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Preparation of a novel molecularly imprinted polymer by the sol–gel process for sensing creatinine

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ABSTRACT

A novel molecularly imprinted polymer (MIP) was prepared and used as an artificial receptor for creatinine (Cre). A sol–gel process was used to prepare the MIP. Tetraethoxysilane (TEOS) was employed as the crosslinker for the formation of a silica matrix for the MIP. Aluminum ion (Al^{3+}) was chosen as the dopant to generate Lewis acid sites in the silica matrix for interactions with Cre. Through the sol–gel process, a polymeric matrix with memory sites for Cre was obtained, and this is mentioned here as the molecularly imprinted polymer for creatinine (MIP_{Cre}). The imprinting efficiency of MIP_{Cre} was evaluated by contrasting the adsorbed amount of Cre by MIP_{Cre} with that by the corresponding non-imprinted polymer (NIP). Creatine (Cn), N-hydroxysuccinimide (NHS), and L-tyrosine (L-tyr) were selected as interferences to study the selectivity of the MIP_{Cre}. The interference studies were also conducted using binary mixtures, such as Cre/Cn, Cre/NHS, and Cre/L-tyr. All these studies reveal that the MIP_{Cre} possess a remarkable affinity for Cre. The crucial role of Al^{3+} in this system is discussed in detail. Furthermore, the effects of concentrations of Al^{3+} and TEOS on the adsorbed amount of Cre by MIP_{Cre} were also investigated.

1. Introduction

Creatine (Cn) is a physiologically essential nitrogenous compound that represents the energy source for muscle tissues. Cn can be synthesized endogenously and stored in skeletal muscle in the form of phosphocreatine (PCn), which maintains high adenosine triphosphate (ATP) levels by losing one phosphate ion rapidly and then reconverting adenosine diphosphate (ADP) back to ATP [1]. During muscle contraction, Cn and PCn are spontaneously converted to creatinine (Cre), a waste product removed from human body by renal excretion at a relatively constant rate [2]. Cre serves as a clinically important index for assessing renal and muscular functions, because of its relatively stable concentration in human serum, compared with the concentration of blood urea nitrogen (BUN). The reference range of Cre in human serum is 0.7–1.5 mg dL^{-1} (*ca.* 60–130 μM) for a male and 0.6–1.4 mg dL^{-1} (*ca.* 50–120 μM) for a female. High Cre level may indicate acute or chronic renal failure, urinary tract obstruction, and glomerulonephritis, while low level may indicate muscular dystrophy and myasthenia [3].

Developing reliable methods for monitoring the concentration of Cre is a task worthy of endeavors, and indeed has been pursued for decades. The methods often adopted are based on Jaffé reaction, in which Cre reacts with picric acid in an alkaline solution and forms a red–yellow complex [4]. This reaction, however, is subject to interference; substances such as acetoacetate, ascorbic acid, glucose, and uric acid are found to interfere with the results. Lloyd's reagent, *i.e.*, aluminum magnesium silicate clay, has been used to selectively adsorb Cre; nevertheless, substances such as pyruvate and indole are the interferences in this case [5]. To enhance the specificity, enzymatic methods are often used [6–11]. The most popular among them is the three-enzyme method, in which creatinine amidohydrolase (CA), creatine amidinohydrolase (CI), and sarcosine oxidase (SO) can be employed to catalyze the hydrolysis of Cre to hydrogen peroxide (H_2O_2), which can then be detected amperometrically [6]. Besides, creatinine iminohydrolase (CIH) was utilized to catalyze the hydrolysis of Cre to ammonia (NH_3); the NH_3 was then detected by potentiometry [9]. Although enzymatic methods are much more specific to Cre, they have some drawbacks, *e.g.*, they are expensive, suffer from instability, and involve complex procedures for immobilization.

There have been very active researches on designing and synthesizing molecularly imprinted polymers (MIP) for molecules of interest (templates). There are generally three steps for preparing a MIP. A functional monomer initially forms a stable complex with the template, and then polymerization proceeds in the presence of a crosslinker and an initiator to form a polymeric matrix. Finally, the template is removed by an adequate solvent to create binding sites that are complementary in size and shape to the template [12,13]. In this way, molecular memory is imparted to the synthetic polymer.

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Table 1
Summary of the reported building blocks for Cre imprinted polymers and the corresponding detection methods.

Building blocks	Detection methods	References
MAA, NVP, HEMA, EGDMA	HPLC and UV–vis spectrophotometer	[14]
Zinc(II) cyclen, EGDMA	HPLC and UV–vis spectrophotometer	[15]
β-CD, EPI	HPLC and UV–vis spectrophotometer	[16,17]
4-Vpy, DVB	HPLC and UV–vis spectrophotometer	[17,18]
EVAL	HPLC and UV–vis spectrophotometer	[19]
EVAL	CV	[20]
Mel, Chl	DPCSV	[21]
AMPS, MBA	Capacitive sensing	[22]
MAA, OPA, EGDMA	Fluorescent sensing	[23]
MAA, 4-MAANI, EGDMA	Fluorescent sensing	[24]
Mel, Chl, SiO_2	DPCSV	[25]
Mel, Chl, TEOS	DPCSV	[26]
AMPS, TEOS	HPLC and UV–vis spectrophotometer	[27]
MAA, MPS, MAA, EGGE, SiO_2	HPLC and UV–vis spectrophotometer	[28]

Notes: AMPS: acrylamido-(2-methyl)-propane sulfonic acid, Chl: chloranil, CV: cyclic voltammetry, DPCSV: differential pulse stripping voltammetry, DVB: divinylbenzene, EGDMA: ethylene glycol dimethacrylate, EGGE: ethylene glycol diglycidyl ether, EPI: epichlorohydrin, EVAL: poly(ethylene-co-vinyl alcohol), HEMA: 2-hydroxyethyl methacrylate, HPLC: high performance liquid chromatography, MAA: methacrylic acid, MBA: N,N-methylenediacrylamide, Mel: melamine, MPS: γ-methacryloyl trimethoxysilane, NVP: N-vinylpyrrolidone, OPA: o-phthalic dialdehyde, TEOS: tetraethoxysilane, UV: ultra-violet, β-CD: β-cyclodextrin, 4-MAANI: 4-methylamino-N-allylnaphthalimide, 4-Vpy: 4-vinylpyridine.

Preparing imprinted polymers for Cre for its determination was initiated by Sreenivasan and Sivakumar in 1997 [14]. Several investigations were carried out, after this work, on the preparation of imprinted polymers for Cre. Table 1 summarizes the building blocks and the detection methods [14–28]. It can be noticed that most of the imprinted polymers for Cre are based on organic compounds. Very recently, a few groups have attempted to prepare hybrid imprinted polymers for Cre, by grafting organic functional monomers onto the surface of inorganic silica [25–28]. As to the methods for detection, high performance liquid chromatography (HPLC) along with ultra-violet–visible (UV–vis) spectrometer is found to be a favorable tool, because it can analyze Cre and interferences in a separated manner [14–19,27,28]. Moreover, detection of electrochemical or fluorescent signal change that represents the adsorbed amount of Cre by its imprinted polymer was also reported [20–26].

Sol–gel process is widely used in the preparation of silica. It includes two fundamental steps: hydrolysis of silane to give silanols (Si–OH) in the presence of an acidic or basic catalyst, and then condensation of the silanols to give a polysiloxane (. . .–Si–O–Si–. . .) network [29,30]. Sol–gel process has become more and more popular in the preparation of MIP, on account of its simple work and mild reaction temperature. Morihara and co-workers [31] reported about the molecular imprinting on a surface of aluminum ion (Al^{3+}) doped silica gel, which was prepared by activating the silica gel with concentrated hydrochloric acid, and then by soaking the activated silica gel in Al^{3+} solution to dope Al^{3+}; subsequently, the doped silica gel was contacted with the target for the imprinting procedure. We have reported a molecularly imprinted silica alumina gel for the recognition of dopamine (DA) [32]. In that study, tetraethoxysilane (TEOS) was hydrolyzed and mixed with Al^{3+} (through the reagent, aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$)) and DA to obtain a homogeneous solution. Subsequently, a condensation reaction was carried out for generating Lewis acid sites in the silica matrix. Although the proposed process was not exactly the same as that reported by Morihara's group, the prepared silica alumina gel possessed good recognition ability toward the target, DA.

In this research, a molecularly imprinted polymer for creatinine (MIP_{Cre}) was synthesized by a sol–gel process. Al^{3+} (from the reagent $AlCl_3{\cdot}6H_2O$) and tetraethoxysilane were chosen as dopant and crosslinker, respectively. Imprinting efficiencies of MIP_{Cre} were evaluated in Cre adsorption experiments. Cn, N-hydroxysuccinimide (NHS), and L-tyrosine (L-tyr) were selected as the interferences for assessing the selectivity of the MIP_{Cre} in aqueous solutions of both single and binary components. Since the application of Al^{3+} as the dopant for the preparation of the MIP_{Cre} was not reported earlier, the crucial role it plays is studied in detail. Moreover, the effects of concentrations of Al^{3+} and TEOS on the adsorbed amount of Cre by MIP_{Cre} were also investigated.

2. Experimental

2.1. Materials

Creatinine, creatine, and L-tyrosine were purchased from Sigma–Aldrich, Inc. (St. Louis, Missouri, USA). N-hydroxysuccinimide was purchased from Fluka, Inc. (Buchs, Switzerland). Aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$) and hydrochloric acid (HCl) were obtained from Riedel-deHaën, Inc. (Seelze, Germany). Tetraethoxysilane was obtained from Showa, Inc. (Tokyo, Japan). Methanol was obtained from Tedia, Inc. (Fairfield, Ohio, USA). Deionized water with a resistance of ≧18.2 MΩ was produced by Purelab Maxima (ELGA, UK). Deionized water (pH value *ca.* 6.5) was used throughout the experiments. All the chemicals were used as received without further treatment.

2.2. Preparation of the molecularly imprinted polymer for Cre

Schematic representation of preparation of the MIP_{Cre} is illustrated in Fig. 1. At first, the precursor for inorganic silica, TEOS (1 mL, 4.48 mmol) and the catalyst, HCl (437 μL, 1 M) were added into 2 mL of deionized water and the solution was stirred to achieve complete hydration. The pH value was *ca.* 0.9. The template, Cre (50.6 mg, 0.45 mmol) and the source of Al^{3+}, $AlCl_3{\cdot}6H_2O$ (324.8 mg, 1.35 mmol) were then added to the stirred solution. The final molar ratio of Cre:Al^{3+}:TEOS in the solution was 1:3:10. The final pH value was *ca.* 1.1. After persistently stirring for 4 h, the homogeneous solution was incubated at 60 °C for 1 day. The gel monolith obtained was crushed and washed with deionized water for 6 times to extract Cre. The extracted amount of Cre was measured by UV–vis spectrophotometer (Jasco, V-670), and the detection wavelength was 233 nm. Finally, the gel was rinsed with methanol, dried at 85 °C, and ground into powders. The corresponding non-imprinted polymer was synthesized in the same manner except for adding Cre. NIP was also washed with deionized water for 6 times.

2.3. Measurement of Al^{3+} in the extraction solutions of Cre

When needed, the concentration of Al^{3+} in each extraction solution was detected by inductively coupled plasma optical emission spectrum (ICP-OES, PerkinElmer, Optima 3100XL).

2.4. Determination of suitable adsorption time

The adsorption kinetics of Cre by MIP_{Cre} was studied as follows: 10 mL of a 100 μM solution of Cre at pH *ca.* 6.6 was contacted with 10 mg of MIP_{Cre}, and the contents were shaken well. The decrease in Cre in the solution as a function of time was detected by UV–vis spectrophotometer. By plotting the adsorbed amount of Cre by MIP_{Cre} versus time, an adsorption kinetic curve could be obtained, and the suitable adsorption time could be determined.