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Iron removal from human plasma based on molecular recognition using imprinted beads

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Abstract

The aim of this study is to prepare ion-imprinted polymers which can be used for the selective removal of Fe^{3+} ions from Fe^{3+}-overdosed human plasma. *N*-Methacryloly-(L)-glutamic acid (MAGA) was chosen as the complexing monomer. In the first step, Fe^{3+} was complexed with MAGA and the Fe^{3+}-imprinted poly(HEMA–MAGA) beads were synthesized by suspension polymerization. After that, the template (i.e., Fe^{3+} ions) was removed using 0.1 M EDTA solution. The specific surface area of the Fe^{3+}-imprinted poly(HEMA–MAGA) beads was found to be 76.4 m^2/g with a size range of 63–140 μm in diameter and the swelling ratio was 75%. According to the elemental analysis results, the beads contained 84.7 μmol MAGA/g polymer. The maximum adsorption capacity was 92.6 μmol Fe^{3+}/g beads. The relative selectivity coefficients of imprinted beads for Fe^{3+}/Zn^{2+} and Fe^{3+}/Cr^{3+} were 17.3 and 48.6 times greater than non-imprinted matrix, respectively. The Fe^{3+}-imprinted poly(HEMA–MAGA) beads could be used many times without decreasing their adsorption capacities significantly.

Keywords: Molecular imprinting; Molecular recognition; Iron removal; Metal detoxification; Affinity binding

1. Introduction

Molecular imprinting is a technology to create recognition sites in a macromolecular matrix using a molecular template [1]. In other words, both the shape image of the target and alignment of the functional moieties to interact with those in the target, are memorized in the macromolecular matrix for the recognition or separation of the target during formation of the polymeric materials themselves [2]. Molecularly imprinted polymers (MIP) are easy to prepare, stable, inexpensive and capable of molecular recognition. Therefore, MIPs can be considered as artificial affinity media. Molecular recognition-based separation techniques have received much attention in various fields because of their high selectivity for target molecules. Three steps are involved in the ion-imprinting process: (i) complexation of template (i.e., metal ions) to a polymerizable ligand, (ii) polymerization of this complex and (iii) removal of template after polymerization. In the ion-imprinting process, the selectivity of a polymeric adsorbent is based on the specificity of the ligand, on the coordination geometry and coordination number of the ions, on their charges and sizes [3–8]. Numerous studies describing such methodology were carried out in order to adsorb metal ions [9–15].

Iron is an essential trace element for almost all organisms [16]. The toxic effects of iron overload are well known especially since the human body has no physiological route for the elimination of excess iron [17]. Chronic iron overload can be caused by a genetic defect, certain types of anemia, by accidental ingestion, repeated blood transfusion, inhalation of tobacco smoke or asbestos, over medication with iron supplements or iron pills

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prescribed by a physician [18]. In acute iron overload, the iron transport proteins are overwhelmed and unbound toxic iron (in excess of the total iron binding capacity) is created, generating highly reactive free oxygen radicals causing lipid peroxidation and cell membrane damage. In chronic iron overload a small non-specific iron pool exists, but most of the iron is deposited in the organs, especially in the spleen, liver and heart, causing widespread organ damage [19].

For transfusional iron overload and acute iron poisoning, the only available supportive treatment is chelation therapy and the only available clinical drug for this treatment is desferrioxamine B (DFO) a linear hydroxamate, a natural siderophore [20]. The use of DFO has already been shown to result in prolonged life expectancy, reduced liver iron and the establishment of negative iron balance. However, the major limitation to the use of DFO is its lack of effectiveness when administered orally, the short half-life time in plasma and its potential toxicity when present in high concentrations [21]. DFO is highly expensive also. For this reason a number of orally active iron chelators are being tested but none of them are still satisfactory [22–24]. To overcome the drawbacks of soluble iron chelators in the treatment of iron overload, attachment of iron chelating ligands has been studied. Comparing to soluble iron chelators, iron chelating resins might have advantages in the stability, reusability and minimal damage to biological substances. Recently, one of the most promising technique for blood detoxification is extracorporeal affinity adsorption. So far only a few affinity adsorbents were reported in literature [25–28]. But, selectivity still remains a great problem in these systems.

In this study, ion-imprinted polymer beads were used for the selective removal of Fe^{3+} ions from human plasma. *N*-Methacryloly-(L)-glutamic acid (MAGA) was used as the metal complexing monomer. Usually, molecularly imprinted polymers are prepared by the bulk polymerization method. The disadvantage of this method is that the obtained block polymer should be crushed, ground and sieved to produce packing materials. In this study, Fe^{3+}-imprinted poly(hydroxyethyl methacrylate-*N*-methacryloly-(L)-glutamic acid) beads were produced by suspension polymerization. Poly(2-hydroxyethyl methacrylate) (PHEMA) was selected as the basic matrix by considering properties which make it useful for possible extracorporeal therapy, including hydrophilic character, good blood-compatibility, minimal non-specific protein interactions, high chemical and mechanical stability and resistance toward microbial and enzymatic attacks [29–32]. After removal of Fe^{3+} ions, ion-imprinted beads were used for the separation of iron from human plasma. Fe^{3+} adsorption and selectivity studies of iron versus other metal ions which are Zn^{2+} and Cr^{3+} are reported here. Finally, repeated use of the ion-imprinted beads is also discussed.

2. Experimental

2.1. Materials

Hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) were obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. L-Glutamic acid hydrochloride and methacryloyl chloride were supplied by Sigma (St. Louis, USA). Benzoyl peroxide (BPO) was obtained from Fluka (Switzerland). Poly(vinyl alcohol) (PVAL; MW: 100.000, 98% hydrolyzed) was supplied by Aldrich Chem. Co. (USA). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). All water used in the adsorption experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed-bed system.

2.2. Synthesis of *N*-methacryloly-(L)-glutamic acid

Details of the preparation and characterization of the *N*-methacryloly-(L)-glutamic acid (MAGA) was reported elsewhere [33]. Briefly, the following experimental procedure was applied for the synthesis of MAGA monomer: 5.0 g of L-glutamic acid hydrochloride and 0.2 g of hydroquinone were dissolved in 100 mL of dichloromethane. This solution was cooled down to 0 °C. Then, 13.0 g triethylamine was added to the solution and 4.0 mL of methacryloyl chloride was poured slowly into this solution under nitrogen atmosphere. This solution was stirred magnetically at room temperature for 2 h. At the end of the chemical reaction period, unreacted methacryloyl chloride was extracted with 10% NaOH solution. The aqueous phase was evaporated in a rotary evaporator and residue (i.e., MAGA) was dissolved in ethanol.

2.3. Preparation of Fe^{3+}–MAGA complex

In order to prepare MAGA–Fe^{3+} complex, solid *N*-methacryloly-(L)-glutamic acid (MAGA) (2.0 mmol) was added slowly into 15 mL of ethanol–water mixture (50/50 v/v) and then treated with iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) (1.0 mmol) at room temperature with continuous stirring for 3 h. Then, the formed metal–monomer complex was filtered, washed with 99% ethanol (250 ml), and dried in a vacuum oven at 50 °C for 24 h (pressure: 100 mmHg).

2.4. Preparation of Fe^{3+}-imprinted poly(HEMA–MAGA) beads

Suspension polymerization method was used for the preparation of spherical poly(HEMA–MAGA) beads. A typical preparation procedure is described below. Continu-