

Development of Electrochemical Nano-Biosensors with Distributed Metal Particles

Cristina Schreiner¹, Theodora Krasia-Christoforou², Cristina Bratescu¹
Ramona Burlacu¹, Maria Demetriou², Ioanna Savva², Romeo Ciobanu¹

¹ Faculty of Electrical Engineering and Applied Informatics, Technical University Iasi, Romania
cschrein@ee.tuiasi.ro

² Department of Mechanical and Manufacturing Engineering, University of Cyprus, Nicosia, Cyprus

Abstract - A new concept of hybrid nano-biosensors was developed based on well-defined methacrylic diblock copolymers and metal nanoparticles (Au and Pd). The nano-composite systems were used as surfactant for modified carbon electrodes. The electrochemical characterization (Potentiostat/Galvanostat method) was performed by using reference redox molecules in order to test the sensors sensitivity and reproducibility at different concentrations of dopamine in acid medium (0.1 M HCl). It was shown that the functionalization of carbon electrodes with well-defined electrically active diblock copolymers and metal nanoparticles offer superior metrological performance for neurotransmitters analyze, comparing to conventional electrodes.

I. Introduction

Nanocomposites, in which nano-metallic and organic components are combined together in a new material at nanoscale have opened up new opportunities for the development of novel, multifunctional components with potential applications in numerous fields, [1]. As example, a palladium-nanoparticle-cored G3 dendrimer, derived from Frechet-type dendritic polyaryl ether disulfide of generation three, was found to possess approximately 300 Pd atoms in an average diameter of 2 nm, to which were attached 14 G3 dendrons. The dendrons inhibited metal agglomeration without adversely affecting its chemical reactivity [2]. The medical market is interested in promoting new concepts of nano-bio-sensors specialised in analysing the biological redox processes at the cellular and subcellular level, allowing subsecond measurements during neurotransmission with minimal tissue damage. The neurotransmitters are involved in the functioning of renal, cardiovascular, hormonal and nervous systems, but were also related to neurological diseases such as Parkinson's, Alzheimer's and schizophrenia, were found relevant in neurodegeneration, neuroblastomas and adrenal gland cancer, and are supposed to play a role in drug addiction and some manifestations of HIV. The use of metallic or carbon fibers/CNTs for dopamine detection was tried by some research groups in the last 5 years, along with different variants of microelectrode functionalisation, including Boron, oxides or conductive polymers deposition, but a large scale solution is still missing. If made available, disposable hybrid nano-biosensors based on well-defined electrically active diblock copolymers and metal nanoparticles may offer clear advantages regarding cost-effective measurements, sensitivity and reliability, comparing to conventional (Pt, Au, Carbon fibre, CNT) electrodes, and represent a consistent step forward.

II. Materials and Methods

Well-defined poly(lauryl methacrylate)-*block*-poly(2-(acetoacetoxy)ethyl methacrylate) (LauMA_x-*b*-AEMA_y) diblock copolymers synthesized by Reversible Addition-Fragmentation chain Transfer (RAFT) controlled radical polymerization [3-4] were employed for the stabilization of Au and Pd metal nanoparticles in n-hexane. In this particular solvent, hybrid micelles are generated consisting of a poly(LauMA) hydrophobic corona and a metal-containing poly(AEMA) core. For the preparation of the LauMA_x-*b*-AEMA_y/Pd systems, initially the polymer was dissolved in n-hexane, see Figure 1. Subsequently, triethylamine was added in the solution to generate the enolate form of the β -ketoester side-chain functionalities of the poly(AEMA) segment, the latter being well-known for the ability to act as strong bidentate ligands for various metal ions of different geometries and oxidation states [3]. For the preparation of the LauMA_x-*b*-AEMA_y/Au systems, after complete dissolution of the polymer in n-hexane, triethylamine was added to the solution that was further mixed with HAuCl₄.3H₂O. The mixture was left to stir at room temperature for one day. Upon dissolution *via* complexation, the color of the solution changed from white transparent to pink-purple transparent, indicating the formation of Au nanoparticles. The polymer-metal (LauMA₂₇₇-*b*-AEMA₈₁/Au, LauMA₂₇₇-*b*-AEMA₈₁/Pd, LauMA₅₀-*b*-AEMA₉/Pd)

nanocomposite films were fabricated upon spin coating at specific amounts of metallic nanoparticles (100 μL and 200 μL) of the corresponding solutions of the nanohybrids on a thin low density polyethylene film (LDPE). After drying, the respective components were submitted to manual screen-printing with a carbon based ink in order to generate the sensor with working (4 mm diameter) and counter electrodes, ideal for analysing 50 μL volume solutions. An AFM image of the $\text{LauMA}_{277}\text{-}b\text{-AEMA}_{81}/\text{Pd}$ film (about 4 μm thick) is presented in Figure 2. According to the final purpose, the technology may be improved, as described in literature [5-7], by exposure to microwave radiation to speed the components curing and maturation, or increase the density of metallic nanoparticles concentration towards the film surface, and/or by annealing the sample up to 300 $^{\circ}\text{C}$ for 10-40 min., in order to reach the glass transition, transforming the polymer from a glassy state to a rubbery state that would increase the mobility of the metallic nanoparticles.

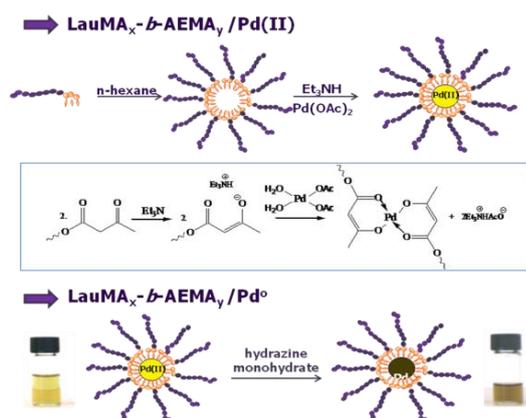


Figure 1. Example of preparation of micellar $\text{LauMA}_x\text{-}b\text{-AEMA}_y/\text{Pd}$ in *n*-hexan

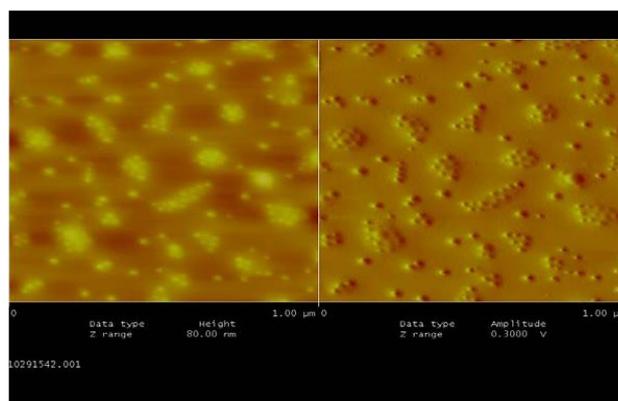
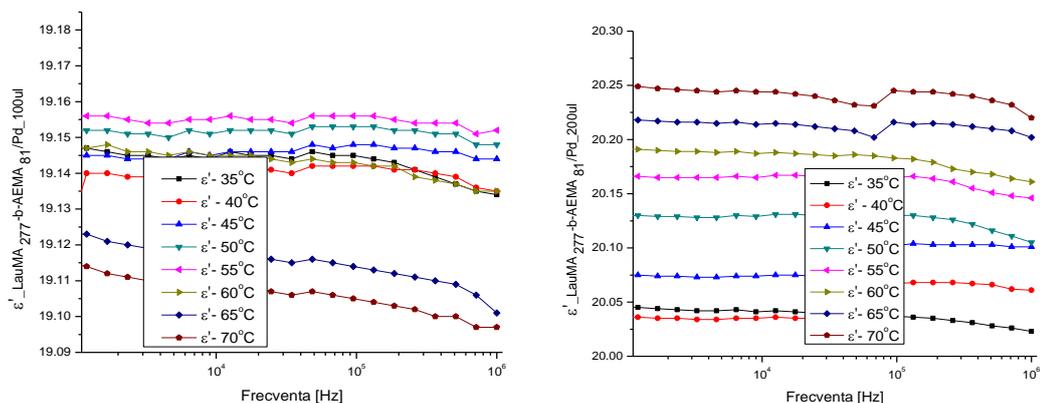


Figure 2. AFM image of the $\text{LauMA}_{277}\text{-}b\text{-AEMA}_{81}/\text{Pd}$



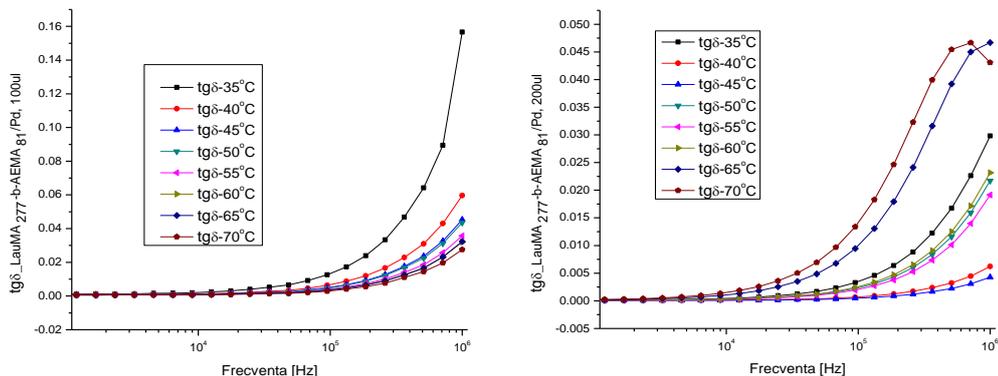


Figure 3. Dielectric properties of *LauMA*₂₇₇-*b-AEMA*₈₁/*Pd* (100 and 200 µL) on LDPE

III. Results and Discussions

The dielectric behaviour of novel nanocomposite films with metal (Au, Pd) nanoparticles has been investigated by means of Broadband Dielectric Spectroscopy, in a frequency range starting from 1 Hz up to 10 MHz and in a temperature range from 35°C up to 70°C. The obtained results for dielectric permittivity and loss factor suggested that the hybrid systems exhibit remarkable stable characteristics in both frequency and temperature till 10 kHz, being suitable for use in different applications of sensors and related signal processing technologies, e.g. in Figure 1. for *LauMA*₂₇₇-*b-AEMA*₈₁/*Pd* for different amount of metallic nanoparticles (100 and 200 µL), [5]. At higher frequency, due to the increase of loss factor, the structures emphasise very relevant shielding effects, aspect very useful in designing the electrochemical sensors with very low sensitivity to electromagnetic interference. The electromagnetic shielding effect depends on micellar features at specific amounts of metallic nanoparticles, e.g. it was found more obvious at *LauMA*₅₀-*b-AEMA*₉/*Pd* structures, Figure 4.

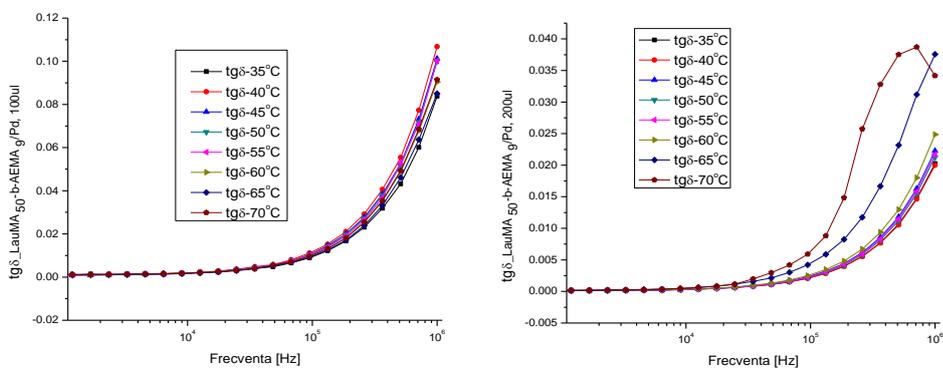


Figure 4. Dielectric properties of *LauMA*₅₀-*b-AEMA*₉/*Pd* (100 and 200 µL) on LDPE

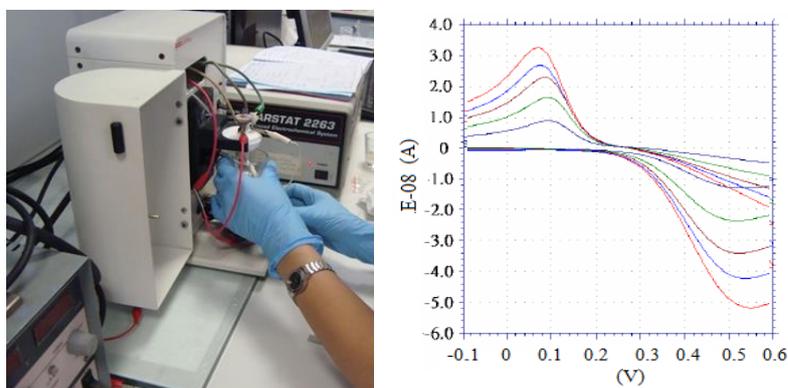


Figure 5. Example of cyclic voltamograms of hybrid sensor for dopamine, 0.1 M HCl

The electrochemical characterization (via Potentiostat/Galvanostat cyclic voltammetry method) was performed by using reference redox molecules such as ferricyanide, p-aminophenol, hydroquinone etc. The tests on different hybrid components, with both nano-dispersed Au and Pd, emphasized a higher sensitivity and reproducibility at different concentrations of dopamine (concentration between 0.02 mM and 20 mM) in acid medium (0.1 M HCl), Figure 5. The results are relevant, being homologue, even if less sensitive, to those obtained with commercial carbon and gold screen-printed sensors. In addition, selectivity to other molecules was studied, and a very selective detection capability was noticed due to metal activity at nanoscale. The results are in line with the results of other research groups, studying other types of micellar/ diblock copolymers microelectrodes with distributed metal particles (Au, Pd), [6,7].

IV. Conclusions

A new method of synthesis was developed for sensing applications, by realising microelectrodes of micellar/ diblock copolymers with distributed metal particles (Au, Pd). A laboratory model was developed, using the micellar thin film deposition on LDPE and submitting it to manual screen-printing with a carbon based ink in order to generate an electrochemical sensor with three electrodes, ideal for analysing up to 50 μ L volume solutions. The concept and preliminary obtained results are very promising, but more detailed experiments related to the influence of nanoparticles density are needed. In addition, it is assumed that the copolymerization with more conductive groups and application of additional treatments to tailor the density of metallic nanoparticles concentration would significantly improve the sensitivity and the selectivity for certain molecular species. Finally, to enlarge the immuno-sensorial applications, it would be useful to directly achieve the working electrode on a dedicated substrate, without using the screen-printing with carbon/CNT-based inks. Compared to the actual methods of fabrication, the present technique has the advantage of simplicity in obtaining a large mass of disposable sensors at a very convenient price. The concept of microelectrodes of diblock copolymers with metal nanoparticles is suitable for electrochemical sensing related to microfluidic applications for a large variety of biomolecules. The preliminary experiments showed a good sensitivity at different concentrations of dopamine.

References

- [1] C. Sanchez, G. J. de A. A. Soler-Illia, F. Ribot, T. Lalot, C. R. Mayer, and V. Cabuil: "Designed hybrid organic-inorganic nanocomposites from functional nanobuilding blocks", *Chem. Mater.*, 13, pp. 3061-3083, 2001.
- [2] K. R. Gopidas, J. K. Whitesell, M. A. Fox: "Synthesis, characterization, and catalytic applications of a palladium-nanoparticle-cored dendrimer", *Nano Lett.*, 3, pp. 1757-1760, 2003.
- [3] M. Demetriou, T. Krasia-Christoforou: "Synthesis and characterization of well-defined block and statistical copolymers based on lauryl methacrylate and 2-(acetoacetoxy)ethyl methacrylate employing raft controlled radical polymerization", *Journal of Polymer Science Part A: Polymer Chemistry*, 46, pp. 5442-5456, 2008.
- [4] K. Iliopoulos, G. Chatzikyriakos, S. Couris, M. Demetriou, T. Krasia-Christoforou: "Preparation and nonlinear optical response of novel palladium-containing micellar nanohybrids", *Optical Materials*, 33, pp. 1342-1349, 2011.
- [5] C. Bratescu, R. Burlacu, R. Ciobanu, T. Krasia-Christoforou, A. Neamtu: "Dielectric characteristics of novel hybrid materials consisting of functional block copolymers and metal oxide nanoparticles at temperature and frequency variation", *Annals of the University of Craiova - Romania / Series: Electrical Engineering*, 35, ISSN 1842-4805, 2011.
- [6] M. Alsawafta, S. Badilescu, A. Paneri, V.-V. Truong, M. Packirisamy: "Gold-poly(methyl methacrylate) nanocomposite films for plasmonic biosensing applications", *Polymers*, 3, pp.1833-1848, 2011
- [7] P. Devi, A.Y. Mahmoud, S. Badilescu, M. Packirisamy, P. Jeevanandam, V.-V. Truong: "Synthesis and surface modification of poly (dimethylsiloxane)-gold nanocomposite films for biosensing applications", *Proceedings of 2010 International Conference on Biosciences (BIOSCIENCESWORLD)*, Cancun, Mexico, March 2010.