A nanogold supported inorganic/organic hybrid 3D sensor for electrochemical quantification of propranolol—effective antagonist of β -adrenergic receptors

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Abstract

The new hybrid inorganic/organic 3D nanogold electrode material composed of gold template, gold nanoparticles, 4-mercaptobutyric acid, and cysteamine has been prepared and for the first time used for electrochemical oxidation and quantitative determination of propranolol, an effective antagonist of β -adrenergic receptors, in the buffered solution and in the simulated body fluids. A high improvement in electrooxidation of propranolol as compared to that observed on the unmodified gold was detected. The prepared sensor was found to give reliable results in the propranolol concentration range between 0.0001 and 0.02 mM with the detection limit of 6.75×10^{-5} mM without any interference from biogenic species. A new inorganic/organic hybrid 3D nanogold sensor displays good sensitivity, repeatability, and stability which make it a promising tool for application both in clinical diagnosis and pharmaceutical analysis.

Keywords Nanogold hybrid material · Self-assembled layer · Voltammetric sensor · β -Adrenergic receptors blocking drug

Introduction

One of a commonly known and used of β -blocking drugs is propranolol (PROP)—an effective antagonist of β -adrenergic receptors. Indications for the use of propranolol include, among others, hypertension, angina pectoris, and cardiac arrhythmia, as well as prophylaxis of myocardial infarction. This drug also effectively eliminates some of the symptoms of neurosis and anxiety attacks. It may also support the treatment of hyperthyroidism. Moreover, it is also used as a remedy for various types of headaches, including migraine pain [1–4]. As a non-selective β -blocker, propranolol can be used to counteract overproduction of catechol amines: adrenaline and noradrenaline. It is often used to alleviate the trauma of

alcohol and drug withdrawal, as well as to relieve the stress associated with stressful events such as exam and public speech [5, 6]. Propranolol has been introduced on the list of prohibited drugs by the World Anti-Doping Agency (www.wada-ama.org/sites/default/files/prohibited_list_2018_en.pdf). Unfortunately, propranolol, like many other drugs, can cause serious side effects. Among them, one should mention: bradycardia, worsening of heart failure, hypotension, peripheral circulation disorder, feeling of bewilderment, mood changes, dizziness, nightmares, hallucinations, and even psychosis [4]. As described above, propranolol may be used for treatment of a wide spectrum of ailments; therefore, it can be easily overdosed. For this reason, the design of different methods for its detection and determination is an important challenge for scientific study. Different techniques have been proposed for the determination of propranolol, such as spectrophotometry [7–15], chemiluminescence [16–18], chromatography [19–21], colorimetry [22], and titrimetry [23, 24]. Although all these methods display good precision and accuracy, meanwhile from the analytical point of view, some of these methods are complex—have required several preparatory steps prior to testing, are time-consuming, and usually needed using toxic solvents. Literature provides evidences that propranolol has an electroactive nature [25], so it

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can be determined electrochemically. This fact is particularly important from an analytical point of view because electroanalytical methods have some important advantages over the methods mentioned above for quantification of propranolol. There is no doubt that these advantages include good selectivity, low detection limit, excellent reproducibility, high stability, rapid response, and simple operating procedures. Moreover, electroanalytical methods are environmental friendly and have relatively low costs of analysis [26]. Literature provides evidences of using electroanalytical methods for propranolol quantification in practice. However, the amount of information on electrochemical behavior of this drug is still rather scarce. The first studies have been made on unmodified electrodes: carbon paste and carbon nanotubes paste electrodes, mercury, platinum, and gold [27–30]. Moreover, a boron-doped diamond electrode has been used for propranolol detection [31]. Nowadays, it has been proved that better results during propranolol oxidation are obtained on modified electrodes; for instance, literature reports on the use of multi-walled carbon nanotube/silicone rubber composite and calixarene/multi-walled carbon nanotubes modified glassy carbon electrodes [32–34]. Propranolol electrochemical was studied also at the glassy carbon electrode modified with multi-walled carbon nanotubes doped with platinum nanoparticles [35, 36]. For the same purpose, the functionalized multi-walled carbon nanotubes within a poly (allylamine hydrochloride) film has been used [37]. Moreover, a carbon paste electrode based on γ -cyclodextrin–carbon nanotube composite (γ -CD–CNT–CME) was developed for the determination of propranolol hydrochloride [38]. It was found that a nitrogen-containing tetrahedral amorphous carbon electrode can be an excellent new carbon material for electrochemical propranolol detection [39]. Some information on propranolol electrochemical can be get when using both the edge plane pyrolytic graphite (EPPG) electrode modified both with poly-(melamine) film [40] and the graphene and conductive polymer (poly-1,5-diaminonaphthalene) [41]. The electrochemical behavior of propranolol has been also analyzed at a graphite screen printed electrode modified with zirconium dioxide [42]. Very recently, the glassy carbon electrode modified both with functionalized graphene, ionic liquid, and silver nanoparticles for detection propranolol has been tested [43].

The way of modification of some of mentioned above electrodes is complicated and needs toxic and expensive modifying compounds and/or solutions. To avoid this, scientists are still looking for new modified electrode materials that could be obtained in a simple, fast, cheap, and green way. From literature, it is known that gold nanoparticles significantly enhance the catalytic properties of conducting materials of electrodes [44]. Following this idea, based on a preliminary result found in literature indicating that gold may be a good catalyst for propranolol electrooxidation [30], the goal of the present study was fabrication of a new inorganic/organic hybrid 3D

nanogold sensor modified with the self-assembled layer (SAM) for electrochemical oxidation and quantification of propranolol in buffered solution at pH close to physiological one. Moreover, the quantification of propranolol pharmaceutical formulation dissolved in the simulated body fluid (SBF) will be checked. Such studies have not been so far reported in literature. The results are very important both in pharmaceutical analysis and clinical diagnosis.

Experimental

Apparatus

The following apparatus were used: Autolab potentiostat/galvanostat analyzer (AUTOLAB PGSTAT 302N); the pH meter (Model-ULAB 2002); a UV-Vis spectrometer; a Bruker 66 v/S FT-IR spectrometer; the transmission electron microscopy (JEM - 1200 EX2); the scanning electron microscopy (ZEISS EVO 40 instrument). Water purification system - TKA MicroPure.

Reagents

All reagents used in the measurements were of high purity and were used as received without their further purification. HCl, KCl, NaCl, NaOH, KNO₃, H₂SO₄, H₂O₂, K₃[Fe(CN)₆], K₄[Fe(CN)₆]·3H₂O, KH₂PO₄, Na₂HPO₄·2H₂O, NaH₂PO₄·H₂O, NaHCO₃, CaCl₂, MgSO₄·7H₂O, trisodium citrate, acetone, and ethyl alcohol were from POCh-Gliwice, buffer solutions of pH: from 1 to 11 were from VWR-Poland. Auric acid, 4-mercaptobutyric acid, cysteamine, and propranolol (tris(hydroxymethyl)aminomethane), ascorbic acid, uric acid, and dopamine were purchased from Sigma-Aldrich. Propranolol WZF tablets (1 tablet contain 40 mg of propranolol) and propranolol solution for injection (1 ampoule containing 1 mg of propranolol per 1 ml) were obtained from Polfa, Warsaw, Poland.

Solutions

All solution used were prepared with ultra-pure deionized water with conductivity of 0.055 μ S. As a supporting electrolyte solution, 0.07 mM phosphate buffer of pH 7.4 was used. Before the analysis, all the laboratory glasses had been washed with the Piranha solution (persulfuric acid - Caro acid). Piranha solution is a mixture of concentrated sulfuric acid (VI) and 30% hydrogen peroxide prepared in the ratio 3 v/v:1 v/v under the fume hood. The Au colloidal solution (Au sol) was prepared according procedure described elsewhere, [45]. The simulated body fluid (SBF) was prepared according to Kokubo's procedure by dissolving in deionized water appropriate amounts of different compounds (mentioned above)

to obtain a solution in which the concentration of Na^+ , K^+ , Ca^{2+} , Cl^- , HCO_3^- , HPO_4^{2-} , SO_4^{2-} ions will be 142 mM, 5 mM, 2.3 mM, 103 mM, 27 mM, 1 mM, and 0.5 mM, respectively. The above mentioned concentrations of each ion are the same as those in human blood plasma maintained under identical physiological pH conditions [46].

To prepare the real samples with propranolol to analysis, 8 tablets of propranolol WZF (each containing 40 mg of the drug) were accurately triturated in a mortar. Next 0.2593 g of triturated drug was transferred up to volumetric flask c.a. 1000 cm^3 and dissolved in SBF. In obtained solution, the propranolol concentration was equal to 1 mM. To obtain different exact propranolol concentrations, different volumes of the prepared solution were dissolved in SBF. Moreover, the real samples with propranolol for analysis were prepared using the propranolol solution for injection. For this purpose, the content of 1 ampoule containing 1 mg cm^{-3} of propranolol was dissolved quantitatively in 100 cm^3 of SBF. In the latter solutions, the propranolol concentration was equal to 3.86×10^{-2} mM. For analysis, 10 cm^3 of this solution was taken. For interference studies, the analytical standard solutions of dopamine (1 mM, 10 cm^3), ascorbic acid (50 mM, 10 cm^3), and uric acid (50 mM, 10 cm^3) were quantitatively put into 100 cm^3 volumetric flasks and were dissolved to the volume of 100 cm^3 with SBF. The obtained concentrations of dopamine, ascorbic acid, and uric acid were 0.1 mM, 5 mM, and 5 mM, respectively. The mixture of four compounds was prepared as follows: 1 cm^3 of 0.1 mM solution of propranolol, 1 cm^3 of 0.1 mM solution of dopamine, 1 cm^3 of 5 mM solution of uric acid, and 1 cm^3 of 5 mM solution of ascorbic acid were put into a 100 cm^3 volumetric flask and dissolved up to this volume with SBF. At the end in such prepared mixture, the concentrations of propranolol, dopamine, uric acid, and ascorbic acid were 1×10^{-3} mM, 1×10^{-3} mM, 5×10^{-2} mM, and 5×10^{-2} mM, respectively.

Electrodes

In this study, either unmodified or modified polycrystalline gold (Au) was used as working electrode. Geometrical areas of the electrodes were evaluated from the Randles–Sevcik equation [26] as 0.064 cm^2 and 0.115 cm^2 , respectively. A polycrystalline gold electrode with a much larger surface area as compared to that of working electrode and a saturated calomel electrode (SCE) were used as a counter and reference electrode, respectively. The unmodified and modified electrodes before use were washed with the Piranha solution, rinsed with distilled and deionized water. The SCE electrode was only rinsed with distilled and deionized water. Then dry electrodes were located into clean the three-compartment electrochemical cell separated by glass frits which was used in electrochemical measurements. Electrochemical activation of the bare polycrystalline gold electrode was carried out as

described earlier [47]. All measurements described were made at room temperature using solutions deoxygenated by 99.998% argon. Between measurements, the rinsed and dry unmodified or modified gold electrodes were stored in an empty tube.

In order to obtain the hybrid inorganic/organic nanogold modified Au electrode (labeled as 3D-Au-NPs-Au), the activated polycrystalline bare Au electrode (labeled as 2D Au) was submerged in the following solutions: Au sol (NPs-Au), 0.01 M alcoholic solution of 4-mercaptobutyric acid (MBA), 0.005 M solution of cysteamine (CA), and once again in Au sol solution. After each modification step, the modified template was rinsed and dried. The exact time of immersion of modified template in each modifying solution was determined in preliminary tests and established as 10 h, 4 h, 2 h, and once again 10 h in the Au sol solution, in the MBA solution, in the CA solution, and second time in the Au sol solution, respectively. It should be noticed that after each modified template immersion in a given modifying agent [Au-NPs, MBA, CA, and once again Au-NPs], a new modified electrode was obtained. In all of these studies, 4 modified electrodes were prepared: 3D-Au-NPs, 3D-Au-NPs-MBA, 3D-Au-NPs-MBA-CA, and 3D-Au-NPs-MBA-CA-Au-NPs. For TEM, UV-vis, and FT-IR characterization of the modified agent used in this paper, see the electronic supporting information Fig. S1. Moreover, Fig. S2 (the electronic supplementary material) schematically presents the four-step modification path from 2D Au electrode to 3D Au-NPs-Au electrode.

Four obtained electrodes were tested in the preliminary measurements and it was concluded that the highest catalytic effect was obtained when the 3D-Au-NPs-MBA-CA-Au-NPs electrode was used as a working electrode. For this reason, the detailed electrochemical studies of propranolol were made only on this electrode. For the sake of clarification, the unmodified (2D Au electrode) and modified (3D Au-NPs-MBA-CA-Au-NPs electrode) electrodes were labeled as 2D Au and 3D Au-NPs-Au, respectively, and such acronyms will be used in the text below.

Results and discussion

Characterization of the modified electrode

The subsequent stages of modification of the 2D Au electrode, which lead to obtaining the 3D Au-NPs-Au electrode, are shown schematically in Fig. S2. A gold nanoparticle modified gold template was obtained without binder molecules [48]. It means that the activated Au electrode (2D Au electrode) was directly dipped in colloidal gold solution. The Au-NPs layer was formed making use of the electrostatic interactions between the gold nanoparticles stabilized with citrate anions, used in the present study, which are endowed with negative

charge [49]. The surface obtained was called three-dimensional (3D) Au electrode and labeled as 3D Au-NPs. It is generally accepted in literature that chemisorption of alkane thiols and other S-functionalized aliphatic compounds on gold is preceded by the oxidative abstraction of hydrogen from S-H bonds, whereas the organic molecules existing in the trans-conformation in solution transform into an ordered structure with alkane chains oriented at a tilt angle of 20° – 30° toward the electrode surface [50, 51]. Per analogy to the SAM formation of alkane thiols on a gold substrate [52–54], the firm attachment of MBA acid to the 3D Au-NPs template surface was realized via developing the covalent bonds between Au-NPs nanoparticles and S atoms after S-H bond has been split in the MBA acid molecule. As a consequence, the 3D Au-NPs-MBA template was formed. The COOH terminated layers of MBA attached to the gold nanoparticles surface can be easily bonded with the amine group of cysteamine when such a modified electrode comes in contact with a solution of the latter compound. By comparison of the pK_a values of MBA ($pK_a = 3.7$ (www.Reaxys.com)) and pK_a of cysteamine (pK_a 8.35 and 10.81) (www.Reaxys.com), it is obvious that the carboxylic groups of MBA are mostly dissociated at $pH = 7.4$ of the electrolyte solution and the COO^- terminated negatively charged layers COO^- of MBA can favorably interact with protonated NH_3^+ group (i.e., the positively charged part CA). Moreover, strong hydrogen bonds may be formed between carboxylic groups of the acid and the amine group of cysteamine [25]. This explains development of a stable MBA-CA connection exposed with the SH group of cysteamine directed toward the top-side of the monolayer formed. As a result, the Au/Au-NPs/MBA/CA template was developed. Consequently, it is possible to modify further the Au/Au-NPs/MBA/CA electrodes with gold nanoparticles, via formation of the covalent bonds between S atoms of CA and Au-NPs. In this way, the Au/Au-NPs/MBA/CA/Au-NP-s electrode (labeled as 3D Au-NPs-Au) with gold nanoparticles on top of the created SAM layer was obtained. As previously reported [55, 56], the sulfur-functionalized aliphatic carboxylic acids form well-organized two-dimensional compact monolayers on the bare gold electrode (2D Au), whereas the three-dimensional SAMs developing on Au electrodes (2D template) coated with Au nanoparticles (3D template) exhibit a more disordered structure. This opinion is supported by the observation that permeability of SAMs increases with increasing substrate roughness [57] which was confirmed by IRRAS spectra [58] from which it appears that the permeability of self-assembled layers increases at surfaces with higher levels of roughness [52, 57].

An electrochemical picture of unmodified and modified electrode is presented in Fig. 1 in the form respective cyclic voltammograms. From electrochemical literature [26], it is clear that due to the electrode modification, its roughness factor $rf = A_r / A_g$ (where rf is the electrode roughness factor, while A_r and A_g are

Fig. 1 Cyclic voltammograms recorded on unmodified (2D Au) and modified (3D Au-NPs-Au) electrodes in phosphate buffer, $pH = 7.4$. $v = 0.1 \text{ V s}^{-1}$

the real and geometrical area of electrode, respectively) increases. Thus, the increase in rf results in increasing of the real electrochemical electrode surface area. Taking into account the fact that the reduction of 1 cm^2 of the oxide film adsorbed on the gold electrode surface requires the charge flow of 0.4 mC/cm^2 through it [59], the calculated rf for the 2D Au and 3D Au-NPs-Au electrodes tested in this paper were 1.89 and 2.41, respectively. The fact that the roughness factor increases after 2D Au electrode modification is confirmed by the SEM images obtained, shown in Fig. 2. By taking into account the obtained rf values, the real electrochemical areas of the tested electrodes were calculated [26] as 0.12 cm^2 for 2D Au electrode and 0.28 cm^2 for 3D Au-NPs-Au.

Oxidation of propranolol at unmodified and modified gold electrodes

Electrooxidation of propranolol both at unmodified (the 2D Au) and modified gold electrode (3D-Au-NPs-Au) is reflected as a single irreversible peak visible in respective cyclic voltammograms (CV) (Fig. 3). For comparison, the CVs recorded at the 2D Au and 3D Au-NPs-Au electrode in the pure supporting electrolyte solution (0.07 mM phosphate buffer, $pH = 7.4$) are presented too. According to general rules adopted in literature [25, 60], the irreversible peak visible in the CVs recorded is assigned to the oxidation of OH group in propranolol molecule to $C=O$ as a result of exchange of both 2 electrons and 2 protons between the propranolol molecule and the electrode surface giving 1-(isopropylamino)-3-(naphthalen-1-yl)oxy) propan-2-one as the product of oxidation. The peak assigned to the irreversible propranolol oxidation to its keto-form on the unmodified gold electrode (2D Au) begins to grow at $E = 1.0 \text{ V}$ vs SCE and reaches the maximum of $E = 1.39 \text{ V}$ vs SCE with the maximum peak current density $j = 0.5 \text{ mA cm}^{-2}$ (Fig. 3). According to the theory of electrochemical kinetics, the increase in current density and decrease in the overpotential of oxidized species should be taken into account

Fig. 2 SEM images of **a** 2D Au and **b** 3D Au-NPs-Au surfaces. Scale bar 2 mm, accelerating voltage: 20 kV; magnification $\times 10000$

in evaluation of the electrocatalytic effect. For the modified 3D Au-NPs-Au electrode, the anodic peak begins to grow up at $E = 0.65$ V vs SCE and reaches the maximum of $E = 1.24$ V vs SCE with the maximum peak current density $j = 1.58$ mA cm $^{-2}$ (Fig. 3). Without doubt, the observed experimental data confirm that at the modified 3D Au-NPs-Au electrode, a high catalytic effect during propranolol oxidation is achieved. It is manifested both as a substantial shift in the voltammetric peak toward the negative potential values and a high increase in the current density response at this peak. One can add that during the experiment described in this work, the fouling effect on the electrode surface during propranolol oxidation was not observed, the current densities were not changed after a number of scans. If the current densities altered after successive cycles, it would indicate poisoning of the electrode surface. It should be underlined that such a high catalytic effect at the modified electrode cannot be associated only with increase in the surface area after the modification process. Taking into consideration the ratio of the real surface area of the modified and unmodified electrodes (0.28 cm 2 / 0.12 cm 2 = 2.33), the current density observed during propranolol oxidation at the 3D Au-NPs-Au electrode should be 2.33 times higher than that observed at the unmodified 2D Au electrode. While at the 3D Au-NPs-Au electrode, j grows up to 1.58 mA cm $^{-2}$ (Fig. 3) and it is 3.16 times higher than expected only on the basis of the increase in the real area

due to modification. The latter value shows that the obtained electrocatalytic effect observed during propranolol oxidation on the 3D Au-NPs-Au electrode depends not only on the increase in the roughness factor (and thus the real area of the electrode surface as a result of its modification with hybrid inorganic/organic material) but also other factors have impact on the rate electron transfer between oxidized propranolol and the 3D Au-NPs-Au surface. There is no doubt that architecture of the prepared SAM layer with the gold nanoparticles immobilized on the outer side of the SAM layers attached to the gold surface effectively protects the electrode metal surface from adsorption of reaction products which may poison the 3D Au/Au-NPs/Au electrode surface. On the other hand, this facilitates accumulation of the substance studied at the electrode/solution interface which has to permit easier penetration of the reactant through the alkane chains to the 3D Au/Au-NPs/Au surface as compared to that at the 2D electrode and can efficiently promote tunneling of electrons between propranolol and the electrode surface [52, 57, 58]. In summary, there is no doubt that the huge electrocatalytic effect observed during propranolol electrooxidation on 3D Au-NPs-Au electrode is affected both by the type and structure of the modifying SAM layer which as follows from the electrochemical aspects of catalysis decreases the energy barrier for oxidative electron transfer [26, 61].

Fig. 3 Cyclic voltammograms recorded in 0.07 mM phosphate buffer, pH = 7.4 at the 2D Au electrode and at the 3D Au-NPs-Au without and with addition of 0.002 mM propranolol. $\nu = 0.1$ V s $^{-1}$

The effect of pH

In order to confirm the above described electrochemical behavior of propranolol on the 3D Au-NPs-Au electrode, the effect of the peak potential and the peak current density was studied by cyclic voltammetry in solutions containing 0.002 mM of propranolol of pH changed in the range 1–11 (Fig. 4). With increasing pH of the solution, as shown in Fig. 4 a, b, the propranolol electrooxidation peak potential shifts toward negative values and the shift can be described by the following equation: $E_p = 1.64 - 0.060 \pm 0.02$ pH ($R^2 = 0.994$). The value 0.060 of the dE_p/dpH slope indicates that in the oxidation of propranolol at the 3D Au/Au-NPs electrode the same number of electrons and protons is exchanged. This is in agreement with the electrochemical kinetics theory [26] and

Fig. 4 **a** Cyclic voltammetric responses of 3D Au-NPs-Au in phosphate buffer at different pH containing of 0.002 mM propranolol. $\nu = 0.1$. **b** Dependence of pH on the current peak potential for 0.002 mM propranolol. **c** Dependence of pH on the current peak density for 0.002 mM propranolol

confirms the above adopted mechanism of propranolol electrooxidation. Furthermore, as shown in Fig. 4c, the j^p of propranolol oxidation increases up to pH about 7–8, then a decrease in the recorded j^p values is seen. This observation may be associated with instability of propranolol at acidic condition. At low pH, isopropyl amino side chain of propranolol can be oxidized. Gradual decreases in the peak currents in

pH range above 8 may be attribute to the deprotonation of propranolol molecules and thus cannot be effectively accumulated at the electrode surface, which leads to observed decrease in current density. The similar observation has been reported earlier [36]. The latter observation was the reason why pH 7.4 (equal to the pH value of physiological condition) was chosen for further studies of propranolol on prepared 3D Au-NPs-Au electrosensor.

Effect of scan rate

The dependence of the potential scan rate on the peak potential value provides some important kinetic information. The peak potential (E^p) which is independent of the scan rate indicates a reversible charge transport process, whereas the irreversible charge transfer process causes the peak potential to vary with the potential scan rate. From the kinetics theory [26], it follows that when an increase in the scan potential rate is accompanied by an increase in the peak current density and moreover when at the same time the peak potential shifts toward more positive value, the electrochemical process is completely irreversible. The effect of scan rate on the electrooxidation of propranolol in phosphate buffer, pH 7.4 on 3D Au-NPs-Au electrode, is shown in Fig. 5a. As follows from the recorded CVs, the E_p value increases with increasing potential scan rate, confirming that propranolol oxidation at the 3D Au-NPs-Au electrode is an irreversible process. Moreover, Fig. 5b presents the variation of peak current density with the square root of the potential scan rate. The increase in the scan rates from 0.001 to 0.2 V s⁻¹ causes an increase in the peak current density. Moreover, it was found that peak current density (j^p) showed a good linear correlation with the square root of the scan rates. This result confirmed that the considered process at the tested electrode is controlled by diffusion. This conclusion is additionally supported by the value of $d\log j^p/d\log \nu$ slope obtained from the linear relation $d\log j^p$ vs $d\log \nu$ (Fig. 5c). From the regression equation: $d\log j^p$ (mA) = 0.710 + 0.501 $d\log \nu$ (Vs⁻¹); ($R^2 = 0.9985$; $n = 17$), it is seen that the value of the $d\log j^p/d\log \nu$ is very close to 0.5, which proves that the propranolol oxidation at the 3D Au-NPs-Au electrode is indeed diffusion controlled, which means that during the considered process, the diffusion of propranolol is slower than both its adsorption and the following electron transfer between electrooxidized substrate molecule and the electrode surface [26].

Analytical application of the prepared electrode for propranolol electrooxidation

As outlined in the “Introduction” section, propranolol plays really an important role in human life so its accurate quantification is justified. Both a decrease in the overpotential of oxidation and an increase in the current density observed

Fig. 5 **a** Cyclic voltammetric responses of 3D Au-NPs-Au in a phosphate buffer containing 0.002 mM propranolol at different scan rates. **b** The variation of anodic peak current density versus square root of scan rate varied 0.001 to 1 V s⁻¹ for 0.002 mM propranolol in phosphate buffer. **c** The variation of the logarithm anodic peak current density versus the logarithm of scan rate varied from 0.001 to 2 V s⁻¹ for 0.002 mM propranolol in phosphate buffer

during propranolol oxidation at the 3D Au-NPs-Au electrode, without a doubt, make the modified sensor suitable for analytical application. The analytical sensitivity of the 3D Au-NPs-Au electrode toward propranolol quantification was determined by using DPV (the differential pulse voltammetry) method with the following parameters: the step potential:

4 mV, the pulse wide 50 ms, the pulse period 200 ms and the pulse amplitude 10 mV. The solute concentration has been changed in range from 0.0001 to 0.035 mM. The differential pulse voltammograms (DVPs) recorded on 3D Au-NPs-Au for selected concentrations of propranolol from the above range are presented in Fig. 6a. As follows from these data, a direct correlation between increasing propranolol concentration in the phosphate buffer and increasing current density on the recorded DVPs is well pronounced.

As follows from Fig. 6b, the linearity between the current peak density j^p corresponding to the electrooxidation of propranolol and its concentration in supporting electrolyte solution is satisfied in the concentration interval from 0.0001 to 0.02 mM. For propranolol, the following linear regression equation is satisfied: $j_p[\text{mA cm}^{-2}] = (412 \pm 17) \cdot 10^{-3} + (570 \pm 11) c_{\text{PROP}} [\text{mM}]$; ($R^2 = 0.999$; $n = 28$). The detection limit c_L , calculated from the formula recommended by IUPAC [62]: $c_L = 3S_b/S$ (where 3 means the numerical constant, S_b , is the standard deviation of the current density in the supporting electrolyte solution, and S is the slope of j_p –

Fig. 6 **a** Differential pulse voltammograms recorded on the 3D Au-NPs-Au gold electrode (DVP parameters: the step potential: 4 mV, the pulse wide 50 ms, the pulse period 200 ms, and the pulse amplitude 10 mV) during electrooxidation of different concentrations of propranolol in 70 mM phosphate buffer, pH 7.4. **b** Analysis of j^p - c_{PROP} . Insert: For clarity, analysis of j^p - c_{PROP} in the narrow propranolol concentration (0.0001 to 0.005 mM)

Table 1 Determination of propranolol in samples obtained by dissolving the propranolol tablet or its solution for injection in the simulated body fluid

Sample number	Nominal content of propranolol [mM] ^a	Propranolol concentration fund [mM] ^a	Nominal content of propranolol [mM] ^b	Propranolol concentration fund [mM] ^b
1	2×10^{-3}	2.1×10^{-3}	3.86×10^{-3}	3.76×10^{-3}
2	4×10^{-3}	3.98×10^{-3}	3.86×10^{-3}	3.91×10^{-3}
3	6×10^{-3}	6.05×10^{-3}	3.86×10^{-3}	3.95×10^{-3}
R.S.D. (%)		2.33%		2.03%
Recovery (%)		99.5–105%		97.0–102%

^a The source of propranolol—a tablet^b The source of propranolol—a solution for injection

c_{PROP} linear dependence, respectively), was equal 6.75×10^{-5} mM. There is no doubt that the 3D Au-NPs-Au modified electrode is suitable for precise determination of propranolol in buffered solution of physiological pH. The c_L value and linearity range of propranolol quantification obtained with the modified electrode prepared in this work were compared with the analogous parameters obtained on the other electrodes described in literature [27, 31–43]; it can be concluded that in the vast majority of tested electrodes, both the linearity and the c_L value were better when 3D Au-NPs-Au electrode was used (see the electronic supplementary material, Table S1). This observations mean that both the selection of the 3D Au-NPs-Au electrode and methods used for the propranolol testing were correct and reasonable.

Real sample analysis

The practical application of the prepared hybrid inorganic/organic voltammetric sensor for quantitative determination of propranolol was tested by differential pulse voltammetry (DVP) using the calibration method [63]. For this purpose, different volumes of the propranolol solution prepared by using commercial pharmaceutical formulations were diluted

with the simulated body fluid (see the “Experimental” section). For each studied propranolol sample at an exact concentration, the measurements were repeated three times. Table 1 presents the arithmetic average values of concentrations obtained from the conducted tests. There is no doubt that both the relative standard deviations (R.S.D.) and the recovery ranges of propranolol determination in the prepared samples proved that both the voltammetric sensor prepared and the proposed method of concentration determination could be useful tool for the determination of propranolol in bioanalytical analysis.

Interfering analysis

Electrochemical quantitative determination of different drugs in real samples is in most cases affected by interference of some biogenic compounds like dopamine, ascorbic acid or uric acid. According to literature, the presence of both AA and UA in the mixture with DA has no effect on the simultaneous quantitative detection of these compounds when measurements are carried out on modified electrodes, regardless of the type of conductive material used in the electrochemical sensor. For example, such results were observed on different carbon, or noble metal electrodes modified with organic sulfur compounds and gold nanoparticles or on reduced graphene oxide modified with bimetallic alloyed nanoparticles [64–68]. In this study, the effect of possible interference of uric acid, ascorbic acid, and dopamine on propranolol determination was examined. Comparison of the pK_a values of uric acid ($pK_a = 5.4$), ascorbic acid ($pK_a = 4.1$), dopamine ($pK_a = 8.87$), and propranolol ($pK_a = 9.45$) (www.Reaxys.com) [68] shows that the pK_a value of propranolol is higher than that of DA, UA, and AA so the peaks of the tested four-component mixture are separated along the potential axis as a result of electrostatic interactions. The results of measurements performed in the four-component mixture (propranolol, uric acid, ascorbic acid, and dopamine) are listed in Table 2. The data collected in Table 2 unquestionably prove that the sensor proposed in this work can be successfully used for the determination of propranolol in the presence of the abovementioned

Table 2 Determination of propranolol (1), uric acid (2), ascorbic acid (3), and dopamine (4) in the four-component mixture in the SBF solution

Sample number	Nominal content of given compound in the four-component mixture [mM]				Sample number	Concentration fund of given compound in the four-component mixture [mM]			
	1	2	3	4		1	2	3	4
1	1×10^{-3}	5×10^{-2}	5×10^{-2}	1×10^{-3}	1	0.99×10^{-3}	5.1×10^{-2}	4.97×10^{-2}	1.05×10^{-3}
2	1×10^{-2}	5×10^{-2}	5×10^{-2}	1×10^{-2}	2	0.98×10^{-3}	5.2×10^{-2}	5.04×10^{-2}	0.96×10^{-2}
3	1×10^{-2}	5×10^{-2}	5×10^{-2}	1×10^{-2}	3	1.01×10^{-3}	4.89×10^{-2}	$4 \times 96 \times 10^{-2}$	0.98×10^{-2}
R.S.D. (%)						1.33%	2.01%	2.7%	3.6%
Recovery (%)						98–101%	98–102%	99–102%	96–106%

1—propranolol; 2—uric acid; 3—ascorbic acid; 4—dopamine

biogenic interfering compounds. The obtained recovery and relative standard deviation are very good, indicating the efficiency of the tested sensor. Moreover, because these measurements were made in the SBF solution which has very close content to the human body fluid [46], the prepared electrochemical sensor is much promising for reliable and sensitive application in medical and pharmacology analysis.

Reproducibility and stability of the 3D-Au-NPs-Au prepared sensor

The reproducibility of the 3D Au-NPs-Au sensor was checked in the experiment in which two prepared in the same way modified 3D Au-NPs-Au electrodes were examined. For each of them, cyclic voltammetric curves (CVs) were recorded with $dE/dt = 0.1 \text{ V s}^{-1}$ in 0.07 mM phosphate buffer solution with addition of 0.002 mM of propranolol. The relative standard deviation (R.D.S.) was about 2.8% and 2.9% after 30 successive CVs scans for two independently tested modified electrodes, respectively. This result indicates that the prepared sensor ensures excellent reproducibility. The stability of the 3D Au-NPs-Au prepared sensor was tested also for two sensors prepared in the same way. For 4 weeks (5 times a week), 5 CVs were recorded with $dE/dt = 0.1 \text{ V s}^{-1}$ in 0.07 mM buffer phosphate solution with addition of 0.002 mM of propranolol. After each daily test, both modified electrodes tested were rinsed with deionized water and dried. Between the measurements, the tested sensors were stored in empty glass tubes. After 4 weeks of the stability testing experiment, the current densities recorded with these two tested electrodes retained about 96% of their initial values. The results have shown that the 3D Au-NPs-Au prepared sensor had good stability.

Conclusion

A hybrid nanocomposite inorganic/organic modified gold template has been presented. The modifying agents were gold nanoparticles, 4-mercaptopbutyric acid, and cysteamine. The proposed method of preparation of the novel modified material is fast simple and green, e.g., does not need using toxic compounds and solutions. The resulting 3D catalyst was used as a conducting material in a voltammetric sensor for electrooxidation and quantification of propranolol—the effective antagonist of β -adrenergic receptors. The prepared sensor displays good sensitivity and stability. Using the differential pulse voltammetry for propranolol quantification, the linear range between 0.0001 and 0.02 mM was found with a detection limit of $6.75 \times 10^{-5} \text{ mM}$. Moreover, it has been established that propranolol can be determined in the presence of some biogenic compounds like dopamine, ascorbic acid, or uric acid without any interferences. All these properties are promising for application of the 3D Au-NPs-Au both in

clinical analysis, quality control and routine determination of propranolol in pharmaceutical formulation. The results obtained on prepared modified electrode are in agreement with the common tendency observed in the last decades that in electrochemical sensing of different pharmaceutical drugs, indeed the modified electrodes are more useful as compared to the unmodified electrodes.

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Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

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