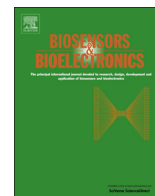




ELSEVIER

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Short communication

Continuous monitoring of Naproxen by a cytochrome P450-based electrochemical sensor

C. Baj-Rossi ^{a,*}, T. Rezzonico Jost ^b, A. Cavallini ^a, F. Grassi ^b, G. De Micheli ^a, S. Carrara ^a^a Laboratory of Integrated Systems, EPFL – École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland^b Institute for Research in Biomedicine – IRB, Bellinzona, Switzerland

ARTICLE INFO

Article history:

Received 10 September 2013

Received in revised form

23 September 2013

Accepted 24 September 2013

Available online 5 October 2013

Keywords:

Cytochrome P450

Continuous drug monitoring

Electrochemical biosensors

Carbon nanotubes

Personalized therapy

ABSTRACT

This paper reports the characterization of an electrochemical biosensor for the continuous monitoring of Naproxen based on cytochrome P450. The electrochemical biosensor is based on the drop-casting of multi-walled carbon-nanotubes (MWCNTs) and microsomal cytochrome P4501A2 (msCYP1A2) on a graphite screen-printed electrode (SPE).

The proposed biosensor was employed to monitor Naproxen (NAP), a well-known anti-inflammatory compound, through cyclic voltammetry. The dynamic linear range for the amperometric detection of NAP had an upper limit of 300 μ M with a corresponding limit of detection (LOD) of $16 \pm 1 \mu$ M ($S/N=3$), which is included in NAP physiological range (9–300 μ M). The MWCNT/msCYP1A2-SPE sensor was also calibrated for NAP detection in mouse serum that was previously extracted from mice, showing a slightly higher LOD ($33 \pm 18 \mu$ M).

The stability of the msCYP1A2-based biosensor was assessed by longtime continuous cyclic voltammetric measurements. The ability of the sensor to monitor drug delivery was investigated by using a commercial micro-osmotic pump. Results show that the MWCNT/msCYP1A2-SPE sensor is capable of precisely monitoring the real-time delivery of NAP for 16 h. This work proves that the proposed electrochemical sensor might represent an innovative point-of-care solution for the personalization of drug therapies, as well as for pharmacokinetic studies in both animals and humans.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

In the last decades, many efforts have been focused on the development of new technologies for personalized medical diagnostics (Hamburg and Collins, 2010). The main reasons are the urgency to increase the efficacy of therapies, with a drastic reduction of adverse drug reactions and to reduce the unnecessary costs associated with ineffective therapies (Aspinall and Hamermesh, 2007).

The therapeutic drug monitoring (TDM) is a discipline based on the precise quantification of drug concentration after administration, and on the individual drug dose adjustment according to the pharmacokinetics profile (Gross, 2002). However, despite its advantages, the TMD is restricted to few facilities mainly because the current analytical techniques (e.g. chromatography, mass spectroscopy and immunoassay) require highly trained personnel and specific equipment, high costs, extensive sample preparation and time consuming pre-concentration steps (Hiemke, 2008).

For this reason, the TDM practice can greatly benefit from the introduction of electrochemical biosensors owing to their portability,

speed, low-cost and enhanced specificity (Wang, 2006), which can both simplify patients' compliance and reduce the cost of hospitalization (D'Orazio, 2011).

Enzymatic amperometric biosensors for drug monitoring have recently attracted considerable attention due to their potential in clinical applications (Wang, 1999; Hendricks et al., 2009). One approach is the use of cytochrome P450 enzyme (CYP), whose best features are its capability to detect non-electroactive drugs and a broad substrate range (Schneider and Clark, 2013).

The limitation with CYP biosensors is the necessity to deal with the low efficiency of the electrochemical coupling between protein and electrode, and to investigate the protein stability in time (Sadeghi et al., 2011).

This article reports an investigation on the performances of an electrochemical biosensor based on multi-walled carbon-nanotubes (MWCNTs), microsomal cytochrome P4501A2 (msCYP1A2) and graphite SPE for the sensitive monitoring of a drug continuously delivered by an osmotic pump. The measured drug is Naproxen (NAP), a non-steroidal anti-inflammatory agent with analgesic and antipyretic properties, widely used for mild to moderate pain relief and in the treatment of osteo- and rheumatoid arthritis (Anttila et al., 1980).

MWCNTs have been recognized as very promising nanomaterials for enhancing electron transfer in biosensing (Wang, 2005).

* Corresponding author. Tel.: +41 21 693 6878.

E-mail address: camilla.baj-rossi@epfl.ch (C. Baj-Rossi).

Previously, MWCNTs were shown to play a key role in enhancing the electrocatalytic current of CYP (Carrara et al., 2011) and also for drugs detection (Baj-Rossi et al., 2012). msCYP1A2 was used since it has been discovered that microsomes are as effective as recombinant CYP in mediating electrocatalysis of substrates (Sultana et al., 2005; Mie et al., 2009), they are easier to produce than recombinant CYP, are largely used in the industry for drug development (Schneider and Clark, 2013), and they do not require specific pre-treatments.

In this work, the duration of enzymatic activity of msCYP1A2 was first assessed by long-time continuous cyclic voltammetric measurements. The ability of the sensor to monitor drug delivery was investigated by means of a commercial micro-osmotic pump. The MWCNT/msCYP1A2-SPE sensor was successfully applied to the real-time monitoring of NAP for 16 h. The results show that this electrochemical sensor provides high sensitivity and extreme simplicity in sensor preparation, all features that are suitable for the development of a point-of-care sensor making this approach a good candidate for the development of a fully operational therapeutic biosensor that might be in future useful in TDM practice.

2. Experimental

2.1. Reagents and chemicals

The biosensors were prepared using commercial carbon paste SPEs (model DRP-C110, DropSens, Llanera, Spain) consisting of a graphite working electrode with an active area of 12.54 mm², a graphite counter electrode and a Ag/AgCl reference electrode. MWCNTs (≈ 10 nm diameter and ≈ 1 –2 mm length) were purchased as a powder from DropSens. A 1 mg/ml solution of MWCNTs, prepared in chloroform, was sonicated for 30 min to obtain a homogeneous suspension (Carrara et al., 2011). The msCYP1A2 (Sigma-Aldrich, St. Gallen, Switzerland) purchased as isozyme microsomes with recombinant human CYP1A2, recombinant rabbit NADPH-P450 reductase, and cytochrome b5, were provided in vials of 0.5 nmol dissolved in 100 mM of pH 7.4 phosphate buffered saline (PBS, from Sigma-Aldrich) and used as received. Milli-Q water (18 M Ω /cm) was used to prepare all aqueous solutions. All experiments were carried out in a 100 mM PBS solution (pH 7.4) as supporting electrolyte. NAP, was purchased as a powder from Sigma-Aldrich. Due to its low solubility in water, NAP solutions at different concentrations (50, 100, 150, 200, 250, 300 μ M) were prepared by dissolving it in chromatography grade methanol ($\geq 99.9\%$ from Sigma-Aldrich) before storing at 4 °C.

2.2. Apparatus

Electrochemical measurements were performed using an Auto-lab electrochemical workstation (N Series Potentiostat/Galvanostat, Metrohm, Switzerland).

An Alzet model 1002 micro-osmotic pump (Durect corporation, USA) of 0.6 cm diameter, 1.5 cm length and 90 ± 10 μ l reservoir volume with a nominal delivery pumping rate of 0.25 ± 0.05 μ l/h (in isotonic solution at 37 °C) was used to continuously deliver NAP.

2.3. Preparation of MWCNT/msCYP1A2 electrodes

MWCNT/msCYP1A2-SPEs were prepared according to Carrara et al. (2011) and Baj-Rossi et al. (2012) by drop-casting 30 μ g of MWCNTs and three drops of msCYP1A2 (9 μ l each drop). The excess cytochrome was then removed by washing with milliQ water. All the functionalized electrodes were stored in PBS at 4 °C until use.

2.4. In vitro testing and serum collection from mice

Male C57BL/6 mice were used to extract serum samples. They were intravenously injected with PBS. After 4 h, mice were euthanized, blood harvested and left 1 h at room temperature. The blood was collected and then centrifuged at 7000 rpm for 10 min at 4 °C; serum and supernatant were stored at -80 °C.

2.5. Electrochemical measurements

The electrochemical response of the biosensor was investigated by cyclic voltammetry between -800 and 300 mV vs. Ag/AgCl at 20 mV/s at room temperature under aerobic conditions. For the sensor calibration to NAP, the electrode was covered with 100 μ l of 100 mM PBS (pH 7.4). Drug samples were added in 1 μ l drops. For the long-time continuous cyclic voltammetric measurements the electrodes were placed in a stirred 10 ml of 100 mM PBS (pH 7.4). For measurements with continuous NAP delivery, the micro-osmotic pump was used in conjunction with the electrode in PBS. The micro-osmotic pump was loaded with a 0.33 M NAP solution and the experimental pumping rate was estimated equal to 0.15 ± 0.03 μ l/h (at 27 °C), which corresponds to a NAP delivery rate of 5 ± 1 μ M/h (details on these calculations can be found in the Supplementary materials).

Sensitivity per unit area was computed from the slope of the straight line obtained by plotting peak current vs. drug concentration. Peak current was estimated according to the procedure reported by Carrara et al. (2009). The limit of detection (LOD) was computed based on three times the signal-to-noise ratio according to the expression (Mocak et al., 1997; Miller and Miller, 2010)

$$\text{LOD} = k \frac{\delta_i}{S} \quad (1)$$

where δ_i is the standard deviation of the blank measurements, S is the detector sensitivity, and k is a parameter accounting for the confidence level ($k=1, 2, \text{ or } 3$ corresponds to 68.2%, 95.4%, or 99.6% of statistical confidence).

3. Results and discussion

3.1. Electrochemical response of the MWCNT/msCYP1A2-based drug sensor

In CYP-biosensors, enzymes are immobilized on the electrode surface that provides the electrons needed to drive the redox reaction of the heme group of CYP, by replacing the natural redox partners (Krishnan et al., 2011a). In cyclic voltammetry the redox reaction of the heme group is recorded as reduction and oxidation current peaks. Fig. S1 reports a typical voltammogram of msCYP1A2 on MWCNT-SPE in aerobic conditions. According to the enzymatic kinetics, the cycle of oxidation/reduction of the heme group is dependent on the substrate concentration. Thus, in cyclic voltammetry the addition of a substrate to a CYP electrode results in a further increase of the CYP reduction current proportional to the substrate concentration (Shumyantseva et al., 2004).

The oxidation/reduction of the heme group of CYP occurs at a potential likely to be subjected to factors such as the electrode preparation and immobilization technique employed for the CYP (Johnson et al., 2005; Schneider and Clark, 2013).

In several reports, CYP1A2 peaks were detected at -380 mV (Krishnan et al., 2011b), -265 mV (Antonini et al., 2003; Paternolli et al., 2004), -280 mV (Estavillo et al., 2003), and -437 mV (Shumyantseva et al., 2007). Even more complex is the situation when microsomal CYPs are immobilized on electrodes. Since microsomal CYPs contain both the P450 enzyme and the NADPH-P450

reductase (CPR), a contribution from each component can be present in a voltammogram as reduction peaks. Recent works by Sultana et al. (2005) and Mie et al. (2009) demonstrated that microsomal CYPs show a CYP reduction peak at -380 mV and a CPR reduction peak at -420 mV. Analogous potentials were found in other individual studies on CPR electrochemistry (Shukla et al., 2006). It is therefore necessary to identify in cyclic voltammetry the peak due to the reduction of CYP that will increase in the presence of a substrate.

Fig. 1A shows the resulting reduction peaks for a MWCNT/msCYP1A2-SPE from the background subtracted voltammograms at various NAP concentrations in an air saturated 100 mM PBS solution (pH 7.4) at room temperature. Here the normalized values of the current variations (positive y-axis) are reported. Cyclic voltammograms were recorded before and after the NAP solution was added to the PBS, in different concentrations (from $50 \mu\text{M}$ to $300 \mu\text{M}$). Each voltammogram was background-subtracted to exclude the contribution of CNT (Shobha Jeykumari et al., 2007). The reduction peak region was isolated and fitted by two Gaussian functions centered at -560 mV and -380 mV (inset Fig. 1A). The two peaks are attributed respectively to the CPR and CYP components of microsome (Mie et al., 2009). The Gaussian functions were then summed, varying height and amplitude to fit the current profile obtained from the raw data, as already reported by Carrara et al. (2009). The peak potentials showed a slight positive shift with increasing NAP concentrations, while the reduction current peak at -380 mV showed a clear linear increase. These are typical behaviors of CYP in the presence of a substrate (Johnson et al., 2005; Joseph et al., 2003), suggesting that the peak at -380 mV is effectively ascribed to the catalytic reduction of CYP1A2. As negative control, we performed cyclic voltammetry with bare-SPE in the presence of NAP $300 \mu\text{M}$ (Fig. S2). The reduction peak typical of a graphite SPE does not significantly change after the addition of the drug.

Fig. 1B shows the dependence of the peak current on NAP concentration in PBS. The y-axis values are the reduction current peaks obtained from cyclic voltammograms. The linear regression equation is $I_{pc} = 0.124 + 0.005C_{NAP}$, where I_{pc} (μA) is the reduction

peak current and C_{NAP} (μM) is the NAP concentration, with a correlation coefficient of 0.99. The dynamic range of the MWCNT/msCYP1A2-SPE sensor had an upper limit of $300 \mu\text{M}$ with a sensitivity per unit area of $0.54 \pm 0.02 \mu\text{A}/\text{mM mm}^2$. The corresponding LOD was estimated to be $16 \pm 1 \mu\text{M}$ based on a signal-to-noise ratio of 3, which is included in NAP physiological range (9 – $300 \mu\text{M}$) (Upton et al., 1984).

The MWCNT/msCYP1A2-SPE sensor was also calibrated for NAP detection in mouse serum (Fig. 1C). We obtained a sensitivity comparable to the one obtained in PBS ($0.6 \pm 0.2 \mu\text{A}/\text{mM mm}^2$), but higher data variability ($R^2=0.98$), a lower linear range (up to $200 \mu\text{M}$) and a higher LOD ($33 \pm 18 \mu\text{M}$). The reason for these differences is the complexity of the serum that generates interferences (Carrara et al., 2011).

3.2. Electrochemical behavior of the MWCNT/msCYP1A2-based sensor in 36 h

An essential feature of a sensor for real-time drug monitoring is the stability during continuous measurements. Few literature reports studied CYP biosensor stability over a long time period (Hendricks et al., 2009; Iwuoha et al., 2000), with the majority showing the storage stability. Furthermore no studies have been conducted on the CYP-based sensor stability during continuous measurements.

The stability of the MWCNT/msCYP1A2-SPE sensor was first studied over 36 h. The sensor was incubated in 100 mM PBS (pH 7.4) for 36 h and cyclic voltammograms were continuously acquired every 90 min, to reduce the scans applied to CYP and saving its catalytic activity. Cyclic voltammograms were recorded in aerobic conditions and in the absence of any substrate. Fig. 2 depicts the CYP1A2 reduction peak behavior at -380 mV in 36 h. The peak shows good stability for the first 16 h, and then starts to gradually decrease, reaching about 70% of its initial height after 22 h, and about 20% after 36 h. It is reasonable to explain the decrease of CYP activity after 16 h with the enzyme degradation due to prolonged use under continuous potential cycling, as previously demonstrated in another study (Millo et al., 2009).

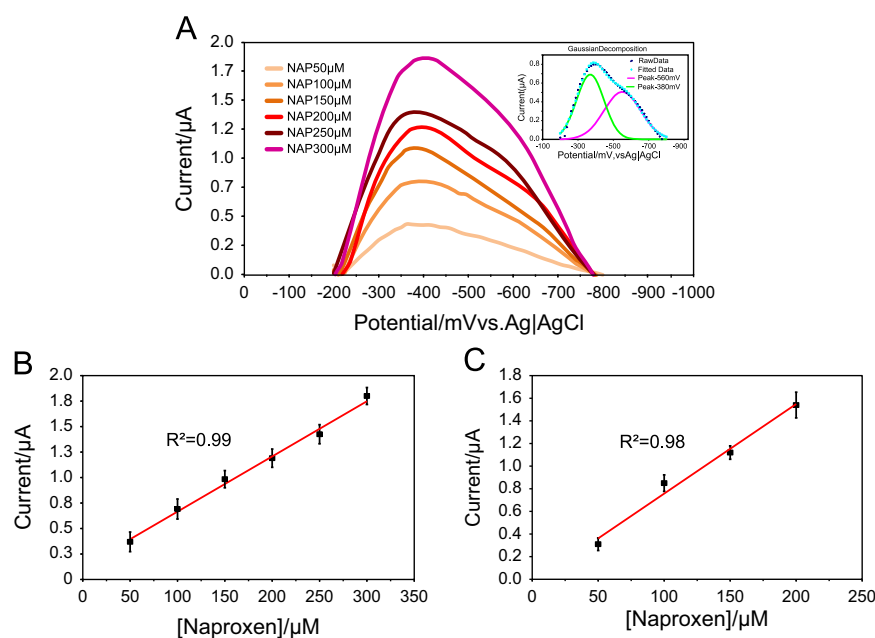


Fig. 1. (A) Cyclic voltammetry reduction peak analysis after background subtraction at various NAP concentrations (from $50 \mu\text{M}$ to $300 \mu\text{M}$) for a MWCNT/msCYP1A2-SPE. The inset shows the reduction peak region (from 0 to -800 mV) fitted by two Gaussian functions centered at -560 mV and -380 mV to describe the different faradic contributes from microsomal CYP1A2 components. (B) Current response of the MWCNT/msCYP1A2-SPE sensor to different concentrations of NAP (confidence interval 95.4%) in PBS and (C) in mouse serum.

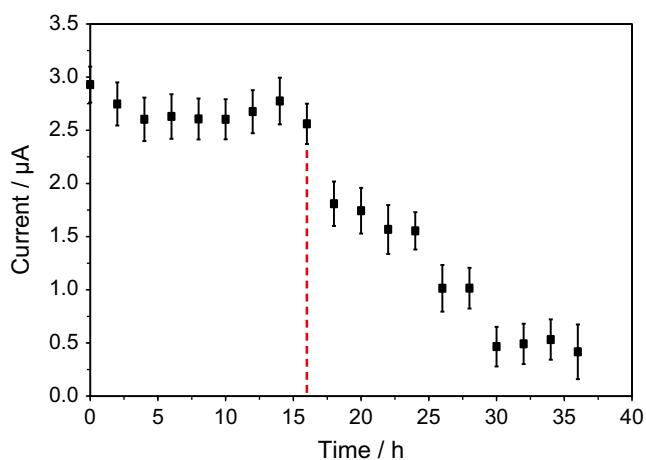


Fig. 2. Stability of the MWCNT/msCYP1A2-SPE sensor during 36 h of continuous measurements. The y-axis reports the currents of the reduction peaks of msCYP1A2 (at -380 mV).

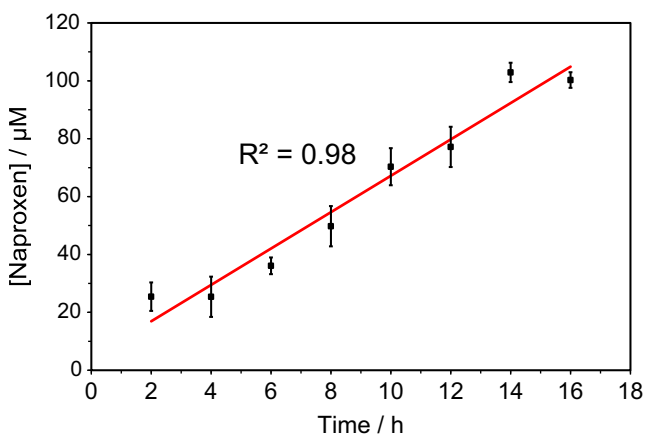


Fig. 3. Monitoring of NAP delivery with the MWCNT/msCYP1A2-SPE sensor. The reduction current peaks measured with the sensor every 2 h were transformed in NAP concentration (y-axis) according to the linear regression function obtained with the sensor calibration (Fig. 1B).

3.3. Determination of NAP continuously delivered by an osmotic pump

The sensor was employed to monitor the concentration variation of NAP, which was continuously delivered in solution by means of a micro-osmotic pump. After the micro-osmotic pump delivery rate reached the steady state, cyclic voltammograms were acquired in PBS, under aerobic conditions. The MWCNT/msCYP1A2-SPE sensor was incubated in PBS for 36 h, while $5 \pm 1 \mu\text{M/h}$ of NAP was constantly delivered (according to Eq. (1) in the [Supplementary materials](#)).

After 16 h the MWCNT/msCYP1A2-SPE sensor starts losing its activity. Fig. 3 shows the detection of NAP delivered by the osmotic pump. The reduction current peaks measured with the sensor every 2 h were transformed in NAP concentration (y-axis in Fig. 3), according to the linear regression function obtained by the sensor calibration (Fig. 1B). A fairly good linearity over a time lapse of 16 h was obtained. The amount of NAP measured per hour estimated from the linear regression analysis of the data shown in Fig. 3 was $6.2 \pm 0.2 \mu\text{M/h}$, which is very close to the NAP delivery rate of the osmotic pump previously estimated, $5 \pm 1 \mu\text{M/h}$. These results proved that the MWCNT/msCYP1A2-SPE sensor was able to monitor the continuous delivery of NAP up to 16 h.

3.4. Repeatability and stability investigation of the MWCNT/msCYP1A2-based sensor

The repeatability of the biosensing process was evaluated by measuring the responses of freshly prepared biosensor to $200 \mu\text{M}$ NAP within the first 6 h of preparation at 1 h time interval, while storing in PBS at 4°C after each measurement. A relative standard deviation (R.S.D.) value of 3.1% was obtained for the six measurements, indicating good repeatability.

The reproducibility in electrochemical signals obtained using three biosensors was evaluated by comparing the peak current on continuous cycling (for 1 h) of the MWCNT/msCYP1A2-SPE sensor. As expected, almost no obvious changes were found (R.S.D. value of 4.4%).

The storage stability of the biosensor was investigated by monitoring its response to $200 \mu\text{M}$ NAP with cyclic voltammograms after storing in PBS at 4°C for 0 h, 12 h, 24 h, 2 d, 4 d and 1 week. The peak current decreased with increase in storage time. Nevertheless the biosensor still retained 75% of its initial response after 1 week of storage. An R.S.D. value of 11.2% was obtained for the 1-week stability study. These results proved that the MWCNT/msCYP1A2-SPE sensor had high reproducibility in the preparation procedure and it could be repeatedly used in voltammetric determination.

4. Conclusions

A biosensor consisting of MWCNTs and microsomal CYP1A2 was developed for the continuous monitoring of NAP. The sensor showed a stable response for 16 h of continuous measurements and it was successfully applied to monitor the real-time monitoring of NAP up to 16 h.

Results show that the electrochemical sensor provides high sensitivity and extreme simplicity in sensor preparation, all features that are suitable for the development of a point-of-care sensor for continuous monitoring of pharmacological compounds. Moreover the results presented in this article represent a novelty since amperometric biosensors for continuous monitoring have already been developed for glucose (Girardin et al., 2009), and other endogenous compounds (Frost and Meyerhoff, 2002), but no enzymatic amperometric biosensors have been reported so far for real-time monitoring of drugs.

It is however important to say that taking into account the complexity of the samples, we are planning for the future a more complete validation of the device which will include the evaluation of interferences, the analysis of real samples and the evaluation of the accuracy of the method.

Acknowledgments

The SNF Sinergia Project, code CRSII2_127547/1 and title "Innovative Enabling Micro-Nano-Bio-technologies for Implantable systems in molecular medicine and personalized therapy" financially supported this research. Michele De Marchi is acknowledged for the proof-reading of the manuscript.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2013.09.058>.

References

Antonini, M., Ghisellini, P., Pastorino, L., Paternolli, C., Nicolini, C., 2003. IET, vol. 150, 31–34.

- Anttila, M., Haataja, M., Kasanen, A., 1980. *European Journal of Clinical Pharmacology* 18 (3), 263–268.
- Aspinall, M.G., Hamermesh, R.G., 2007. *Harvard business review* 85 (10), 108.
- Baj-Rossi, C., Micheli, G.D., Carrara, S., 2012. *Sensors* 12 (5), 6520–6537.
- Carrara, S., Cavallini, A., Erokhin, V., De Micheli, G., 2011. *Biosensors and Bioelectronics* 26 (9), 3914–3919.
- Carrara, S., Cavallini, A., Garg, A., De Micheli, G., 2009. *Proceedings of the ICME International Conference on Complex Medical Engineering*, April 2009. pp. 1–6.
- D'Orazio, P., 2011. *Clinica Chimica Acta* 412 (19), 1749–1761.
- Estavillo, C., Lu, Z., Jansson, I., Schenkman, J.B., Rusling, J.F., 2003. *Biophysical Chemistry* 104 (1), 291–296.
- Frost, M.C., Meyerhoff, M.E., 2002. *Current Opinion in Chemical Biology* 6 (5), 633–641.
- Girardin, C.M., Huot, C., Gonthier, M., Delvin, E., 2009. *Clinical Biochemistry* 42 (3), 136–142.
- Gross, A.S., 2002. *British Journal of Clinical Pharmacology* 46 (2), 95–99.
- Hamburg, M.A., Collins, F.S., 2010. *New England Journal of Medicine* 363 (4), 301–304.
- Hendricks, N.R., Waryo, T.T., Arotiba, O., Jahed, N., Baker, P.G., Iwuoha, E.I., 2009. *Electrochimica Acta* 54 (7), 1925–1931.
- Hiemke, C., 2008. *European Journal of Clinical Pharmacology* 64 (2), 159–166.
- Iwuoha, E.I., Kane, S., Ania, C.O., Smyth, M.R., Ortiz de Montellano, P.R., Fuhr, U., 2000. *Electroanalysis* 12 (12), 980–986.
- Johnson, D., Lewis, B., Elliot, D., Miners, J., Martin, L., 2005. *Biochemical Pharmacology* 69 (10), 1533–1541.
- Joseph, S., Rusling, J.F., Lvov, Y.M., Friedberg, T., Fuhr, U., 2003. *Biochemical Pharmacology* 65 (11), 1817–1826.
- Krishnan, S., Schenkman, J.B., Rusling, J.F., 2011a. *The Journal of Physical Chemistry B* 115 (26), 8371–8380.
- Krishnan, S., Wasalathanthri, D., Zhao, L., Schenkman, J.B., Rusling, J.F., 2011b. *Journal of the American Chemical Society* 133 (5), 1459.
- Mie, Y., Suzuki, M., Komatsu, Y., 2009. *Journal of the American Chemical Society* 131 (19), 6646–6647.
- Miller, J.N., Miller, J.C., 2010. *Statistics and Chemometrics for Analytical Chemistry*, Sixth Edition, Prentice Hall England, pp. 110–135.
- Millo, D., Pandelia, M.-E., Utesch, T., Wisitruangsakul, N., Mroginski, M.A., Lubitz, W., Hildebrandt, P., Zebger, I., 2009. *The Journal of Physical Chemistry B* 113 (46), 15344–15351.
- Mocak, J., Bond, A., Mitchell, S., Scollary, G., 1997. *Pure and Applied Chemistry* 69 (2), 297–328.
- Paternolli, C., Antonini, M., Ghisellini, P., Nicolini, C., 2004. *Langmuir* 20 (26), 11706–11712.
- Sadeghi, S.J., Fantuzzi, A., Gilardi, G., 2011. *Biochimica et Biophysica Acta (BBA)–Proteins and Proteomics* 1814 (1), 237–248.
- Schneider, E., Clark, D.S., 2013. *Biosensors and Bioelectronics* 1 (39), 1–13.
- Shobha Jeykumari, D., Ramaprabhu, S., Sriman Narayanan, S., 2007. *Carbon* 45 (6), 1340–1353.
- Shukla, A., Gillam, E.M., Bernhardt, P.V., 2006. *Electrochemistry Communications* 8 (12), 1845–1849.
- Shumyantseva, V., Bulko, T., Rudakov, Y.O., Kuznetsova, G., Samenkova, N., Lisitsa, A., Karuzina, I., Archakov, A., 2007. *Biochemistry (Moscow) Supplemental Series B: Biomedical Chemistry* 1 (4), 327–333.
- Shumyantseva, V.V., Ivanov, Y.D., Bistolas, N., Scheller, F.W., Archakov, A.I., Wollenberger, U., 2004. *Analytical Chemistry* 76 (20), 6046–6052.
- Sultana, N., Schenkman, J.B., Rusling, J.F., 2005. *Journal of the American Chemical Society* 127 (39), 13460–13461.
- Upton, R., Williams, R., Kelly, J., Jones, R., 1984. *British Journal of Clinical Pharmacology* 18 (2), 207–214.
- Wang, J., 1999. *Journal of Pharmaceutical and Biomedical Analysis* 19 (1), 47–53.
- Wang, J., 2005. *Electroanalysis* 17 (1), 7–14.
- Wang, J., 2006. *Biosensors and Bioelectronics* 21 (10), 1887–1892.