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Computational-aided design of magnetic ultra-thin dummy molecularly imprinted polymer for selective extraction and determination of morphine from urine by high-performance liquid chromatography

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ABSTRACT

In this work, a novel magnetic ultra-thin dummy molecularly imprinted polymer (MMIP) for morphine (MO) was prepared. In order to obtain highly selective recognition cavities, the MMIP has been designed using semi-flexible docking to screen the optimal monomer and its ratio to morphine from six representative monomers. Furthermore, the dummy template was creatively screened by semi-flexible docking method from opioid drugs. The system of dihydrocodeine (DI) as dummy template, methacrylamide (MAC) as founctional monomer, ethyleneglycol dimethacrylate (EGDMA) as crosslinker was chosen for MO imprinting. The morphological and magnetic properties of MMIP were characterized by FT-IR, TEM and VSM. The results suggested that molecularly imprinted polymer (MIP) was synthesized evenly on Fe_3O_4 surface. The adsorption experiments revealed that MMIP showed better extraction capacity and selectivity toward MO and its analogues than the non-imprinted polymer (NIP). The MMIP possessed adsorption capacity of 14.71 mg/g for MO and the imprinting factor was 2.10 at separate adsorption and 1.87 at competitive adsorption. A magnetic molecularly imprinted solid phase extraction coupled with HPLC method (M-MISPE-HPLC) has been established for the analysis of MO in urine sample. The developed method was validated for its linearity (0.038–100 mg L^{-1} R^2 = 0.9937), precision (1.07%–3.72%) and accuracy (83.62%–100.37%).

1. Introduction

Morphine (MO) is a powerful central nervous system stimulant that offered to patients in relieving severe pain [1–4]. But it can cause psychological dependence. Major health problems arising from MO use include miscarriages, heart infections, and death from overdose [5,6]. A number of analytical methods have been developed for the determination of MO which includes radioimmunoassay [7], high performance liquid chromatography (HPLC) [8–11], gas chromatography (GC) coupled with mass spectrometric detection [12–14], electrophoresis with UV or mass spectrometric detection [15–19]. and electro-chemical detection [20,21]. However, the determination of morphine was still great challenging to these due to the complicated biological matrices interferences and low concentration of these structure similar metabolites. Therefore, a practical, selective and sensitive method to determine MO in biological samples is urgently needed.

Molecularly imprinting polymer (MIP) is synthetic material that is designed to have a molecular memory for a particular target molecule (template) [22]. It has showed high selectivity and affinity towards the template [23–25]. Due to their outstanding molecular recognition characteristics, the MIP have drawn much attention in various fields, such as purification of racemic mixtures [26], separation media [27], chemical sensing [28], catalytic control of chemical reactions [29], and enzyme mimics [30]. Nevertheless, the main challenges have limited their successful and widespread use, such as time-consuming separated, template leakage, and slow mass transfer. Moreover, traditional MIP in which molecules imprint layer is very thick having the disadvantages of large diffusion resistance, hard eluting, low binding rate and deep embedded template in the internal. By solving this problem, the promising strategy of magnetic ultra-thin molecular imprinted polymer using a dummy

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template has been developed. Magnetic molecularly imprinted polymer (MMIP) can be easily isolated/collected and recycled by an external magnetic field without additional centrifugation or filtration [31,32]. An elegant solution to template leakage is the utilisation of a dummy template during polymerisation. With this approach, any leakage of the dummy template will not interfere with the analysis, provided that a procedure capable of separating the two analogue molecules is applied prior to analyte quantification [33,34] . Moreover, the use of dummy template also can avoid the poisonous, hydrophobic and expensive target analyte as template [35,36].

The nature of the MIP and its binding properties are influenced by many factors, such as the type and ratio of functional monomer and templates. However, the selection of founctional monomer and dummy template of MIP is still mainly relying on tedious trials and errors or reported methods. For rational design and selecting an appropriate synthesis method for MIP, a growing number of research groups have paid attention to computer simulation and computer-aided design [37–39] . Screening the ingredient of MIP by computer simulation was first introduced by the group of Sergeyeva can be traced back to 1999. They used HyperChem 3.0 to select the functional monomers and determine the ratio of functional monomers to the template [40] . From then on, with various software such as Gaussian [41] , PRODRUG SERVER [42], and Discovery Studio, more and more studies of molecular recognition mechanisms, screening functional monomers and optimized imprinting system were carried out by computer simulation. In addition, there is no publication to report selecting the dummy template by the computer-aid method.

In this study, a novel magnetic ultra-thin molecularly imprinted polymer for morphine was prepared coupled with a computer simulation approach. A flexible docking method was successfully combined to select the most suitable dummy template, functional monomer and its ratio to dummy template. According to the theoretical calculation results, the MMIP was prepared by surface imprinting method. The adsorption behaviour of the material was studied by adsorption statics, adsorption kinetics, isotherm and selectivity after optimizing the adsorption conditions. Then, a magnetic molecularly imprinted solid phase coupled with HPLC (M-MISPE-HPLC) method has been established and validated for the detection of MO in human urine sample.

2. Experimental section

2.1. Chemicals

Dimethyl sulfoxide (DMSO), sodium chloride (NaCl), polyvinyl pyrrolidone (PVP), iron (III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), potassium dihydrogen phosphate, sodium acetate trihydrate (NaAC) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Methacrylamide (MAC), ethyleneglycol dimethacrylate (EGDMA), azodiisobutyronitrile (AIBN), Polyethylene glycol-10000 (PEG 10000) were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). Diethylene glycol, ethylene glycol (EG), triethylamine were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd (Shanghai, China). AceticAcid (CH_3COOH) was purchased from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). Methanol (CH_3OH) was purchased from Cinc High Purity Solvents Co., Ltd (Shanghai, China). Ethanol (C2H5OH) was obtained from Shanghai Titan Scientific Co., Ltd (Shanghai, China). Morphine (MO), tramadol (INN) and dihydrocodeine (DI) from National Institutes for Food and Drug Control (Beijing, China). Gatifloxacin (GA) was obtained from Hubei Xing Galaxy Chemical Co., Ltd (Wuhan, China).

2.2. Instrumentation and software

All calculations have been carried out using the Discovery Studio (DS, v2.5). UV–vis spectrophotometer (UV-1800) was purchased from Shimadzu (Kyoto, Japan). Fourier transform infrared spectrophotometer (FT-IR-8400s) was purchased from Shimadzu (Kyoto, Japan). HPLC analyses were done using a LC-20AT pump (Kyoto, Japan) and a Shimadzu SPD-M20A UV–vis detector. All separation was achieved on Hedera ODS-2 (4.6mm × 250 mm; Hanbon Sci & Tech, Jiangsu, China). FEI Tecnai G2 F20 transmission electron microscope (TEM) were used to characterize the morphology of the materials and the magnetic properties were tested by a LDJ 9600 -1 vibrating sample magnetometer (VSM) at room temperature with applied fields up to 10 kOe.

2.3. Computational simulation

MO, template candidates thebaine, dihydrocodeine, naloxone, morphine and six representative functional monomers methacrylic acid (MAA), MAC, acrylic acid (AA), acrylamide (AM), 2-vinylpyridine (2-VP), 4-vinylpyridine (4-VP) were constructed and preliminary optimization in DS 2.5. Steepest descent method and conjugate gradient method were applied 2000 steps respectively to obtain the minimal energies of each 3D structure in DS.

Depending on the optimization results, A flexible docking programme CDOCKER was used to simulate the interaction between MO and the functional monomers with the ratio 1: n ($n \leq 8$) in the force field of CHARMm and MMFF94 in DS. Then the chosen founctional monomer and its ratio to MO was interacted with MO and left the cavity interacted with the template candidates. The interaction energies were calculated using following equation [43]:

$$\triangle E = E_{template-monomercomplex} - (E_{template} + nE_{monomer}) \quad (1)$$

Where the △E is the docking energy, $E_{template-monomercomplex}$, $E_{template}$ and $E_{monomer}$ represents the energy of the template–monomer complex, template and monomer respectively. Where n refers to monomer number in the template–monomer complexes. The △E is including van der Waals forces, hydrogen bonding, hydrophobic bonding and electrostatic forces.

2.4. Preparation of the MMIP and MNIP

2.4.1. Preparation of magnetic particles

The core Fe_3O_4 was synthesized using solvothermal method. Briefly, $FeCl_3{\cdot}6H_2O$ (6.08 g), PEG 10000 (1.0 g) and NaAC (3.6 g) were dissolved in mixed solution of EG and diethylene glycol (90 mL 1:1, v/v) under vigorous stirring for 30 min at room temperature. The mixture was sealed in a Teflon lined stainless steel autoclave (50 mL capacity) and heated at 200 °C for 8 h, and then cooled to room temperature. The black products were collected by magnet, washed for several times with purified water and C2H5OH, followed by dried in vacuum.

2.4.2. Preparation of magnetic ultra-thin molecularly imprinted particle

For the preparation of MMIP and MNIP, functional monomer MAC (0.7 mmol) and dihydrocodeine (0.1 mmol) were dissolved in DMSO (5 mL) under shaking for 0.5 h at room temperature, while magnetic particles (0.25 g) in DMSO (2.5 mL) under ultrasonic for 10 min. Then EDGMA (5 mmol) was mixed with the above-mentioned solutions. The mixture was shaken for 12 h to self-assembly. The pre-polymerization solution was transferred to a three-neck flask. Under the nitrogen gas protection and vigorous mechanical stirring, PVP (0.1 g) in 25 mL DMSO-H2O (9:1 v/v) and AIBN (0.025 g) were added, and the reaction was maintained for