Biosensors and Bioelectronics 54 (2014) 515–520

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Potentiometric sensors doped with biomolecules as a new approach to small molecule/biomolecule binding kinetics analysis

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ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form
11 November 2013
Accepted 12 November 2013
Available online 23 November 2013

Keywords:
Binding kinetics
Potentiometry
Biosensor
Dopamine
Aptamer

ABSTRACT

The most successful binding kinetics analysis systems at this moment include surface plasmon resonance (SPR), quartz microcrystal balance (QMB) and surface acoustic wave (SAW). Although these are powerful methods, they generally are complex, expensive and require the use of monolayers. Here, we report on potentiometric sensors as an inexpensive and simple alternative to do binding kinetics analysis between small molecules in solution and biomolecules (covalently) attached in a biopolymer sensor coating layer. As an example, dopamine and an anti-dopamine aptamer were used as the small molecule and the biomolecule respectively. Binding between both follows a Langmuir adsorption type model and creates a surface potential. The system operates in Flow Injection Analysis mode (FIA). Besides being an interesting new binding kinetics tool, the approach allows systematic design of potentiometric biosensors (in the present study a dopamine sensor), and gives new insights into the functioning of ion-selective electrodes (ISE's).

1. Introduction

Some 20 years after its introduction, surface plasmon resonance (SPR) has become the successful standard for studying interactions between (bio)molecules (Jönsson et al., 1991; Homola et al., 1999; Schasfoort and Tudos, 2008). The method relies on the measurement of adsorption/desorption rates of "prey" molecules injected in a microfluidics flow system, to "bait" molecules linked to the surface of a sensor. The prey molecules are injected in different concentrations, as square concentration pulses. This provokes typical response patterns on the sensor, which are called sensorgrams. The sensorgram shows the speed at which molecules adsorb to–, and desorb from molecules which are immobilized on the sensor surface. From these adsorption/desorption patterns, k_{on} and k_{off} rate constants, and K_d equilibrium constants (equal to k_{off}/k_{on}) are derived. These K_d values can be different from K_d values measured in solution. SPR has a high market penetration, and is used in over 1000 publications per year. Its most competitive alternatives are based on the quartz microcrystal balance (QMB) (Liu et al., 2011) and on the surface acoustic wave (SAW) principle (Gronewold, 2007). These methods have a strongly growing field of applications, ranging from guiding the design of new drugs to the study of a variety of biopolymer interactions in molecular biology. They all use surface derivatisation to obtain molecular monolayers or film layers with nm dimensions.

Electrochemical sensors have until now played only a minor role in binding kinetics analysis. We have shown recently for the first time that potentiometric sensors with a high impedance voltmeter readout system can be used to measure the analyte/sensor surface interaction. An adsorption/desorption kinetics approach was used, comparable to SPR methodology. We will demonstrate in the present work that the response of potentiometric sensors can also be described with the model developed by our group , when an aptamer is used as the recognition element in the (bio)sensor. The specificity and selectivity of the potentiometric biosensors is determined by the strength of the intermolecular attraction between the bait recognition element and the prey analyte molecule, as expressed via a K_d value.

The working principles of potentiometric sensors were discussed critically recently (Wojciechowski et al., 2010). It was shown that analyte adsorption at the sensors surface plays an underestimated role in potential formation for many potentiometric sensors. These sensors can work with thick (μm scale) ionically conductive and biocompatible coatings, in which the biomolecules can be covalently attached. As other binding kinetics measuring devices work with nm scale bait layer structures, potentiometry opens new roads as the biomolecules can be attached equally well with the classical nm scale method, as with a thick (μm scale) surface coating. In earlier work (De Wael et al., 2012a),

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http://dx.doi.org/10.1016/j.bios.2013.11.045

we were able to calculate the K_d values for the interaction of drug substances and organic acids with the surface of a potentiometric sensor. In the present work, we describe a method to study the interaction of DNA and aptamer molecules with basic drugs injected via a flow injection analysis (FIA) setup. The DNA and aptamer molecules are embedded covalently in the natural polymer collagen, which forms the top coating layer of the sensor. Collagen forms a robust gelatin layer which is used in many industrial applications (Gareis and Schrieber, 2007). We used a dopamine (DA)/anti-dopamine aptamer interaction as a model to study this method.

This report describes for the first time that a potentiometric sensor can be used to study an analyte/biomolecule interaction. We are confident that the method can be extended to examine the binding kinetics of other molecule/biomolecule combinations. In contrast to existing binding kinetics methods, the bait layer is not restricted to (but can also be used in) the nm scale. We also believe that these findings will give a boost to the understanding of potentiometry, and that it will lead to a more rapid development of potentiometric biosensors.

2. Experimental

2.1. Instrumentation

2.1.1. Flow injection analysis (FIA)

The FIA recordings were performed using a LC-10ADvp pump (Shimadzu Liquid Chromatography) and a Rheodyne 7125 six port external sample injector (VICI, US). A 2.00 mL sample loop was used to generate square concentration pulses for potentiogram recordings. The PEEK tubing (Alltech, US) of the injection loop and the injector–detector connections had an internal diameter of 0.18 mm. The flow rate was 1.00 mL min^{-1}. Poiseuille peak broadening effects were kept to a minimum using short injector–detector connections (150 mm). To avoid such effects at the end of the square concentration pulse of 80 s duration, the injector was switched from inject to load after 80 s (well before the sample loop volume was totally emptied). This results in a sharp pulse with negligible broadening as well at the start as at the end of the pulse.

The eluent was 10.0 mM 2-(N-morpholino)ethanesulfonic acid (MES), pH 7.0. The column outlet was directed perpendicularly towards the sensitive membrane of the coated wire electrode in a "wall-jet" flow cell (see, Fig. 1). The distance from the LC tubing outlet to the electrode was 100 μm. This home-made flow-cell is fully described in an earlier publication form the group (Daems et al., 2013).

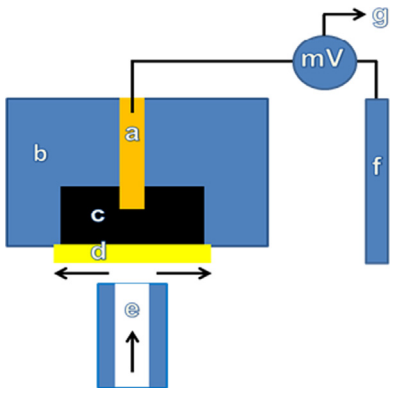

Fig. 1. Section of the large-volume wall-jet flow cell used. It comprises a copper wire (a), a technical PVC cylinder (b), a "substrate" electrode (c) an electrically conducting composite), and a gelatin B coating layer (d). The outlet of the FIA system (e) polyether ether ketone HPLC tubing) is directed perpendicularly to the sensor surface, at a 100 μm distance. "mV" denotes a high impedance voltmeter, "*f*" is a reference electrode and "*g*" is a data station. The figure is not drawn to scale. See experimental for details.

The membrane potential was measured against an Orion 800500Ross® reference electrode (Ag/AgCl) using a high impedance (10^{13} Ω) homemade amplifier. The detection signals were recorded on a data station composed of a PC equipped with a 6013 NI DA converter and LabVIEW 7 (National Instruments, US) based software. The overall RC time constant of the high impedance amplifier plus data station was set to 0.200 s. The sampling rate was 1 point per 2 s. These conditions were in accordance with the time scale of the FIA experiments (several minutes).

2.1.2. Confocal microscopy

Cy3 dye labeled anti-DA aptamers were visualized with a Nikon C1 laser scanning confocal unit (D-eclipse-C1, Nikon, Melville, NY) equipped with an argon and a helium/neon laser line fitted onto an upright microscope (Eclipse E600, Nikon, Melville, US) in combination with a 10× planfluor (NA: 0.50) objective manufactured by Nikon (Melville, US).

2.1.3. Potentiometric electrode

The indicator electrode is made of a PVC cylinder. It contains a cylindrical substrate electrode (3.0 mm diameter × 1.0 mm length), which is an electronically conducting graphite/PVC composite material (Daems et al., 2013). The composite substrate electrode was polished with Carbimet grid 600 (Buehler Ltd, US).

To coat the gelatinous hydrogel on the electrode, 10.0 μL of a mixture, which consists of 25.0 mg gelatin B dissolved in 0.50 mL 10.0 mM MES (5% w/v) pH 7.0 at 40 °C, was brought onto the electrode surface with a micropipette and exposed to air for 2 h at 4 °C (drop drying) (De Wael et al., 2012b).

A 58-mer aptamer selected to specifically detect DA (5'-GTCTCTGTGTGCGCCAGAGAACACTGGGGCAGATATGGGCCAGCACAGAATGAGGCCC-spacer-NH_2-3') (Zheng et al., 2011) was studied. The fluorescently labeled aptamer had the same sequence, and a Cy3 group was coupled to the 5' end: 5'Cy3SP- GTCTCTGTGTGCGCCAGAGAACACTGGGGCAGATATGGGCCAGCACAGAATGAGGCCC-spacer-NH_2-3'. The 25 mer non-specific DNA structure that was used was: 5'- NH_2-spacer-TAGGAAACACCAAAGATGATATTTT-Cy3Sp-3'.

To immobilize the gelatin hydrogel with the anti-DA aptamer (or the non-specific DNA) on the electrode, a mixture of gelatin B, 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) and the oligonucleotide solution was made. A hydrogel solution was made by dissolving 75.0 mg of gelatin B in 500 μL of 0.01 M MES buffer at pH 7.0. A coupling agents solution was made by dissolving 15.32 mg of EDC and 2.32 mg of NHS in 100 μL of 0.01 M MES buffer at pH 7.0. The hydrogel solution (6 μL) was mixed with the coupling agents solution (6 μL) and the 10^{-4} M oligonucleotide solution (12 μL). 10 μL of this mixture was dip-coated on the electrode. This procedure ensures a homogeneous distribution of the aptamer in the hydrogel coating. After evaporation for 2 h at 4 °C, the electrode was kept in 0.01 M MES running buffer pH 7.0 for at least 3 h until a stable baseline was obtained.

The potentiograms of each investigated analyte were measured using 3 electrodes. At least 2 injections were recorded on each electrode after conditioning and stabilization in the running buffer.

2.2. Chemicals

All chemicals were of analytical reagent grade. The FIA eluents were prepared daily by dissolving 2-(N-morpholino)ethanesulfonic acid (MES), obtained from Acros Organics (filter Service NV, Eupen, Belgium). Type B gelatin (IEP=5, Bloom strength=257), isolated from bovine skin by the alkaline process, was obtained from Tessenderlo Chemie (Tessenderlo, Belgium). Basic drugs promazine, lidocaine and ritodrine were obtained from Sigma-Aldrich (Bornem,

Belgium), dopamine (DA) was available from Fluka. To dissolve the drugs, small amounts of ethanol (Fluka, Analytical grade) were used. The coupling agents 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. The oligonucleotides (aptamers and DNA) were synthesized by Integrated DNA Technologies (IDT, Leuven, Belgium).

3. Results and discussion

3.1. Potentiometric response of a gelatin coating doped with an aptamer

An important component of the potentiometric sensor is the ionically conductive coating layer, labeled "d" in Fig. 1, which is doped with the bait biomolecule. In the present study, gelatin B is used as a coating material. It is a proteinaceous collagen derived material, well-known for its robust film-forming properties (Garies and Schreiber (2007)). In potentiometric sensors, this film can be used as a thick layer (μm size range), a thin layer (nm size range), or it can even be omitted (gold surface). SPR does not have this choice: only nm bait layer approaches are possible, and the choice of surface materials is limited (gold being the standard material). We first choose to work with an anti-DA aptamer as the bait molecule immobilized in a collagen film, for the construction of a potentiometric DA sensor to study its interaction with small prey molecules. This aptamer was described in the literature, where it was selected with the SELEX procedure to form a complex with DA (Zheng et al., 2011).

After injection of an analyte in the FIA system, the voltage output varies with time, due to the kinetics of complex formation between DA and the anti-DA aptamer. A positively charged analyte molecule such as DA has the tendency to adsorb to the sensor coating, if a DA binding aptamer is present in this material. This provokes a surface potential change, which is our analytical signal. In its most simple representation, the eluent/gelatin interface is expected to have a Boltzmann type distribution of positive charges as the gelatin behaves as a cation exchange-like material in the given conditions. An adsorption type model was also favored and discussed for this type of potentiometric sensors (Wojciechowski et al., 2010). Our former (De wael et al., 2012a) and present results also strongly point in this direction. In the present study, we calculate the interaction energies between the analyte molecule (DA), and a biological recognition element (an anti-DA aptamer). Adsorption of a cation like DA changes the ionic "capacitor" characteristics of the eluent/gelatin interface. This capacitor is unlikely to be discharged during the measurement of its potential, due to the $10^{13}\ \Omega$ impedance of the voltmeter, allowing a quasi currentless measurement. The generated potential is directly related to the Gibbs free energy of interaction of the analyte with the surface (Daems et al., 2013; Nagels, 2004) as predicted by the well-known $\Delta G = -n$FE equation. Herein, ΔG is the difference in Gibbs free energy of the analyte between the eluent buffer phase and the sensor coating surface. E is the change in potential which results from this interaction.

Gelatin B has been used previously (De Wael et al., 2012b) in amperometric sensor applications. It has a good adhesion to our substrate electrode material (Fig. 1c), which is an electrically conducting carbon composite. The covalent binding of aminated and fluorescently labeled oligonucleotides (see Section 2.1.3) to the Gelatin B (which contains carboxyl groups), was confirmed with confocal microscopy.

In further experiments, the fluorescent label was omitted. As gelatin B is a proteinaceous material which contains functional groups such as carboxyl groups, covalent coupling of biomolecules with many different known coupling methods is possible. Therefore, doping of this (or an equivalent) ionically conducting coating material offers a good method to incorporate biomolecules. As gelatin B is a hydrophilic material, it will generally have a negligible interaction with the analyte (prey) molecule which is under study, and the response of the sensor will be mainly due to the strongly binding biomolecule. A blank experiment (non-doped gelatin material) should therefore always be conducted.

3.2. Testing the potentiometric sensor for dopamine

After checking the coupling of the anti-DA aptamer to the biopolymer as described above (see Section 3.1), the electrodes were placed in a FIA potentiometric setup. The DA molecules were injected as square concentration pulses, comparable to the block pulses in SPR. Potentiograms (mV responses as a function of time) were recorded (see Fig. 2). Using a hydrodynamic method and square concentration pulses has the advantage that we can examine adsorption as well as desorption curves, and calculate the adsorption/desorption kinetics.

Fig. 2 shows the potentiogram of DA on the sensor which contains the anti-DA specific aptamer, for a series of injected concentrations. The concentration dependent response heights (mV obtained after 80 s of injection) are considerable. If the maximum responses of the potentiograms (in mV, see Fig. 2) are plotted against the logarithm of the concentration, the typical Nicolskii–Eisenmann curve is obtained (Fig. 3).

In Fig. 3, the response of the sensor (the voltage) is set to zero for $c_{DA}=0$ ($\log c_{DA}=-\infty$). This condition is experimentally easily obtained by offsetting the voltage reading of the high impedance voltmeter to zero for a zero concentration of analyte (pure buffer solution). The Nicolskii–Eisenmann (NE) equation reduces to a simple form for the case where no other interfering compounds are present except the ions from the elution buffer (Sekula et al., 2006): see Eq. (1)

$$E = E^{\circ} + S \log (c_{DA} + \mathrm{Cst}) \quad (1)$$

In this equation, S is the slope of the calibration curve (see Fig. 3), in the part where the response curve is linearly related to the logarithm of the concentration. In this part of the curve, the DA concentration (c_{DA}) is large as compared to Cst, and E is log c_{DA} dependent. The value of Cst ("Constant") depends on the extent of interference which is provoked by the buffer solution (which is constant). If the DA concentration (c_{DA}) becomes small as compared to Cst, the curve starts to go asymptotically to a constant value which is arbitrarily offset to zero to keep the equations simple. Highly interfering buffers will provoke high values of Cst, yielding inflection points in the curve of Fig. 3 at relatively high concentrations, and vice versa. The smooth curve in Fig. 3 is obtained from a nonlinear least-squares curve fit to a

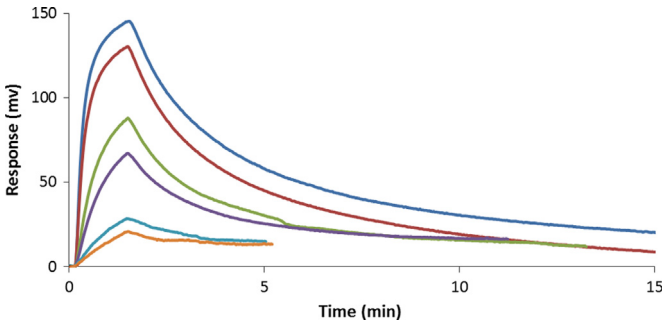

Fig. 2. Potentiogram recordings for DA on a biosensor with a Gelatin B membrane with a covalently linked aptamer. Square concentration pulses were injected for 80 s. The start of the pulse is marked by the onset in the above curves, the end of the pulse is marked by the top of the above curves. The concentration varied from 5×10^{-7} M (lower curve) to 10^{-6} M, 5×10^{-6} M, 10^{-5} M, 5×10^{-5} M, and 10^{-4} M (upper curve).

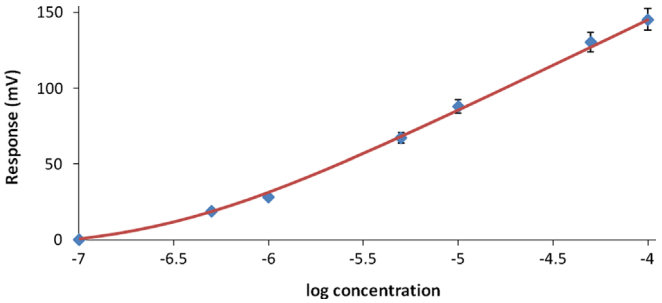

Fig. 3. Nicolskii-Eisenmann-type calibration graph for DA. Each point was the mean of 3 measurements (%RSD ≤ 5).

Nicolskii–Eisenmann function of the type of Eq. (1), using the Solver function in Excel software (Microsoft corp).

The values $E^{\circ}=387$ mV, and $S=60.4$ mV were obtained, and $\mathrm{Cst}=3.90\times10^{-7}$ M.

Figs. 2 and 3 clearly show that the responses provoked by the aptamer-based sensors are large. In comparison to the response of several classical PVC-based electrodes which were thoroughly tested in our group towards this rather hydrophilic compound (Vissers et al., 2006), the responses of the aptamer-based sensors are 15 × higher (on a mV basis). In potentiometry, detection limits are usually calculated by drawing straight lines asymptotically to the linear part in the higher concentrations region, and to the horizontal part in the lower concentrations region (in Fig. 3 this coincides with the *x*-axis) of the NE plot (Fig. 3). The intersection of both lines is taken as the detection limit. In our case (Fig. 3), this was 3.41×10^{-7} M.

The transpose of the Nicolskii–Eisenmann equation (see Eq. (1), and Fig. 3) was derived in a former article from our group (Zheng et al., 2011) resulting in Eq. (2):

$$c_{analyte}=(10^{\mathrm{mV}/S}-1)\mathrm{Cst} \tag{2}$$

Eq. (2) converts the voltage output signal ("mV") of the sensor to a signal which is linearly related to the analyte concentration, $c_{analyte}$. S is the slope of the NE calibration curve in the linear part (see Fig. 3). Therefore, to obtain a concentration dependent output signal, we can use $10^{\mathrm{mV}/S}-1$, which we will call the "transformed Response", tR see Eq. (3).

$$\text{Transformed Response}=\mathrm{tR}=10^{\mathrm{mV}/S}-1=\frac{c_{analyte}}{\mathrm{Cst}} \tag{3}$$

The transformed response values (Eq. (3)) of the potentiometric sensors with coupled aptamer were compared with the values obtained with electrodes which do not have an aptamer coupled to the hydrogel: see Fig. 4, left part. Three electrodes were tested. Three injections were done for each concentration, yielding an intra-electrode variation of 5%. From 5 to 12 times higher signals were measured with the aptamer containing electrodes. Not only the height, but also the shape of the potentiogram was different when comparing hydrogel membranes with and without coupled aptamer: see Fig. 4, left part.

In Fig. 5, the "on" curves from the potentiograms of Fig. 3 are shown, after transformation of the mV axis to a $10^{\mathrm{mV}/S}-1$ axis (tR), and normalization of the plateau values (all curves evolve to 100%). The curves were fitted to the experimentally measured data (nonlinear least-squares method), but we plot the fittings up to 100 min. This figure shows the typical slowing down of the adsorption process as the injected concentration lowers from 10^{-4} M (upper curve) to 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, and 5×10^{-7} M (lowest curve). Note that at the lower concentrations, an equilibrium is not reached even after 100 min.

3.3. Kinetics analysis for the aptamer/DA complex.

In a recent publication De Wael et al., 2012a we developed a method to determine K_d values for the interaction (binding) between a potentiometric sensor membrane (acting as the "bait"), and an analyte (the "prey") molecule. In the present case, the gelatin sensor membrane material itself has little or no interaction with the analyte molecule, as shown above (see e.g. Fig. 4). The main interaction therefore is due to the immobilized biomolecule.

Potentiograms were recorded at different analyte concentrations (see Fig. 2). The mV *y*-axis in these potentiograms was first converted to the concentration dependent response, called transformed Response (see Eq. (2)), tR. The transformed Response (tR) of the sensor is linearly related to the number of occupied adsorption sites, $R_{occupied}$, i.e.: transformed Response (tR) $\sim R_{occupied}$.

The rate of adsorption of the DA, v_{on}, can be regarded as a reaction rate which is first order in the DA concentration in the bulk of the solution ($c_{analyte}$) and in the concentration of free adsorption sites (or aptamers) on the sensor surface, $R_{max}-R_{occupied}$.

The rate of desorption, which is occurring as soon as adsorption takes place, is given by $k_{off}R_{occupied}$. The rate of adsorption is given by Eq. (4), which was derived earlier (De Wael et al., 2012a).

$$\frac{dR_{occupied}}{dt}=k_{on}c_{analyte}R_{max}-(k_{on}c_{analyte}+k_{off})R_{occupied} \tag{4}$$

This equation can be rewritten by substituting the concentration of DA molecules which adsorbed onto (or "occupy") the aptamer derivatized surface, $R_{occupied}$, by the sensor's transformed Response, tR (and by replacing R_{max} by tR_{max}). The rationale behind this is the fact that tR is linearly related to $c_{analyte}$, which in its turn is linearly related to the concentration of adsorbed species, $R_{occupied}$. This yields Eq. (5):

$$\frac{d(tR)}{dt}=k_{on}c_{analyte}tR_{max}-(k_{on}c_{analyte}+k_{off})tR \tag{5}$$

The differential Eq. (5) can be solved to yield Eq. (6):

$$tR(t)=tR_{max}(1-e^{-k_{obs}t}) \tag{6}$$

Where $k_{obs}=k_{on}c_{analyte}+k_{off}$

Eq. (6) describes the curves from Fig. 5. Plotting these k_{obs} values versus $c_{analyte}$ for the set of analyte (DA) concentrations then yields a straight line of the form $y=0.00181x+0.00223$, with R^2 equal to 0.992 ($n=6$).

The slope and intercept of this straight line yields calculated values of 1810 $\mathrm{M}^{-1}\,\mathrm{min}^{-1}$ for k_{on}, and 0.00223 min^{-1} for k_{off} respectively. Therefore, $K_d=1.22\times10^{-6}$ M and $\Delta G(=-\mathrm{RT}\ln K_{ass}=-\mathrm{RT}\ln 1/K_d)$ is −7.93 kcal mol^{-1}. In binding kinetics, K_d values are used for discussing the strength of interaction. These values were determined for three different electrodes (2 measurements per electrode), yielding K_d values with a mean of 1.63×10^{-6} M and an inter-electrode standard deviation of 3.19×10^{-7} M. The obtained K_d is somewhat higher than the value determined colorimetrically in the literature (Zheng et al., 2011), i.e. 0.7×10^{-6} M. As far as we know, this is the first time that molecular interaction data were obtained for the interaction of a biomolecule (the aptamer) with its target molecule, via a potentiometric sensor.

The same experiment was performed in the same conditions, with a non-selective oligonucleotide. This yielded a K_d value of 5.63×10^{-6} M. This means there is still a considerable binding between a random oligonucleotide and dopamine, although the interaction is not as strong as with the selected aptamer, for which K_d is 3.45 × lower. This binding of dopamine with DNA is a well-known phenomenon, which is described in several publications (see e.g. Vissers et al., 2006), and which is confirmed by the present study. Three other basic drugs which were tested did not

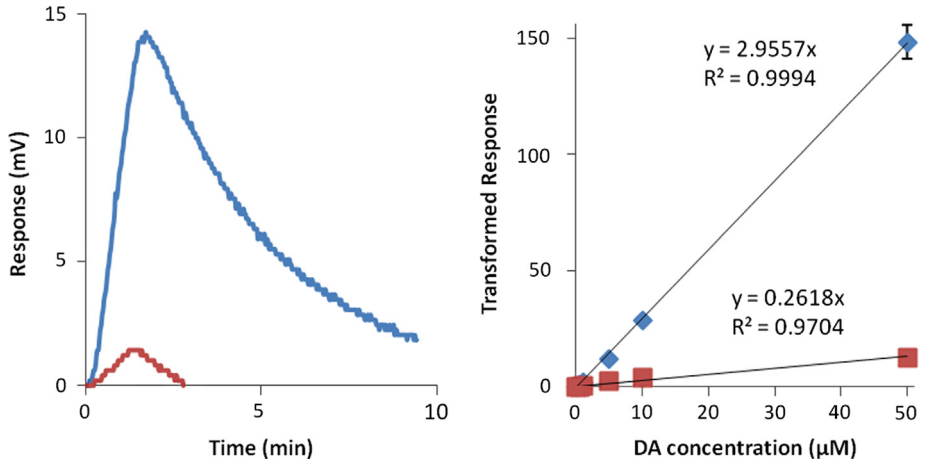

Fig. 4. Left: Injections of DA 10^{-6} M on a sensor which contains an aptamer (upper curve) and on a sensor which does not contain an aptamer (lower curve). Right: Potentiometric responses of 5×10^{-5} M to 10^{-7} M DA injections in FIA after transformation to a concentration related signal (Transformed Response, given in arbitrary units, see text and Eq. (3)). The upper trendline corresponds to biosensors with aptamer, the lower trendline corresponds to negative control potentiometric sensors (no aptamer). Each point was the mean of 3 measurements (%RSD ≤ 5).

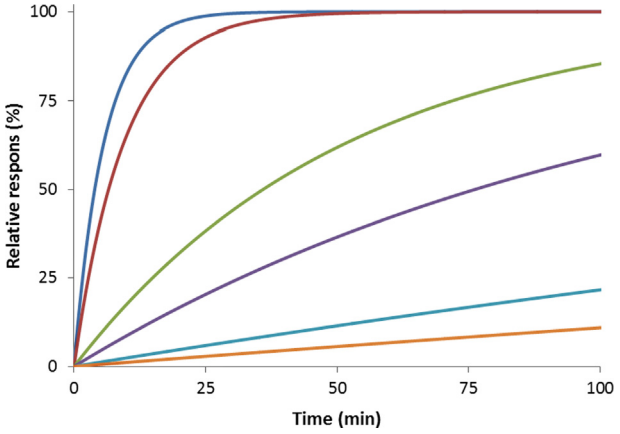

Fig. 5. "On" phases of the measurements shown in Fig. 2, after curve fitting, transformation to a concentration related response (tR), normalization, and extrapolation from 80 s (measured values) to 100 min (calculated data).

show such interactions. These lipophilic cationic drugs have much better responses on conventional lipophilic PVC/plasticiser based potentiometric sensors (Liu et al., 2002) than the hydrophilic DA. Sensors based on the hydrophilic gelatin B however are quasi insensitive towards these drugs. There is also no increase in sensitivity for these compounds, when the DA binding aptamer biorecognition element is coupled to the hydrogel.

It is clear from Figs. 2 and 3 that DA responds extremely sensitive to the Gelatin B+aptamer coated sensors (92.9 mV response−non transformed−for an injection of 10^{-5} M DA). Molecules like Ritodrine, Lidocaine and Promazine have totally negligible responses (respectively 1.57 mV, 1.57 mV and 2.44 mV) as compared to the response of DA on the anti-DA aptamer doped sensor. When performed with a more "classical" potentiometric sensor coating, made from a polyvinyl chloride (PVC)/plasticizer and e.g. potassium tetrakis(p-chlorophenyl)borate (TCPB) mixture, the responses would follow a lipophilicity order. The log *P* values of dopamine, ritodrine, lidocaine and promazine are respectively −0.48, 1.22, 2.44 and 4.36. In that case, the order of response is promazine > lidocaine > ritodrine > dopamine: see(Vissers et al., 2006). This is so-called "Hofmeister" behavior. It is very difficult to escape from Hofmeister behavior for organic analytes for most presently available potentiometric sensors which cannot rely on being equipped with a selectively interacting host molecule and a non-interacting sensor coating material (the hydrophilic gelatin in our case). Some macrocyclic or podand organic molecules have a reasonable effect, but generally, there is a lack of good organic receptor molecules to direct the selectivity (and sensitivity) of the present potentiometric sensors for detecting organic molecules.

4. Conclusions

Hydrophilic natural coating materials of the collagen B type are very interesting materials to couple bio-recognition elements such as aptamers to sensors. These become the immobilized "bait" molecules, interacting with the dissolved "prey" molecules. Covalent immobilization of the aptamers was proven with confocal microscopy. When used in a potentiometric sensor, the sensor responds sensitively and selectively to the target analyte, or prey, DA, and binding characteristics can be determined. The method is restricted to charged prey molecules, whereas charge is not expected to be important for the immobilized bait molecules. This could be a disadvantage, however an important fraction of the metabolome, and of e.g. pharmaceutical drugs consists of charged molecules. As these small molecules are difficult targets for e.g. SPR, they could possibly become the most important application for potentiometrically performed binding kinetics.

This study shows for the first time that potentiometric sensors can be used to obtain quantitative binding kinetics data such as K_d in biomolecular interaction studies. The gelatin B coating provides a biocompatible layer which is easily doped with the biomolecule, and which allows μm structures instead of nm monolayers. We believe that the described method is a serious candidate to complement the present existing binding kinetics analysis tools. Other biomolecule/analyte combinations can be studied using this method. The adsorption based model can be used for the systematic design of potentiometric biosensors, in this case a DA sensor.

Acknowledgments

Financial support for this work was provided by the University of Antwerp by granting L.N. and G.V.C. a BOF interdisciplinary research project.

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