Synthesis of nano-sized timolol-imprinted polymer via ultrasonication assisted suspension polymerization in silicon oil and its use for the fabrication of timolol voltammetric sensor

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ABSTRACT

A novel timolol voltammetric sensor based on the nano-sized molecularly imprinted polymer (nano-MIP)-modified carbon paste electrode was introduced. Timolol-imprinted polymers (MIP) were synthesized by the ultrasonic assisted suspension polymerization in silicon oil. The MIP nanoparticles were then embedded in a carbon paste (CP) electrode in order to prepare the nano-MIP-CP electrode. Timolol was extracted in the electrode for a definite time and then it was analyzed by square wave voltammetry, found to be an effective determination method. The electrode showed higher response to timolol, compared to the CP electrode, and CP electrode modified with non-imprinted polymer (nano-NIP-CP). Various factors, known to affect the response behavior of the nano-MIP-CP electrode, were investigated and optimized. The sensor exhibited distinct linear response ranges of 1.0×10^{-7}–2.1×10^{-6} M with the sensitivity of 71.523 $\mu A\,\mu M^{-1}$. The lower detection limit of the sensor was calculated to be 2.3×10^{-8} M (S/N = 3). The sensor was applied successfully for timolol determination in pharmaceutical formulations, blood serum and urine samples.

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1. Introduction

Timolol (1-(*tert*-butylamino)-3-[(4-morpholin-4-yl-1, 2, 5-thiadiazol-3-yl)oxy]propan-2-ol) is a non-selective beta-adrenergic antagonist drug which could be used for treating of glaucoma, hypertension, heart attacks, and migraine headache [1]. Timolol is one of the most effective β-blockers for treating glaucoma that acts with reduces intraocular pressure [2]. Several analytical methods such as: high-performance liquid chromatography [3–7], gas chromatography [8], thin-layer chromatography [9] and spectrophotometry [10–13] have been applied for timolol determination in different samples. However, for the majority of these methods, some sample pretreatment involving separation, extraction and adsorption is generally necessary.

Electroanalytical methods were also used in determination of timolol [14–21]. These techniques are more simple, cheap and fast in comparison with the above mentioned methods and also there is no need for high quality expensive solvents. One of the preferred options is using common low price electrode materials (e.g. carbon paste) with some modifications. Modified electrodes are being used frequently in the voltammetric determination of organic compounds because of their effectiveness and selectivity.

Molecularly imprinted polymers (MIP's) are one of the most promising materials that are currently being implemented in sensor applications as recognition elements or modifying agents (instead of other common modifiers) [22]. The MIP is usually synthesized by polymerization of monomer mixture in the presence of template. This process results in the production of highly cross-linked polymer, containing artificially created receptors in the polymer which are relatively accessible for target molecules to be recognized. This recognition occurs due to cavity-shaped active sites that are complementary to the template, regarding its functional groups type and position as well as its molecular shape [23]. This biomimetic modifier functions as both selectivity inducing agent and pre-concentrator for the analyte determination. The sensors equipped with the MIP-based recognition element converts the analyte-MIP binding event into a physically readable signal in order to identify and quantify the target species [24–28].

To the best of our knowledge, there is not any published report on preparing timolol sensor based on a modified carbon paste electrode. So in this research, a new nano-sized MIP-modified carbon paste electrode was prepared for timolol determination. Nano-structured, imprinted materials have a small dimension with extremely high surface-to-volume ratio. Thus, the majority of imprinted sites are situated at the surface or in the proximity of surface. Therefore, the forms of imprinted materials are expected to greatly improve the binding capacity, affinity, rebinding kinetics and site accessibility of imprinted

materials [29–31]. In this work, the nanoparticles of molecularly imprinted polymer, containing recognition sites of timolol, was used to fabrication the modified carbon paste electrode that possessed some improved characteristics such as: simplicity in sensor preparation, high selectivity against the structurally similar molecules, a low detection limit (DL), high sensitivity and long term stability. The prepared electrode was used for timolol determination in pharmaceutical samples by a two-step procedure, including analyte extraction in the electrode and electrochemical measurement of timolol.

2. Experimental

2.1. Instruments and reagents

Electrochemical data were obtained using a potentiostat/galvanostat model PGSTAT302, Metrohm. The measurements were performed in a three-electrode system: working electrode (The MIP/NIP modified carbon paste electrodes), counter electrode (platinum) and reference electrode (Ag/AgCl). Zeta potential variation as a function of pH for the polymeric nanoparticles was measured using a zeta potential meter instrument (particlemetrix PMX 200C StabiSizer.)

Methacrylic acid (MAA), obtained from Sigma–Aldrich (Munich, Germany), was purified by passing it through a short column of neutral alumina, followed by distillation under reduced pressure. Ethylene glycol dimethacrylate (EDMA), obtained from Fluka (Buchs, Switzerland), was distilled under reduced pressure in the presence of a hydroquinone inhibitor and stored at 4 °C until use. Timolol and 2,2′-azobisisobutyronitrile (AIBN) and n-eicosane were supplied by Sigma–Aldrich (Munich, Germany) and used as received. Graphite powder was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of the nano-sized-timolol imprinted polymers

For the MIP synthesis, 1 mmol of timolol, 4 mmol of functional monomer (MAA) and 3 mL of acetonitrile were mixed together and kept motionless for 5 min. Subsequently, 16 mmol of cross-linker (EGDMA) and 0.2 mmol of initiator (AIBN) were added to the mixture. The solution was purged by N_2 for 5 min and then, it was dispersed in silicon oil at 800 rpm for 2 min. Afterwards, the new mixture was purged again with N_2 for 10 min. The flask, containing the mixture, was sealed and sonicated for 10 min (at 0 °C). This was done in order to break the polymerizable suspensions into the smaller droplets. The sealed flask was put in a water bath for 12 h, (fixed 65 °C). The synthesized polymer was washed several times with petroleum ether, to dissolve and remove silicon oil from the polymer. Then, the template and unreacted monomers were removed by washing of the polymer with chloroform and methanol respectively. For every washing step, the polymer was contacted with the aimed solvent for a definite time and centrifuged to separate the polymer nanoparticles from the solvent. Finally, the particles were dried in vacuum at 50 °C overnight. The non-imprinted polymer nanoparticles were prepared and treated with the same manner, but in the absence of timolol.

2.3. Preparation of the sensors

For the sensor preparation, 23 mg of MIP was added to 100 mg of graphite and homogenized in a mortar for 10 min. Subsequently, 40 mg of n-eicosane (used as binder) was melted at 45–50 °C. Then, the graphite/MIP blend was added to the melted binder and mixed with a stainless steel spatula. The final paste was used to fill a hole (2.00 mm in diameter, 3 mm in depth) at the end of an electrode body, previously heated at 45 °C. After cooling at room temperature, the excess of solidified material was removed by the aid of a paper sheet. The electrode can be reused after each experiment by polishing of the electrode surface on a paper in order to rub out a thin layer of the electrode surface.

2.4. General method for electrochemical measurements

Electrochemical measurement of timolol concentration was performed according to the following procedure:

Extraction step: The prepared carbon paste electrode was placed into the analyte solutions in which the pH was fixed by buffer solution. In the extraction step, all solutions were stirred at a fixed stirring rate and for a determined period of time.

Analyzing step: The electrode was then inserted into an electrochemical cell containing 20 mL of KCl solution (0.05 M) with determined pH and then the square wave voltammetry (SWV) technique was applied for determination of timolol. For all SWV experiments, the potential range of 0.5–1.3 V, amplitude of 150 mV and frequency of 50 Hz, was used.

2.5. The measurement of timolol in real samples

In order to determine the timolol in real samples, the prepared sensor was immersed into the spiked solution, adjusted pH to 6 by the use of NaOH solution. After incubation for 15 min, the electrode was transferred to the electrochemical cell containing 20 mL of KCl solution (0.05 M) as a supporting electrolyte, adjusted to pH of 8, using appropriate solution of NaOH. Then, square wave voltammetry was fulfilled by scanning of the potential from 0.5 to 1.3 V and the resulting maximum current response was recorded and used for quantitative measurement of timolol, with respect to the previously established calibration curve. The scanned potentials had amplitude of 150 mV and frequency of 50 Hz.

3. Results and discussion

3.1. Synthesis of nano-sized imprinted polymer

Fig. 1 illustrates the FT-IR spectra of the MIP and NIP. As can be seen, there is a complete similarity between the spectra of these materials, suggesting that the chemical structures of both polymeric materials are the same. This is expectable, since, in both MIP and NIP the same functional monomer and cross-linker agent are used. Furthermore, from the described similarity in the FT-IR spectra it can be deduced that template molecules are removed wholly from the MIP material as the washing steps.

The scanning electron microscopy (SEM) images of the prepared MIP and the relevant NIP are shown in Fig. 2(I) and (II), respectively. As can be seen, the nano-sized polymeric particles are obtained in both cases. This is because of using a well-designed suspension polymerization, in which both high speed mechanical mixing and ultrasonic
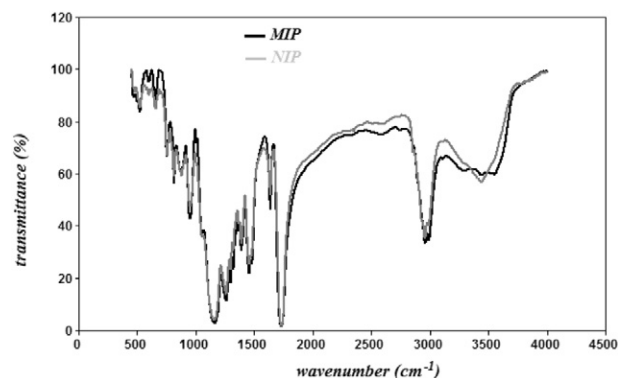

Fig. 1. FT-IR spectra of the MIP and NIP, prepared via ultrasonication-assisted suspension polymerization in silicon oil.