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Gold nanoparticle mediated designing of non-hydrolytic sol–gel cross-linked metformin imprinted polymer network: A theoretical and experimental study

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ABSTRACT

A sensitive and selective electrochemical sensor based on molecularly imprinted polymers was developed for trace level detection of metformin—an antidiabetic drug. For the first time, we have applied non-hydrolytic sol–gel matrix as a cross-linking agent in the field of molecular imprinting. To create the sol–gel matrix and enhance the electro-conductivity of the proposed sensor citrate-capped gold nanoparticle were used. The morphologies and properties of the sensor were characterized by scanning electron microscopy, cyclic voltammetry, electron impedance spectroscopy, chronocoulometry and differential pulse voltammetry. Energy of the HOMO and LUMO orbitals and Mülliken's atomic charges of template molecule were also calculated using density functional theory utilizing B3LYP with 3-21G-basis set. The theoretical results allied to the diagnostic criteria of the cyclic voltammetry indicate that the metformin redox mechanism is associated to the irreversible oxidation process of metformin-imino-group to N-hydroxyimino-group. The results demonstrated that the prepared sensor had excellent selectivity and high sensitivity for metformin in the linear range from 0.02 to 80 ng ml^{-1} with a detection limit of 0.005 ng ml^{-1} ($S/N=3$). The sensor was also successfully employed to detect metformin in pharmaceutical sample.

1. Introduction

Metformin (N, N-dimethylimido dicarbonimidic diamide monohydrochloride) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with normal kidney function [1]. Metformin is the only anti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It helps in reduction of LDL cholesterol and triglyceride levels. In 2010, World Health Organization enlisted metformin among one of the only two oral antidiabetic drugs in the model list of essential medicines [2]. Therefore, metformin is an effective antidiabetic drug for the treatment of Type II diabetes. It is necessary and important to monitor the concentrations of the drug in blood plasma and their pharmacokinetics for the optimization of dose with accuracy.

Various analytical methods have been described for the measurement of metformin in biological fluids, including high performance liquid chromatography coupled with several detectors and extraction techniques [3–5], gas chromatography (GC) with electron-capture [6], solid phase extraction [7], fast Fourier continuous cyclic voltammetry [8], voltammetry [9–12], capillary electrophoresis (CE) [13] and conductometry [14]. But the major problem associated with the detection of this drug is the high polarity of molecule. So for the metformin analysis the GC methods require a complex and time-consuming derivatization procedure [15], while CE method use an ion-pair extraction to remove ionic substances from blood plasma samples [16]. The reported HPLC methods also suffer from several disadvantages, such as lack of sensitivity [17], complex extraction procedures [18,19], use of ultrafiltration, column-switching system [20], expensive instrumentation, running costs and long chromatographic time. Therefore, a very sensitive, selective, rapid, and cost-effective technique is required for the estimation of metformin in biological and pharmaceutical samples.

Electrochemical analysis is an excellent technique for the sensitive determination of drugs and related compounds in pharmaceutical samples and biological fluids [21]. Now-a-days the popularity of electrochemical techniques in the field of drug analysis is due to their simplicity, high sensitivity, low cost, and relatively short analysis time, although it lacks selectivity.

To improve selectivity and specificity during electrochemical analysis the most popular technique is synthetic molecular recognition, which is also termed as molecularly imprinted polymers (MIP) [22]. It is a polymer network created by the appropriate combination of monomer, template and cross-linker. After polymerization,

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the template molecule is removed from the polymer matrix, leaving behind a cavity specific to the shape and size of template molecule. The resultant MIPs exhibits high selectivity, excellent mechanical strength, durability to heat, acid and basic conditions and better engineering possibility than their biological counterparts.

Although the conventional imprinting protocol is simple and effective, there are several critical factors to overcome viz., uncontrollable random polymerization (resulting in the heterogeneity of the imprinted sites), and high cross-linking (causes slow diffusion and poor rebinding of template) [23]. In the same pursuit, we have tried to overcome all these problems of MIP by incorporation of gold nanoparticle (AuNP) and silica compound.

Till date, sol–gel materials are commonly applied either as adhesive material or in compact matrix preparation. However, in this paper, we have explored the brightest part of silica compounds i.e. in thin film preparation and as a cross-linking agent to strengthen the imprinted polymer network. For the preparation of sol–gel matrix, other than conventional hydrolytic route, non-hydrolytic sol–gel (NHSG) synthesis procedure is opted in this work. In aqueous systems, metal alkoxides are the most widely used precursors, and their chemical transformation into the oxide network involves hydrolysis and condensation reactions. In aqueous sol–gel processes the water molecules supply the oxygen for the formation of the oxide compound. In non-aqueous systems, where intrinsically no water is present, the question arises, where the oxygen for the metal oxide comes from. Analogous to the non-hydrolytic preparation of bulk metal oxide gels [24], the oxygen for nanoparticle formation is provided by the solvent (ethers, alcohols, ketones or aldehydes) or by the organic constituent of the precursor (alkoxides or acetylacetonates), herein citrate-capped AuNPs work as oxygen donor. Usually NPs are explored for their electronic, catalytic, and many more properties [25].

In this work, for the first time, we have utilized and explored dual behavior of AuNPs i.e. (1) as oxygen donor for hydrolysis and condensation of silane and (2) as enhancer for electro-conductivity. Our main aim in this work is to overcome the problem of difficult template washing from the compact imprinted polymer matrix by the combination of sol–gel synthesis and nanotechnology. Furthermore, the synthesized nanomaterial was used for the fabrication of electrochemical sensor capable of metformin detection in real sample matrix at trace level.

2. Experimental section

2.1. Reagents

Gold (III) chloride hydrate ($HAuCl_4$), tetraethyl orthosilicate (TEOS), sodium citrate, acrylic acid (AA), acrylamide (AM), 2,2′ azoisobutyronitrile (AIBN), ethylene glycol dimethyl bisacrylate (EGDMA), metformin and other interferents were purchased from Aldrich (Steinheim, Germany) and Fluka (Steinheim, Germany). Solvents, dimethyl sulphoxide (DMSO) and ethanol were procured from Spectrochem Pvt. Ltd. (Mumbai, India). Standard stock solution of metformin (5.0 mg mL^{-1}) and others were prepared by using distilled water. The analyzed pharmaceutical sample was Cetapin XR (500 mg) and was purchased from Sanofi Diabetes, India.

2.2. Apparatus

All the electrochemical analysis [cyclic voltammetry (CV), electron impedance spectroscopy (EIS), chronocoulometry and differential pulse voltammetry (DPV)] were performed on CH instrument (USA, model number 660 C), using a three electrode cell assembly consisted of a MIP-modified Pt electrode, a platinum wire, and an Ag/AgCl (3.0 M KCl) as working, counter, and reference electrodes, respectively. Morphological images of bare and modified electrode surfaces were recorded using a scanning electron microscope (SEM), Hitachi, model S-3400N. For the binding characterization IR analysis was carried out on Varian Fourier Transform Infrared [FTIR (USA)] spectrometers. For the AuNPs characterization UV analysis was performed using a Perkin Elmer Lambda 35 (Singapore) spectrophotometer. All the experiments were performed at room temperature (25 ± 1 °C) under ambient conditions.

2.3. Computational study

Gaussian 03 was used as a tool to study binding interaction between various monomers and template molecule. All the structures of monomer, template and monomer–template complex were drawn with the help of chemdraw ultra and gauss view 4.0 software. Firstly, all the structures were optimized using Hartree-Fock theory with 6-311G** basis set and were further optimized using the density functional theory (DFT) approach utilizing hybrid Becke three-parameter exchange-correlation functional (B3LYP) with 3-21G basis set. The binding energy of template–monomer complexes, ΔE, were calculated via following equation:

$$\Delta E = E(\text{template–monomer complex}) - E(\text{template}) - nE(\text{monomer}) \quad (1)$$

Based on the results obtained from computational study of monomer–template interaction final synthesis was performed. Because polymerization is occurred in solution, we must take into account the effect of solvent, during energy calculations because it leads to changes in energy and stability of the template–monomer complexes in solvent phase than gaseous phase. In this work, Polarizable Continuum Model (PCM) was applied to calculate the energy of complex. In this model solvent was taken as a uniform polarizable medium with a dielectric constant of ε, while the solute is placed in a suitably shaped cavity in the medium [26]. Herein, DMSO (dielectric constant, $\varepsilon = 46.7$) was selected for the modeling because it was used as the porogen during polymer preparation. Based on the results obtained from computational study of monomer–template interaction final synthesis was performed.

2.4. Synthesis of citrate capped-AuNPs

Citrate capped-AuNPs were synthesized using sodium citrate, following earlier reported method [27]. In brief, first of all, 250 μL of 1.0% $HAuCl_4$ was added in a 250 mL Erlenmeyer flask containing 25 mL of deionized water, and the mixture was heated with continuous stirring. To the boiling mixture, 750 μL of 1.0% sodium citrate was added followed by color changes from purple to blue and finally to wine red, indicating formation of citrate capped-AuNP. Once the AuNPs were formed, the solution was stirred for another 15 min (without heating) for the completion of the reaction. The colloidal gold solution then centrifuged at 1500 rpm and dried at room temperature to obtain citrate capped AuNPs. The characterization of AuNPs and their role in polymer synthesis are discussed in Supplementary material (SI-Section 1.1).

2.5. Synthesis of metformin imprinted polymer

Herein, various types of imprinted polymer were prepared using different concentrations of the capped-AuNPs, TEOS, monomer and template and are shown in Table 1. For the synthesis of optimized polymer (optimization of polymer composition is discussed in SI-Section 1.2), to separate solutions were prepared. Firstly, the template molecule (metformin, 0.1 mmol, 0.0129 g dissolved in 0.5 ml of DMSO) was mixed with the selected functional monomers acryl amide (0.1 mmol, 0.0071 g dissolved in 60 μL DMSO) and