

“SCREEN PRINTING” FOR CHEMICAL SENSOR AND BIOSENSOR PRODUCTION

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Abstract: “Screen printing” is one of the most promising technology allowing chemical sensors and biosensors to be largely on the market in the next future as analytical devices. Amperometric or conductometric electrodes, printed on ceramic or polymeric substrates and coupled with screen printed layers of enzymes and polymers, can be easily assembled for obtaining biological or chemical sensors. The chances of mass and low cost production and reproducibility are critically reviewed examining some devices developed in ENEA laboratories.

Keywords: Screen printing, enzymes, biosensors, molecular imprinting.

INTRODUCTION

Biosensors obtained a significant commercial success in the field of clinical chemistry and diagnostics in the last two decades. One of the most successful and largely known example

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is represented by the so called "*glucose pen*", allowing the direct determination of glycaemia at any time and any place and the consequent infusion of the right dosage of insulin. Biosensors for glucose, lactate and pyruvate were developed for continuous in vivo monitoring of blood [1] and finally artificial pancreas based on glucose biosensor is now commercially available (β -Like, Esacontrol Ansaldo Elettronica Biomedicale). The number of devices based on biosensors really on the market is relatively low in spite of the big effort of the scientific community, the large spectrum of the purified biomediators and the variety of the available chemical or physical transducers. The limiting step for commercialization is mainly due to the preparation of the transducer and to the immobilization procedures of the biomediator, which don't fit, in most of the cases, with mass production and manufacturing costs. Screen printing was chosen as one of the most promising technology which allows biosensors to be largely on the market [2-13]. As a matter of fact Thick Film Technology (TFT) allows the deposition of metal conductors, dielectric insulators and polymeric pastes, even in a multilayer configuration, on inorganic (mainly alumina) or polymeric (PVC) substrates. It is based on the use of a "screen printer" (figure 1) which allows to stratify inks or pastes in several layers on an insulating substrate with a process similar to the serigraphic one. The deposition is realised by forcing the paste (figure 2) through the open meshes of a net mounted on an aluminum frame (screens) using a sylicon spatula (squeege). The layout drawn on the open meshes of the screen is then transferred on the

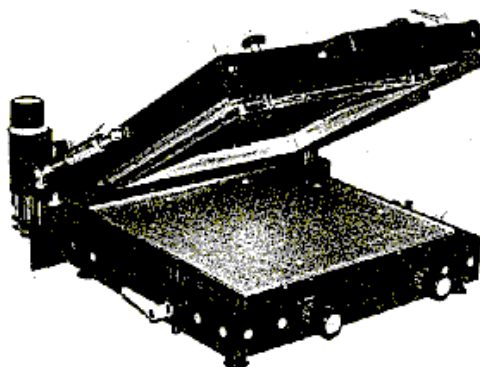


Figure 1: The screen printer used in our laboratory is the most simple and cheap on the market

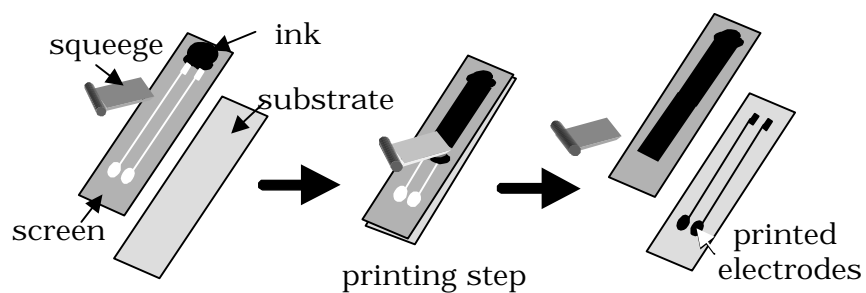


Figure 2: The printing process allows the deposition of pastes or inks containing fine powders of the electrode material dispersed in an organic media (solvent, polymers)

substrate allowing simple or even complex plan microcircuit as well as physical or chemical sensors.

Glucose oxidase, Tyrosinase, PhotoSystem II [14] have been previously immobilised on our screen printed electrodes by using several traditional techniques. As a matter of fact, many techniques are available for deposition of sensitive layers on the transducers, ranging from physical absorption or entrapment, covalent bonds between the functional groups on the transducer surface and the mediator, crosslinking with glutaraldehyde and bovine serum albumin, covalent immobilization on activated membranes etc. Some of these methods requires tedious and even complex chemical steps for introducing functional groups on the surfaces or immobilizing the mediator, which have to be performed in a chemistry lab. For this reason, transferring these treatments to a large scale production is difficult and expensive. The deposition of enzyme or polymer layers [6] could be performed at the same time with the same technology (TFT) used for printing of the electrode materials. Several screen printed electrodes can be easily and unexpensively produced in the ENEA laboratories: PVC based devices with graphite electrodes, or ceramic alumina based ones with platinum electrodes. Conductor paths and contact pads of both devices are printed with silver based inks. Amperometric biosensors have been obtained by using glucose oxidase as a model biomediator. Conductometric sensors [15] have been coupled with molecular imprinted materials [16-27] for obtaining advanced chemical sensors directed toward atrazine-like herbicides to be used in environmental analyses. As a matter of fact, experiments are still in progress in the frame of a community project [Brite Euram BE

(95) 1745] which involves molecularly imprinted polymers (methacrylate) deposited on the electrode surface for atrazine analytical determination in environmental samples. The aim of the MIMICS project (Molecularly Imprinted Materials for Integrated Chemical Sensors) is to investigate the potential use of molecularly imprinted materials (MIMs) as the basis for a new generation of chemical sensors.

MATERIALS AND METHODS

Ceramic and PVC based device preparation: Ceramic substrates were prepared by tape casting from non-aqueous slurries of polyvinylbutyrals resins, plasticized with alkyl phthalates and polyethylene glycol as previously reported [28]. Pt and Ag/Pd based inks were printed on ceramic substrates, obtaining a Pt working electrode and an Ag reference electrode with an electrochemically deposited AgCl layer. Finally an insulating layer was printed on the conducting paths. A firing step at 900°C was performed after each printing step. Graphite and Ag/Pd based inks were printed on the polymeric substrate (a PVC sheet). The conducting paths and pads were printed with the same Ag/Pd ink. Electrodes printed on PVC was left overnight at room temperature or treated for 20 min at 80°C. Graphite based inks were doped with Rh/graphite or Pt/graphite powders in the range 5-50% w/w to obtain the working electrode to be sensitive to H₂O₂ and available for oxidase enzymes. Figure 3 summarizes the lay-out used for both the polymeric and the ceramic devices.

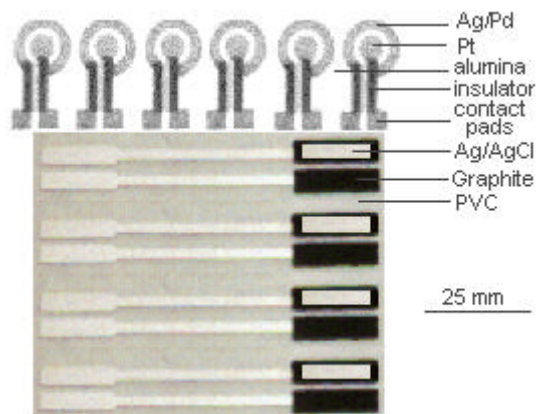


Figure 3: Lay-out for alumina (above) and PVC based devices (below). See fig.4 for additional lay-outs of PVC based devices for conductometric or amperometric measurements

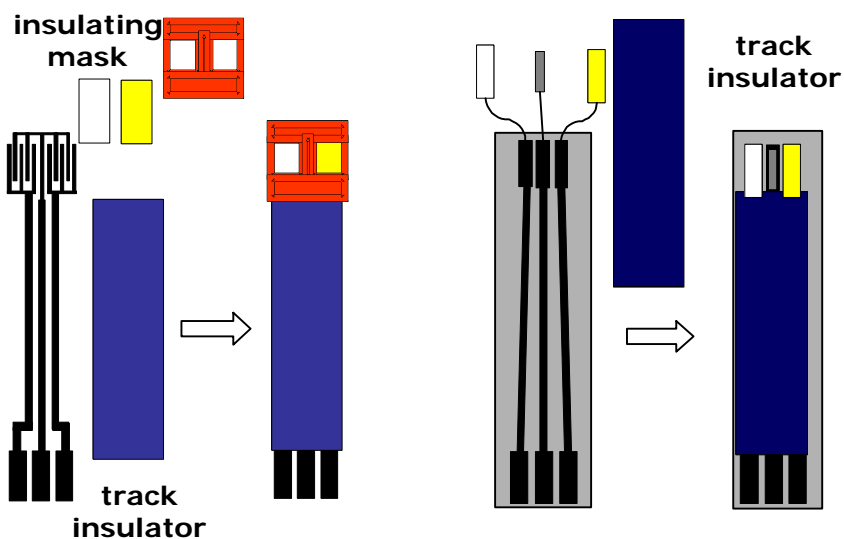


Figure 4: additional lay-out of conductometric interdigitated and amperometric devices. Each printed layer is shown separately on the left and the resulting devices are shown on the right

Several sensors lay-out have been tested in addition to the above mentioned ones, allowing the measurements to be performed in several experimental setup ranging from Flow Injection Analysis to steady state flow systems to very simple in field assay formats (drop-on analyses), for amperometric or conductometric measurements, and have been reported in figure 4.

Printing of layers sensitive to Hydrogen Peroxide: Graphite based inks are commercially available for printing on polymer substrates but, as a result of high operating potentials, hydrogen peroxide, the product of oxidase enzymes, cannot be detected by means of screen printed graphite electrodes. Graphite fine powders with adsorbed noble metals (Pt,Rh) on the surface (SIGMA) can be used with the aim of obtaining screen printed amperometric electrodes sensitive to hydrogen peroxide and therefore able to be coupled with oxidase enzymes.

Printing of biosensing layers: Glucose oxidase powder was mixed with an epoxy resin (araldite) and then printed with different thickness. Hardening of the epoxy resin was performed either in an oven at 40°C or under vacuum. The last method allowed to completely remove solvents of the epoxy resin without using a thermal treatment which could be dangerous for the enzyme activity.

Printing of molecularly imprinted layers: Molecular imprinted polymers (MIP) were printed on activated surfaces: electrodes were initially dipped in a solution containing 5% of 3-acryloxypropyltrimethoxysilane in toluene for 15 min., dried under nitrogen flow, heated at 80°C for 10 min. and finally washed in ethanol for 5 min. The preparation of the surface could be also performed by mixing 3-acryloxypropyltrimethoxysilane with the graphite ink before printing the electrodes. A pre-polymerization step of the monomer mixture (15 min.) under an UV lamp was

needed to achieve the right viscosity for printing. The monomer mixture was prepared as reported in the following table:

Table 1: Mixture for MIP preparation

Template molecule	Atrazine (1 eq. mol.)
Functional monomer	Methacrylic acid [MAA] (4 eq. mole)
Cross-linker	Ethylene glycol dimethacrylate [EGDMA] (16 eq. mol.)
Photoinitiator	Irgacure
Solvent	Toluene

Finally the pre-polymerized mixture was printed on the activated electrode surface and polymerization was completed with an UV lamp under nitrogen flow. An example of thickness profiles of the deposited layers is reported in figure 5. In figure 6 pictures by SEM observation are reported. Bulk polymers "against" atrazine were also obtained by complete polymerization of the mixture. The bulk polymer was milled and sieved obtaining polymer particles of different size which were then mixed with graphite based inks and finally printed. The scheme of the preparation of these sensors is reported in figure 7.

Tools for operating with screen printed electrodes:
Contacting the sensors to the electronics is an important step to perform effective and reproducible measurements with screen printed devices as well as flow cell design. Contacts and flow cells (wall jet configuration) were developed in our laboratory as shown in figure 8.

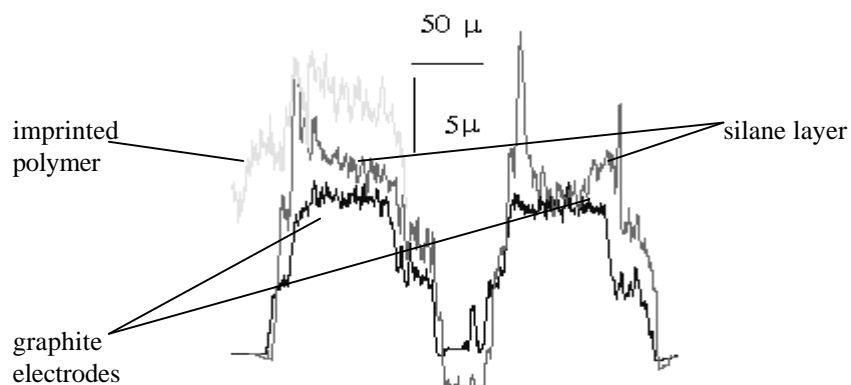


Figure 5: Surface analysis of graphite electrodes, with subsequent deposition of silane layer and molecular imprinted film (on the left)

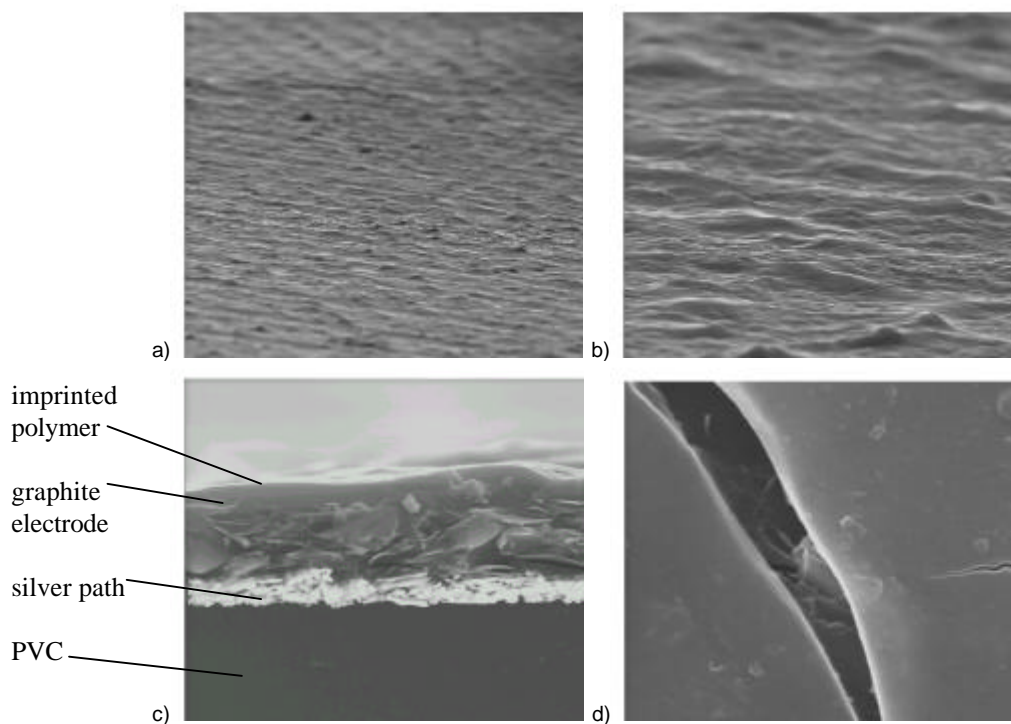


Figure 6: Observation of the MIP printed layers by SEM: a) (1:10) and b) (1:50): Landscape of the surface of the MIP printed on graphite electrodes; c) (1:200) cross section of a fractured device: PVC, Ag, Graphite and polymer (3 μm thick) layers assembled in a sandwich format can be observed; d) (1:1000) An intentionally obtained fracture on the MIP surface.

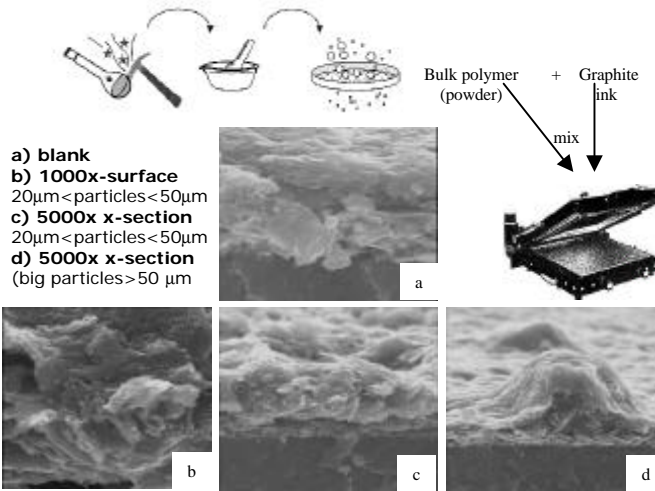


Figure 7: Printing of bulk polymer particles mixed with the graphite paste. Observation by SEM of the resulting surfaces

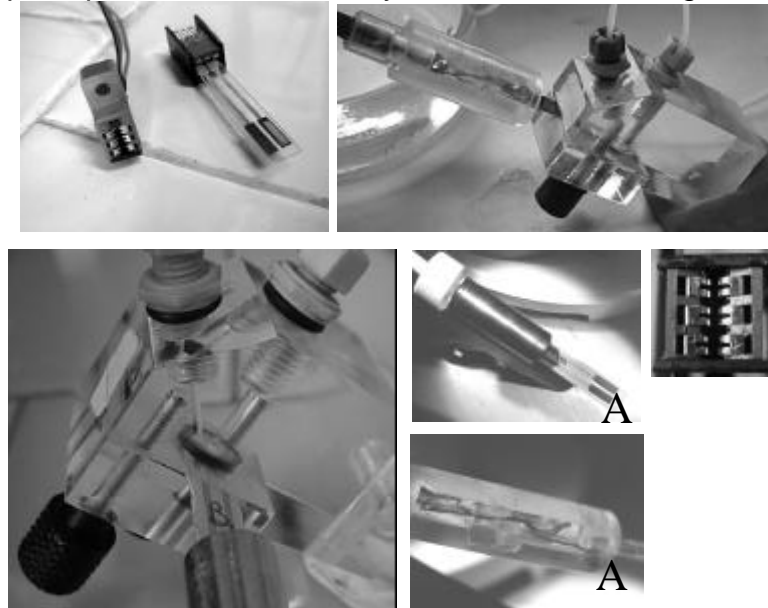


Figure 8: The contactors and the flow cell developed in our laboratory by courtesy of Mr.A.Lucchi. Contactor A was obtained from Krejci Engineering

RESULTS AND DISCUSSION

Hydrogen peroxide sensors based on graphite inks: Higher sensitivity to hydrogen peroxide ($6.5 \text{ nA}/\mu\text{M}$) was obtained with a 20% w/w mixture of Rh/graphite ink while decreased sensitivity was observed with higher content of the powder. 30% and 50% gave sensitivity respectively of $2.3 \text{ nA}/\mu\text{M}$ and $1.5 \text{ nA}/\mu\text{M}$. With Pt based graphite powder, sensitivity was lower but the optimum ($1.8 \text{ nA}/\mu\text{M}$) was reached at lower concentration (about 11%). As a result, the Pt/graphite powder can be considered more convenient than Rh based powder because of its lower price and the lower concentration needed. The bell shaped curve observed for both Rh and Pt graphite powders could be due to opposite effects on sensitivity: the higher the concentration of noble metal, the higher the sensitivity of the electrode, but also, the higher the concentration of the graphite powder added to the ink with a corresponding decrease of its performances.

Printing of biomediator based pastes: Glucose oxidase was chosen as the model protein, because it is commercially available and because this enzyme is the best known and the most spreadly used for biochemical analyses in the broad class of oxidases [29-30]. As recently reported in several papers, organic solvents affect the enzyme activity less than the commonly believed [31-40]. The enzyme (GOD) was then dispersed in graphite based pastes, or in epoxy resins, or in cellulose acetate solutions (water/acetone). In addition, adsorption or entrapment in silica aerogels as suitable materials to be mixed with graphite inks

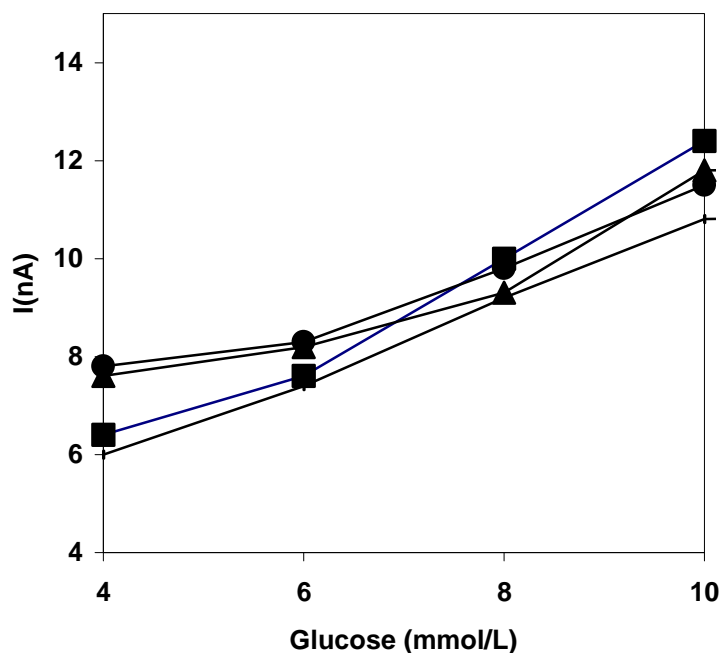


Figure 9: Calibration curves for several electrodes obtained by printing araldite/GOD composites on Pt-graphite electrodes.

and printed were also tested. Best results were obtained with the enzyme dispersed in epoxy resins as following reported. Our experiments started from examples in the literature [41-45] which used araldite as dispersing polymer to obtain graphite/GOD based rod electrodes. In spite of the good behaviour from the literature, mixing graphite ink with Pt or Rh graphite powder, araldite and GOD didn't give the expected results even if several formulations of the final pastes were tested. An additional layer of

araldite/GOD composite was then printed on the previously obtained graphite/Pt-graphite based electrodes. Good reproducibility was recorded as reported in figure 9 where calibration curves for 4 electrodes are plotted.

Unexpected result was the good linearity at very high glucose concentration (up to 80-100 mM) as reported in figure 10.

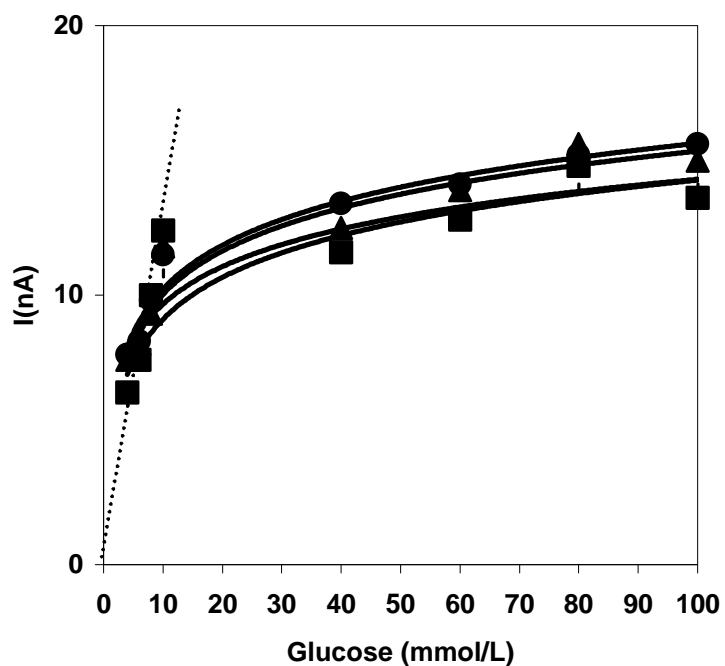


Figure 10: Calibration curve at higher glucose concentration of the araldite/GOD composite screen printed electrodes

This behaviour should be due to a reduced transport of the glucose molecule with respect to oxygen diffusion through the araldite membrane. The reduced diffusion didn't affect greatly the response time which is about 3 min.. In addition, GOD was slowly released during the first hours of operation and at the 3rd day the biosensor sensitivity remained stable at above 70% of the initial value as reported in figure 11.

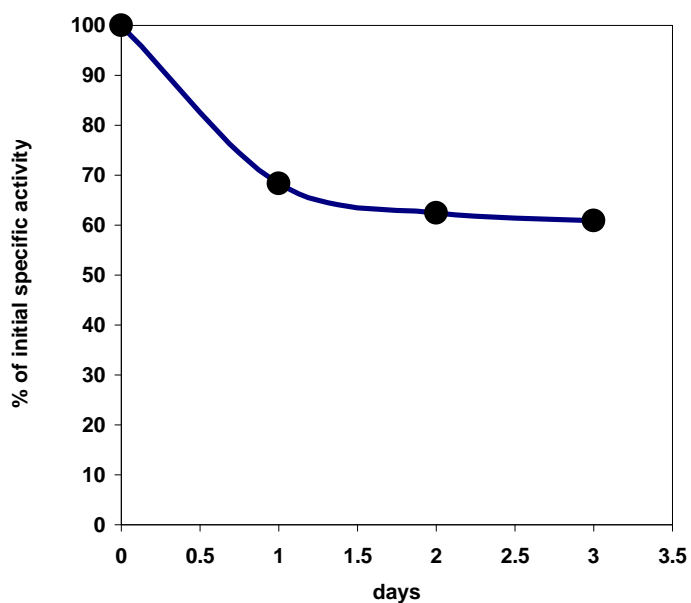


Figure 11: Lifetime of GOD immobilised and printed with araldite epoxy resin

The layer thickness was also investigated. The expected bell shaped curve (figure 12) was obtained as a result of two opposite effects on the signal due to the diffusion and the enzyme quantity on the electrode in the araldite layer.

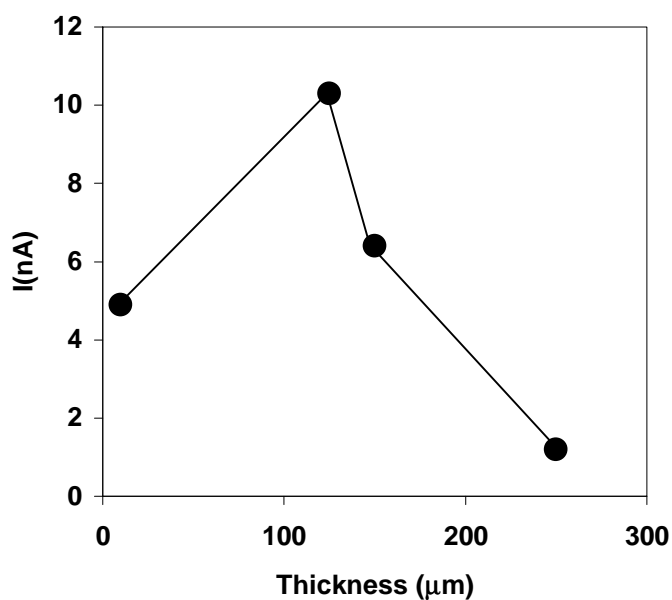


Figure 12: Effect of thickness of the araldite/GOD layer

Printing Molecularly Imprinted Polymers: Molecularly imprinted based sensors were tested by conductivity measurements which were performed with graphite (PVC) or Pt (ceramic) electrodes. The optimization of the conductivity

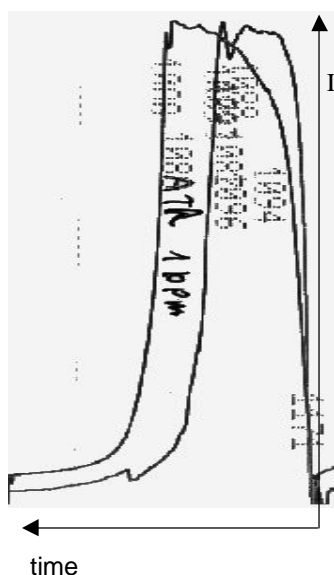


Figure 13: Response time of a MIM before and after incubation with an atrazine solution

measurements was made with bare electrodes as the first approach. Signal vs frequency (from 1 to 10kHz), vs potential (from 9 to 84 mV peak to peak) and vs buffer (Tris) concentration were tested with the aim to find the most sensitive experimental conditions. Electrodes were then coated with MIM (Molecularly Imprinted Membrane) using the techniques described above. Amperometric detection with the above mentioned sensors printed with bulk polymer fine particles, showed, after incubation with an atrazine aqueous solution (1ppm), a decreased slope of the signal obtained with an ascorbic acid solution (1mmol/l) as reported in figure 13. Graphite electrode was at 200 mV vs Ag/AgCl.

Conductivity of the electrode coated with the imprinted polymer membrane decreased when the selective cavities were occupied from the target molecule (atrazine). As a matter of fact, immediately after the polymerization step and before washing with ethanol, lower conductivity was recorded. Treatment with ethanol can reasonably be considered as responsible for washing out the entrapped atrazine from the cavities of the MIM surface and, as a consequence, an increased conductivity is observed.

A protocol for real analyses, including timing of all the treatments was set up (data to be published). Briefly, the measurements are performed by washing with ethanol for removing initial atrazine content in the polymer, measuring baseline, extracting and concentrating (about 1:100) the water sample with toluene by using SPE C₁₈-cartridges, incubating the resulting toluene solution with MIM on the sensor, washing to remove the not specifically adsorbed atrazine on the surface and, finally, measuring the resulting baseline. Analyses performed on standard solution of atrazine in toluene have given the calibration curve reported in figure 14.

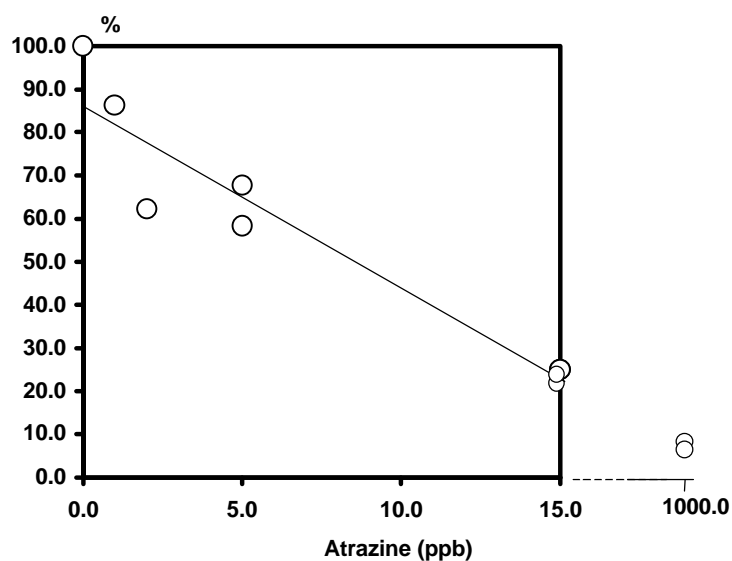


Figure 14: Calibration curve with atrazine standard solutions in toluene

A limit of detection of 2-5 ppb can be stated considering that 1 ppb atrazine solution gave the same signal of the blank (only toluene). Considering the concentration factor (1:100) obtainable with the SPE cartridges a tentative limit of detection could be hypotised to be below 100 ppt of atrazine in water samples. Analyses on cross reactivity with other triazine based herbicide are in progress.

CONCLUSIONS

The use of commercially available or home made inks for electrode printing and the wide range of possibilities with respect to the substrate material allow the development of low cost/high performances transducers. Ceramic devices showed characteristics of robustness and were mechanically and chemically more stable than PVC devices. On the contrary PVC devices are preferable because of their simple preparation.

The use of noble metal as well as Pt or Rh adsorbed on graphite powders allowed the possibility to apply our graphite electrode to the very broad class of oxidase enzymes. In addition, epoxy resins represented a good dispersing polymer for obtaining enzyme immobilization and printing on thick film electrodes. The effect of the specific binding of the target molecule (atrazine) in the cavities of a MIP printed on amperometric or conductometric thick film electrodes was shown. By conductometric measurements the construction of a calibration curve in toluene at

ppb level was performed and a protocol to be applied in environmental analysis at 0.1 ppb level is setting up. The tests reported in this paper show the applicability of thick film technologies in a wide range of configurations from the enzyme based biosensor to a new generation of chemical sensors based on molecularly imprinted materials. The deposition of the sensing layer by TFT was demonstrated to be a possible way for obtaining mass production of chemical or biochemical sensors.

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