PH stimuli [34]. In addition, the use of high surface to volume ratios magnetic nanoparticles, nanocapsules, nanowires and nanotubes can also be related to the MIP, which also increases the binding capacity and kinetics [35,36]. The aim of this study was the preparation of Tizanidine imprinting polymer as well as its application for selective solid phase extraction. UV spectrophotometer was used for determination of Tizanidine after pre-concentration by MMIP-NPs in urine and ultrapure water samples.

2. Experimental

2.1. Reagents and solutions

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2.2-azobisisobutyronitrile (AIBN), acetic acid (HOAC), sodium hydroxide (NaOH), phosphoric acid (H_3PO_4), methanol (MeOH), tetra ethoxysilane (TEOS) purchased from merck chemical company, 3-(Trimethoxysilyl) propyl methacrylate 98% purchased from Sigma–Alderich company, were utilized for the purpose of this study. The drug used in this study, Tizanidine or Zanaflex, were obtained from daroupakhsh (Tehran, Iran). All the reagents used were of analytical grade. Ultrapure water was prepared by using a milli-Q* system (Millipore, Milford, MA, USA). A stock standard solution of Tizanidine 0.0034 M was prepared in methanol. The working solution 1.72×10^{-5} M was prepared daily with an appropriate dilution of Tizanidine stock. Urine was also collected from healthy volunteers.

2.2. Instrumentation

A double beam spectrophotometer (UV–vis) PerkinElmer model lambda 25 was used for all measurements. The thermo gravimetric analysis (TGA, model PL, UK) was used to determine the thermal properties of synthesized polymers, and a scanning electron microscopy (SEM, LEO 1430 VP, UK) was used to characterize the nano-sized MMIP and MNIP. pH of solutions were adjusted using a pH meter of model 630 digital metrohm which was equipped with a combined glass–calomel electrode. Magnet 1.3 T with dimensions $20 \times 40 \times 50$ cm as an external magnetic field was used for separating MMIP-NPs from the solutions.

2.3. Synthesis of magnetic (molecularly imprinted and non imprinted) polymer nanoparticles

In order to synthesis of MMIP-NPs, 43.2 mmol $FeCl_3 \cdot 6H_2O$ and 21.6 mmol $FeCl_2 \cdot 4H_2O$ were dissolved in 200 mL deionized water and 20 mL of $NH_3 \cdot H_2O$ (30%) under N_2 gas at 70 °C, then the black product Fe_3O_4 NPs was dried in the vacuum [37]. Silica was then coated on the Fe_3O_4 NPs by 5 mL of $NH_3 \cdot H_2O$ (30%) and 2 mL of TEOS [38]. Subsequently, the C=C groups were grated onto the silica-modified Fe_3O_4 surface by adding 5 mL of 3-(Trimethoxysilyl) propyl methacrylate. Finally, the MIPs films were formed on the surface of Fe_3O_4@SiO_2 by the copolymerization of C=C end groups with 1.3 mmol of MAA (the functional monomer), 2.6 mmol of EGDMA (the cross-linker), 0.3 mmol of AIBN (the initiator), and 0.2 mmol of Tizanidine (the template molecule). This system was allowed to react at 62 °C for 24 h under N_2 gas [39]. Furthermore, magnetic non imprinted polymer nanoparticles (MNIP-NPs) was prepared using the same process of magnetic molecular imprinted polymer nanoparticles but without the addition of the template molecule.

2.4. Preparation of standard solutions

A stock standard solution of Tizanidine was prepared at a concentration level of 0.0034 M in methanol, and for the preparation of standard solutions (6.89×10^{-6}, 1.72×10^{-5}, 3.44×10^{-5}, 5.17×10^{-5}, 6.89×10^{-5}) M, amounts (20, 50, 100, 150, 200) μL from synthesized nano-MMIP was diluted to 10 mL with ultrapure water. For analysis of urine samples, 7 mL of spiked urine (without Tizanidine) to 2 mL standard solutions with a concentrations of (3.44×10^{-5}, 8.60×10^{-5}, 1.72×10^{-4}, 2.58×10^{-4}, 3.44×10^{-4}) M was diluted to 10 mL with ultrapure water.

3. Extraction procedure

Extraction procedure was investigated using sorption and desorption steps. In the sorption step, the pH of sample solution was adjusted to 8 by drop wise addition of 0.2 mol L^{-1} sodium hydroxide solutions. Then, 100 mg of dried MMIP-NPs was suspended in aqueous solution 10 mL containing 1.72×10^{-5} M concentra-

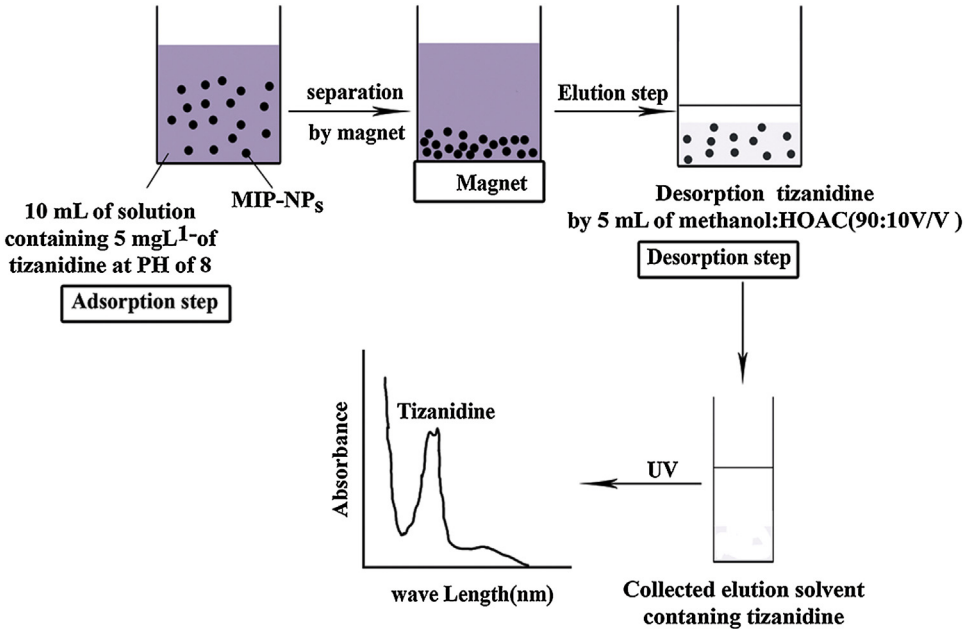

Fig. 1. Schematic illustration of extraction procedure.