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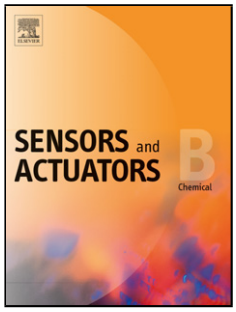

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Solid-phase synthesis of electroactive nanoparticles of molecularly imprinted polymers. A novel platform for indirect electrochemical sensing applications

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Abstract

Electroactive nanoparticles of molecularly imprinted polymers (MIP NPs) specific for a non-electroactive template (i.e. the antibiotic vancomycin) were for the first time synthesized by solid-phase synthesis adding two ferrocene-derivative monomers (namely, vinylferrocene and ferrocenylmethyl methacrylate) in different amount to polymerization mixture. MIP NPs were characterized by dynamic light scattering and by cyclic voltammetry studies. This latter allowed identifying the synthetic conditions determining the highest MIP NP electroactivity. The content of electrochemical label was verified by X-Ray Photoelectron Spectroscopy, which provided an estimation of the amount of ferrocene moieties in nanoparticle structure. In the attempt to apply MIP NPs for sensing applications, nanoparticles were anchored to a Nafion modified electrode by a simple self-assembly process and the indirect electrochemical detection of vancomycin was allowed by the change of ferrocene group redox properties upon the exposure to vancomycin. The observed behavior is believed to be due to hindering of the electron transfer process of the ferrocene redox sites within nanoparticles by their interaction with non-electroactive vancomycin. A novel sensing platform is thus developed by directly anchoring to the electrode surface an electroactive probe integrated within the imprinted polymer thus allowing the selective, easy and rapid electrochemical detection of non-electroactive target molecules.

Keywords molecularly imprinted polymers (MIPs), electroactive MIP nanoparticles, vancomycin, indirect electrochemical detection

1. Introduction

In recent years, Molecularly Imprinted Polymers (MIPs) have been increasingly recognized as a useful tool for studying molecular recognition processes and in the development of sensing systems due to their remarkable selectivity and affinity. By using imprinting technique, low cost materials containing cavities able to selectively recognize analytes ranging from biologically relevant markers, to drugs and agrochemicals[1,2] are easily prepared. MIPs represent an alternative to natural receptors with the main advantages being robustness, versatility and cost effectiveness. For the successful application of MIPs in sensors, it is necessary to improve their binding kinetics, shorten analysis times and achieve complete template removal. The possibility of designing MIPs at nanodimensional scale has been demonstrated to have a key effect on these issues by enhancing surface-to-volume ratio thus making binding sites more accessible to analytes. Different MIP nanostructures have been prepared[3] and successfully applied in the design of sensing devices. Among these, MIP nanoparticles (MIP NPs) have received particular attention being prepared through several chemical and electrochemical strategies[3,4] and successfully applied in different analytical fields, in particular in sensing applications[5]. Among different protocols proposed for the synthesis of MIP NPs[5], the solid-phase synthesis[6] has been found particularly useful leading to the fabrication of MIP NPs with pseudo-monoclonal binding properties, uniform binding sites and high affinity for the target used as template. One of the benefits of such imprinting strategy is the possibility of easy modification of the composition of polymerization mixture by adding specific monomers with the aim to confer particular properties to the resulting nanoparticles without limitations coming from solubility conditions which exist in precipitation and in mini-emulsion polymerization. Moreover, contrarily to core-shell approaches, solid-phase synthesis allows both to introduce *ad-hoc* functionalities in MIP NPs in a one-step polymerization and to obtain high affinity nanoparticles with strong integration of the desired functionalities within nanoparticle structure and not by depositing an external layer on a preformed core.

In the present work, for the first time, solid-phase synthesis was used for preparing electroactive MIP NPs by adding two electroactive monomers, vinylferrocene (VF) and ferrocenylmethyl methacrylate (FMMA) to the polymerization mixture. The redox properties of the ferrocene moiety confer electroactivity to MIP NPs, which was used for the electrochemical detection of the non-electroactive template molecule, the antibiotic vancomycin. The possibility of simply modifying MIP NP composition without affecting the binding properties was further exploited by introducing primary amino groups in nanoparticle structure, achieved by adding N-(3-aminopropyl)methacrylamide hydrochloride as functional monomer in the polymerization mixture.

This provides nanoparticles containing functionalities, which can be easily anchored to the electrode surface using simple coupling chemistry. The selective interaction of MIP NP-modified electrode with non-electroactive target molecule was shown to provoke a decrease of ferrocene groups redox current, proportional to vancomycin concentration.

It should be highlighted that the role of MIP NPs in the developed sensing platform is different from usually proposed schemes in MIP NP-based electrochemical sensors which exploit accumulation and concentration of electroactive targets by MIP[3,7], rather consisting in active generation and modulation of analytical signal by MIP-analyte interaction.

Vancomycin was here selected as a case study non-electroactive analyte as it is a powerful antibiotic used in treatment of various serious gram-positive infections, which in high doses can be toxic to the ears and kidneys, while at low doses can cause hypersensitivity reactions. Also the monitoring of vancomycin production by bacteria in culture media could be useful for checking antibiotic biosynthesis[8]. Electrochemical strategies commonly used for MIP-mediated recognition of non-electroactive species are based on the attenuation of the electrochemical signal of a probe in solution, whose diffusion to the electrode surface is limited by the target molecules occupying the imprinted cavities of MIP coating the electrode surface[9]. Two-step analysis is thus required consisting in preliminary MIP incubation with the template molecule and in subsequent electrochemical measurement in probe solution. In the proposed sensing platform, the electroactive probe is anchored onto the electrode surface as a component of the imprinted material. This determines a dual advantage consisting in a significant shortening of time analysis due to the elimination of incubation steps and in a more direct MIP-analyte interaction, not influenced by probe diffusion processes in solution.

MIP NPs prepared with different amounts of ferrocene-derivative monomers (Fig. 1a-b) were compared in terms of size distribution by dynamic light scattering (DLS), chemical composition by X-ray photoelectron spectroscopy (XPS) and electrochemical properties by cyclic voltammetry (CV). Two different schemes have been exploited for MIP NP integration with the electrode surface, one based on simple casting and the other on self-assembly on a Nafion membrane coated electrode. As a proof of concept, sensing performances of the as prepared MIP NP-modified electrode in the electrochemical detection of vancomycin were evaluated as well as its selectivity, tested against other commonly prescribed antibiotics as ciprofloxacin, levofloxacin and ampicillin.

2. Materials and methods

2.1 Reagents

Vancomycin, ciprofloxacin, levofloxacin, ampicillin, methacrylic acid (MMA), trimethylolpropane trimethacrylate (TRIM), ethyleneglycol dimethacrylate (EGDMA), 3-aminopropyltrimethyloxysilane (APTMS), sodium hydroxide, glutaraldehyde (GA), vinylferrocene (VF), pentaerythritol-tetrakis-(3-mercaptopropionate) (PETMP), ferrocenylmethyl methacrylate (FMMA) and acetone were from Sigma-Aldrich, UK. N-(3-Aminopropyl) methacrylamide hydrochloride (APMA) was from Polysciences Europe GmbH, Germany. *N*-diethyldithiocarbamic acid benzyl ester (DABE) was from TCI Europe, Belgium. Acetonitrile (ACN) was obtained from Acros, UK. Phosphate buffered saline (PBS) was prepared from PBS buffer tablets (Sigma-Aldrich, UK) and consisted of phosphate buffer (0.01 M), potassium chloride (0.0027 M) and sodium chloride (0.137 M), pH 7.4. Double-distilled (DD) ultrapure water (Millipore, UK) was used for the experiments. All chemicals and solvents were of analytical or HPLC grade and were used without further purification.

2.2 Synthesis of MIP NPs imprinted with vancomycin

The first step of synthesis of MIP NPs by solid-phase imprinting consisted in the immobilization of vancomycin on glass beads, as described elsewhere[6]. Briefly, glass beads were activated by boiling in 1 M NaOH for 10 min. They were then washed with DD water until neutral, followed by acetone and dried. The beads were then incubated in a solution (0.4 ml/g glass beads) of 2 % (v/v) APTMS in dry toluene overnight, washed with acetone, dried at 60 °C and subsequently incubated during 2 h in a solution of GA in PBS pH 7.4. The immobilization of vancomycin was achieved by incubating the beads with a solution of the antibiotic (5 mg mL^{-1}) in PBS, pH 7.2, overnight at 4 °C, using 0.4 mL solution/g glass beads. Finally, the glass beads were washed with DD water, dried under vacuum and stored at 4 °C until used. The glass beads with immobilized template were then utilized for the synthesis of MIP NP with different amount of electroactive monomers. The polymer composition was adapted from a previous work[10]. Monomer mixture was prepared by mixing 1.44 g MAA (16.7 mmol) as functional monomer, 1.62 g EGDMA (8.17 mmol) and 1.62 g TRIM (4.78 mmol) as cross-linkers, 0.37 g DABE (1.57 mmol) as initiator and PETMP 0.09 g (0.18 mmol), as chain transfer agent. Then 0.5 or 3% molar (0.15 to 0.89 mmol) of either VF (31 to 188 mg) or FMMA (42 to 253 mg) were added to the mixture, which was then dissolved in ACN (10.52 g). Monomer mixture was bubbled with N_2 for 10 min to remove dissolved oxygen. Vancomycin-derivatized glass beads (25 g) were placed in a 200 mL flat-bottomed glass beaker (with a flat glass

cover) and degassed *in vacuo* for 20 min in a desiccator and then the atmosphere replaced with N_2. The polymerization mixture was poured onto the solid-phase (under an N_2 stream) and the vessel then placed between two UV light sources (one on top and one below the beaker) Philips model HB/171/A, each fitted with 4 15 W lamps, for 2 min. After polymerization, the contents of the beaker were transferred into an SPE cartridge fitted with a polyethylene frit (20 mm porosity) in order to perform the temperature-based affinity separation of MIP NPs. Washing steps (8 washings with 20 mL ACN each) were carried out with at 0 °C in order to remove non-polymerized monomers and low affinity nanoparticles. High affinity nanoparticles were recovered washing the cartridges with ACN at 60 °C, five 20 mL washes. This allows high affinity MIP NPs to be eluted from the solid phase. The total collected volume of high affinity fraction of MIP NP was about 100 mL.

2.3 Characterization of MIP NPs

The size of the nanoparticles was determined by dynamic light scattering (DLS) using a Zetasizer Nano (Nano-S) from Malvern Instruments Ltd. (Malvern, UK). Prior to DLS measurements, the solution of MIP NPs was ultra-sonicated for 5 min to remove any possible aggregate. 1 mL of MIP NPs in water was then tested.

For XPS analysis, a drop (100 μL) of a suspension of MIP NPs in ACN was deposited on a silicon wafer and left in air for solvent evaporation. XPS measurements were performed using an Axis ULTRA DLD Spectrometer (Kratos Analytical, UK) with a monochromatic Al Kα source operating at 225 W (15 kV, 15 mA). For each sample a wide-scan spectrum was acquired in the binding energy range 0−1200 eV with a pass energy of 160 and 1 eV step, while high-resolution regions were acquired with a pass energy of 20 and 0.1 eV step. The area of analysis was about 700x300 □m^2. The base pressure in the instrument was 1×10^{-9} mbar. Data analysis was performed by CasaXPS software. Surface charging was corrected considering adventitious C 1s (BE = 285 eV).

The electrochemical characterization was carried out on MIP NPs deposited on a glassy carbon (GC) electrode (3 mm diameter) by two ways, namely by depositing a drop (40 μL) of a suspension of MIP NPs in ACN on GC and left to evaporate at room temperature, and by self-assembly with a Nafion membrane coated GC (Scheme 1). To this aim, 1 mL of MIP NP suspension in ACN was evaporated and re-suspended in water pH 3; meanwhile 20 μL of Nafion 117 (1% in ethanol) were deposited on the GC electrode, which was then immersed in the acidic solution of MIP NPs for 1 hour under stirring. Electrochemical experiments were carried out with a CHI660D Potentiostat (CH Instruments, USA). A one-compartment cell was used consisting of a glassy carbon modified

electrode, a platinum wire and a saturated calomel electrode (SCE) as working, counter and reference electrodes, respectively.

2.4 Electrochemical detection of vancomycin on MIP NP modified electrodes

The electrochemical detection of vancomycin was performed in TRIS buffer pH 7 by CV between 0 and 0.65 V (vs SCE) (scan rate 100 mV/s) in the concentration range 83-410 μM. Sensor response was evaluated in terms of current percentage decrease as $(i_0-i)/i_0 \times 100$, where i_0 and i are the peak current values in the absence and in the presence of vancomycin, respectively, evaluated for both anodic and cathodic scans. i_0 and i current values were evaluated after preliminarily stabilizing MIP NP modified electrode by CV between 0 and 0.65 V (vs SCE) (scan rate 100 mV/s) for 250 cycles in TRIS buffer pH 7. The same experimental conditions used for testing vancomycin were used for evaluating sensor response to other antibiotics such as ciprofloxacin, levofloxacin and ampicillin (Fig. 1d-f) and interference ratios (between percentage decrease of cathodic current in presence of interference and of vancomycin, both at concentration 410 μM) were evaluated.

3. Results and discussion

3.1 DLS and XPS characterization of MIP NPs

DLS measurements showed sizes of 266 nm and 316 nm for MIP NP prepared with 0.5% and 3% of VF, respectively. The nanoparticles containing FMMA were slightly smaller with sizes of 206 nm and 159 nm for MIP NPs with 0.5% and 3% of the electroactive monomer, respectively. Acceptable polydistribution indexes (around 0.3-0.4) were found for all samples indicating a uniform distribution of nanoparticles. These results are in agreement with previously reported TEM data on MIP NP for vancomycin prepared by solid-phase imprinting[11,12]. They also demonstrate that the presence of the electroactive monomer influences the size of the nanoparticles to a certain degree, with the VF producing larger MIP NP than FMMA.

Considering the key role that the ferrocene derivatives play in determining the electroactivity of the resulting nanoparticles, an estimation of the iron content within MIP NPs was obtained by XPS analysis. Detailed spectra relevant to Fe 2p region for each MIP NP sample are compared in Fig. 2. Two well resolved Fe $2p_{3/2}$ and Fe $2p_{1/2}$ spin-orbit split components at 707.6 eV and at 721.5 eV can be observed for MIP NP samples prepared with FMMA 3% and 0.5% (spectra (a) and (b), respectively), indicating a predominant Fe(II) population[13,14]. An estimation of the iron content was thus performed for MIP NP samples containing FMMA monomer, by considering the Fe 2p/N

1s atomic ratio and obtaining values of 0.032 and 0.065 for samples with FMMA 0.5% and 3%, respectively, thus confirming the higher iron content in the latter case. What is interesting to observe is the lack of detectable signals in Fe 2p region in the case of MIP NP prepared with VF 0.5% and 3% (spectra (c) and (d), respectively). This result could be interpreted either as due to the entrapment of a low amount of VF monomer or to a reduced surface distribution of ferrocene-derivatives making them not accessible by XPS analysis. The different incorporation of two ferrocene derivatives within the structure of MIP NPs could be explained by slow reactivity of vinyl derivative as compared with methacrylate-substituted analogue[15] thus determining a lower polymer yield.

3.2 Electrochemical characterization of MIP NPs

With the aim to evaluate the effect of different ferrocene-monomers on the electroactivity of resulting MIP NPs, their electrochemical behavior was evaluated by CV, after casting nanoparticles on the electrode surface. A remarkable electroactivity was observed for nanoparticles prepared with FMMA 0.5% and 3% (Fig. 3a), showing, respectively, the separation between the anodic (E_a) and cathodic (E_c) peak potentials of about 60 and 100 mV and the formal potential ($E^{0'}$) of about 360 and 400 mV, respectively, in agreement with previously reported value for ferrocene-derivative assemblies on electrode surface[16]. Interestingly, different values of current peaks were observed for MIP NPs containing different quantities of FMMA, with nanoparticles prepared with 3% of the monomer showing about five-fold higher anodic currents than those prepared with 0.5% FMMA. Considering that such electrochemical signals could be ascribed only to FMMA moieties, these results could suggest that almost the total amount of FMMA used for their synthesis is incorporated within MIP NPs and is involved in electrochemical processes. Completely different results were obtained with nanoparticles prepared with 0.5% and 3% of VF as both types of MIP NPs exhibited a lack of well distinguishable signal ascribable to faradic processes (Fig. 3b). These findings are in agreement with XPS data and might implicate that only a very low amount of vinylferrocene is effectively polymerized and entrapped in MIP NPs and/or that it is not accessible to redox processes due to higher NP dimensions. On this basis, only MIP NPs prepared with FMMA 3% were selected for further electrochemical experiments.

After depositing MIP NPs by drop casting on the electrode surface, it was noticed that a drastic decrease of peak current, both in direct and inverse scan, occurred in subsequent cycles, leading to the almost complete disappearance of redox signals (Fig. 3c). This was probably due to the weak adsorption of MIP NPs on the electrode surface, causing their easy dispersion in electrolytic solution thus preventing the use of such system for sensing purposes.

With the aim to improve the stability of the MIP NPs modified electrode, their assembly on a Nafion coated GC electrode was performed by exploiting the electrostatic interaction between negatively charged sulfonic groups of Nafion and protonated amino groups *ad hoc* introduced in MIP NPs for this purpose. Interestingly, the as-prepared electrodes revealed a quite different CV profile (Fig. 3d) with well-defined peak-shaped curves, both in direct and inverse scans, with higher peak current values and lower formal potential ($E^{0'}$ = 245 mV) suggesting an enhanced electron transfer process. As in the case of drop casted electrodes, the total width at half-height of either the cathodic or anodic wave (fwhm) was found to be higher than the value reported for ideal Nernstian behavior[17], possibly because of non-negligible interactions between neighboring ferrocene sites[16]. As clearly evidenced in Fig. 3d, this immobilization does not prevent substantial decrease of peak current (of about 85%) after about 250 scans. It was verified that such a decrease was not due to the weakening of the electrostatic interactions between Nafion and MIP NPs as a consequence of pH change when passing from pH 3.0 (self-assembly process) to pH 7.0 (CV experiments), since when cycling experiments were carried out in buffer at pH 3.0 the same current decrease was recorded. It should be highlighted that contrarily to what observed on drop casted electrode, in this case redox signals were still clearly evident after 250 cycles, and current values reached a steady state condition without exhibiting any further decrease in the last 50 cycles, as shown by the comparison of the insets in Fig. 3c and 3d. This suggests that in spite of the loss of a certain amount of MIP NPs, an equilibrium condition can be reached making the system suitable for sensing applications.

3.3 Vancomycin electrochemical detection

With the aim to provide a proof of concept of sensing platform applications, the electrochemical detection of vancomycin was tested by monitoring the current peak decrease after the exposure of the system to the target molecule. Fig. 4a reports CV curves on MIP NPs modified electrode, after being subjected to 250 cycles for current stabilization, in clean buffer and in the presence of vancomycin. It can be observed that the increase in vancomycin concentration produces a proportional decrease of both anodic and cathodic peak currents, as evidenced in Fig. 4b where calibration curves are obtained by plotting percentage current decrease evaluated at 310 mV (i_{pa}) and at 240 mV (i_{pc}) as a function of vancomycin concentration. Such a behavior was ascribed to the effect of the non-electroactive target molecule on ferrocene moieties redox processes. It could be hypothesized that the attenuation of current upon vancomycin binding determines a restriction of counter ions accessing the anchored redox probe to balance the charge, thereby hindering the electron transfer[18]. A linear range can be identified in the tested concentration range (83-410 μM)

for both anodic and cathodic currents with R=0.9957 and R=0.9930, respectively. Having in mind further applications of the proposed system for amperometric detection of vancomycin, the cathodic current was here selected as analytical signal due to the lower potential value, which should minimize the interference of coexisting electroactive oxidizable compounds. A satisfactory sensitivity was achieved (0.146 μM^{-1}) as well as a good reproducibility (RSD 6.7%, n=3) evidencing the high reliability of the developed sensor. In addition, the lowest detectable concentration (83 μM) could allow the prospective use of the sensor for clinical measurements of vancomycin (recommended therapeutic concentration range in plasma 15-20 mg L^{-1} or 10-15 μM[11]) following a detection scheme employing a simple cleanup/pre-concentration step.

3.4 Selectivity studies

The specificity of the MIP-based sensor toward vancomycin was evaluated by testing and comparing its responses to vancomycin and some possible interfering substances. The electrochemical response to three other non-electroactive antibiotics, namely ciprofloxacin, levofloxacin and ampicillin, was tested (Fig. 5). By comparing current responses, interference ratios (expressed as the ratio between percentage decrease of cathodic current in presence of interference and of vancomycin, both at concentration 410 μM) of 0.17, 0.20 and 0.44 were obtained for levofloxacin, ciprofloxacin and ampicillin, respectively. These results suggest the presence of a minimal non-specific contribution from these molecules, which could derive from ferrocene moieties located outside of imprinted cavities. This may be also explained by the fact that these compounds are smaller than vancomycin in molecular size and have some chance of accessing the imprinted sites. In particular, the higher sensor response to ampicillin in respect to levofloxacin and ciprofloxacin could be explained by considering the higher availability and flexibility of functional groups responsible for hydrogen bonding with imprinted nanoparticles, which could promote a certain interaction with the imprinted sites subsequently hindering ferrocene electron transfer.

Conclusions

Electroactive molecularly imprinted polymer nanoparticles were prepared for the first time by solid-phase synthesis and used in a novel sensing platform based on an indirect electrochemical detection scheme after their easy anchoring to the electrode surface. The antibiotic vancomycin was selected as a case study non-electroactive template molecule. Different amounts of two ferrocene-derivative monomers were introduced into the polymerization mixture and spectroscopic and electrochemical

properties of the resulting MIP NPs were compared, thus selecting optimal synthetic conditions for sensing applications. As a proof of concept, the application of the developed system to electrochemical vancomycin detection was successfully carried out, gaining satisfactory sensor performance in terms of sensitivity and selectivity.

The proposed sensing platform, based on current attenuation upon the specific interaction of electroactive MIP NPs with vancomycin, can be extended to the detection of any non-electroactive target molecule, for which imprinted nanoparticles can be easily and cheaply produced using the solid-phase synthesis approach.

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Elisabetta Mazzotta (PhD) is a permanent Researcher at the University of Salento since 2011. In 2005 she received her MSc degree in Environmental Sciences at the University of Salento and in 2009 a PhD in Chemistry of Innovative Materials at the University of Bari. From 2009 to 2011 she was a post-doctoral research fellow at the Department of Materials Science of the University of Salento. The scientific activity of E. M. is devoted to the synthesis of novel materials and to their use in the design of biosensors and biomimetic sensors. In particular, her research activity is focused on micro- and nanostructured materials (nanoparticles, conducting polymers and molecularly imprinted polymers) and on the use of XPS methodology for their chemical characterization.

Antonio Turco (PhD) is a Researcher at the University of Salento. He received his MSc degree (2009) in Biotechnology Science from University of Salento and his PhD (2014) in chemical and pharmaceutical sciences and technologies from Center of excellence for nanostructured materials (CENMAT) of University of Trieste. His research focuses on innovative nanocomposite materials for tissue engineering and environmental remediation, electrochemical sensor and biosensor and surface functionalization with nanostructured materials, polymers and molecularly imprinted polymers for application as sensitive layers in sensors.

Iva Chianella (PhD) was awarded her first degree in Chemistry in 1998 from the University of Florence, Italy. She gained her Ph.D. in 2003 from Cranfield University working on the development on sensors based on artificial receptors. In 2002, she was employed by Cranfield as Research Officer and was promoted to Lecturer in 2007. Iva has nearly 50 publications in the area of natural and artificial receptors and related aspects of their use in analytical systems. In the past 10 years she has been involved with a number of industrial and European Union funded projects within this area.

Antonio Guerreiro (PhD) is a senior research fellow. He received his BSc and MSc degrees from Universidade do Algarve (Portugal). He did his PhD in the area of Supramolecular Chemistry in Cranfield University (UK) before starting as research fellow in same university. His current research is focused on applied polymer science and molecular recognition, in addition to the design of processes for the manufacture of nano-materials.

Sergey A. Piletsky (PhD) is a professor in Bioanalytical Chemistry. Piletsky supervised work of numerous MSc and PhD students and published over 250 peer-reviewed papers and patent applications. Prof. Piletsky is visiting Professor in Jinan and Huazhong Universities, China, member of Editorial Board of ACS Combinatorial Sciences, Scientific Board of Society of Molecular Imprinting, Fellow of the Society of Biology, and Vice-President of the International Union of Advanced Materials.

Cosimino Malitesta (PhD) received his MSc degree cum laude in Chemistry in 1983 and his PhD in Analytical Chemistry in 1989 from Università di Bari. Since then he has there covered lecturer and associate professor positions in Analytical Chemistry. In 2001 he has been appointed as full professor of Analytical Chemistry at Università del Salento. He is member of Italian Chemistry Society and served as Vice-President of Analytical Chemistry Division and in the Board of Biotechnology Group. His main research interests include XPS development and application, biosensors and biomimetic sensors based on electrosynthesized polymers, electrosynthesis of molecularly imprinted polymers, nanostructures.

Figure 1 – Molecular structures of electroactive tested monomers: FMMA (a) and VF (b), and of vancomycin (c) and chemicals used for selectivity tests: cyprofloxacin (d), levofloxacin (e), ampicillin (f).

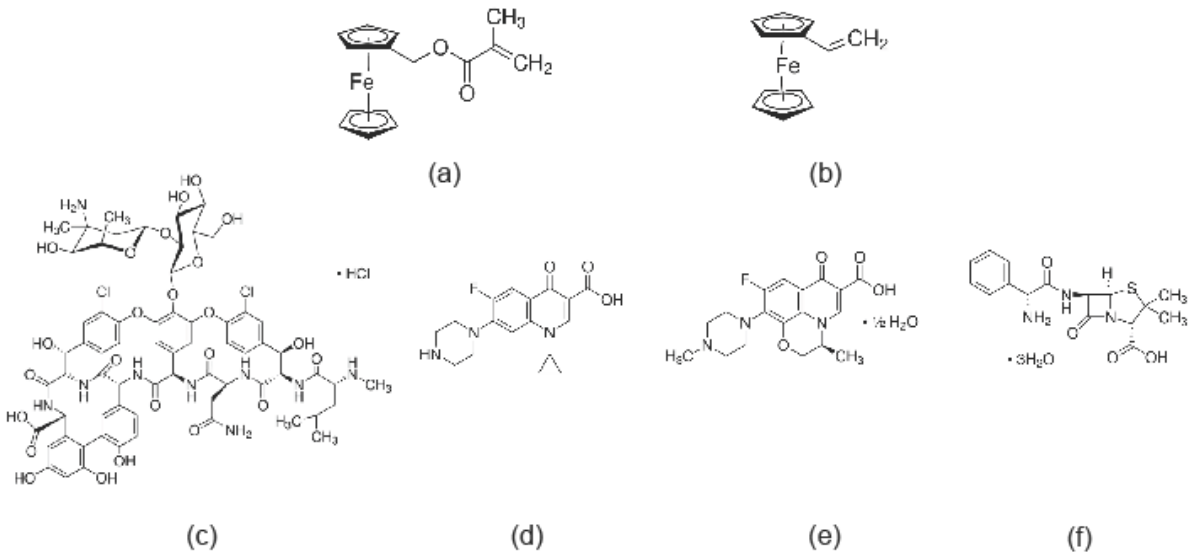


Figure 2 – Comparison of Fe 2p XPS detailed spectra of MIP NP samples prepared with FMMA 3% (a), FMMA 0.5% (b), VF 0.5% (c), VF 3% (d).

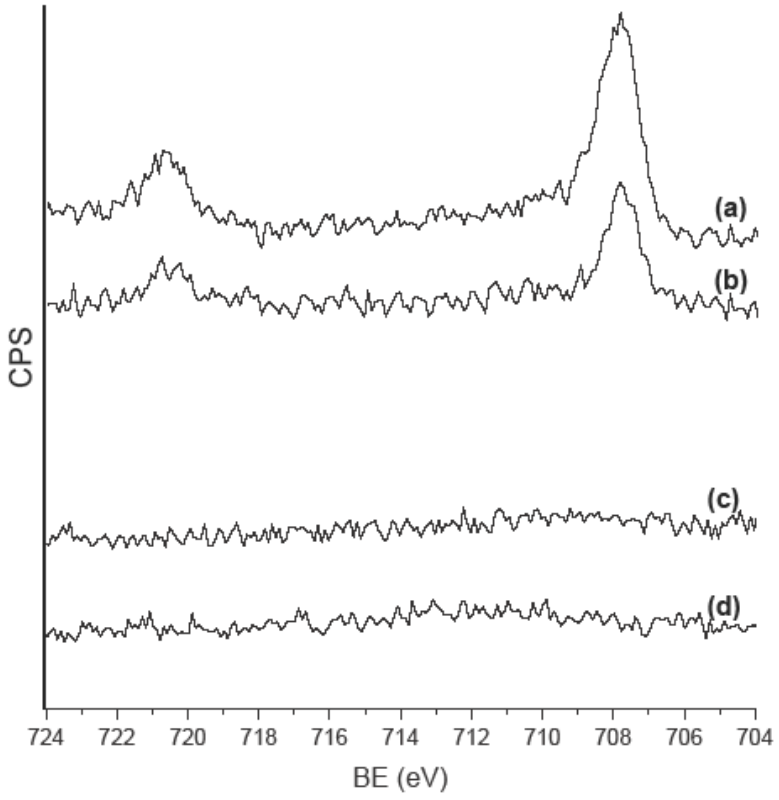


Figure 3 – CVs relevant to MIP NP prepared with FMMA 3%-0.5% (a) and VF 3%-0.5% (b) deposited by drop casting on a GC electrode surface. CVs relevant to MIP NP prepared with FMMA 3% deposited by drop casting (c) and by self-assembly on a Nafion coated GC electrode (d) showing current variation from 1st to 250th cycle. The insets report CV curves from cycle 200 to cycle 250. CVs are recorded in TRIS buffer pH 7, scan rate 100 mV/s.

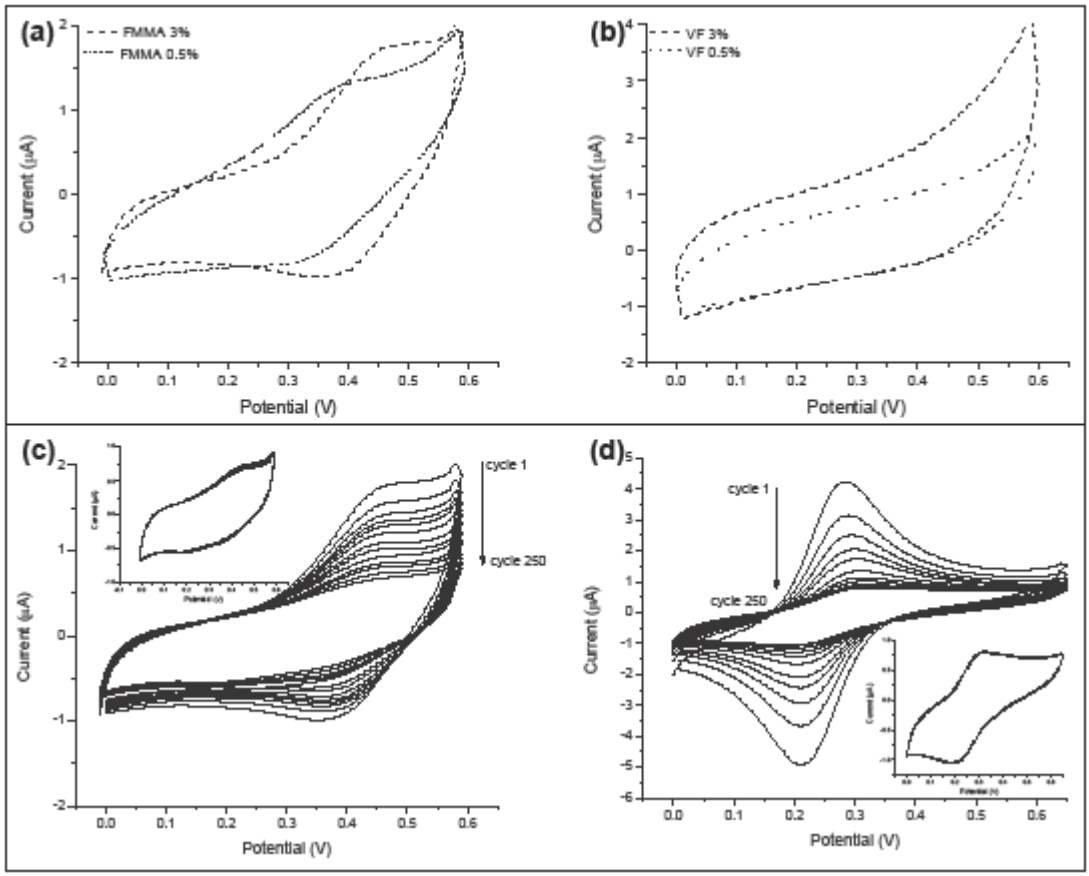


Figure 4 – (a) CV response of MIP NP modified electrode to increasing vancomycin concentration and (b) calibration curves plotting percentage decrease of anodic (i_{pa}, diamond) and cathodic (i_{pc}, circle) peak current, evaluated after 250 CV cycles stabilization of MIP NP electrode (experimental conditions as in Figure 3).

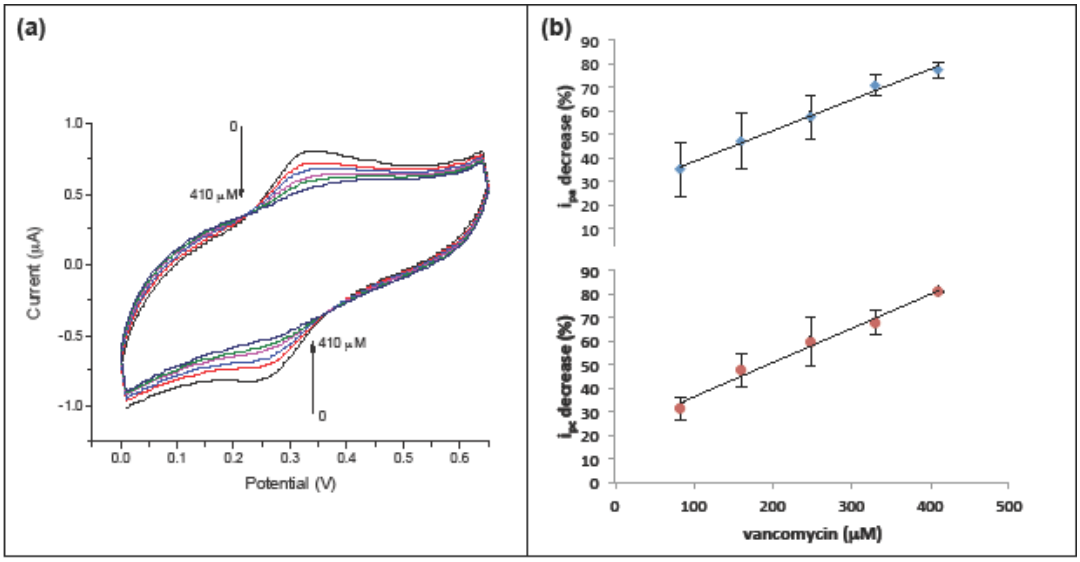
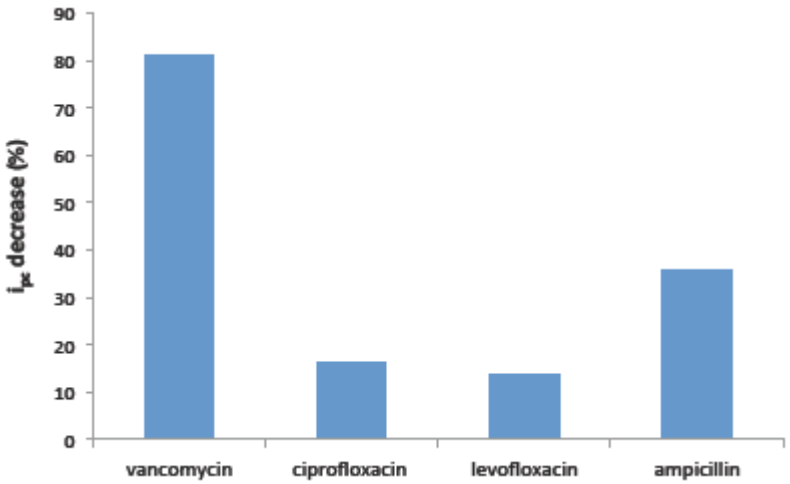


Figure 5 – Bar chart showing results of selectivity studies. Data are referred to percentage decrease of cathodic peak current (i_{pc}).



Scheme 1 – Scheme of solid-phase synthesis of MIP NPs for vancomycin and of their self-assembly on a Nafion coated glassy carbon electrode.