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QbD approach by computer aided design and response surface methodology for molecularly imprinted polymer based on magnetic halloysite nanotubes for extraction of norfloxacin from real samples

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ABSTRACT

This article describes the development, optimization, and evaluation of a novel composite imprinted polymer, on the basis of magnetic halloysite nanotubes (MHNTs-MIPs) using "Quality by Design (QbD)" approach combining computer simulation and response surface methodology. Norfloxacin, methacrylic acid, and ethylene glycol dimethacrylate were used as template, functional monomer and cross-linker, respectively. As a comparison, two MHNTs-MIPs have been prepared with the most suitable functional monomer methacrylic acid (MAA) along with acrylamide (AM). To explain the adsorption behavior, adsorption kinetics and isotherms were studied. Magnetic halloysite nanotubes molecularly imprinted polymers prepared from MAA (MHNTs-MIP_1) displayed a high adsorption capacity (349 $\mu g\, mg^{-1}$) toward NOR. A magnetic imprinting solid phase extraction method coupled with high performance liquid chromatography (MHNTs-MISPE-HPLC-UV) was developed for the determination of NOR in serum and water samples, by applying MHNTs-MIP as a sorbent. The recoveries from 83.76% to 103.30% in water and from 90.46% to 99.78% in serum were obtained. Besides remarquable mechanical properties and specific recognition of MHNTs-MIP toward template molecule. It could be also collected and separated fastly by external magnetic field. Moreover, MHNTs-MIPs could be reused for several cycles with the recovery range from 83.25% to 100.96% for water sample and from 85.65% to 100.33% for serum sample. These analytical results of serum and water samples showed that the proposed method based on MHNTs-MIPs is applicable for fast and selective extraction of therapeutic agents from biological fluids and environmental water.

1. Introduction

Robust molecular recognition elements with antibody-like ability to bind and discriminate between molecules or other structures can today be synthesized using molecular imprinting techniques, which is used to create molecularly imprinted polymers (MIPs) [1]. MIPs have been used for a wide variety of applications, such as solid phase extraction [2–4], drug-controlled release [5,6], and sensor devices [7–9]. As the extraction of template molecules embedded inside the thick polymer network is quite difficult, traditionally MIPs suffer the drawbacks of incomplete template removal, low binding capacity and slow mass transfer [10]. The surface location of the imprinted sites and caves lead surface imprinted technique become an alternative and one of the most effective ways to improve the MIP preparation. Due to the easy accessibility to recognition sites and the homogeneous repartition of binding sites, surface imprinted polymers reveal fast mass transfer, high binding capacities and rapid binding kinetics [11]. Several supporting substrates such as silica nanoparticles [12,13], carbon nanotubes (CNTs) [14,15] and Fe_3O_4 [16,17] have been developed to prepare MIP.

Halloysite nanotubes (HNTs) are clay aluminosilicate mineral. They are chemically similar to the structure of kaolinite. The general stoichiometry of halloysite is $Al_2Si_2O_5\,(OH)_4.nH_2O$ [18], and has a similar geometry to carbon nanotubes [19]. The HNTs range in length from 500 to 1000 nm [20]. Due to a small number of hydroxyl groups on its surface, halloysite has exclusive properties [21]. HNTs have layered, well crystallized structure consisting of aluminum oxide octahedral in the inner layer and silicon dioxide tetrahedra in the outer layer [22,23]. Recently, HNTs have gained growing interest because of their strong interactions, stability under acidic conditions, good dispersibility [24], lack of swelling, and large surface area [11]. In contrast with other

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nanosized materials, especially carbon nanotubes, HNTs available in thousands of tons at low price [25]. For that reason, this natural biocompatible nanomaterial could be a promising candidate for the nanosized support and nanoarchitectural composites.

To simplify the extraction process, magnetic molecularly imprinted polymers (MMIPs) have overcome the shortage of traditional MIPs. The combination of Fe_3O_4 and HNTs can form the magnetic HNTs (MHNTs) due to the abundant hydroxyl groups on its surface, which has been applied in adsorption technology [26]. Pan and co-workers used MHNTs combined with MIPs as sorbent for solid-phase extraction and separation of 2, 4, 6-trichlorophenol from environmental water samples [10,11,19], Zhong and co-workers also prepared a novel MHNTs-MIPs for rapid enrichment of 2,4-dichlorophenoxyacetic from water [27]. Several previous works has been made to attempt for designing MHNTs-MIPs to investigate the adsorption behavior of some antibiotics as tetracycline and Chloramphenicol [28–30]. However, no report has described the practical application of the prepared imprinted polymers on the surface of MHNTs for detection and extraction of drug in biological fluid samples. In our previous work [31], same principal was used to prepare polymer grafted magnetic HNTs loaded norfloxacin through imprinting polymer mimic procedure. But, no template washing step was needed because the prepared nanomaterial was used as nanocarrier for sustained release of norfloxacin. The sustained release was influenced by EGDMA cross linker density. The difference between the previous and present work is that the present nanomaterial have good selectivity as all MIPs preparation steps were applied to obtain polymer with an imprinting cavity for extraction purposes. To the best of our knowledge, This research represents the first attempt to prepare molecularly imprinted polymers on the surface of magnetic halloysite nanotubes (MHNTs-MIPs) for the detection and extraction of norfloxacin (NOR) from two complex systems (water and serum sample) using “Quality by Design (QbD)” approach. Norfloxacin is one kind of fluoroquinolones antibiotics belong to synthetic antibacterial drugs, which are widely used for prevention and treatment of bacterial infection [32].

This research aims to enhance the quality of MIPs from the synthesis step and reduce the cost, time consuming and number of experiments. For that reason Quality by design approach has been successfully applied. In the present study, the QbD involved the Computer aided design followed by Response surface methodology approaches to obtain a nanomaterial with the desired quality in term of adsorption capacity. Rational MIP design necessitates knowledge of the molecular-level events occurring in prepolymerization mixtures [33]. With the development of quantum chemistry and computer technology, computer-aided design have been widely used in MIP, specifically for functional monomer screening [34]. Recently our group has designed a novel magnetic molecularly imprinted polymer (MMIP) using flexible docking [34,35] and molecular dynamics in computer simulation. Six kinds of representative functional monomers have been screened to obtain the optimal one, as well as optimizing its ratio to the template (Amlodipin). The results showed that the good ratio of template to MAA and AM was 1:4. In this research, five kinds of monomers were selected and eight ratios were optimized by computer simulation.

Since the adsorption behavior of MIPs is influenced by numerous factors, a variety of approaches have been proposed by the researchers including the use of Design of Experiments (DOE) to identify optimal conditions, efficiently, rapidly and accurately [36]. Orthogonal experimental design was used to screen several combinations to obtain a better value instead of the best within the given range of conditions [34]. Response surface methodology (RSM) consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design. A very useful element of the QbD is the understanding of various factors (variables) and their interactions by a small number of experiments using a statistical tools [37,38]. From the fact that computer modeling as well reduces the time and number of experiments to be realized to the minimum, for designing final material with high quality, it could also be considered as one of the QbD approach in this work. The selected QbD strategy allowed an efficient selection of the suitable monomer to design MIPs and good conditions for high adsorption capacity [39].

In this work, the superparamagnetic NOR-imprinted Polymers based on MHNTs were prepared using simple precipitation polymerization. Associated with a computer simulation approach, two MHNTs-MIPs were synthesized under the suitable functional monomers and ratio. The molecular imprinted polymer based MHNTs was optimized in terms of adsorption capacity by three-factors (amount of MHNTs-MIPs (mg), drug concentration ($\mu g\,mL^{-1}$) and pH). The adsorption and separation properties of the MHNTs-MIPs were further evaluated by the adsorption statics, adsorption kinetics and selectivity. After optimization of adsorption by the above mentioned QbD strategies and optimization of elution conditions, the practical applications of MHNTs-MIPs nanoparticles for real sample was further assessed by the enrichment of NOR from bovine serum and lake water samples.

2. Experimental section

2.1. Materials

Halloysite clay was supplied from DanjiangKou, China. Norfloxacin, Ethylene glycol dimethacrylate (EGDMA), polyvinylpyrrolidone (PVP), 3-(methacryloyloxy)propyl trimethoxysilane (MPS), azobisisobutyronitrile (AIBN), methacrylic acid (MAA) and acylamide (AM) were obtained from Aladdin Industrial Corporation (Shanghai, China). $FeCl_3{\cdot}6H_2O$ (Fe^{3+}) and dimethyl solfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). $FeSO_4{\cdot}7H_2O$ (Fe^{2+}), ammonium hydroxide ($NH_3{\cdot}H_2O$) and acetic acid were obtained from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). Bovine serum samples were purchased from Alibaba Company. All these chemicals and solutions used were of analytical reagent grade.

2.2. Instruments and softwares

An FEI Tecnai G2 F20 transmission electron microscope (TEM) was used to characterize the morphology of the materials. The magnetic properties were tested by a LDJ 9600–1 vibrating sample magnetometer (VSM) operating at room temperature with applied fields up to 10 kOe. Computer simulation was carried out via Molecular Operating Environment (MOE, v2008) and Discovery Studio (DS, v2.5). Design-Expert (v8.0) was used in response surface methodology.

Chromatographic conditions: HPLC analysis system is composed by four G1311C liquid pump, G1329B automatic sampler, G1316A thermostat with G4212B UV detector. Chromatographic column: Eclipse Plus C18, 4.6 mm x 150 mm; Column temperature: 35 °C. Detection wavelength: 276 nm. Mobile phase: phosphate buffer solution (1%, pH 3.20)-methanol (70: 30, V/V). Flow rate of Mobile phase: 1 $mL\,min^{-1}$; Injection volume: 10 μL.

2.3. Computer simulation

In order to comprehend the properties of MIP at molecular level, Discovery studio program was used. The drug norfloxacin and five representative monomers methacrylic acid (MAA), acrylic acid (AA), methacrylamide (MAM), acrylamide (AM) and 4-vinyl pyridine (4-VP) were constructed and minimized using force field parameter Merck Molecular Force field (MMFF94X) implemented in Molecular Operating Environment (MOE), at a gradient of 0.1. One thousand steps were applied by steepest descent and conjugate gradient method in minimization module to obtain the minimal energies of each 3D structure in Discovery studio (DS). To simulate the interaction between NOR and the monomers in the complex force field of Chemistry at Harvard Macromolecular Mechanics (CHARMm) and MMFF94X in DS, a flexible