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# A novel electrochemical nanocomposite imprinted sensor for the determination of lorazepam based on modified polypyrrole@sol-gel@gold nanoparticles/ pencil graphite electrode

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ABSTRACT

A new sensitive and selective imprinted electrochemical sensor was developed for lorazepam determination, which is based on a pencil graphite electrode (PGE) modified with one-step electropolymerization of the molecularly imprinted polymer (MIP) composed from polypyrrole (ppy), sol-gel, gold nanoparticles (AuNPs), and lorazepam. AuNPs were introduced into the polymer composite for the development of electrical response by facilitating charge transfer of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ which was used as an electrochemical active probe. The fabrication process of the sensor was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Several significant parameters controlling the performance of the MIP sensor were examined and optimized. Under the optimized condition calibration curve of the imprinted sensor has two linear concentration ranges from 0.2 to 2.0 nM and 2.0 to 20.0 nM, with the limit of detection (LOD) of 0.09 nM. The imprinted sensor has the advantages of high porous surface structure, ease of preparation, good reproducibility and repeatability, high selectivity and sensitivity. Furthermore, the proposed method was successfully intended for the determination of lorazepam in real samples (tablet, plasma, and urine).



## 1. Introduction

Lorazepam (7-chloro-5-(2-chlorophenyl)-1, 3-dihydro-3-hydroxy-2H-1, 4-benzodiazepine-2-one) belongs to a class of drugs known as benzodiazepines which act on the central nervous system to produce a calming effect. The benzodiazepines have become the most worldwide commonly prescribed medicines in the therapy of anxiety, insomnia, acute seizures, and convulsive attacks. However, high amounts of lorazepam in the body may cause in fatal cases of drug intoxication. Hence, the identification and quantification of lorazepam in biological fluids can be used for the bio-pharmacological, clinical, and toxicological studies of this drug. Lorazepam is widely metabolized to its inactive glucuronide conjugate; therefore a rapid, sensitive, and selective analytical method for the determination of lorazepam in biological fluids and pharmaceutical formulations is necessary [1–3]. Several analytical techniques have been reported for the determination of benzodiazepines, such as adsorptive stripping voltammetry [4,5], gas chromatography-tandem mass spectrometry [1], liquid chromatography [6,7], and ultraviolet spectrophotometry [3].

Electrochemical methods, especially electrochemical MIP based sensors, have attracted more attention in recent years for reasons of their recognition properties, simplicity, high sensitivity, good stability, high selectivity, low cost, fast response and real time detection [8–10]. MIP has been demonstrated as a powerful technique in designing and synthesizing some artificial receptor molecules which is based on the copolymerization of functional and cross-linking monomers in the presence of the template molecule [11]. Bulk polymerization MIPs have encountered many limitations including incomplete template removal, poor site accessibility for template molecules, slow interaction kinetics, low affinity, and heterogeneous nature of the binding sites [12,13]. To resolve these concerns, the use of thin layer molecular imprinted on the surface was suggested [14,15]. While traditional bulk polymerization has been widely used in MIPs preparation, the thin imprinted polymer can be directly synthesized on the electrode surface by electropolymerization. In comparison with conventional methods, electrochemical deposition offers a simple, reproducible, convenient, and friendly to environment method for the deposition of MIP film on the support surface. Moreover, the thickness, homogeneity, and morphology of deposited layer can be controlled by

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varying polymerization conditions (e.g. applied potential and cyclic scan) [10,12].

The sol-gel technology has been widely applied to improve the performance of MIPs film. Sol-gel processing provides a simple way to produce a rigid three dimensional silicate network, through efficient incorporation of organic-inorganic hybrid network. The sol-gel based MIPs possess some advantages, such as a simple fabrication process, low temperature reaction condition, and matrix porosity due to highly cross-linked structure [16,17]. Also, the sol-gel procedure replaced general toxic solvents (chloroform, toluene, and so on) that used in the conventional polymerization process with the eco-friendly solvent (water or ethanol). However, the electrical insulation property of sol-gel coatings is undoubtedly an undesirable property, in order to improve the electrical or electrochemical properties; the ppy, sol-gel, and Au nanoparticles nanocomposite (polypyrrole@sol-gel@gold nanoparticles MIP/PGE) was prepared.

Conducting polymers consist of a group of compounds (such as polypyrrole, polythiophene, and polyaniline) with very specific properties, which have penetrated many fields of electrochemical research. They can be synthesized under mild conditions which it is ideal for immobilizing of biomolecules into the polymer structure. Also, they have unique electronic properties, good stability, and can easily be deposited electrochemically on substrate [18–20]. Polypyrrole has become one of the most commonly used conductive polymers in design of sensors and biosensors due to their significant electrical conductivity, good biocompatibility, and facile polymerization by electrochemical or chemical methods [10,21].

AuNPs have been widely used in fabrication of different kinds of sensors due to their unique properties such as strong adsorption ability, large specific surface area, good biocompatibility and conductivity. The MIPs doped with AuNPs can be simply fabricated by some approaches including covalent linking, direct electrostatic assembly, polymer entrapment, and electrodeposition methods [22–25].

In the present paper, a simple approach is suggested to improve the electrical conduction from sol-gel layer to the electrode surface with the entrapment of ppy and AuNPs within sol-gel networks. The integration between AuNPs, conductive polymers, and sol-gel technology is a way to take most advantage of the applied materials. This polypyrrole@sol-gel@gold nanoparticles MIP/PGE sensor has the advantages of low cost, ease of preparation (one-step electropolymerization), more porous surface structure, higher functional groups and recognition capacity, and better conductivity. In addition, the MIP film properties and polarity can be designed through proper selection of the sol-gel precursor and conductive polymer derivatives. The experimental parameters that affect the performance of the MIP sensor were studied and optimized. The polypyrrole@sol-gel@gold nanoparticles MIP/PGE sensor was evaluated to verify its electrochemical properties such as conductivity, linearity, selectivity, stability, repeatability and reproducibility. Moreover, the developed sensor was applied for the analysis of the lorazepam in real samples.

## 2. Experimental

### 2.1. Materials

Tetraethoxysilane (TEOS), phenyltriethoxysilane (PTEOS), trifluoroacetic acid (TFA), sodium dodecyl sulfate (SDS), hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$), and all other solvents and salts were purchased from Merck (Darmstadt, Germany). Lorazepam was obtained from Zahravi Pharmaceutical Co. (Tabriz, Iran). Diazepam and Phenobarbital were purchased from Caspian Tamin Pharmaceutical Co. (Tehran, Iran). Ethanol was obtained from Bidestan Co. (Qazvin, Iran). Pyrrole (Merck, Darmstadt, Germany) was distillated and stored in a dark bottle in a refrigerator before use. Double distilled water was used in all experiments.

Lorazepam stock solution was prepared and stored at 4 °C prior to use. An intermediate standard solution was prepared weekly, by dilution of the stock standard solution. More diluted working solutions used in further experiments, were prepared daily by diluting different amounts of the intermediate standard solution with phosphate buffer (pH 7.0) to the required concentrations.

### 2.2. Instrumentation

Electrochemical measurements were performed using a Potentiostat-Galvanostat μ-AutoLab (Echo Chemie, B.V., Netherlands, NOVA software). A three-electrode system consisted of a KCl saturated Ag/AgCl electrode, a platinum rod, and a polypyrrole@sol-gel@gold nanoparticles MIP/PGE was used as the reference electrode, auxiliary electrode, and working electrode, respectively. Autolab system (PGSTAT 12, Eco Chemie B.V., Utrecht, Netherlands) was applied for the electrochemical impedance spectroscopic measurements. The system was performed on a PC by GPES and FRA 4.9 software.

The pencil graphite was available as pencil lead from Rotring Co. Ltd. (Germany, type 2B, 0.7 mm diameter). A mechanical pencil was used as a holder for graphite leads. Electrical contact with the lead was obtained by soldering a metal wire to the metal part of mechanical pencil. Metrohm pH meter (Model 827) with a glass electrode (Corning) was used to measure the pH value of the solution. An atomic force microscope (BRUKER, Germany) was used to consider AuNPs electrodeposited into the nanocomposite MIP.

### 2.3. Preparation of polypyrrole@sol-gel@gold nanoparticles MIP/PGE

Before the electropolymerization, the surface of the pencil lead was pretreated by applying a constant potential of +1.40 V for 300 s in 0.5 M acetate buffer (pH 4.8) containing 0.02 M sodium chloride.

A typical method to prepare nanocomposite film is introduced as follows. The sol solution was prepared using 75 μL of PTEOS (0.15 μM), 75 μL of TEOS (0.17 μM), 700 μL of water, 1100 μL of ethanol, 10 μL of TFA, and lorazepam (5.0 mM) in a vial. The solution was stirred at room temperature for 2 h. Instantly following, 20 μL of the pyrrole solution (final concentration of 10.0 mM), 5.0 mg of SDS (as the counter ion), and 50 μL of 0.01% $HAuCl_4$ solution (final concentration of $2.5 \times 10^{-4}$%) were added to the solution. The resulting solution was sonicated for 10 min. Then, the pretreated electrode was immersed in polymerization solution and the potential range of -0.80 and +0.60 V (versus Ag/AgCl) was applied, the polypyrrole@sol-gel@gold nanoparticles MIP film was prepared by electrodeposition using CV at the scan rate of 50 mV/s for seven cycles. After electrodeposition the modified electrode was dried at room temperature for 2 h. The lorazepam was extracted from the MIP film by immersion in methanol-acetic acid (50% v/v) solution with stirring magnetically for 30 min.

For the comparison purpose a sol-gel/AuNPs nanocomposite and a ppy/sol-gel/AuNPs layer-by-layer imprinted modified electrode was prepared. The non-imprinted polymer (NIP) electrode was prepared under the same experimental conditions but no template molecule was used.

### 2.4. Electroanalytical measurements

Considering that lorazepam is not an electroactive compound over the studied potential range, $[Fe(CN)_6]^{3-/4-}$ was chosen as the redox probe in the determination procedure. It is based on