
Erik Jonsson School of Engineering and Computer Science

2013-07-16

*An Electrochemical Sensor for the Detection of
Antibiotic Contaminants in Water*

UTD AUTHOR(s): Shalini Prasad, Michael Jacobs and Anjan Panneer
Selvam

©2013 The Royal Society of Chemistry. Further distribution of this
article is prohibited.

An electrochemical sensor for the detection of antibiotic contaminants in water

Cite this: *Anal. Methods*, 2013, **5**, 4325

Michael Jacobs,^{†a} Vinay J. Nagaraj,^{†b} Tim Mertz,^c Anjan Panneer Selvam,^a Thi Ngo^b and Shalini Prasad^{*a}

Received 17th June 2013

Accepted 15th July 2013

DOI: 10.1039/c3ay40994e

www.rsc.org/methods

A nanochannel-based electrochemical sensor for the detection of trace amounts of erythromycin has been developed. The sensor is capable of specifically detecting erythromycin, at a sensitivity of 0.001 parts per trillion, in various water samples and has potential utility in the assessment of environmental water quality.

Introduction

In 2009 alone, approximately four billion prescriptions were distributed to Americans containing a variety of pharmaceuticals.¹ These drugs rarely get fully metabolized and are excreted by the body.² Often times they get discarded by consumers and pharmaceutical companies as well.³ For this reason, sewage and drinking water treatment plants are utilized throughout the United States in an effort to keep water clean for human use. However, evidence has recently emerged showing that millions of Americans are being supplied with water containing trace amounts of pharmaceutical contaminants.⁴ Even at very small concentrations, these drugs pose a huge risk not only to humans, but also to the aquatic wildlife inhabiting local rivers, lakes, and reservoirs.⁵ This issue illustrates a significant need for the creation of a reliable technology that allows for continual, rapid detection of these hazardous chemicals prior to allocation of water to the environment and to individuals for consumption.

The most widely used method for analyzing possible contaminants in water suffers from numerous drawbacks that make constant quality monitoring difficult. Isolation of the drugs is accomplished with solid phase extraction techniques, followed by either liquid or gas chromatography combined with

mass spectrometry for identification and quantification. This technique is fairly sensitive (LOQ \sim 0.1 ppt) and accurate, however issues with the procedure include the use of bulky expensive equipment, trained personnel, as well as lengthy experimental and preparation times. Gas chromatography requires even longer setup due to derivatization steps for polar compounds.⁶ Although gas chromatography requires additional steps, it is more efficient than liquid chromatography which can have variability in measurements associated with changes in ambient pressure and temperature in addition to issues of signal suppression and matrix effects.^{7,8} To alleviate the current problem, a sensor is needed that exceeds the detection reliability of the existing methods with significantly lower cost, ease of use, portability, and a faster method of analysis.

Electrochemical sensors have become increasingly utilized in the fields of industry, medicine, and agriculture for their robustness as a sensitive, cost-effective, and rapid form of molecular detection. This transduction mechanism has allowed for the detection of gases, biological molecules, pesticides, and other chemicals by measuring changes in electrical charge transfer within the system.⁹ One such method of electrochemical detection is electrochemical impedance spectroscopy (EIS), which has been shown to effectively detect surface morphologies associated with either molecular corrosion or molecular binding to the transducing element. EIS has proven to be a more efficient strategy of analyte identification and quantification at low concentrations levels compared to other electrochemical techniques. A nanoporous membrane can be incorporated with the sensing portion of a platform to decrease background interferences and amplify electrical signals.¹⁰ Compared to cyclic, square wave, and differential pulse voltammetry, EIS allows for lower limits of detection while at the same time providing better sensitivity.¹¹ Also, other electrochemical modalities require the use of a redox probe which adds restrictions of time, cost, and lack of portability to the analytical process.

A microdevice, coupled with a nanoporous membrane, has been designed to effectively and reliably detect a common

^aDepartment of Bioengineering University of Texas, Dallas, 800 W Campbell Road, EC 39, Richardson, TX 75080, USA. E-mail: Shalini.Prasad@utdallas.edu

^bDepartment of Biochemistry, Midwestern University, 19555 North 59th Avenue, Glendale, Arizona 85308, USA

^cBioengineering Program, College of Engineering, Wichita State University, 1745 N. Fairmount St., Wichita, KS 67260, USA

[†] Equal contribution.

pharmaceutical contaminant in various water sources. Erythromycin, an antibiotic frequently administered to patients with penicillin allergies, was chosen as the drug of interest for this study because it has been shown to occur in small quantities in local water supplies.^{5,12} Experimental results using the EIS strategy show that the platform can quickly and efficiently identify small concentrations of this drug in deionized water, bottled water, and river water. Integration of the nanoporous membrane with the sensor is the key to significant enhancement in detection, proving to be a robust methodology for application to label-free sensors.

Materials and methods

Sensor design

The sensor platform consists of a printed circuit board with an electroplated two-electrode gold pattern. As seen in Fig. 1(a), the sensing site is comprised of working and reference electrodes configured into an inter-digitated concentric circular design. All electrodes have a width of 1 mm with 1 mm edge-to-edge spacing. The outer and innermost electrode rings connect to form the reference electrode, maintaining diameters of 13 mm and 5 mm, respectively. The working electrode is composed of the intermediate ring, with a diameter of 8 mm, branched from a central stem. This specific design was established to enhance the changes in impedance resulting from molecular binding by creating a large surface area for the reference electrode compared with the working electrode (10 : 1) and maintaining small gaps between the electrodes. It has been previously established that circular patterns maximize the surface area of the sensing site while helping to minimize edge effects.¹³

The circular electrode pattern was overlaid with a nanoporous alumina membrane (Anodisc – Whatman, NJ) having

the same diameter as the outer electrode ring, which enabled full coverage of the sensing site. The 60 μm thick AnoporeTM inorganic membrane has an average pore density of 37.5%, with each individual pore measuring 200 nm in diameter. This pore size allows for proper diffusion of molecules from the bulk solution onto the gold electrode surface.

Lastly, a polydimethylsiloxane (PDMS) manifold was created in order to secure the membrane in place and encapsulate the fluid on the sensor. The manifold has a 150 μL volume capacity, with two 1 mm inlet/outlet ports to allow for introduction of solution into the space above the membrane. The manifold was attached to the PCB substrate using a UV-curable, silicon adhesive.

Assay preparation

The process of functionalizing the erythromycin antibody conjugate onto the sensor surface was accomplished by first adding 150 μL of a 10 mM solution of dithiobis succinimidyl propionate (DSP) dissolved in dimethyl sulfoxide (DMSO) onto the electrodes (Thermo Scientific – Waltham, MA). After the DSP molecule gets cleaved in the DMSO, the available thiol groups self-adsorb to gold, leaving the *N*-hydroxysuccinimide (NHS) groups available for the antibody's primary amine to bind. After a 30 minutes incubation at room temperature, unbound DSP was washed with DMSO followed by phosphate buffer saline (PBS) and removed from the encapsulating area. Next, the erythromycin conjugate antibody (Abcam – Cambridge, MA) was diluted to 100 $\mu\text{g mL}^{-1}$ in 150 μL of PBS and allowed to incubate at room temperature on the sensing area for 15 minutes. An antibody calibration study was performed previously to isolate the incubation time and concentration that would allow for optimum saturation of the sensing site. After incubation, another wash step was performed with PBS to remove excess antibody. Finally, 150 μL of Superblock (Thermo Scientific) was injected into the sensing area for 15 min at room temperature. This step is imperative for eliminating non-specific binding by obstructing any available NHS groups on the linker molecule that did not bind an antibody. Assay preparation was concluded by removal of the blocking buffer and subsequent washing of the sensor with deionized water.

Erythromycin detection

Prior to measurement, erythromycin was first diluted into three different water sources: deionized water (Corning – Corning, NY), bottled drinking water (Kroger – Cincinnati, OH), and water collected from the Arkansas River in Wichita, KS. The only additional sample preparation step consisted of filtering the river water of suspensions using a 0.22 μm filter. The concentrations of antibiotic prepared in each fluid sample were 0.001, 0.05, 0.25, 0.5, 1, 250, 1000 and 10 000 parts per trillion (ppt).

Detection of the antibiotic was achieved using the technique of EIS. Impedance measurements were obtained by first attaching the leads of the sensor platform to a Gamry Reference 600 potentiostat. Analysis of the water samples was achieved by applying a 10 mV AC voltage while sweeping the frequency from 50 to 1200 Hz. When biological molecules are involved in

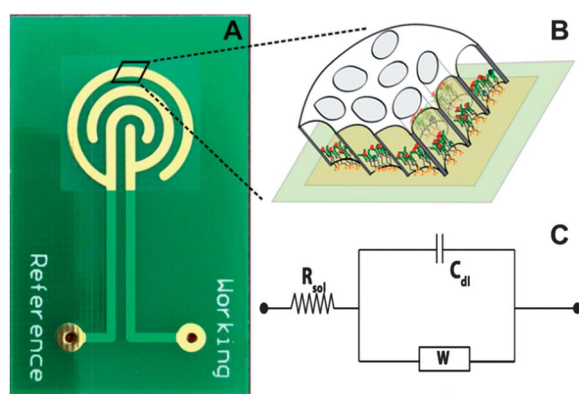


Fig. 1 Sensor platform for detection of antibiotic contaminants in water. (A) Gold electroplated printed circuit board for antibiotic detection using EIS; voltage is applied between the working and reference electrodes and the measured impedance is used to estimate the concentration of erythromycin. (B) Biochemical reactions occurring inside the channels of the overlaid nanoporous membrane form the electrical double layer; the nanoporous membrane behaves as a molecular sieve as well as a confined space for effective biochemical interactions. (C) Modified Randle's equivalent circuit for non-faradaic electrochemical bio-sensing assay – C_{dl} : double layer capacitance, R_{sol} : solution resistance, W : Warburg impedance for ion diffusion reactions.

impedance measurements, the voltage is kept low to reduce the risk of structural denaturation.¹⁴ The impedance readings were collected during each step of assay preparation as well as all erythromycin dilutions by measuring the resultant voltage to current ratio at 100 Hz. This specific frequency was chosen by first plotting frequency vs. impedance over a range of frequencies from 0.1 Hz to 1 MHz. This allowed for identification of the frequency range playing the most significant role in impedance changes due to charge perturbations at the electrical double layer (50–1200 Hz). It was then possible to further narrow down the frequency to the one that provided the largest changes in impedance between each concentrated sample (100 Hz).

All erythromycin detection assays began by introducing water without analyte onto the sensor and taking an impedance measurement. The sample was removed and the sensing site was washed with deionized water. Then the lowest dilution of erythromycin was added onto the sensor and allowed to incubate at room temperature for 15 minutes, followed by another impedance reading. This process was repeated with increasing doses until all concentrations of the drug were measured. Fig. 1(b) shows a detailed schematic of the electrode-functionalized immunoassay. After impedance measurements were obtained for each concentration in all three water sources, the procedure was carried out again using the antibiotic spectinomycin. These experiments were performed to analyze the specificity of the sensor for erythromycin. Spectinomycin was chosen for cross-reactivity studies since it is a commonly prescribed alternative to erythromycin due to its similar structure and function compared to the target molecule.

Results and discussion

The research described here involves the development of a nanotechnology-enabled EIS sensor platform for the highly sensitive detection of a commonly used pharmaceutical antibiotic – erythromycin – as a contaminant of water derived from various sources.

EIS and sensor performance

Fig. 1(c) depicts the modified Randle's circuit model for the sensor. Because the platform executes a non-faradaic form of EIS due to the lack of a redox probe, the charge transfer resistance is negligible and thus left out of the circuit.¹⁵ Identification of erythromycin concentrations is accomplished by measuring changes in the surface charge distribution at the electrical double layer (E_{dl}) of the sensing site. As erythromycin molecules bind to the antibodies at the boundary between the solution and the metal electrode, a disruption of the charge distribution at the E_{dl} occurs. This results in a capacitive change that can be measured by impedance. The impedance used when representing the data is the Z modulus values. The Z modulus is a combination of resistance and capacitance measured at a particular frequency. We observed capacitance as a dominating factor in our experiments. The change in capacitance was directly correlated to the change in impedance. As erythromycin binding increases at the surface of the electrodes, the resultant

impedance decreases.¹⁶ A change in impedance can then be plotted on the positive axis by subtracting the measured impedance of each sample from the impedance correlating to the buffer lacking any antibiotic. These changes in impedance can be mapped directly to different concentrations of erythromycin in solution. By measuring the changes in impedance over a range of erythromycin concentrations, it is possible to generate a calibration curve with which to compare unknown samples.¹⁷

Based on our previous studies, sensor sensitivity is greatly enhanced through the placement of a nanoporous membrane on the surface of the sensing site.^{18,19} The pores in the alumina membrane create a network of tiny channels for molecules to diffuse through prior to binding on the electrode. In this way the membrane acts as a type of filter, excluding large molecules that could potentially cause an unreliable signal. In addition, the nanopores provide a unique confined environment for the interaction of the functionalized antibody with its target antigen.^{20,21} This nanoscale space assists in enhanced binding, which results in amplified impedance measurements.¹⁸

Detection of erythromycin

Varied concentrations of the antibiotic erythromycin were prepared in three different water sources to analyze the reliability of the sensor for water quality assessment. Detection of the drug was determined by first taking an impedance measurement of the medium without any analyte prior to addition of the erythromycin samples. Evaluation of each concentration of the drug could then be represented as the difference in measured impedance between the samples with and without erythromycin.^{11,19} Another antibiotic – spectinomycin – was analyzed similarly in parallel in order to test the specificity of the sensor to erythromycin. The range of sample concentrations chosen coincides with the amount of antibiotics commonly found in environmental water sources, which is in the 1 to 1000's of ppt range depending on the source.²² The sensor was also evaluated for samples with concentrations below the environmental range to attempt sensitivity of detection at lower limits compared with current standard methods.

The samples prepared with deionized water were first used as a control for this study. Considering this medium does not contain any ions or molecules, the only contributing factor to the electrical signal measured by the sensor is the binding of erythromycin to its conjugate antibody. The results of these tests are illustrated in Fig. 2(a). The change in impedance can be seen to directly correlate to the amount of analyte in the fluid over a range of concentrations from 0.1 to 1000 ppt. At low concentrations of the drug there are few bound erythromycin molecules that are able to contribute to E_{dl} perturbations, causing only minor changes to impedance values. As the concentration of the antibiotic in solution increases there are more molecules binding to the surface of the electrodes, resulting in a larger impedance change. However as the antibody binding sites for erythromycin get saturated, further increases in concentration do not contribute to changes in impedance.²³

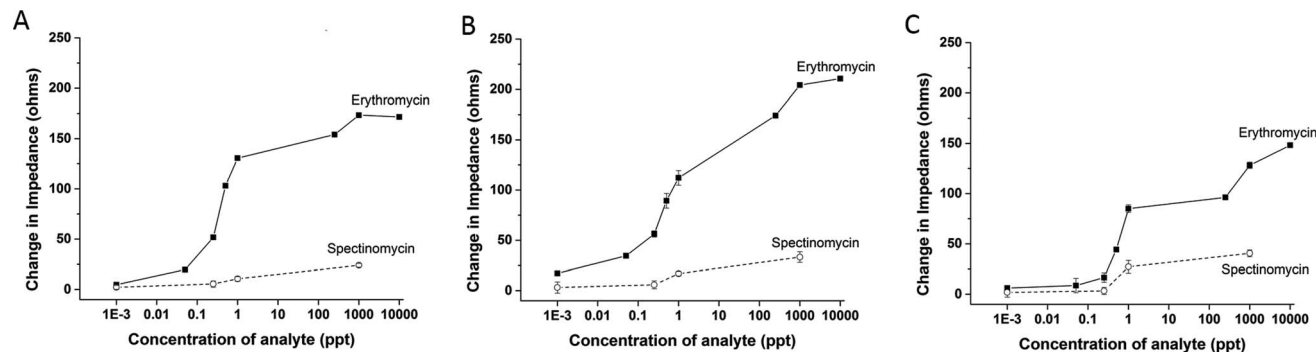


Fig. 2 Dose response studies for erythromycin and spectinomycin in deionized water, drinking water, and river water. The data represented in the graph was obtained from 3 independent replicates. The nanosensor calibration response for detecting erythromycin and its cross-reactivity to spectinomycin have been demonstrated. Concentrations in the range of 1×10^{-3} parts per trillion to 1×10^4 parts per trillion were tested on the nanosensor. (A) The lower limit of detection for erythromycin was identified to be 0.1 ppt. The upper limit of detection was identified to be at 1×10^3 ppt. Below and above these limits, the sensor did not measure any significant change in impedance to applied doses of erythromycin. The observed change in impedance was between 4 ohms and 172 ohms for erythromycin and between 2 ohms and 24 ohms for spectinomycin. (B) The lower limit of detection for erythromycin was identified to be 0.1 ppt. The upper limit of detection was identified to be at 1×10^4 ppt. The observed change in impedance was between 17 ohms and 215 ohms for erythromycin and between 3 ohms and 34 ohms for spectinomycin. (C) The lower limit of detection for erythromycin was identified to be 1 ppt. The upper limit of detection was identified to be at 1×10^4 ppt. The observed change in impedance was between 6 ohms and 150 ohms for erythromycin and between 2 ohms and 41 ohms for spectinomycin.

After performing the control tests, the same range of erythromycin concentrations prepared in bottled water and river water. The results of these tests can be seen in Fig. 2(b) and (c). The trend of these two experiments coincides with that of the previous test. However the exact impedance values for the experimental samples are slightly different, which may be attributed to the presence of other substances. For instance, it has been found that bottled water typically contains minute quantities of added ions such as Cl^- , Na^+ , Ca^{2+} , and Mg^{2+} . Although only slightly, these ions may influence the resultant impedance measurements given by the sensor.²⁴ The river water, on the other hand, will contain a large quantity of organic matter that can interfere with erythromycin binding and therefore lead to reduced sensitivity.²⁵

The lower limit of detection (LOD) with deionized water and bottled water was found to be around 0.1 ppt whereas the river water samples showed a slightly higher limit of detection around 1 ppt. The LOD from each type of water source was established by first addressing the issue of noise. This was estimated by identifying the electrical signal created as a result of non-specific analyte (spectinomycin) interaction with the functionalized antibodies on the electrode surface. The LOD was then determined by locating the lowest erythromycin concentration that resulted in a signal to noise ratio of at least 3 : 1.²⁶ The upper end of the dynamic range for the sensor in all media types peaked between 1000 and 10 000 ppt. This demonstrates a point of saturation where there are no additional unbound antibodies available on the sensor surface for free erythromycin molecules to bind.

Although EIS was not able to distinguish antibiotic concentrations below 0.1 ppt, the sensor can still be applicable into the 1000's of ppt. When the samples get into the higher concentration ranges, the changes in impedance between subsequent samples are not as significant. However, the overall impedance change is large enough to show that the concentration of erythromycin is in a range unsuitable for drinking.

Results for the cross-reactivity experiments performed with spectinomycin are shown in Fig. 2. Compared with the measurements associated with erythromycin, spectinomycin showed negligible changes in impedance, which corroborates the claim that the sensor is highly specific to erythromycin detection. The changes in impedance seen with spectinomycin can be attributed to the inherent noise of the system as a result of having a concentrated fluid sample added to the sensing site. Specificity of the sensor for erythromycin can be visualized through the large separation of electrical signal that is apparent when comparing the impedance changes associated with the two antibiotics at the higher concentrations.

Conclusion

A number of studies have been conducted showing that contamination of environmental and potable water with pharmaceuticals and other chemicals is of serious concern. The aim of this work was to devise a sensor that exhibits potential as a reliable platform capable of detecting these chemicals in various types of water samples. Our results indicate that the integration of a nanoporous membrane, combined with EIS, is an effective strategy for the identification of a wide range of concentrations of erythromycin in water. The measured impedance changes resulting from erythromycin binding on the sensor indicate that there is a non-linear relationship between sample concentration and the generated electrical signal. Further computational analysis must be completed to achieve linear transformation of the raw data.

Compared with current water analysis methods, this sensor provides similar sensitivity but significantly reduces the time, cost, and complexity associated with chromatography and mass spectrometry techniques. The sensor described here has the potential to be developed into a multiplexed device for the highly sensitive and selective detection of water samples

contaminated with various pharmaceuticals. Future work will focus on modifying the sensor for identification of other pharmaceutical contaminants as well as detection of multiple drugs simultaneously.

Acknowledgements

Krishna Mohan Vattipalli, Whitney Wilson, and Eric Romney for technical help with experiments and manuscript preparation.

References

- Agency for Healthcare Research and Quality, Medical Expenditure Panel Survey Component Data, "Prescription Medicines – Mean and Median Expenses per Person With Expense and Distribution of Expenses by Source of Payment," Table 2, <http://www.meps.ahrq.gov/mepsweb/>.
- P. K. Jjemba, Excretion and ecotoxicity of pharmaceutical and personal care products in the environment, *Ecotoxicol. Environ. Saf.*, 2006, **63**(1), 113–130.
- C. G. Daughton and I. S. Ruhoy, Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers, *Environ. Toxicol. Chem.*, 2009, **28**(12), 2495–2521.
- N. M. Vieno, H. Härkki, *et al.*, Occurrence of Pharmaceuticals in River Water and Their Elimination in a Pilot-Scale Drinking Water Treatment Plant, *Environ. Sci. Technol.*, 2007, **41**(14), 5077–5084.
- M. D. Hernando, M. Mezcua, *et al.*, Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments, *Talanta*, 2006, **69**(2), 334–342.
- J. Radjenović, M. Petrović, *et al.*, Advanced mass spectrometric methods applied to the study of fate and removal of pharmaceuticals in wastewater treatment, *TrAC, Trends Anal. Chem.*, 2007, **26**(11), 1132–1144.
- B. K. Choi, D. M. Hercules, *et al.*, Effect of liquid chromatography separation of complex matrices on liquid chromatography–tandem mass spectrometry signal suppression, *J. Chromatogr., A*, 2001, **907**(1–2), 337–342.
- P. J. Taylor, Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry, *Clin. Biochem.*, 2005, **38**(4), 328–334.
- E. Bakker and Y. Qin, Electrochemical Sensors, *Anal. Chem.*, 2006, **78**(12), 3965–3984.
- A. P. Minton, The Influence of Macromolecular Crowding and Macromolecular Confinement on Biochemical Reactions in Physiological Media, *J. Biol. Chem.*, 2001, **276**(14), 10577–10580.
- A. Panneer Selvam and S. Prasad, Nanosensor electrical immunoassay for quantitative detection of NT-pro brain natriuretic peptide, *Future Cardiol.*, 2012, **9**(1), 137–147.
- W. J. Morales, S. S. Dickey, *et al.*, Change in antibiotic resistance of group B Streptococcus: Impact on intrapartum management, *Am. J. Obstet. Gynecol.*, 1999, **181**(2), 310–314.
- K. B. Oldham, Electrode "edge effects" analyzed by the Green function method, *J. Electroanal. Chem.*, 2004, **570**(2), 163–170.
- E. P. Randviir and C. E. Banks, Electrochemical impedance spectroscopy: an overview of bioanalytical applications, *Anal. Methods*, 2013, **5**(5), 1098–1115.
- A. Panneer Selvam and K. M. Vattipalli, *et al.*, Design of a high sensitive non-faradaic impedimetric sensor. Engineering in Medicine and Biology Society (EMBC), 2012 Annual International Conference of the IEEE, 2012.
- C. Berggren, B. Bjarnason, *et al.*, Capacitive Biosensors, *Electroanalysis*, 2001, **13**(3), 173–180.
- M. Bothara, V. Venkatraman, *et al.*, Nanomonitors: electrical immunoassays for protein biomarker profiling, *Nanomedicine*, 2008, **3**(4), 423–436.
- S. Brandigampala and P. Feikert, *et al.* Designing better nano biosensors: role of pore confinement of biomolecules. Nanotechnology (IEEE-NANO), 2011 11th IEEE Conference on, 2011.
- K.-C. Lin, V. Kunduru, *et al.*, Biogenic nanoporous silica-based sensor for enhanced electrochemical detection of cardiovascular biomarkers proteins, *Biosens. Bioelectron.*, 2010, **25**(10), 2336–2342.
- N. A. Chebotareva, B. I. Kurganov, *et al.*, Biochemical effects of molecular crowding, *Biochemistry*, 2004, **69**(11), 1239–1251.
- S. B. Zimmerman and A. P. Minton, Macromolecular Crowding: Biochemical, Biophysical, and Physiological Consequences, *Annu. Rev. Biophys. Biomol. Struct.*, 1993, **22**(1), 27–65.
- F. Bloetscher and J. D. Plummer, ENVIRONMENTAL REVIEW & CASE STUDY: Evaluating the Significance of Certain Pharmaceuticals and Emerging Pathogens in Raw Water Supplies, *Environ. Pract.*, 2011, **13**(03), 198–215.
- P. Pichetturnthorn, K. Vattipalli, *et al.*, Nanoporous impedimetric biosensor for detection of trace atrazine from water samples, *Biosens. Bioelectron.*, 2012, **32**(1), 155–162.
- M. A. Vorotyntsev, J.-P. Badiali, *et al.*, Electrochemical impedance spectroscopy of thin films with two mobile charge carriers: effects of the interfacial charging, *J. Electroanal. Chem.*, 1999, **472**(1), 7–19.
- J. H. Jensen, Calculating pH and salt dependence of protein–protein binding, *Curr. Pharm. Biotechnol.*, 2008, **9**(2), 96–102.
- A. Hassibi, H. Vikalo, *et al.*, On noise processes and limits of performance in biosensors, *J. Appl. Phys.*, 2007, **102**(1), 014909–014912.