SELECTIVE AND SENSITIVE POLY–*ORTHO*-PHENYLENEDIAMINE-SHIELDED MICROSENSORE AND BIOSENSORS FOR *IN VIVO* NEUROCHEMICAL MONITORING

Pier A. Serra[1], M.Speranza Desole[1], Gaia Rocchitta[1], Rossana Migheli[1], John P. Lowry[2], Robert D. O'Neill[3]

[1]Department of Neuroscience, Medical School, University of Sassari, Viale S. Pietro 43/b, 07100 Sassari, Italy

[2]Department of Chemistry, National University of Ireland, Maynooth, Co. Kildare, Ireland

[3]UCD School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland

Different methodologies are being developed, such as imaging, spectroscopy and electrochemistry, to study neurochemical dynamics in cell cultures or in intact brain [1-2]. One of these techniques involves the *in-situ* detection of biologically active molecules, including nitric oxide (NO) [3], glucose [4], glutamate (GLUT) [5-6] and lactate [1,7], in brain extracellular fluid (ECF), using implanted microsensors and biosensors. NO is a water-soluble free radical that readily diffuses through membranes and its actions in the CNS are largely studied. While low concentrations of NO modulate normal synaptic transmission, excess levels of NO may be neurotoxic. The constant-potential oxidation of NO occurs on Nafion poly-*ortho*-phenylenediamine (PPD)-coated carbon fibers at +865 mV vs Ag/AgCl reference electrode (RE) with good selectivity against electrochemically oxidizable anions [3]. When direct electrochemical oxidation is not possible under standard experimental conditions, a biosensor may then be used instead. Glucose, for example, is oxidized by the enzyme glucose oxidase (GOx), an oxidoreductase with a covalently-linked flavin adenine dinucleotide (FAD) cofactor. The reconversion of $FADH_2$ to FAD produces H_2O_2 in the presence of O_2. The application of a potential of +700mV to a platinum (Pt) working electrode, relative to a RE, causes the generation of a measurable current that is proportional to the H_2O_2 produced. The high specificity and stability of GOx makes this enzyme suitable for biosensor construction when immobilized on the surface of Pt electrodes with PPD [4]. The substitution of the GOx with glutamate or lactate oxydases, and the modification of biosensor design, allow to detect, respectively, glutamate [1,2,5-6,8-10] and lactate [1,7], normally present in the ECF. While glucose and lactate are involved in the neural energetic metabolism, glutamate (GLUT), is considered the most common excitatory neurotransmitter in the brain. The long term potentiation in the glutamatergic transmission, indeed, determines an increasing of GLUT and NO concentrations in the extracellular compartment with excitotoxic effects.

Therefore, measuring real-time chemical events in the living brain, however, is an extreme technical challenge, even in animal models, and involves many biocompatibility issues [1]. Unfortunately, the remarkable specificity of enzyme-based amperometric biosensors can be seriously undermined by interference from electroactive species present in the target medium, compromising the selectivity of the device. This problem is particularly pronounced for microsensors and biosensors implanted in biological tissues for real-time monitoring, because separation techniques cannot be exploited to eliminate the interferences. Despite this negative aspect, considerable efforts have been made over the past two decades to overcome interference in sensors signals. PPD is a widely used permselective polymer in the construction of biosensors for a range analytes and can be deposited electrochemically from *o*-phenylenediamine (*o*PD) solutions at neutral pH to produce a thin self-sealing insulating polymer on the electrode surface [1-10]. Film thickness estimations for PPD generated under these conditions are typically in the region of 10-35 nm [1]. As well as possessing excellent interference rejection characteristics, PPD also serves as a method of immobilising enzymes and protects the electrode surface from enzyme proteases and fouling, making it ideal for microsensor/biosensor applications *in vivo* [1,7]. The *in vitro* characterization of PPD-based sensors involves not only sensitivity e selectivity studies, but also biosensor oxygen dependence [8-10] and aging processes before [10] and after *in vivo* implantation [1-7].

Bibliography

[1] O'Neill R.D., Lowry J.P., Rocchitta G., McMahon C.P. and Serra P.A. (2008) Designing sensitive and selective polymer / enzyme composite biosensors for brain monitoring *in vivo*. *Trends Anal. Chem*. vol. 1, pp.78-88.

[2] KIRWAN SM, ROCCHITTA G, MCMAHON CP, CRAIG JD, KILLORAN SJ, O'BRIEN KB, SERRA P., LOWRY JP, O'NEILL RD. (2007). Modifications of poly(o-phenylenediamine) permselective layer on Pt-Ir for biosensor application in neurochemical monitoring. SENSORS (on line). vol. 7, pp. 420-437 ISSN: 1424-8220.

[3] GAIA ROCCHITTA, ROSSANA MIGHELI, SONIA DEDOLA, GIAMMARIO CALIA, MARIA S. DESOLE, EGIDIO MIELE, JOHN P. LOWRY, SERRA P. (2007). Development of a distributed, fully automated, bidirectional telemetry system for amperometric microsensor and biosensor applications. SENSORS AND ACTUATORS. B, CHEMICAL. vol. 126, pp. 700-709 ISSN: 0925-4005.

[4] SERRA P., GAIA ROCCHITTA, GIANFRANCO BAZZU, ANTONIO MANCA, GIULIA M. PUGGIONI, JOHN P. LOWRY AND ROBERT D. ONEILL. (2007). Design and construction of a low cost single supply embedded telemetry system for amperometric biosensor applications. SENSORS AND ACTUATORS. B, CHEMICAL. vol. 122, pp. 118-126 ISSN: 0925-4005.

[5] MCMAHON CP, ROCCHITTA G, KIRWAN SM, KILLORAN SJ, SERRA P., LOWRY JP, O'NEILL RD. (2007). Oxygen tolerance of an implantable polymer/enzyme composite glutamate biosensor displaying polycation-enhanced substrate sensitivity. BIOSENSORS & BIOELECTRONICS. vol. 22, pp. 1466-1473 ISSN: 0956-5663.

[6] MARTIN HEBEL and SERRA P. (2007). Development of a Parallel-Computing embedded telemetry system for voltammetric microsensor and Biosensor applications. In: MARIE-ISABELLE BARATON. Sensors for Environment,Health and Security: Advanced Materials and Technologies. NATO-ASI Series. NATO Advanced Science Institute Series Book. NATO REFERENCE: CBP.NR.ASI 982324. DORDRECHT: Springer (NETHERLANDS).

[7] F.B. BOLGER, SERRA P., M.DALTON, R.D.O'NEILL, M.FILLENZ, J.P. LOWRY. (2006). REAL-TIME MONITORING OF BRAIN EXTRACELLULAR LACTATE. Monitoring Molecules In Neuroscience. 19-22 May 2006. (pp. 286-288).

[8] MCMAHON CP, ROCCHITTA G, SERRA P., KIRWAN SM, LOWRY JP, O'NEILL RD. (2006). Control of the oxygen dependence of an implantable polymer/enzyme composite biosensor for glutamate. ANALYTICAL CHEMISTRY. ISSN: 0003-2700.

[9] G.ROCCHITTA, C.P.MCMAHOM, SERRA P., S.M. KIRWAN, F.B. BOLGER, J.P. LOWRY, R.D. O'NEILL. (2006). Significant Enhancement of Glutamate Biosensor Sensitivity, using a Polycation-Modified Polymer/Enzyme Composite Sensing Layer. Monitoring Molecules in Neuroscience. 22-26 May 2006. (pp. 331-333).

[10] MCMAHON CP, ROCCHITTA G, SERRA P., KIRWAN SM, LOWRY JP, O'NEILL RD. (2006). The efficiency of immobilised glutamate oxidase decreases with surface enzyme loading: an electrostatic effect, and reversal by a polycation significantly enhances biosensor sensitivity. ANALYST. ISSN: 0003-2654.