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Electrochemical studies of self-assembled monolayers composed of various phenylboronic acid derivatives

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ABSTRACT

Gold electrodes were functionalized with thiolated phenylboronic acids or by sequential immobilization of alkanethiols with terminal carboxylic groups and conjugation with aminophenylboronic acids. The stepwise assembly of organic molecules was characterized by several voltammetric techniques with systematic monitoring of surface coverage of organic molecules anchored to the gold electrodes. The receptor properties and applicability of the obtained monomolecular layers for the voltammetric detection of fructose and fluoride ions in solution were evaluated. The MPBA and APBA modified electrodes can be applied for the voltammetric determination of chosen non-electroactive analytes on the basis of the changes in peak currents of ferricyanide/ferrocyanide couple in the presence of the detected species.

1. Introduction

Boronic acids are trivalent boron-containing organic compounds applied widely as selective molecular receptors for the development of chemical sensors based on various transduction schemes [1–4]. First of all, they are known to react with 1,2- and 1,3-diols (e.g. carbohydrates, catecholamines), leading to the reversible formation of five- or six-membered cyclic boronic esters in aqueous solution [5]. The simultaneous binding of hydroxyl groups provides negatively charged tetrahedral species with an electron-rich sp^3 boron atom [6,7]. Moreover, boronic acids can act as Lewis acids and form complexes with hard bases, like F^-, CN^- or OH^- since the sp^2-hybridized boron atom possesses a vacant p orbital [3,4,8]. Boronic acid conjugated colorimetric and fluorescent probes for Hg^{2+}, Cu^{2+} and other transition metal cations were also recently reported [4]. These unique features of boronic acids were exploited for the construction of optical and electrochemical sensors for the detection of biologically important species, such as carbohydrates and neurotransmitters [2–4,9,10].

The first fluorescent receptors based on boronic acid moiety – anthrylboronic acids – were reported by Yoon and Czarnik in 1992. The fluorescence quenching of these derivatives was observed upon binding of saccharide, dopamine or L-DOPA according to the photoinduced electron transfer (PET) process [11]. (Interestingly, the internal charge transfer mechanism (ICT) was postulated for this interaction in [12]). The PET principle of saccharide detection was also exploited in phenylboronic acids modified by an aliphatic amino group, developed by Shinkai and co-workers [13,14], where the formation of B–N bond upon saccharide binding led to the significant fluorescence recovery [14]. Similar diboronic acid derivatives provided the selective recognition of D-glucose against D-fructose and D-galactose [15]. A series of ICT arylboronic acid analogues were designed by Shinkai [16]; whereas stilbene derivatives of boronic acid, exhibiting red or blueshift in the emission wavelength after boronate formation (depending on the presence of either electron donor or acceptor groups in their structure), were proposed by Lakowicz and DiCesare [17]. The stilbenes derivatives as well as boronic acid fluorophores based on quaternized form of the quinolinium nucleus were introduced into contact lens for non-invasive monitoring of physiological glucose [18,19]. Finally, colorimetric diazoboronic acids sugar receptors [20,21] and multi-component systems of higher sensitivity, applying e.g. Alizarine Red S as an indicator, were proposed [22]. Since this time a broad range of fluorescent and colorimetric boronic acids probes of various sensing properties have been reported over the last two decades [2–4,9,10,12,23].

The electrochemical strategies for sensing boronic acid-saccharide interaction were significantly less frequently reported [9,12]. Since boronic acids are themselves electrochemically inactive, their structure was modified with a redox-active marker. As an example, ferrocene derivatives have been synthesized and evaluated; the complexation of carbohydrates induced changes in their redox potential, whereas intramolecular B–N bonding facilitated saccharide binding at physiological pH [24–26]. Further studies involved the synthesis of ferrocene based diboronic acids of various architecture [27–29]. Despite the good enantioselectivity for the binding of

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ferrocenyl diboronic acids with D- and L-sugar [28], reasonable affinity and selectivity of glucose complexation were not achieved [29].

Boronic acids have also been directly immobilized on the surface of metal or glassy carbon electrodes forming self-assembled monolayers (SAMs) or polymer thin films. Platinum electrode coated with a phenylboronic acid-bearing copolymer film was used for amperometric sensing of glucose [30]. Nakashima and co-workers proposed a molecular-level modification of the surface of gold electrodes by means of a SAM of phenylboronic acid-terminated viologen, exhibiting different shift of redox potential depending on the type and concentration of saccharides in sample [31]. Voltammetric sensing of saccharide and glycoproteins using Au electrodes modified with SAMs of dithiobis(4-butyrylamino-*m*-phenylboronic acid) (DTBA-PBA), forming closely packed monomolecular films, was presented by Kanayama and Kitano [32]. Further studies compared Au electrodes coated with SAMs composed of 4-mercaptophenylboronic acid (MPBA) and the mentioned above dithiobutyric acid derivative (DTBA-PBA) [33a]. The response mechanism involved the "ion-channel sensor" principle—the formation of the negatively charged electrode surface (due to the fructose binding and addition of OH^- ion), blocked the access of the redox probe $Fe(CN_6)^{3-/4-}$ to the electrode. Nevertheless, a rather limited dynamic range of the electrode response for glucose (3–100 mM) and fructose (1–30 mM) was recorded. Other examples of more complex biosensors based on the immobilization of enzymes or proteins on the surface of SAMs containing boronic acid moieties were reported in the literature [9].

Finally, the potentiometric detection of carbohydrates on glassy carbon electrodes electrochemically modified with poly(aniline boronic acid) film was reported. The electrode potential, measured in open circuit, was sensitive to the changes in p*K*a of the polymer as a result of boronic acid-diol complexation [34]. Poly(aniline boronic acid) layers were further applied to develop saccharide-imprinted film-modified electrodes [35], impedance sugar [36] and conductimetric dopamine sensors [37].

The high affinity of fluoride binding to boronic acids was the basis of many selective analytical methods. The electrochemical sensing by a redox active ferroceneboronic acid in aqueous media was reported by Shinkai and co-workers [38]. The ferroceneboronic acid coupled with appropriate dye molecules enabled the colorimetric detection of fluoride [39]. James and co-workers elaborated the first fluorimetric method based on the fluorescence quenching of simple phenylboronic and 2-naphthylboronic acid upon fluoride complexation in aqueous-methanol solution [40]. Significantly lower detection limit (in DMSO) of fluorimetric method based on the similar 1-naphthylboronic acid was obtained by Yuchi [41]. Naphthylboronic acid was also applied as an ionophore in polymeric membrane of ion-selective electrodes. Further detailed potentiometric and ^{19}F NMR spectroscopy studies elucidated the response mechanism of fluoride-selective electrodes containing phenylboronic acid derivative [42]. Numerous examples of specific fluoride recognition by various boronic acid derivatives can be found in the recent reviews [3,4,8].

In this work, self-assembled monolayers of phenylboronic acids derivatives were formed on the surface of Au electrodes using various methodologies. Electrochemical studies on the structure and sensing properties of the obtained receptor monolayers towards chosen analytes were carried out and discussed.

2. Experimental

2.1. Chemicals and instruments

4–mercaptophenyloboronic acid (MPBA), 4–aminophenylboronic acid (APBA), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 2-(*N*-morpholino) ethanesulfonic acid (MES), 16-mercaptododecylohexanoic acid (MDHA), 6-mercaptohexanoic acid (MHA) and octanethiol (OT) were obtained from Sigma-Aldrich. D-fructose, tris(hydroxymethyl)aminomethane (TRIS), sodium fluoride, potassium ferrocyanide and toluene were purchased from FLUKA. Absolute ethanol and potassium ferricyanide were received from Polish Chemical Reagents.

Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) experiments were conducted with CHI 1030 Electrochemical Analyzer. The Electrochemical Impedance Spectroscopy (EIS) analysis was performed with CHI 650B Electrochemical Workstation. All electrochemical experiments were carried out using a conventional three-electrode cell, with Pt coated titanium rod as an auxiliary electrode, an Ag/AgCl reference electrode and gold disc electrode (purchased from Mineral) as a working electrode. The reference electrode potential was calibrated by using electrode process in equimolar ferricyanide/ferrocyanide solution.

2.2. Preparation of the electrodes

Before modification with chemical compounds all gold electrodes were sequentially polished mechanically with 1.0, 0.3 and 0.05 μm alumina powder and rinsed thoroughly with distilled water. In order to remove remaining powder, the electrodes have been sonicated for 5 min in water. Afterwards, they were electrochemically cleaned by potential cycling in 0.5 M NaOH in the potential range between −1.5 V and −0.2 V followed by electrochemical etching in 0.5 M H_2SO_4 between 0.2 and 1.6 V at scan rate of 0.1 V/s, until typical stable voltammograms of clean gold were obtained. Finally, the gold electrodes were rinsed thoroughly with distilled water and acetone and dried with nitrogen. After this procedure the electrodes were transferred to the coating solution. Six electrode specimens were tested in each experiment.

2.3. Formation of MPBA monolayer

SAMs were obtained by immersing previously prepared electrodes in 1 mM ethanolic solution of MPBA. Before solving MPBA in ethanol, the alcohol was deoxidized by saturating with nitrogen. The incubation took place overnight at room temperature without access of light. Before electrochemical measurements, the modified electrodes were rinsed with ethanol and then distilled water.

2.4. Immobilization of APBA on MHA monolayer

Immobilization of APBA consisted of tree steps: obtaining MHA monolayer, activation of carboxylic groups with EDC and NHS reagents, and a conjugation reaction between alkanethiol monolayer and APBA. The first step was similar to the immobilization of MPBA, the only difference was the use of other thiol-functioned compound. The activation of the carboxylic group was carried out by immersing the electrode with MHA monolayer in a stirred solution of 50 mM EDC and NHS. The solution was buffered with 0.1 M MES and adjusted to pH ~5.0. The activation lasted 2 h at room temperature. The conjugation reaction was performed by immersing the electrodes previously activated in 1 mM APBA solution. The applied solvent was pure toluene. Activation took place overnight at room temperature.

2.5. Immobilization of APBA on MDHA monolayer

The procedure of immobilization of MDHA and coupling it with APBA was the same as in the case of MHA. However, due to the densely packed structure of MDHA monolayer, another step of preparation consisting of conditioning of prepared APBA-MDHA monolayer, was required. The conditioning solution had the same

composition as during electrochemical measurements. Conditioning took place overnight at room temperature.

2.6. Electrochemical measurements

During all measurements, 50 μM solution of the $Fe(CN)_6^{3-/4-}$ couple was applied as a redox marker, whereas 0.1 M NaCl was used as a supporting electrolyte. The solutions were buffered with 0.01 M MES (pH 4.0) and 0.01 M TRIS (pH 9.0) for fluoride ions and fructose analysis, respectively. Each measurement was performed 5 min after an immersion of electrodes into a stirred solution. The electrode responses towards chosen analytes were analyzed in 0.01 M MES or 0.01 M TRIS buffer solution containing 50 μM Fe $(CN)_6^{3-/4-}$, increasing gradually the concentration of fructose or fluoride anions in steps of log *c* over the range: 10^{-8}–10^{-2} M.

The scan rate amounted to 0.1 V/s in CV measurements. The frequency was set at 50 Hz and the amplitude value was 25 mV for SWV. In the case of EIS experiments, the frequency range varied from 0.1–10^5 Hz, with initial potential 0.23 V and amplitude 5 mV.

3. Results and discussion

3.1. MPBA and ABPA monolayers

The formation of all types of molecular layers was confirmed and characterized by voltammetry and electrochemical impedance spectroscopy. The surface coverage of MPBA molecules (Γ_{MPBA}) evaluated from the charge of the reduction peak corresponded to $3.9\ (\pm 1.0) \times 10^{-10}$ mol/cm^2, and consequently, the area per molecule in the MPBA monolayer was approximately 42.6 ± 8.7 Å^2. The data differed from those described by Takahashi and co-workers, who reported the packing densities of 7.5×10^{-10} mol/cm^2 derived from the desorption experiments [33b]. However, the results obtained for thiophenol (TP) molecules adsorbed on monocrystalline gold Au(1 1 1) showed that aromatic molecules with –SH group linked directly with phenyl ring provided layers without high control over the molecular organization. In this case, the surface coverage of TP monolayers was equal to 4.4×10^{-10} mol/cm^2 (one molecule occupied an area of 37.8 Å^2) [43], which is comparable with our results obtained for the polycrystalline gold surfaces modified with MPBA. These values suggested that both TP and MPBA do not form well-ordered monolayers on the gold electrodes. Probably, the molecules were aggregated on the surface, due to the hydrophobic interactions of the phenyl moieties from neighboring particles. It is well-known that the intermolecular interactions between the neighboring aromatic residues may cause deviations from the ideal parallel orientations [44]. However, it must be emphasized, that the value of packing densities based on the desorption experiments should be considered carefully, since they are usually overestimated. This is related to the fact that the double-layer charging current can contribute significantly to the desorption charge. As a result, the value of Γ calculated from the area of the desorption peak on the cyclic voltammogram can be up to 20% higher than the real value [45].

The electrodes were also modified by sequential immobilization of alkanethiols with terminal carboxylic groups (MHDA) and conjugation with aminophenylboronic acid (APBA). The value of surface coverage of MHDA molecules ($\Gamma = 6.4\ (\pm 1.3) \times 10^{-10}$ mol/cm^2), and thus the area occupied by one MHDA molecule immobilized on gold (25.8 ± 8.7 Å^2), suggested that the APBA conjugated with MHDA layers were packed more densely than MPBA. The real values of Γ for APBA were not accessible using electrochemical techniques, since probably not all MHDA sites are coupled to APBA molecules. Therefore, the direct comparison of the surface coverage of MPBA and APBA/MHDA layer should be done with caution.

In order to get a more ordered and upright oriented molecular layer, the electrode surface was covered by a mixed APBA-MHDA/OT monolayer (1:10 MHDA:OT molar ratio in the self-assembly solution). According to the literature reports, the mixed monolayer could be more uniformly organized in terms of the orientation of molecules and probably one favored, nearly perpendicular orientation will be achieved. Moreover, the longer molecules extend above the diluent octanethiol and therefore are more exposed and susceptible to interactions with detected species.

3.2. Interactions between MPBA monolayers and fructose

Fig. 1a presents the cyclic voltammograms of the electrode before and after modification with MPBA, measured in solution of varying concentration of fructose. The experiments were performed only in pH 9.0, since the effect of pH on the peak current and peak potential of MPBA modified electrodes was examined carefully by Takahashi et al. [33a]. The immobilization of the boronic acid resulted in decreasing of the peak current height and increasing of a distance between reduction and oxidation peaks. This was attributed to a hindering effect of molecules anchored to the gold surface i.e. the formation of SAM limited

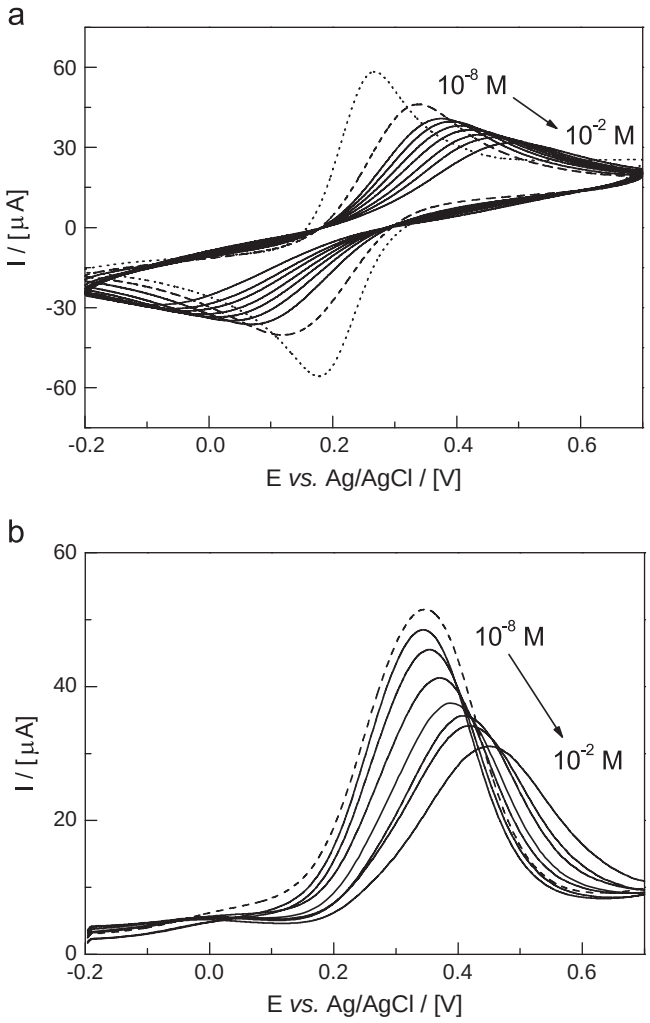

Fig. 1. (a) Cyclic voltammograms recorded for electrode in solution of $Fe(CN)_6^{3-/4-}$ ions (pH 9.0) before (dotted line) and after immobilization of MPBA in the absence (dashed line) and in the presence (solid lines) of fructose; (b) square wave voltammograms of the same electrode measured in the similar conditions.

the access of $Fe(CN)_6^{3-/4-}$ to the electrode surface and increased the electron transfer resistance. Additionally, since boronic acids exist in neutral and anionic forms at pH 9.0 the negatively charged monolayer on gold caused electrostatic repulsion of the negatively charged redox probe.

The cyclic voltammograms exhibited a further decrease in both reduction and oxidation peak currents (as well as the increased peak separation) after the addition of fructose. The difference between reduction and oxidation peak potentials raised from 0.376 ± 0.123 V to 0.635 ± 0.153 V for 10^{-8} and 10^{-2} M fructose solution, respectively. For the same concentrations, the oxidation peak current density was 1.17 ± 0.22 mA/cm^2 and 0.93 ± 0.17 mA/cm^2, respectively. The similar regularity was observed in the case of square wave experiments (Fig. 1b). The reduction peak at 0.266 V for fructose-free solution shifted to the value of 0.566 ± 0.067 V for 10^{-2} M fructose, and the peak current decreased from 0.97 ± 0.03 mA/cm^2 to 0.65 ± 0.02 mA/cm^2. Finally, the linear dependence of the SWV peak current on fructose concentration over a wide range i.e. from 10^{-8} to 10^{-2} M (slope: -2.81 μA/decade) must be emphasized (see Fig. 2), since milimolar detection range of fructose response was achieved for MPBA modified electrodes in [33b].

The explanation of this behavior is based on the fact, that the binding of fructose leads to the conversion of the boronic acid to an ester of a lower p*K*a, increasing its affinity for hydroxyl ions present in solution [7]. Therefore, the anionic species of boronic acid dominated in pH 9.0, which in consequence reduced the accessibility of negatively charged redox marker to the electrode surface. The electrostatic repulsion of $Fe(CN)_6^{3-/4-}$, combined with the sterical effect caused by the presence of bonded fructose, was proportional to fructose concentration in solution.

In order to confirm the voltammetric results described above, electrochemical impedance spectroscopy (EIS) technique was used to characterize the properties of the modified electrodes. Randles equivalent circuit model was used to evaluate the monolayer parameters, which were fitted to experimental data using *n*-linear least square method. As it is shown in Fig. 3, the addition of fructose to tested solution caused a rise of charge transfer resistance, which varied from 9584 Ω to 22,028 Ω for 10^{-8} and 10^{-2} M solution, respectively. This observation is consistent with the data acquired during voltammetric measurements.

After each electrochemical experiment, the regeneration of the electrodes was performed by soaking them in 10 mM acetate buffer solution (pH 4.5) for 10 min, according to the procedure proposed previously for DTBA-PBA modified gold electrodes [33b]. The experiments involving consecutive steps of decomposition of MPBA sugar ester and reaccumulation of fructose in 0.5 mM

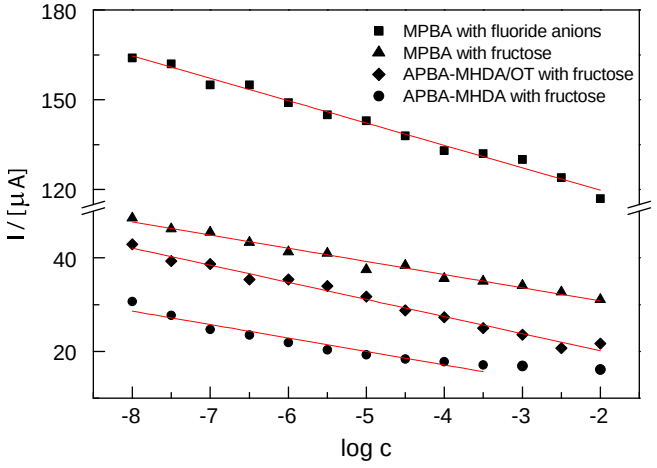

Fig. 2. The electrode responses—SWV peak current plotted against given analyte concentration for various monolayers.

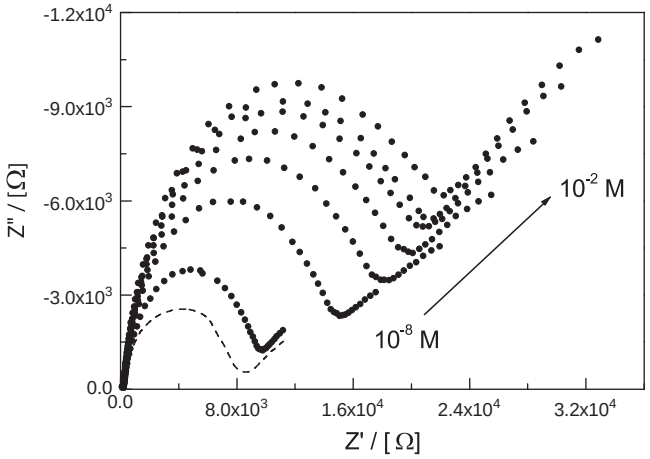

Fig. 3. Electrochemical impedance spectra recorded for electrode modified with MPBA in solution of $Fe(CN)_6^{3-/4-}$ ions (pH 9.0) in the absence (dashed line) and in the presence (dotted lines) of fructose.

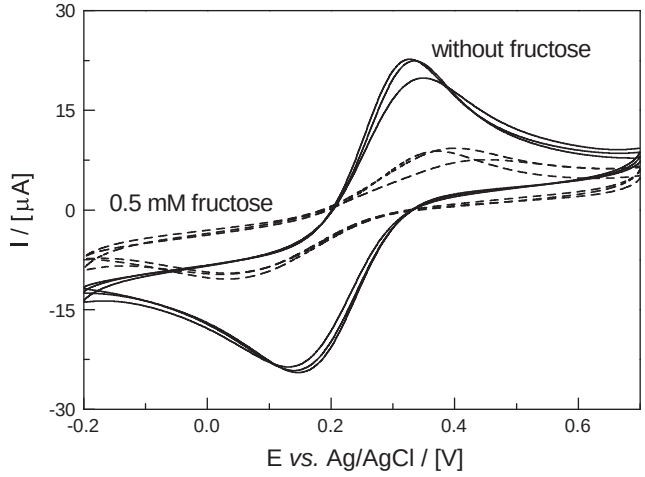

Fig. 4. Cyclic voltammograms recorded for electrode modified with MPBA in solution of $Fe(CN)_6^{3-/4-}$ ions (pH 9.0) before (solid lines) and after (dashed lines) exposure to 0.5 mM fructose in the supporting electrolyte. The regeneration of the MPBA surface were realized by immersing the working electrode in 10 mM acetate buffer solution (pH 4.5) for 10 min.

solution gave promising results. The analyte molecules were almost fully removed from the electrode by acetate buffer-mediated process i.e. the CV responses of the regenerated MPBA electrode were nearly indistinguishable from those of fresh electrode measured in the solution at chosen concentration (Fig. 4). There was no apparent loss in signal after three cycles of regeneration, and only about 2.5% decrease in reduction peak current and 10% decrease in oxidation peak current compared to the fresh prepared MPBA electrode was observed. This effect indicated that the regenerated electrodes can be used for fructose detection in a reproducible manner, without significant loss of sensitivity (similar results were noticed for 6 examined electrodes). The obtained results confirmed that the MPBA sugar ester immobilized on gold electrode is instable in the acidic medium and thus were consistent with those reported in [33a].

3.3. *Interactions between MPBA monolayers and fluoride anions*

Next, similar voltammetric measurements were performed to study the suitability of the MPBA monolayers for the electrochemical detection of fluoride anions. Since the substitution of hydroxyl groups by fluoride anions should not change the charge

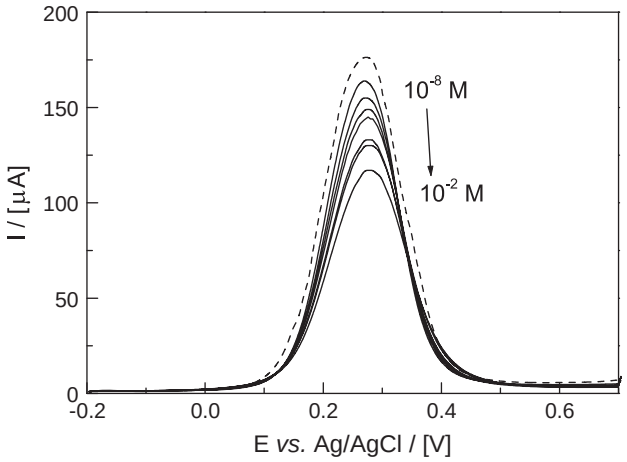

Fig. 5. Square wave voltammograms recorded for electrode modified with MPBA in solution of $Fe(CN)_6^{3-/4-}$ ions (pH 4.0) in the absence (dashed line) and in the presence (solid lines) of fluoride ions.

of the monolayer, acidic conditions (pH 4.0) were applied to retain the neutral form of the boronic moiety before analyte binding. Fig. 5 presents the square wave voltammograms recorded for the modified electrode in the presence of fluoride ions at various concentration. A decrease of peak current density has been observed (from 5.22 mA/cm^2 to 3.73 mA/cm^2 for 10^{-8} and 10^{-2} M fluoride solution, respectively). Moreover, a small positive potential shift has been noticed (from 0.268 V to 0.272 V for 10^{-8} and 10^{-2} M solution, respectively), which confirmed boron-fluoride binding. The results suggested that electrostatic repulsion of the redox probe was responsible for lowering the current intensity, while the reaction potential was mostly affected by steric hindering.

In analogy to the fructose response of MPBA based electrodes, a linear relationship between peak current and fluoride concentration was obtained in the same range (see Fig. 2); however, in this case the sensitivity was higher (-7.47 μA/decade). The presented studies led to the development of a sensitive and inexpensive method for voltammetric detection of fluoride ions. Although various derivatives of boronic acids, boronic esters or boronate-functionalized polymer films enabled the electrochemical sensing of fluoride ions [38,46,47], functional sensors exhibiting low detection limit were not reported.

3.4. Interactions of APBA anchored to alkanethiols monolayers with fructose

The studies on the development of functional voltammetric sensor for the detection of fructose were extended to the use of aminophenylboronic acid (APBA), chemically linked with alkanethiols monolayers obtained on gold electrodes. Two types of alkanethiols with terminal carboxylic group differing in length of hydrocarbon chain (MHDA and MHA) were used as a platform for the anchoring of boronic acid molecules. The main goal was to improve the structure and quality of the APBA monolayer, and thus the sensitivity and stability of the sensor. However, the electrochemical approach proposed in this work provided only the direct evaluation of the performances of the sensors.

The conditioning of the APBA-MHDA and APBA-MHA modified electrodes in the electrolyte solution was a necessary step to ensure the proper operation of the sensor. Without the conditioning procedure, the anodic and cathodic peaks of the redox probe were not identified and the current signal did not undergo significant changes increasing the concentration of the analyte. This effect can be explained by the high degree of organization of the molecules in the monolayer, which blocked the access of $Fe(CN)_6^{3-/4-}$ couple to the electrode surface. It can be assumed that after the conditioning procedure, the molecules of electrolyte were accumulated between the alkyl chains, facilitating the access of the redox probe to the electrode surface.

In contrary to MPBA monolayers, the self-assembly of APBA-alkanethiols resulted in greater decreasing of the peak current height and increasing of the distance between reduction and oxidation peaks (exemplary cyclic voltammograms recorded for APBA-MHDA modified electrodes in fructose solution were depicted in Fig. 6). Different efficiencies of electron tunneling proceeded through APBA linked to various alkanethiols immobilized on gold surface. The longer APBA-MHDA exhibited a higher electron transfer resistance than APBA-MHA and in consequence higher values of the peak currents were measured for the latter. The oxidation peak current density of redox probe was equal to 1.23 ± 0.09 mA/cm^2 and 0.93 ± 0.04 mA/cm^2 (for 10^{-8} and 10^{-2} M fructose solution) at APBA-MHDA modified electrode, whereas 1.54 ± 0.08 mA/cm^2 and 1.27 ± 0.11 mA/cm^2 at APBA-MHA electrode. The values of formal potentials of $Fe(CN)_6^{3-/4-}$, estimated in the presence of fructose, were more positive for electrode modified with MHDA than MHA SAMs i.e. the studied redox processes were slower and more irreversible in the case of APBA-MHDA based electrode.

Mixed monolayers, containing not more than 10% of APBA molecules, were obtained in order to minimize the intermolecular interactions inside the layer. The shape of the voltammogram for reductive desorption of APBA-MHDA/OT layers confirmed the formation of mixed monolayers i.e. two peaks corresponding to the reduction of respective components of various chain lengths were noticed at different potentials (OT at -1.00 V, and APBA-MHDA at -1.27 V). Lower content of the receptor component can limit the repulsion of boronic acid moieties evidenced by a decrease of half-height width of the voltammetric peaks (see Fig. 7). Moreover, the functionalization of the gold electrode with mixed layer (APBA-MHDA/OT) induced ~1.5 times higher current densities of redox marker oxidation (recorded by SWV in fructose solution) compared to those obtained for pure APBA monolayer.

The responses of APBA-MDHA modified electrodes towards fructose were collected in Fig. 2. The smallest sensitivity (-2.33 μA/decade) and limited linear range of the calibration plot, reflected in the case of electrode functionalized with pure APBA, was probably associated with molecular structure of this type of monolayer. More compact and less permeable layer prevents the binding of a large number of fructose molecules, since the receptor

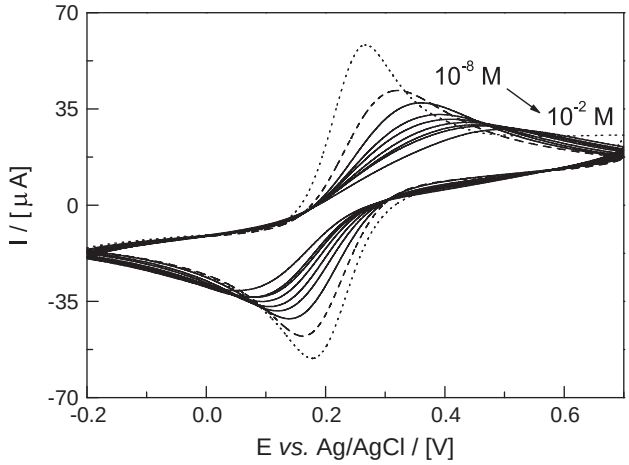

Fig. 6. Cyclic voltammograms recorded for electrode in solution of $Fe(CN)_6^{3-/4-}$ ions (pH 9.0) before (dotted line) and after modification with APBA-MHDA in the absence (dashed line) and in the presence (solid lines) of fructose.

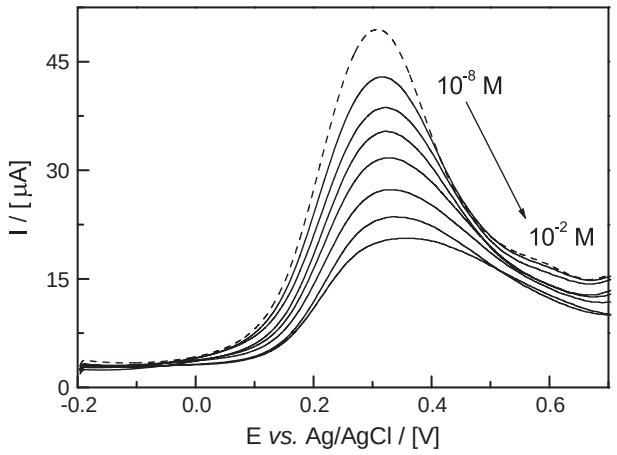

Fig. 7. Square wave voltammograms recorded for electrode in solution of $Fe(CN)_6^{3-/4-}$ ions (pH 9.0) modified with mixed APBA-MHDA/OT monolayer in the absence (dashed line) and in the presence (solid lines) of fructose.

molecules are too close to each other and interactions within the layer impede the access of fructose to boronic acid units, followed by the saturation of the layer with analyte. On the other hand, the electrode based on mixed APBA-MHDA/OT monolayer displayed better sensitivity (-3.66 μA/decade) in a wide linear range, which confirmed that proper separation of molecular receptor moieties allowed optimizing the sensitivity of analyte detection.

4. Conclusions

Several methodologies of the modification of gold surface with phenylboronic acids, anchored directly to the electrode or chemically linked with alkanothiolate monolayers with terminal amino groups were presented and compared in this work. The receptor molecules were assembled in monocomponent and mixed monolayers, where the boronic acid moieties were embedded in a octanethiol matrix. The electrochemical sensing properties of the molecular platforms were examined by voltammetry and the packing densities of immobilized compounds on the gold surface were determined and discussed.

The experimental results indicated lower surface concentration of 4-mercaptophenyl-boronic acid (MPBA) on gold electrodes in comparison to 4-aminophenylboronic acid (APBA). Moreover, APBA monolayers exhibited a higher degree of structural order involving higher electrode sensitivity towards fructose. On the other hand, higher sensitivity of detection of fluoride anions was noticed for electrodes modified with more defective and permeable MPBA monolayers. The differences in sensitivity of voltammetric sensors based on MPBA and APBA monolayers for given analytes can also be explained by different resistances of electron transfer.

Concluding, both MPBA and APBA attached to the alkanethiols matrix formed by spontaneous organization on gold surface, can act as molecular receptors of fructose and fluoride anions providing promising voltammetric sensors for the detection of the analytes in real samples, even in micromolar range.

Acknowledgments

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