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Potentiometric sensor fabrication having 2D sarcosine memories and analytical features

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ABSTRACT

In this study, a simple, rapid and sensitive method based on novel molecular imprinted polymeric sensor has been developed and validated for the determination of prostate cancer metabolite biomarker. The molecularly imprinted polymer (MIP) has been synthesized by emulsion polymerization, using sarcosine as template molecule, methacryloylamido histidine (MAH) as functional monomer and ethylene glycol dimethacrylate (EDMA) as cross-linking agent. The performance of the developed sarcosine sensor has been evaluated, and the results have indicated that a sensitive potentiometric sensor has been fabricated. The sarcosine sensor has showed high-selectivity, shorter response time (<2 min), wider linear range (10^{-2}–10^{-6} mM), lower detection limit (1.35×10^{-7} mM), and satisfactory long-term stability (>5.5 months).

1. Introduction

Prostate cancer is an important cause of mortality and morbidity in the world and is the most common noncutaneous malignancy in men [1].

Major interest in the potential application of metabolomics in prostate cancer was resulted in a recent work by Sreekumar et al. [2], who reported an unbiased metabolomic profiling method using LC/GC–MS to identify six metabolites including sarcosine, uracil, kynurenine, glycerol-3-sarcosine, leucine, and proline. They found that the metabolites levels increased with cancer progression. Among these metabolites, sarcosine (a methylated derivative of glycine) was introduced as a potential marker for prostate cancer aggressiveness in a small metabolomic study.

Sarcosine determination in urine has been carried out using different approaches mainly based on mass-spectrometric detection both using GC and LC as separation techniques [3–5]. Very recently, Biavardi et al. [6] reported a supramolecular approach for the specific detection of sarcosine on the basis of design of a sarcosine detection chip. Moreover, Burton et al. [7] presented a fluorimetric technique for the determination of sarcosine in urine. However, these techniques have been found to have practical limitations with respect to cost, efficiency, instrumental complexity, and manipulation. Therefore, an innovative method for sarcosine determination is necessary.

Electrochemical sensors in the analysis of biological sample have been developed due to their simplicity, reasonable accuracy and precision, low cost, and rapidity [8–14]. There is no need for derivatization or time-consuming extraction steps in comparison with other techniques because electroanalytical methods are less sensitive to the matrix effects. Potentiometric sensors offer several advantages, such as ease of preparation and procedures, simple instrumentation, relatively fast response, wide dynamic range, reasonable selectivity and low cost [15]. Nowadays, conventional potentiometric carbon paste ion selective electrodes are highly selective, highly sensitive, and of low detection limit. The operation mechanism of such chemically modified carbon paste electrodes (CMCPEs) depends on the properties of the modifier materials used to import selectivity and sensitivity towards the target species. Initially, non-conductive reagents, such as mineral oil or paraffin oil were used as binders, but their electrochemical capability was poor due to their low conductivity [16].

Potentiometric sensors are generic and highly successful approaches for chemical sensing, and molecularly imprinted polymer (MIP) based potentiometric sensors have shown to be very promising [17]. Unlike sensors based other transduction techniques, potentiometric sensors do not require the template molecules to diffuse through the electrode membranes for generation of membrane potentials [17], thus dramatically reducing the response time. However, reports on MIP based potentiometric sensors are still rather rare [18–21].

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Molecular imprinting is effective in encoding molecular information (shape, size and functional groups orientation) in bulk materials [22,23]. The synthesis of molecularly imprinted polymers (MIPs) involves the formation of a complex between a template molecule and the functional monomers in an appropriate solvent. After polymerization, the template molecule is removed, leaving microcavities that are complementary to the template molecule [24–27]. Great efforts have been directed to apply the molecular recognition capability of MIPs to biosensors [28,29], antibody and enzyme mimics [30,31], chiral separation [32] and solid phase extraction [33,34].

To the best of our knowledge, there is no report about MIP based potentiometric sensor to monitor sarcosine. We reported that imprinted polymer for sarcosine was prepared with methacryloylamido histidine (MAH) as the functional monomer and the polymers showed high affinity and selectivity for sarcosine. The histidine group residues originating from the functional monomer, could bind to the carboxyl group of sarcosine through electrostatic interaction, and the suitable binding sites complementary to sarcosine could be constructed by molecular imprinting. In the current work, a simple, rapid, selective, and efficient quantitative method was developed for the determination of sarcosine.

2. Experimental

2.1. Chemicals

Dibutyl phthalate, (DBP), was supplied from Aldrich Chemical (USA). Methacryloyl chloride was obtained from Sigma Chemical Co. (St. Louis, USA). Ethylene glycol dimethacrylate (EDMA) and 2,2-azobisisobutyronitrile (AIBN) were purchased from Fluka A.G. (Buchs, Switzerland). KBr (IR Grade), HNO_3, NaOH and other all chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Apparatus

Mettler Toledo Seven Multi pH-ion meter was used to measure pH values at 25.0 ± 0.1 °C and ion concentration. Fourier Transform Infrared (FTIR) spectroscopy (FTIR 100 series, Perkin Elmer, USA) was used in the 4000–400 cm^{-1} range for the characterization of functional monomer, pre-organized complex, and imprinted beads in the solid state. The imprinted beads were characterized by Field Emission Scanning Electron Microscope (FESEM, ZEISS Ultraplus). SONOPULS HD 2070, BANDELIN, (Germany) model homogenizer and MPW-251, MPW Med-Instruments (Poland) centrifuge were used for homogenization and centrifuge processes, respectively.

2.3. Synthesis of sarcosine-imprinted polymer

Histidine-functional monomer, MAH, was synthesized according to the previously published procedure [35]. Sarcosine imprinted beads were prepared by two phase mini-emulsion polymerization method according to the previously published procedure [36]. Emulsion polymerization is an ideal method for making high-surface area small sized particles and its incorporation into the field of molecular imprinting made the production of novel 2D surface imprinting materials possible. Therefore, the organic phase, which contains 1.48 mmol MAH, 3.5 mmol EDMA, 80 μL of hexadecane and 30 mg AIBN, was prepared and then ultrasonicated for 1 min. The aqueous phase was prepared by dissolving of 0.5 mmol sarcosine and 38.5 mg SDS in 18 mL of water. The organic phase was slowly added to the aqueous phase. In order to obtain mini-emulsion, the mixture was homogenized at 25,000 rpm by a homogenizer. Polymerization was carried out for 24 h at 70 °C in a water bath. The sarcosine imprinted beads were washed three times for 5 h with water and water/ethyl alcohol mixtures in order to remove unreacted monomers, surfactant and initiator.

The imprinting of the sarcosine molecule can be explained by non-covalent interactions between the template and polymer matrix. This non-covalent interaction may the formation of electrostatic interactions and hydrogen bonding between the imidazole groups of MAH monomer and polar groups of sarcosine molecules. The template was extracted by incubating three times 2 h in methanol:acetic acid (4/1) and three times in pure methanol, followed by centrifugation steps. For each step, the solution was centrifuged at 30,000 rpm for 30 min; then, the beads were dispersed in fresh washing solution.

2.4. Preparation of sensor and emf measurements

The bare carbon paste was prepared by thoroughly mixing analytical grade graphite and DBP, in 65:35 (w/w %) ratio. The modified carbon paste was also prepared by mixing different percentages of graphite powder, DBP, and MIP (or non-imprinted polymer, NIP). This mixture was mixed in a mortar for at least 10 min for homogeneity. The paste was packed into an end of a Pyrex glass tube in which electrical contact was made with a copper rod that runs through the center of the electrode body. The electrode surface was polished using a butter paper to produce reproducible working surface.

The MIP based potentiometric sensor was conditioned for 3 days in a 1 mM sarcosine solution and then stirred in formaldehyde for 2 h to remove bound sarcosine. The measurements were carried out with saturated Ag/AgCl electrode (SCE) as reference electrode with the following cell assembly. The performance of each sensor was investigated by measuring its potential in sarcosine solutions prepared in the range of 1×10^{-2} to 1×10^{-7} mM by serial dilution of the 0.1 M stock solution at constant pH. The solutions were stirred and potential readings were recorded when they reached steady state values. The data were plotted as observed potential versus the logarithm of the sarcosine concentration.

2.5. Characterization of beads

The imprinted beads were characterized by FESEM. Also, FTIR spectroscopy was used in the range of 4000–400 cm^{-1} for the characterization of chemistry of sarcosine-imprinted polymer in the solid state. The beads (about 0.1 g) were thoroughly mixed with KBr (0.1 g), and pressed into a pellet and then, FTIR spectrum was recorded.

3. Results and discussion

3.1. Characterization

Sarcosine imprinted beads were prepared by two phase mini-emulsion polymerization method. The principle of the technique is that the polymerization is conducted in stable oil droplets in an aqueous dispersion. As seen in SEM photograph, each of the beads has spherical shape and size distribution is between 200 and 400 nm (Fig. 1a). SEM photographs showed that template removal process changed surface morphology of beads (Fig. 1b). As seen from FTIR spectra of sarcosine imprinted and non-imprinted (NIP) beads (Fig. 1c), specific bands (–CH band at 2957 cm^{-1}, carbonyl band at 1730 cm^{-1} and carboxylic acid band at 2958 cm^{-1}) for the polymeric structure have been detected. Both spectra indicated the presence of carbonyl groups. The interaction between template and monomer gave changeable peaks in the MIP spectrum, which showed CH stretching at 2957–2927 cm^{-1}. Also, the symmetric methylene stretching of C—H bonds mainly due to the alkyl chain were recorded at 2855 cm^{-1} for NIP. The broadening at 3400 cm^{-1} indicated that a hydrogen bonding interaction took place between the carboxyl groups from the amido groups.

3.2. Effect of pH

In order to investigate the effect of pH on the potential response of the sensor, the potentials were measured for a 10^{-4} mmol L^{-1} sarcosine solution having different pH values (2–8). The pH changes