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Synthesis and characterization of molecularly imprinted polymers with metallic zinc center for enrofloxacin recognition

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ABSTRACT

A molecularly imprinted polymer with a metallic center (MIPc) was prepared and characterized. The template molecule for MIPc was a complex $[Zn(Aza\text{-}f)(Enr)]NO_3{\cdot}H_2O$, Com, based on the ligands: enrofloxacin (Enr) and azabis(oxazoline) functionalized with styrene. Com was characterized before preparing MIPc. Moreover, the polymer prepared *via* non-covalent (MIPe) was prepared using Enr as the template molecule while no template molecules were used to prepare the non-imprinted polymer (NIP).

MIPc was characterized using different spectroscopic techniques. In addition, the polymer molecular recognition capacity was studied by using rebinding kinetic studies. The kinetics shows that MIPc reach the equilibrium before the MIPe and NIP and the amount of Enr adsorbed is approximately 5 times higher that MIPe and NIP.

Scatchard plots analysis for NIP, MIPe and MIPc shows K_d = 0.4768, 0.020, 0.0067 (mmol L^{-1}) respectively. In comparison with the MIPe the binding affinity is nearly 100 times higher taking into account the high affinity sites. The selectivity of MIPc for Enr was higher than that for ofloxacin or flumequin, isotherms were fit to the Langmuir–Freundlich model, and the Enr showed a value of K_o = 21.38 mM^{-1}, while the ofloxacin had a value of 2.2×10^{-8} mM^{-1} and the flumequin of 1.6×10^{-5} mM^{-1}.

1. Introduction

In recent years, the contamination by antibiotic residues in animal meat for human consumption [1], has become a serious health concern. Quinolones (Qs) are a widely used broad-spectrum antibiotic family in order to prevent and treat a wide variety of diseases in both human and veterinary medicine. The majority of Qs for clinical use belongs to the fluoroquinolone group [2] (FQ).

In addition, the interest in the determination of antibiotic, in particular FQs [3], residues in the environment arises from the fact that they are suspected of being responsible for the appearance of antibiotic-resistant bacterial strains [4–6]. There is also concern that FQs may inhibit photosynthesis in plants [7].

The FQs and their metabolites [8] have already been identified as risky and persistent pollutants in water, sludge, soils and sediments [9–11]. FQs have been found in hospital wastewaters [12] (at concentrations ranging from approximately 60–120,000 ng/L), in wastewater treatment plant effluents (~2–580 ng/L) and in surface waters (~5–1300 ng/L) throughout the world. The persistence [13] of the antibacterial agents like enrofloxacin (ENR), flumequine (FLU), sarafloxacin (SAR), could be a serious problem, for instance, in marine sediments where the initial concentrations of these compounds remain almost intact after 180 days, whereas the residues in the top layer of the sediment depurated more rapidly.

For these reasons, it is important to develop analytical methods for detecting these drugs. However, most of the analytical methods established for the FQ detection or separation are not efficient at directly detecting or removing the trace drug amount. Current methods for the FQ analysis in biological matrices are based on liquid chromatography; mainly ultraviolet (LC-UV) [14], fluorescence (LC-FD) [15], or mass spectrometry (MS) [16,17].

Molecular imprinting [18] allows the formation of specific recognition sites in synthetic polymers through the use of templates. The template molecule organizes functional and cross-linking polymerizable monomers during the polymerization. Subsequently it is extracted from the insoluble network. Then, domains of complementary size, shape, and functional group orientation are created. On the other hand, the most widely used technique for preparing MIPs is the non-covalent, in which template and functional monomer form a pre-organized structure through weak interactions [19]. This method is used with aprotic and low polarity organic solvents; however, this causes a poor level of recognition of the template in aqueous environments. The polar solvents, especially water, could destroy the imprinted recognition

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site, which obviously should limit their further application in environmental and biological fields. To improve the affinity of MIP in aqueous environment, porogenic solvent such as CH_3OH was recommended in MIP preparation [20].

The formation of a template-monomer complex under aqueous conditions is not efficient enough, owing to the fact that the hydrogen-bonding interactions between template and monomers can be interrupted by the polar solvent. In order to avoid the disturbance, the metal ion was introduced as a mediator during the pre-polymerization to form the complex of template-metal ion-monomer. In fact, coordination interaction is a strong interaction and metal ion MIP has been demonstrated to have high selectivity and used in several types of selective recognition systems [21–23].

Several studies of MIPs for FQ have been reported, however most of them were focused on non-covalent MIPs [5,24–30]. The affinity in polar solvents could be increased by introducing stronger interactions, such as those exhibited by metal coordination centers. For instance, Hart and Shea [31] reported synthetic peptide receptors based on imprinted Ni(II)-nitrilotriacetic acid complexes, which have affinity to N-terminal histidine (His) residues. Subat et al. [32] reported imprinting of zinc (II) cyclen complexes yielding MIPs with affinity for imides in aqueous solution.

It is important to highlight the work of Lv et al. [33], who studied the synthesis of imprinted polymers (MIP1) templated with Enr. The recognition between MIP and Enr occurs by means of a coordination bond in a protic solvent. The MIP was developed by copolymerization of Co(II)-Enr complex that was formed *in situ* with 4-vinylpyridne (4-Vpy) and EDGMA.

The binding studies were performed, and the binding parameters were obtained through Scatchard plots suggesting that the binding sites in MIP1 are heterogeneous with respect to the affinity for $[(Co)\text{-}Enr]^{2+}$. The MIP1 has two kinds of sites with specific binding properties. K_d and Q_{max} of higher affinity binding sites can be calculated to be 0.770 μmol mL^{-1} and 102.762 μmol g^{-1}. K_d and Q_{max} of lower affinity binding sites were 0.295 μmol mL^{-1} and 65.225 μmol g^{-1}, respectively.

In this paper, we report the synthesis and characterization of Enr imprinted polymers by using the coordination interaction for recognition of Enr in protic solvents. The MIPc was synthesized using a mixed complex of zinc (II), Com. as a template molecule. The template molecule was synthesized and characterized before preparing polymers in order to have homogeneous recognition sites, which should lead to materials with improved binding and specificity characteristics.

2. Experimental

2.1. Materials

Enrofloxacin, $C_{19}H_{22}FN_3O_3$, (1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid), (Enr, 98%); was bought of Fluka. $Zn(NO_3)_2{\cdot}6H_2O$ (98%); CH_3CN (98%); allyl alcohol (AA; 98.5%), ethylene glycol dimethacrylate (EDGMA, 98%); CH_3OH and CH_2Cl_2 were purchased from Aldrich. 2,2′-azo-bis-isobutyronitrile, (AIBN, 98%) was kindly donated by AKZO NOBEL. Water was purified by Milli-Q system. On the other hand, azabis(oxazoline) was functionalized $C_{21}H_{29}N_3O_2$, ((4S)-4-isopropyl-N-[(4R)-4-isopropyl-4,5-dihydro-1,3-oxazol-2-yl]-N-(4-vinylbenzyl)-4,5-dihydro-1,3-oxazol-2-amine) (Aza-f, 85% w:v), was synthetized in accordance with reports by Reiser et al. [34–36].

2.2. Synthesis of $[Zn(Aza\text{-}f)(Enr)]NO_3{\cdot}H_2O$

The complex $[Zn(Aza\text{-}f)(Enr)]NO_3{\cdot}H_2O$ was synthesized based the following procedure: 200 μL of Enr (0.2028 mmol, 74.4 mg) solution is prepared by using NaOH 1 M as solvent (solution A). After, a solution of Aza-f (0.2028 mmol, 85 mg) in 10 mL of CH_3CN/H_2O (70:30, v:v) is prepared (solution B) and then, solution B was added on solution A, mixing during 5 min. Subsequently, a solution of $Zn(NO_3)_2{\cdot}6H_2O$ (0.2028 mmol, 61 mg) was added to the last mixture. Finally a white powder was obtained.

2.3. Characterization of $[Zn(Aza\text{-}f)(Enr)]NO_3{\cdot}H_2O$

IR spectra were recorded with a Perkin Elmer spectrophotometer. Solid ^{13}C high performance decoupling magic angle spinning nuclear magnetic resonance (HPDEC MAS NMR) was carried out with NMR Brucker Avance-II 300 spectrometer operating at 75.46 MHz. The characterization of the Com solution was done using a Varian NMR, 300 MHz in $CH_3OH\text{-}d_4$ solution. The elemental analysis of the Com was done using Fisons EA1108.

2.4. Preparation of molecularly imprinted polymer with metallic center (MIPc), and Enr (MIPe) and non-imprinted polymer (NIP)

The procedure for the preparation of MIPs or NIP was adapted from the method of precipitation polymerization reported by Ye et al. [37]. AA (0.81 mmol, 48 mg), EGDMA (4.056 mmol, 820 mg) and AIBN (0.21 mmol, 35 mg) were mixed together in 12 mL of CH_3OH. The template molecule was different for each polymer; MIPc, (Com, 232.7 mg, 0.2028 mmol); MIPe, (Enr, 74.4 mg, 0.2028 mmol); and, NIP, without template molecule.

The reaction mixture was purged with N_2 and sonicated for 10 min in a sealed glass tube. Then, the tube was placed in a 10 °C water bath. The polymerization reaction was initiated with a mercury lamp at $\lambda = 365$ nm, and maintained for 24 h. Afterwards, all the polymers were washed in a Soxhlet apparatus with two solvents: CH_3OH and CH_2Cl_2 for 12 h with each solvent.

Additionally, 4.0 g of MIPc was washed with 50 mL of 0.2 M NaCN solution for 12 h, and then rinsed with water and EtOH several times. Finally, the metallic center was regenerated with 25 mL of aqueous solution 0.06 M $Zn(NO_3)_2$, $6H_2O$. The synthesis scheme is illustrated in Fig. 1.

2.5. Characterization of the MIPc, MIPe and NIP

IR spectra were recorded with a Perkin Elmer spectrophotometer. Solid ^{13}C high performance decoupling magic angle spinning nuclear magnetic resonance (HPDEC MAS NMR) was carried out with NMR Brucker Avance-II 300 spectrometer operating at 75.46 MHz. The polymer morphology was studied by using a JEOL-5900LV scanning electron microscope. The samples of polymers were Au sputter-coated for 40 s at 20 mA. The SEM micrographs were acquired with a SEI (detector of secondary electrons) using an acceleration voltage of 20 kV or 10 kV. The elemental analysis of polymers was done using a Fisons EA1108.

2.6. Evaluation of binding properties of the polymers

Kinetic studies were performed to determine the time to attain the binding equilibrium. 10 mg of polymer was added to 1.125 mL CH_3OH solution of Enr (3.7×10^{-4} M). The vial was sealed and maintained at 25 °C in a controlled water bath. The drug adsorption at different times (0–300 min) was determined by UV-spectrophotometry at 280 nm.

In order to determine the binding capacity of MIPs in protic solvents, equilibrium measurement were made using UV-spectroscopy. The experiment was carried out by adding 10 mg of MIPs or NIP in a glass tube containing 1.125 mL of Enr solution with