



Molecular imprinting of boronate functionalized polyaniline for enzyme-free selective detection of saccharides and hydroxy acids

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ABSTRACT

The majority of reagentless affinity sensors suffer from poor selectivity because specific binding and non-specific interactions generate responses in the same direction, commonly causing resistance increase. We report on the advanced transducer, synthesized in course of molecular imprinting, which generates conductivity increase as a result of specific binding. Electropolymerization of 3-aminophenylboronic acid (3-APBA) known to yield conductive polyaniline only in the presence of F⁻, has been carried substituted the latter for hydroxy acids. The achieved synthesis of conductive polymer in course of fluoride-free 3-APBA electropolymerization indicates successful imprinting of hydroxy acids. Imprinting poly(3-APBA) provides as 10–15 times increased, as even decreased binding constants towards target analytes as compared to the non-imprinted polymer. This allows one to achieve the desired selectivity among polyols. Tuning binding properties of boronate functionalized polyaniline we've demonstrated the enzyme-free selective detection of saccharides and hydroxy acids via conductivity increase of the conductive polymer.

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1. Introduction

Saccharides (glucose, fructose etc.) and hydroxy acid residues (lactate, tartrate) are apparently the most important low molecular weight analytes in clinical diagnostics and food industry. Despite both scientific and commercial successes of glucose biosensors has been achieved, the inherent instability of proteins causes a search for non-enzymatic glucose sensors and tests. Lactate oxidase, the enzyme most widely used in lactate biosensors, is less stable [1] and 10–100 times more expensive compared to glucose oxidase [2,3]. Biochemical analysis of fructose is even more complicated.

Biomimetics (mimicking of biological recognition) involves a search for synthetic receptors recognizing certain functional groups. Among such receptors the phenylboronic acid is particularly attractive providing binding selectivity to compounds possessing 1,2- or 1,3-diol functionalities, common structural elements of saccharides and hydroxy acids. The underlying chemistry was thoroughly investigated [4–7] and the area of the corresponding sensors was extensively reviewed [8–11].

Electropolymerization of 3-aminophenylboronic acid (3-APBA) yielding conductive polymer was already reported [12], and the corresponding sensors have already been elaborated [13–15] suffering, however, from poor analytical performance characteristics. Indeed, the sensors reported in [13–15] were potentiometric with the maximum response for glucose of 1.5–2.0 mV, which considering poor stability of the polyaniline redox potential made them hardly applicable.

We've already reported that boronate-substituted polyaniline is able to generate an increase in its conductivity in course of binding with polyols [16]. Taking into account that both polymer degradation and non-specific interactions result in increase of resistance, the discovered raise in conductivity as a result of specific bindings allows the differentiation of the latter from parasitic reactions. However, the obtained material suffered from broad specificity to polyols making impossible their selective detection.

As known, affinity can be improved by imprinting specific analytes during polymerization with their subsequent removal from the resulting material. The so-called molecularly imprinted polymers (MIPs) after their discovery [17,18] have comprised an important field of analytical chemistry. Indeed, even for boronate functionalized polyaniline the saccharide imprinted polymer has been reported [19]. However, saccharides are unsuitable imprinting molecules for synthesis of conductive boronate-substituted

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polyaniline due to the following reason. Whereas conductive polyaniline can be synthesized only from acidic media [20], the affinity of phenylboronic acid towards saccharides, like glucose, decreases as solution pH is lowered below pH 8.9 (pK_a of phenylboronic acid) [4,21]. As a result, there is no overlapping in pH ranges for polyaniline synthesis and for phenylboronic acid-to-saccharide complex formation. Not surprisingly, an attempt for imprinting of boronate functionalized polyaniline with D-fructose produced only a marginal effect of 25% increase in selectivity [19].

While saccharides are unsuitable for imprinting of conductive polyaniline with boronate functionality, binding constants of hydroxy acids with phenylboronic acid display their highest values below pK_a of phenylboronic acid and remain large in weakly acidic solutions [4,21]. Hence, among polyols the only suitable molecules for imprinting are hydroxy acids. Molecular imprinting of conductive poly(aminophenylboronic acid) is first shown in the present article. Tuning affinity of the synthetic receptor towards different polyols it is possible to achieve their selective detection. The concept has been proved analyzing fructose-lactate mixture with the two sensors of different selectivity, which allows enzyme-free selective detection of fructose and lactate.

2. Experimental

2.1. Materials

Experiments were carried out with Millipore Milli-Q water. Potassium L-lactate, 2- and 3-aminophenylboronic acid hydrochloride were purchased from Sigma Aldrich (Germany). D-glucose, D-fructose, sodium tartrate, inorganic salts and acids were obtained of the highest purity from Reachim (Moscow, Russia).

2.2. Instrumentation

Cyclic voltammetry was carried out using PalmSens potentiostat (PalmSens Instruments BV, Netherlands) interfaced to PC. Impedance spectra were recorded using Solartron 1255 frequency response analyzer (Solartron, UK) with home-made low noise electrochemical interface.

2.3. Methods

Three-compartment electrochemical cell contained a platinum net auxiliary electrode and Ag/AgCl reference in 1 M KCl were used. Glassy carbon disk (2.0 mm in diameter) working electrodes were used. Prior to use, they were mechanically polished with alumina powder (Al_2O_3 , 10 μm) up to a mirror finish observed. Impedance spectra were recorded in electrochemical cell with the common space of working and auxiliary electrodes. The latter was a platinum cylinder encircling working electrode. Impedance spectra were recorded in 0.05 M phosphate buffer solution with 0.1 M KCl at a room temperature taking sine-wave voltage amplitude of 5 mV in the frequency range from 1 Hz to 20 kHz; dc potential was in the range from -50 to 150 mV.

Boronate functionalized polyaniline was synthesized by electropolymerization of 3-APBA from its 0.1–0.15 M solution in 0.1–0.2 M sulfuric acid containing different templating agents (0.1–0.75 M). Growing solution pH was from 3.0 to 3.5. The electropolymerization was carried out by cycling the applied potential at a sweep rate of 40 $mV s^{-1}$, the anodic switching potential was in the range 0.85–0.9 V. Growing solution pH was kept close to pK_a of the hydroxy acid ensuring ionization of the latter for successful complexation with aminophenylboronic acid [4,21]. Sulfate has been chosen as counter-anion providing the highest rate of polyaniline growth [22]. Polymer-coated electrodes were gently rinsed

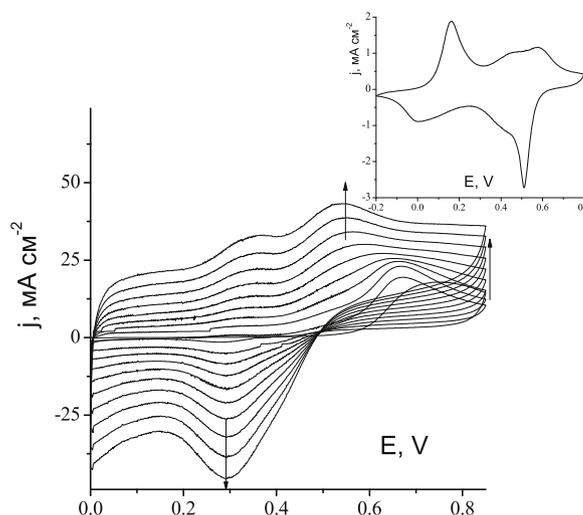


Fig. 1. Electropolymerization of 3-APBA (0.1 M) in the presence of 0.2 M sodium tartrate and 0.1 M sulfuric acid, pH 3; sweep rate 40 $mV s^{-1}$. Inset: Cyclic voltammogram of the 3-APBA electropolymerized in the presence of tartrate, recorded in monomer-free acidic solution: 0.1 M HCl with 0.1 M KCl, sweep rate 40 $mV s^{-1}$.

with sulfuric acid after preparation and with buffer solution before impedance measurements.

3. Results and discussion

Polyaniline synthesis is known to occur through electrophilic *para*-substitution relatively amino group [23]. In line with this, polymerization is favored by electron donor substituents particularly in *meta*-position to $-NH_2$ [24]. When boronic acid residue, a weak electron acceptor [25], forms a complex with hydroxy acid in weakly acidic solutions, it is converted into an electron donor group due to negative charge allocated at the boron atom [26]. Hence for imprinting of boronate functionalized polyaniline by hydroxy acids, the monomer with boronic acid substituent in *meta*-position relatively amino group should be taken.

In contrast to unsubstituted aniline, the electropolymerization of 3-APBA is known to yield conducting polymer only in the presence of fluoride ions [12]. Electropolymerization in fluoride-free solution points to synthesis of non-conductive polymer (Fig. S1, Supporting information). Hence, fluoride-free 3-APBA electropolymerization yielding conducting polymer would indicate successive imprinting of F^- substituting molecules.

Imprinting of different hydroxy acids has been investigated. As seen in Fig. 1, fluoride-free synthesis of conductive polymer in course of electropolymerization of 3-APBA is achieved. Specifically, tartrate is able to promote the growth of conducting polymer in the absence of fluoride ion. Indeed, a continuous growth of conductive polymer noticed from an increase in current of the monomer irreversible oxidation at the anodic switching potential (0.85 V) is observed (Fig. 1). Formation of electroactive covering is concluded from both increase of anodic currents in an entire potential range and raise of cathodic currents below 0.5 V. The inset in Fig. 1 displays cyclic voltammogram of the resulting polymer in acidic solution. As seen, it is very similar to cyclic voltammograms of the common conductive polyaniline. The observed fluoride-free synthesis of conductive polymer in course of 3-APBA electropolymerization indicates successful imprinting of tartrate.

Similarly, the growth of conductive polymer in course of fluoride-free 3-APBA electropolymerization in the presence of lactate has been observed (Fig. S2, Supporting information). Also considering that in the absence of fluoride ion the only non-conducting polymer can be synthesized, we conclude a

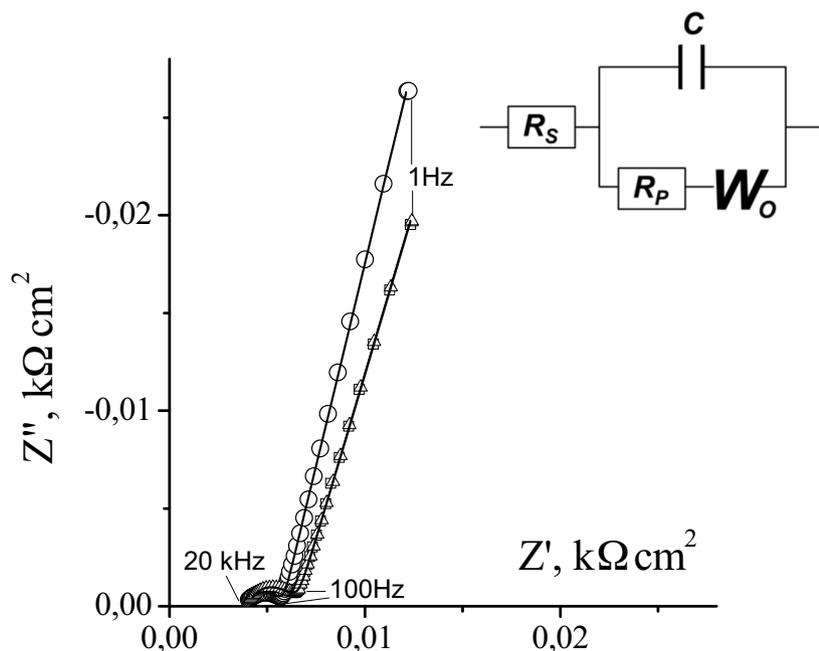


Fig. 2. Impedance spectra of the electropolymerized 3-APBA in the presence of 0.2 M sodium tartrate: 0.05 M phosphate pH 7.0 with 0.1 M KCl, $\Delta E = 5$ mV, $E_{dc} = 20$ mV: (\square) – initial, (\circ) – in the presence of 55 mM glucose, (Δ) – in buffer after glucose washing out; solid lines illustrate fitting to the equivalent circuit. Inset: the equivalent circuit used.

successful imprinting of lactate in course of 3-APBA electropolymerization.

Usually template molecules form strong complexes with MIPs because each functional group has to remain its ligand upon involvement the latter in polymer network. This causes template removal to be rather difficult. In our case regeneration of the electropolymerized 3-APBA from bound saccharides and hydroxy acids occurs even in neutral phosphate buffer [16]. Accordingly, washing with sulfuric acid and equilibration in phosphate buffer (see Experimental) is enough to remove templating molecules from poly(3-APBA).

It is highly attractive to investigate transducing properties of the resulting conducting polymers. Impedance spectra of the tartrate imprinted boronate functionalized polyaniline are displayed in Fig. 2. Similarly to both poly(2-APBA) [27] and 3-APBA electropolymerized without hydroxy acids [16], the impedance spectra are successfully fit to the simplest equivalent circuit valid for conducting polymers (Fig. 2, inset) [28]. Here R_s is solution resistance, R_p is film resistance, C is double layer and film capacitance, W_o is the diffusion impedance with reflective boundary conditions [29]. We note, that except for variation of the exponent in W_o , which is possible for fitting of real conductive polymer spectra [28], all other elements of the circuit are real resistances and capacitance rather than constant phase elements.

Transducing properties of poly(3-APBA) imprinted with tartrate are illustrated in Fig. 2 displaying impedance spectra with and without saccharide (glucose). As seen, the diameter of the high frequency semicircle (equal to the film resistance) is decreased after addition of glucose. Fitting to the equivalent circuit (Fig. 2, inset) confirms an increase in film conductivity as a result of the specific binding. After saccharide removal the impedance spectrum returns to its initial shape confirming that the response is completely reversible (Fig. 2). Similar behavior has been observed for lactate imprinted boronate functionalized polyaniline (Fig. S3, Supporting information).

A confirmation, that an increase in conductivity of boronate functionalized polyaniline is a result of the specific binding

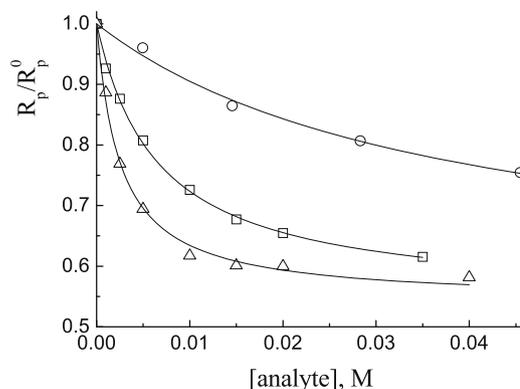


Fig. 3. Calibration graphs for (\circ) glucose, (\square) lactate and (Δ) fructose of the lactate imprinted boronate-substituted polyaniline (grown from 0.15 M 3-APBA, 0.75 M lactate and 0.2 M H_2SO_4 , pH 3.5); 0.05 M phosphate containing 0.1 M KCl, pH 7.0 for glucose and fructose and pH 6.0 for lactate.

between phenylboronic acid residue and polyols has been found considering impedance spectra of unsubstituted polyaniline. As observed, saccharide (glucose) addition causes an increase in resistance of the common polyaniline (Fig. S4, Supporting information).

We thus conclude that poly(3-APBA) imprinted with a hydroxy acid generates conductivity increase as a result of specific binding. The main advantage of this transducer over the majority of the reported systems is the possibility for differentiation of the specific interaction (resulted in conductivity increase) over non-specific bindings or transducer degradation causing a decrease in film conductivity [30].

To illustrate advantages of the imprinted polymers it is necessary to investigate binding constants for different polyols. Concentration dependencies of the film resistance for lactate imprinted poly(3-APBA) are shown in Fig. 3.

Table 1
Apparent binding constants (M^{-1}) for different boronate-substituted polyanilines with saccharides and hydroxy acids.

	phenylboronic acid	poly(2-APBA)	poly(3-APBA) imprinted with		
			fluoride ion	tartrate	lactate
glucose, pH 7	2 [5]	2.5 ± 1.5 [27]	15 ± 4 [30]	35 ± 3	32 ± 4
fructose, pH 7	92 [5]	98 ± 10 [27]	220 ± 20	420 ± 30	410 ± 40
lactate, pH 6	18.5 [4]	19 ± 4 [27]	32 ± 6	14 ± 3	150 ± 20
tartrate, pH 7	–	260 ± 40	770 ± 30	420 ± 30	480 ± 30

Table 2
Analysis of the model lactate–fructose solution.

Concentration added, mM		Concentration found, mM		Recovery, %	
Fructose	Lactate	Fructose	Lactate	Fructose	Lactate
1.5	1.5	1.6 ± 0.4	1.7 ± 0.4	96.6	88.8
4.0	4.0	3.9 ± 0.9	3.8 ± 0.9	97.1	95.6
1.0	1.5	1.1 ± 0.2	1.6 ± 0.4	93.5	92.8

From these plots it is possible to evaluate the corresponding binding constants using Eq. (1) [4,16]

$$R_p = \frac{R_p^0 + R_p^\infty \cdot K_{app} \cdot C}{1 + K_{app} \cdot C}, \quad (1)$$

where C is polyol concentration, R_p is polymer resistance, and R_p^0 and R_p^∞ are resistance at zero and infinite polyol concentration, respectively, and K_{app} is an apparent binding constant. We note, that according to Eq. (1) sensitivity determined as a slope of calibration graph at low (vanishing) analyte concentrations is equal to the apparent binding constant multiplied by $(R_p^0 - R_p^\infty)/R_p^0$. Since the latter does not vary significantly (Fig. 3), we conclude, that the sensitivity of the corresponding sensor is determined by the binding constant K_{app} .

Table 1 summarizes binding constants of different polyanilines with saccharides and hydroxy acids. Among boronate functionalized polyanilines poly(2-APBA) demonstrates the closest behavior to phenylboronic acid [27]. Indeed, the K_{app} values in the corresponding columns of the Table 1 are in a good agreement.

We note, that since aniline electropolymerization occurs through *para*-position the electropolymerized 2-APBA and 3-APBA formally should have similar structures. Compared with phenylboronic acid, 3-APBA electropolymerized in the presence of fluoride ion demonstrates higher binding constants for all polyols under study. This can be explained in terms of conformational changes of boronate substituent after complexation with fluoride ion. Indeed, neutral boronic acid residue, $-B(OH)_2$, displays flat geometry. After interaction with fluoride ion it turns into $-B(F)_3-$ form having tetrahedral configuration similar to boronate-polyol ester. Hence, fluoride ion changes configuration of boronate substituent into the form, optimal for complexation with polyols. The presence of F^- in course of 3-APBA electropolymerization can be thus considered as fluoride ion imprinting. Accordingly, the only poly(2-APBA) synthesized in the absence of fluoride ion or any other templating agent be considered as non-imprinted polymer.

In terms of sensitivity the highest imprinting effect for both hydroxy acids is observed in case of complexation with saccharides: the corresponding binding constant for glucose is increased 10–15 times (Table 1). In addition, the 4–5 times improved binding constants towards fructose after imprinting with both hydroxy acids are observed (Table 1). Even compared with the polymer grown in the presence of fluoride ion, the imprinting with both hydroxy acids provides further twice improvement of the binding constants for both glucose and fructose.

The highest selectivity gain due to molecular imprinting is observed in lactate detection. The polymer imprinted with lactate displays 5 times higher binding constant than boronate

functionalized polyaniline imprinted with fluoride ion. Moreover, tartrate imprinting results in two-fold decreased binding constant compared to F^- imprinting (Table 1). Accordingly, imprinting with various template molecules one can vary lactate binding constant 10 times achieving thus the desired selectivity.

For practical applications the analytical parameters of the poly(3-APBA) based sensors have to be derived from the calibration graphs (Fig. 3, for example). The dynamic ranges and the detection limits are displayed in the tables S1 and S2 (Supporting information), respectively. The dynamic ranges always exceed one order of magnitude of analyte concentrations. The detection limits vary from less than 0.1 mM for fructose (lactate and tartrate imprinted polymers) to almost 10 mM for glucose (non-imprinted boronate functionalized polyaniline).

A lack of satisfaction considering molecular imprinting is observed in case of tartrate. Whereas both tartrate and lactate imprinted polymers display twice improved binding constants compared to non-imprinted polymer, the fluoride ion imprinting provides even higher affinity (Table 1). We note that tartrate is a dibasic acid. Its second ionization constant is too high ($pK_a > 4$) [31] to provide complete tartrate ionization during synthesis of conductive polyaniline. Accordingly, in course of aniline polymerization we are imprinting a mono tartrate anion. This is enough to improve tartrate binding constant only over non-imprinted polymer (Table 1). Moreover, both lactate and tartrate around pH 3 used for polyaniline synthesis are large monobasic templates. Hence, due to steric constrains boronate functionalities are only partially etherified in course of imprinting of either lactate or tartrate. On the contrary, small templating agent, fluoride ion, is able to change geometry of nearly all boronate substituents. Hence, it is not surprising, that affinity towards ions with the two diol functionalities (tartrate, pH 7) is higher for fluoride ion imprinted poly(3-APBA) (Table 1).

The variation in binding constants achieved in course of imprinting should allow selective detection of polyols in their mixture. Obviously, detection of several compounds in the same solution requires several sensors, and the sensors quantity should be not less, than the quantity of analytes. As a model solution we've chosen the fructose–lactate mixture recommended by Agilent Ink. for validation of chromatographic columns [32]. Accordingly, the two sensors have to be used. We note that to achieve the best analytical performance characteristics the sensor materials with the highest variation in their selectivity, in our case, binding constants (Table 1), have to be chosen. As one sensing material the lactate imprinted poly(3-APBA) should be chosen, as the other – either fluoride ion or tartrate imprinted polymer. Despite the latter displays lower binding constant towards lactate, it is much less reproducible.

Accordingly, the two sensors based on the poly(3-APBA) imprinted with lactate and fluoride ions have been used. Assuming there is no cooperative effect of polyols on sensor response, the response of each sensor can be considered as a sum of the two Eq. (1) – type terms. The two equations with the two unknown quantities have to be solved.

The chosen model solutions were 1.5 mM lactate with 1.0 mM or 1.5 mM of fructose and an equimolar (4.0 mM) lactate–fructose solution. The relative responses of the poly(3-APBA) imprinted with lactate and fluoride ions towards the models solutions are shown in table S3 (Supporting information). Sensor responses in different mixtures under investigation differ significantly. To find the recovery values, the parameters of the calibration graphs for both sensors shown in the table S4 (Supporting information) have been used.

The recovery values are displayed in Table 2. We note that they are always not lower than 89%, the latter is the lowest recovery value for 1.5 mM lactate from equimolar fructose–lactate solution. The successful recovery proves a possibility for non-enzymatic selective detection of saccharides and hydroxy acids.

4. Conclusions

Imprinting with hydroxy acids in course of 3-APBA electropolymerization yields conductive boronate functionalized polyaniline generating conductivity increase as a result of specific binding and thus allowing the discrimination of the specific signal from non-specific interactions. Imprinting with hydroxy acids providing up to 10–15 times increased binding constants between the synthetic receptor and the target molecule, may, however, result in decreased affinity towards specific analyte.

Sensors on the basis of hydroxy acids imprinted boronate functionalized polyaniline can find applications not only in selective detection of polyols using several sensors with different selectivity, as shown in the article. Among important application areas are (bio)chemical processes causing significant variations in concentration of a particular polyol. We don't see practical limits to elaborate an enzyme-free non-invasive hypoxia monitor aiming to improve both storage and operational stability of such device operated through lactate monitoring in sweat and based on engineered enzyme lactate oxidase [33].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.snb.2017.02.073>.

References

- [1] E.I. Yashina, A.V. Borisova, E.E. Karyakina, O.I. Shchegolikhina, M.Y. Vagin, D.A. Sakharov, A.G. Tonevitsky, A.A. Karyakin, Sol-gel immobilization of lactate oxidase from organic solvent: toward the advanced lactate biosensor, *Anal. Chem.* 82 (2010) 1601–1604.
- [2] Sigma-Aldrich, Lactate Oxidase, <http://www.sigmaaldrich.com/catalog/product/sigma/i0638?lang=en&ion=RU>, 2017 (Accessed 16 January 2017).
- [3] Sigma-Aldrich, Glucose oxidase, <http://www.sigmaaldrich.com/catalog/product/sigma/g2133?lang=en&ion=RU>, 2017 (Accessed 16 January 2017).
- [4] M.A. Martínez-Aguirre, R. Villamil-Ramos, J.A. Guerrero-Alvarez, A.K. Yatsimirsky, Substituent effects and pH profiles for stability constants of arylboronic acid diol esters, *J. Org. Chem.* 78 (2013) 4674–4684.
- [5] G. Springsteen, B. Wang, A detailed examination of boronic acid–diol complexation, *Tetrahedron* 58 (2002) 5291–5300.
- [6] L.I. Bosch, T.M. Fyles, T.D. James, Binary and ternary phenylboronic acid complexes with saccharides and Lewis bases, *Tetrahedron* 60 (2004) 11175–11190.
- [7] J.A. Peters, Interactions between boric acid derivatives and saccharides in aqueous media: structures and stabilities of resulting esters, *Coord. Chem. Rev.* 268 (2014) 1–22.
- [8] Q. Wu, L. Wang, H. Yu, J. Wang, Z. Chen, Organization of glucose-responsive systems and their properties, *Chem. Rev.* 111 (2011) 7855–7875.
- [9] X. Yang, Y. Cheng, S. Jin, B. Wang, Boronic acid-based receptors and chemosensors, in: V.M. Mirsky, A.K. Yatsimirsky (Eds.), *Artificial Receptors for Chemical Sensors*, Wiley-VCH Verlag GmbH & Co. KGaA, 2010, 2017, pp. 169–189.
- [10] X. Wu, Z. Li, X.-X. Chen, J.S. Fossey, T.D. James, Y.-B. Jiang, Selective sensing of saccharides using simple boronic acids and their aggregates, *Chem. Soc. Rev.* 42 (2013) 8032–8048.
- [11] S.D. Bull, M.G. Davidson, J.M.H. van den Elsen, J.S. Fossey, A.T.A. Jenkins, Y.-B. Jiang, Y. Kubo, F. Marken, K. Sakurai, J. Zhao, T.D. James, Exploiting the reversible covalent bonding of boronic acids: recognition, sensing, and assembly, *Acc. Chem. Res.* 46 (2012) 312–326.
- [12] M. Nicolas, B. Fabre, G. Marchand, J. Simonet, New boronic-acid- and boronate-substituted aromatic compounds as precursors of fluoride-responsive conjugated polymer films, *Eur. J. Org. Chem.* 2000 (2000) 1703–1710.
- [13] E. Shoji, M.S. Freund, Potentiometric sensors based on the inductive effect on the pK(a) of poly(aniline): a nonenzymatic glucose sensor, *J. Am. Chem. Soc.* 123 (2001) 3383–3384.
- [14] E. Shoji, M.S. Freund, Potentiometric saccharide detection based on the pK(a) changes of poly(aniline boronic acid), *J. Am. Chem. Soc.* 124 (2002) 12486–12493.
- [15] M.S. Freund, E. Shoji, Sensors and sensing methods for detecting analytes based on changes in pKa of a sensing polymer, United States Patent US 6797152, 2004.
- [16] E.A. Andreyev, M.A. Komkova, V.N. Nikitina, N.V. Zaryanov, O.G. Voronin, E.E. Karyakina, A.K. Yatsimirsky, A.A. Karyakin, Reagentless polyol detection by conductivity increase in the course of self-doping of boronate-substituted polyaniline, *Anal. Chem.* 86 (2014) 11690–11695.
- [17] B. Sellergren, M. Lepistö, K. Mosbach, Highly enantioselective and substrate-selective polymers obtained by molecular imprinting utilizing noncovalent interactions – NMR and chromatographic studies on the nature of recognition, *J. Am. Chem. Soc.* 110 (1988) 5853–5860.
- [18] K. Mosbach, Molecular imprinting, *Trends Biochem. Sci.* 19 (1994) 9–14.
- [19] B. Deore, M.S. Freund, Saccharide imprinting of poly(aniline boronic acid) in the presence of fluoride, *Analyst* 128 (2003) 803–806.
- [20] A.G. MacDiarmid, A.J. Epstein, Polyanilines: a novel class of conducting polymers, *Faraday Discuss.* 88 (1989) 317–332.
- [21] J. Yan, G. Springsteen, S. Deeter, B. Wang, The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols—it is not as simple as it appears, *Tetrahedron* 60 (2004) 11205–11209.
- [22] G. Zotti, S. Cattarin, N. Comisso, Cyclic potential sweep electropolymerisation of aniline. The role of anions in the polymerisation mechanism, *J. Electroanal. Chem.* 239 (1988) 387–396.
- [23] W. Huang, B.D. Humphrey, A.G. MacDiarmid, Polyaniline, a novel conducting polymer, *J. Chem. Soc., Faraday Trans.* 82 (1986) 2385–2400.
- [24] S. Cattarin, L. Doubova, G. Mengoli, G. Zotti, Electrosynthesis and properties of ring-substituted polyanilines, *Electrochim. Acta* 33 (1988) 1077–1084.
- [25] D.G. Hall (Ed.), *Boronic Acids: Preparation and Application in Organic Synthesis and Medicine*, Wiley-VCH Weinheim, 2005, p. 1.
- [26] J.P. Lorand, J.O. Edwards, Polyol complexes and structure of the benzenboronate ion, *J. Org. Chem.* 24 (1959) 769–774.
- [27] V.N. Nikitina, I.R. Kochetkov, E.E. Karyakina, A.K. Yatsimirsky, A.A. Karyakin, Tuning electropolymerization of boronate-substituted anilines: fluoride-free synthesis of the advanced affinity transducer, *Electrochem. Commun.* 51 (2015) 121–124.
- [28] G. Inzelt, G.G. Lang, Electrochemical impedance spectroscopy for polymer characterization, in: S. Cosnier, A.A. Karyakin (Eds.), *Electropolymerization: Concepts, Materials and Applications*, WILEY-VCH, Weinheim, 2010, pp. 51–76.
- [29] E. Barsouk, J.R. Macdonald, Impedance Spectroscopy, J. Wiley&Sons, New York, 1987, pp. 80–117.
- [30] M.A. Komkova, E.A. Andreyev, V.N. Nikitina, V.A. Krupenin, D.E. Presnov, E.E. Karyakina, A.K. Yatsimirsky, A.A. Karyakin, Novel reagentless label-free detection principle for affinity interactions resulted in conductivity increase of conducting polymer, *Electroanalysis* 27 (2015) 2055–2062.
- [31] J.-M. Kassaian, Tartaric acid, in: *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co KGaA, 2000, pp. 671–678.
- [32] S. Ball, S. Bullock, L. Lloyd, K. Mapp, Analysis of Carbohydrates, Alcohols, and Organic Acids by Ion-exchange Chromatography, In: *Agilent Technologies, Inc.*, 5990–8801EN, USA, 2011.
- [33] M.M. Pribil, G.U. Laptev, E.E. Karyakina, A.A. Karyakin, Noninvasive hypoxia monitor based on gene-free engineering of lactate oxidase for analysis of undiluted sweat, *Anal. Chem.* 86 (2014) 5215–5219.

Biographies



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