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# Neuro-genetic multi-objective optimization and computer-aided design of pantoprazole molecularly imprinted polypyrrole sensor

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ABSTRACT

A molecularly imprinted polymer (MIP) of pantoprazole (PNZ) was prepared through electropolymerization of pyrrole on a functionalized multi-walled carbon nanotube modified pencil graphite electrode. The preparation of MIP and quantitative measurements were performed by cyclic voltammetry and differential pulse voltammetry (DPV), respectively. Several important parameters controlling the performance of polypyrrole film. The factors, i.e. pH of buffer solution, cyclic voltammetric scan rate in polymerization step, number of cyclic voltammetric scans, monomer and template concentrations in prepolymerization mixture, nanotube concentration in functionalized multi-walled carbon nanotubes-coating step, uptake time after MIP preparation and uptake step stirring rate were expected to affect MIP preparation and voltammetric measurements. The optimization of parameters was performed using Plackett–Burman design, central composite design, artificial neural network and genetic algorithm. The Pareto plot showed that effects of monomer concentration and pH are most important to the process. The best MIP to NIP response ratio was obtained 17.4. The selection of monomer was performed computationally using ab initio calculations. The calibration curve demonstrated linearity over a concentration range of 5–700 μM with a correlation coefficient ($r$) of 0.9980. The detection limit of PNZ was obtained $3.75 \times 10^{-7}$ M. The minimum and maximum recovery (%) through the spiking 0.1–0.4 mM PNZ to a biological and some pharmaceutical matrices were obtained 95.9% (human blood serum) and 106% (PNZ tablet), respectively.



## 1. Introduction

PNZ (6-(difluoromethoxy)-2-((3,4-dimethoxypyridin-2-yl)methylsulfinyl)-1H-benzo[d]imidazole) is a highly gastric potent proton pump inhibitor being introduced for the treatment of disorders of gastric acid hyper secretion, ulcer disease and relief of symptoms and healing of lesions in gastro esophageal reflux disease [1]. Due to the electrochemical activity of PNZ, some voltammetric techniques have been used to determine PNZ in pharmaceutical and biological samples [1–3].

Over the past two decades, MIPs have attracted a broad interest from researchers engaged in sensor development [4–7]. The general principal of molecular imprinting is based on such a process where functional monomers are copolymerized in the presence of a target analyte (the imprint molecule) which acts as a molecular template. On the other hand, molecular imprinting is a technique for the creation of highly efficient synthetic molecular receptors [8]. The polymeric films can be synthesized in situ at an electrode surface by electropolymerization technique [4–7,9]. This technique has some advantages e.g. the easy adherence of polymeric films to the surface of conducting electrodes of any shape and size and the ability to control thickness of films under different deposition conditions [9]. Various types of electrosynthesized polymers based on molecular imprinting have been reported in the literature including poly(o-phenylenediamine) [10] and polypyrrole [4–7,9,11,12]. Electropolymerization has been successfully utilized for the preparation of electroactive and electro-inactive polymers on a variety of conductive surfaces including platinum, indium tin oxide, oxidizable metals, stainless steel, zinc [13] and pencil graphite electrode (PGE) [4–7,9,11] as examples. PGE was applied in this work because of its advantages as a carbon electrode: low cost, wide potential window and chemical inertness [14] and its special features such as good mechanical rigidity, ease of modification and miniaturization [14] and disposability. Multi-walled carbon nanotubes

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(MWNTs), owing to their unique structures, high stabilities, low resistivity, and high surface-to-volume ratios, are extremely attractive in the field of electrochemical sensors because increasing the surface areas of the electrodes, enhance conductivity and facilitate the electron transfers [12].

The term "experimental design" comprises a series of procedures used to plan experiments, with several aims: (i) to understand which variables influence the performance of the investigated system; (ii) to model the effects of such factors, also taking into account their interactions; (iii) to optimize the experimental conditions and (iv) to reach these goals with a limited number of experiments [15]. The application of experimental design for the optimization of MIP was reported primarily by Davies et al. [16] and Navarro-Villoslada et al. [17] for the determination of sulfonamides and bisphenol A, respectively. In statistical-based approaches, response surface methodology (RSM) has been used to optimize MIP [7]. Multi-objective optimization has been used by Kunath et al. [18] for MIP design based on the desirability approach of Derringer and Suich [19] which illustrate how several response variables can be transformed into a desirability function, which can be optimized by univariate techniques [19]. However, one of the basic shortcomings of this approach is the subjectivity in the selection of the minimum and maximum acceptable values for each response. That is, improper values of minima and/or maxima may lead to inaccurate solutions for the optimum formulation [20].

Artificial neural networks (ANNs) are inspired by biological neural systems. In this approach weighted sum of inputs arriving at each neuron is passed through an activation function (generally nonlinear) to generate an output signal [21]. Genetic algorithms (GAs) in the broadest sense are model techniques used by simple biological systems. These biological systems use reproduction to produce offspring that can better survive in the current environment [22]. Solving multi-objective optimization problems using ANN-GA have been applied extensively [21,23–26]. To the best of our knowledge there is no report of electrochemical MIP sensor optimization of PNZ in the literature.

The development of a MIP still relies on many experiments, including the preparation of the media and the use of adsorption to evaluate the media. This makes the design and development of MIP time-consuming [27]. So, computational chemistry was used as well as reported before [4–7,27–30] to evaluate the best available functional monomer for the preparation of PNZ MIP sensor.

## 2. Experimental

### 2.1. Chemicals and reagents

Sodium perchlorate monohydrate (99–102%), boric acid (99.9999 Suprapur), acetic acid (99.5%), pyrrole (≥97%) and methanol (99.5%) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (98%, Lobachemie, Mumbai, India), 2-mercapto-1-methylimidazole (≥99%, Aldrich, Steinheim, Germany), Benzimidazole (98%, Aldrich, Steinheim, Germany), piroxicam (≥98%, Sigma–Aldrich, Steinheim, Germany), tetracycline hydrochloride (≥95%, Sigma–Aldrich, Steinheim, Germany), Acetaminophen (≥99%, Sigma–Aldrich, Steinheim, Germany), pantoprazole sodium sesquihydrate (Temad Co., Iran) and other reagents were commercially available and used without further purification. MWNTs were purchased from Research Institute of Petroleum Industry (Tehran, Iran). Stock solution of PNZ was prepared weekly in methanol and was stored refrigerator away from light. Freshly standard solutions of PNZ were prepared each day by diluting the stock solution of PNZ using locally made pure deionized water.

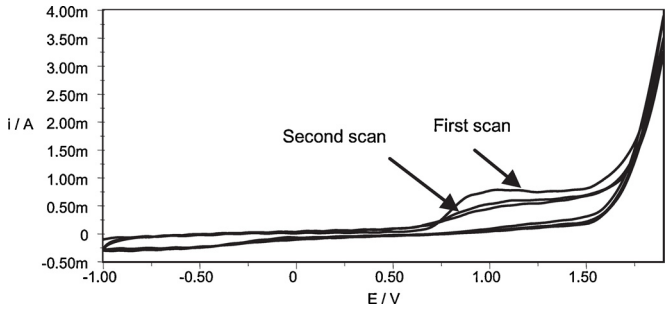


**Fig. 1.** Three CV cycles of MIP preparation at optimal conditions.

### 2.2. Instrumentation

Electrochemical studies were performed using Autolab PGSTAT 12 potentiostat-galvanostat controlled by GPES 4.9 software (Ecochemie, The Netherlands). A three-electrode system was used for all measurements; a PGE as the working electrode and a platinum auxiliary electrode. All measurements carried out with a silver/silver chloride reference electrode. A Metrohm 744 pH-meter (Metrohm, Switzerland) was employed for pH measurements. Sonication of functionalized multi-walled carbon nanotubes in water was performed using Hielscher (UP400S HIELSCHER, Germany) ultrasonic processor.

### 2.3. Hardware and software

All computations were carried out on an ASUS Notebook equipped with 16 GB of random access memory and Intel Core i7-2.2 GHz central processing unit. Molecular structures were drawn by ChemBioDraw Ultra and ChemBio3D Ultra 2008 (Cambridgesoft). Quantum calculations have been carried out using Gaussian 09 [31] software. The optimized 3 dimensional molecular structures were produced by Gauss View 5.0 (Gaussian, Inc.). Experimental design and analysis of the experiments were done using Minitab Release 14 Statistical Software (Minitab Inc.). Matlab R2009a (The Mathworks, Inc.) computing environment was applied to generate a neural network model from the experimental data and GA optimization.

### 2.4. Preparation of MIP and non-imprinted polymer (NIP) electrodes

PGE (Owner, HB, 0.7 mm diameter, Korea) was washed by distilled water and dried at room temperature before the experiments. The MWNTs (0.5 g) were oxidized with 60 ml of concentrated nitric acid solution at 100 °C for 12 h [7]. After cooling down to room temperature, the mixture was filtered and washed thoroughly with distilled water for several times till the pH of the final wash (filtrate) posed to be neutral. The filtered dried under vacuum to obtain carboxylic acid functionalized multi-walled carbon nanotubes (MWNTs-COOH). After sonication, a chronoamperometric method was employed to deposit the latter nanotubes onto the bare PGE surface using 1.7 V for 400 s [7,12] in a solution containing 1.0 g/l of functionalized multi-walled carbon nanotubes. The mentioned process resulted in fabrication of a MWNTs-COOH -modified PGE. Then, the modified PGE was immersed in the polymerization solution. The MIP was obtained by electrodeposition on the surface of modified PGE using cyclic voltammetry in the potential range between −1.0 and 1.9 V during 50 cycles (scan rate 200 mV/s) in an aqueous solution of 0.1 M $NaClO_4{\cdot}H_2O$, 0.00430 M pyrrole and $1.0 \times 10^{-4}$ M PNZ. The electrooxidation of pyrrole monomers was occurred at the anode, and the resulting polymer was deposited on the surface of PGE. Fig. 1 demonstrates three cycles obtained by