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New Techniques in Monitoring Water Pollution - Development of Sonochemically Fabricated Microarrays for the Determination of Pollutants

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ABSTRACT

The focus of this article is to describe a simple-to-use, disposable sensor suitable for the rapid determination of pollutants in aqueous media, utilising a novel sonochemical microelectrode fabrication technique. The use of screen-printing, electrochemical and sonochemical methods allows the production of microelectrode arrays capable of stir-independent determination of chlorine in water. These arrays permit the simultaneous measurement of free and total chlorine at concentrations between 0-20 ppm. Developments leading to production on a mass scale will be briefly discussed. A further system incorporating enzyme containing conductive polymers to give microelectrode arrays capable of detection of ultra-low levels of organophosphate pesticides will also be described. Acetylcholine esterase could be entrapped within conductive polyaniline protrusions and the effects of pesticides on its activity determined. Ultra low concentrations of pesticide were found to reduce the enzymes activity as measured electrochemically. These systems allow the detection of organophosphates at concentrations as low as (10^{-17} M).

INTRODUCTION

Water is fundamental to life on earth - and reliable supplies of clean, safe water are required not only for human consumption (potable water) but also for farm animals and the growing of crops. The environment is also very susceptible to pollution should water supplies become contaminated. It follows that the importance of reliable methods for analysis cannot be overstated - and indeed determinations for a number of pollutants still need to be developed. Many analytical methods depend on highly skilled staff, cumbersome sample pre-treatment regimes and expensive dedicated equipment. Chemical sensors can offer alternative analytical approaches, helping to satisfy the increasing demand for precise analytical information, together with cost benefits - via devices that require relatively simple instrumentation and little, if any, sample pre-treatment (1). Sensor technology is still a rapidly evolving and ever expanding field with many chemical sensors for various applications having been proposed over the last twenty years.

The main requirements for an applicable sensor are good precision, a broad dynamic analytical range, ease of use, fast response times and a good specificity for the analyte to be analysed. Furthermore, a low unit price is often required for disposable sensors.

Another problem that often requires addressing is the variation in sensor response that can be caused by stirring or agitation of the analyte sample. One approach to developing sensors with stir-independent responses has been via the use of microelectrode arrays. Microelectrodes have several properties which make them attractive as active elements within sensors for the determination of analytes in, for example, flowing water streams. Specifically, microelectrodes exhibit enhanced

diffusion in comparison to larger sensors and this leads to enhanced sensitivity – as well as independence from the effects of convectional mass transport and therefore solution flow or other movement (2). Individual microelectrodes however, offer very small responses and one approach for overcoming this problem is via the use of many microelectrodes coupled together in the form of an array to allow a larger cumulative response to be measured. Despite this no commercially successful sensors produced, to date on a large scale have employed microelectrode arrays, largely due to the cost of conventional fabrication routes such as photolithography or laser ablation.

DISCUSSION

Previous work by this research group (3) has provided a novel and patented (4) procedure for the fabrication of microelectrodes, involving the sonochemical ablation of thin poly(1,2-diaminobenzene) film coatings that insulate planar electrode surfaces. This format lends itself to mass fabrication due to the simplicity and inexpensiveness of the approach. We have utilised this approach to construct sensor arrays capable of detecting a wide range of chemical and biochemical species, two of which, chlorine and organophosphate pesticides will be described within this work.

Chlorine is a powerful oxidising agent that is used widely as a disinfectant in the treatment of industrial, recreational and potable drinking water. A variety of industrial processes are heavily dependant on the use of chlorine because of its potency as a sterilising agent for water, and it is essential that individual users and companies are able to measure chlorine concentrations to determine if adequate levels for disinfection are present. However, in addition to its benefits, the use of chlorine

does incur some disadvantages and the presence of excessive concentrations of chlorine can in some cases be detrimental to human health and aquatic life.

Concern over the environmental and health effects of chlorination have led to a raft of legislation relating to its determination (viz: European Economic Community, 1998; US Environmental Protection Agency, 2000). It is clear that accurate, sensitive and simple procedures are required for the monitoring of chlorine by users and regulatory agents in order to assure compliance with regulations.

The majority of chlorine testing within water is performed by colourimetric wet chemistry approaches and a wide range of commercial tests are available for different applications. These however suffer from a number of limitations including bleaching of the colour and a limited analytical range due to fluctuations in colour change. Colourimetric approaches also requires skilled operators for use, the equipment is cumbersome and sampling/testing is time consuming (5). A technique which would permit easy, rapid, accurate and qualitative analysis of chlorine for both free and total determination over a wide analytical range would clearly be advantageous.

There has been much concern recently concerning the levels of pesticides, both as residues within food and also within the environment. A significant proportion of the pesticides used within agriculture become washed off or are otherwise lost from the large areas of agricultural land treated surfaces - and for this reason an excess of pesticide is commonly applied (6). Organophosphate pesticides (OPs) are now commonly used instead of the organochlorine pesticides due to their lower half lives in the environment. It should be remembered that OPs are, however, neurotoxins and

can present a serious risk to human health. These compounds may still find their way into our food and water supplies, which necessitates the use of analytical approaches for the reliable detection of pesticides for environmental protection and food safety purposes. Legislation has now been passed to help control the levels of pesticides that are permitted within water supplies; European Commission: EU Water Framework Directive 2000/60/EC, European Commission: Drinking Water Directive 98/83/EC, which demand that of levels of individual pesticides cannot exceed 0.1 mg l^{-1} and 0.5 mg l^{-1} for the total pesticide concentration. At the time of writing, monitoring for these pesticides is problematic since conventional analytical approaches do not offer the required lower limits of detection for some of the OPs in question. We have recently developed a series of enzyme based microelectrode arrays which are capable of detecting extremely low levels of pesticides.

Production of microelectrode arrays

A schematic of the sonochemical fabrication production process we have developed within our laboratory is shown in figure 1. Commercial screen printed electrodes may be used as the basis for these sensors and consist of screen-printed carbon working and counter electrodes together with an Ag/AgCl reference electrode. The working electrodes are initially coated with a thin film (50-70 nm thickness) of an insulating polymer formed by the electrochemical deposition of 1,2-diaminobenzene (3). This process is self-limiting - and thus reproducible. Sonochemical ablation is then used to ablate or “drill” holes in this insulating material with sizes of 0.1 to several microns and a density of up to 120000 pores cm^{-2}. Scanning electron micrographs for a 60 s sonicated electrode assembly are shown in figure 2(a). There are several features of interest within these micrographs. Firstly the distribution of the pores is random since

ultra-sonic cavitation is a chaotic process. It is also evident that almost all of the cavities are bimodal in size, possessing either 3 μm (±1 μm) or sub-micron diameters. We believe that the smallest of the cavities observed are formed by the initial impact/ablation of the micro−jets of fluid (7). These cavities are known to act as nucleation sites for further bubble formation (7) and it is thought that the cavity grows as new bubbles implode within the confines of the original cavity. This process gives rise to a quantum enlargement in the diameter of a cavity. Since no larger pores are seen it is believed that the 3 μm diameter pores no longer act as nucleation sites. In some instances there is evidence of a few pores joining to form dumbbell shaped cavities when two pores form in close proximity to each other.

Obviously the longer the sonication time, the more pores will be formed and the greater any signal response, as reported in previous studies (8). However, as the number of pores increases, the diffusional profiles will overlap and microelectrode behaviour will be lost, as studied experimentally and theoretically by other workers (9) who studied arrays of carbon microdisc electrodes in an epoxy matrix. By utilising similar electrochemical methods we previously determined (8) that sonication times of 20 seconds were optimum. Increasing sonication time lead to loss of microelectrode behaviour; for example electrochemical measurements on arrays made by sonication for 30s or more did not display stir-independence (8).

Other methods utilising sonication have also been used to generate microelectrode arrays. For example the sonic irradiation of glassy carbon electrodes suspended within slurries of metal nanoparticles in organic solvents lead to the nanoparticles being melted onto the electrode to form microarrays of bismuth, copper and other metals

(10). Bismuth microarrays of this type have for example been used for the determination of As(III) concentrations (10).

These conductive microelectrodes can be utilised as substrates for the deposition of further recognition layers. Another method however utilises a second electropolymerisation step to grow “mushroom-like” protrusions of a conductive polymer (such as polyaniline) from these pores (3, 8). Scanning electron micrographs (figure 2b,c) clearly show formation of these “mushroom” like protrusions - and this allows species capable of chemical or biochemical recognition to be incorporated directly within the conducting polymer. As can be seen these protrusions can be easily grown to at least 10 microns in diameter, the size of these being controlled by the number of deposition cycles.

Production of a chlorine sensor

The microelectrode pore array could be utilised within a electrochemical sensor for chlorine as described above. Chlorine in aqueous solution can exist as free chlorine (Cl_2) or in a variety of combined forms such as hypochlorous acid (HOCl) or other forms upon reaction with organic materials. Ammonia will for example react with chlorine to give NH_2Cl. It is advantageous to simultaneously measure both forms, so experimental electrodes are based on commercial screen printed electrodes supplied by Microarray Ltd (Manchester) and contain two working electrode microarrays; one dedicated to the measurement of free chlorine and the other for measuring total chlorine. The electrochemical determination of chlorine is based on known titrimetric approaches. Chlorine (both free and combined) reacts with iodide ion to produce triiodide ion which can then be titrated using sodium thiosulphate. Free chlorine can

be directly detected electrochemically whereas combined chlorine will not directly reduce at an electrode surface and so acidified potassium iodide is added to the solution, preferentially oxidising both free chorine and combined chlorine according to equation 1.

$$2I^- + Cl_2 \rightarrow I_2 + 2Cl^- \qquad 1$$

The iodine generated can be reduced at the working electrode to produce iodide, according to Equation 2, thereby allowing electrochemical detection.

$$I_2 + 2e^- \rightarrow 2I^- \qquad 2$$

Provided sufficient iodine is made available, the current measured will be proportional to the concentration of total residual chlorine in the mixture. For this reason the microelectrode arrays were coated with a thin film of potassium iodide containing formulation using a BioDot AD3200 dispensing platform, incorporating a BioJet Plus™ 3000 dispensing system. Electrodes were allowed to dry and stored in the dark until required. These electrodes were then used to measure free and total chlorine respectively. Subtraction of the free chlorine value from the total chlorine level gives the level of combined chlorine. A schematic of the final sensor construction is displayed in figure 3a, along with a photograph of a complete electrode (figure 3b). Although when placed in aqueous sample solutions, the iodide will dissolve, diffusion away from the electrode is relatively slow and a result for chlorine can be obtained with our system in 30 seconds, long before loss of iodide becomes a problem. Since these electrode assemblies have been developed for single-use only, it does not matter if the iodide is removed during the first measurement.

Once the fabrication processes for the sensors had been optimised, it became necessary to determine the range of amperometric responses from 0 to 20 ppm free and total chlorine in order to provide calibration data. Figure 4 shows calibration profiles obtained for total chlorine solutions interrogated with chemically modified microelectrode array sensors (concentrations of 0, 0.01, 0.02, 0.03, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 ppm). Given the range of the current responses obtained, data is presented in low- and high-range calibration curves. Error bars represent the relative standard deviation (RSD) from the 5 sensors taken at each chlorine concentration.

The full sensor calibration data exhibits a quasi-linear response to total chlorine solutions, resulting in diminishing escalation in response with increasing concentration. A response of approximately 3 nA per 0.01 ppm for low range total chlorine and 1 nA per 0.01 ppm for high range concentrations was obtained - sufficiently large to permit the differentiation of 0.01 ppm total chlorine.

The unusually large baseline signal of approximately 53 nA indicated for a 0 ppm total chlorine concentration - is the result of oxidised iodide present in the chemical modification layer, deposited at the electrode surface during processing.

Figure 5 demonstrates similar calibration profiles for concentrations of 0-20 ppm free chlorine interrogated with modified screen printed carbon-ink based microelectrode array sensors. Once again, a quasi-linear calibration profile is obtained for the BioDot modified microelectrode arrays sensors with a response of approximately 2 nA per 0.02 ppm increment for low range free chlorine and 1 nA per 0.02 ppm for high range concentrations. A much lower background current of approximately 10 nA is obtained

in this case, since there are no electroactive constituents present in the free chlorine chemical modification layer. As expected, there is a considerably smaller amperometric response for free chlorine when compared to the total chlorine response.

This process has proved suitable for scaling up for commercial production. Large sheets of screen-printed sensors containing hundreds of individual four electrode (two working, a counter and an Ag/AgCl reference) units can be easily constructed by industrial partners. Insulating layers can be simultaneously deposited onto all the working electrodes and the use of large sonic tanks allows the ablation of the electrode sheets. Thousands of chlorine sensors could be produced daily within our pilot scale facility.

Detection of pesticides

Organophosphorus pesticides can pose a threat to human health if ingested either through foodstuffs or water supplies. This is problematical since many direct methods for pesticide detection do not have the required sensitivity to determine ultra-low levels of these compounds. However, it is known that many enzymes are susceptible to poisoning by pesticides at very low concentrations. One enzyme which is extremely sensitive to the effect of many OPs is acetylcholine esterase. We have developed a novel electrochemical method utilising sonochemically fabricated microarrays for the detection of pesticides. This work has been published in detail elsewhere (11,12) - and so only a brief overview will be given here.

Screen-printed carbon ink transducers doped with cobalt phthalocyanine (CoPC) with a working electrode area of 9 mm^2 were printed on alumina substrates in sheets of 20. These were purpose-produced for this project and purchased from Gwent Electronic Materials Ltd., (Gwent, Wales, UK). These electrodes also comprised an on board reference electrode (Ag/AgCl) and counter electrode (platinum) (see figure 6). Arrays of conducting polyaniline "mushrooms" can be constructed containing entrapped acetylcholine esterase (11). When exposed to acetylthiocholine, the entrapped enzymes catalyse the formation of thiocholine which can be detected electrochemically. Exposure of these arrays to low level concentrations of pesticides leads to poisoning of the enzymes and impairment of their ability to catalyse this reaction. Figure 8 shows the effect of paraoxon solutions on the sensitivity of microelectrode enzyme arrays to thiocholine (12). As can be seen, extremely low detection limits of 10^{-17} M can be obtained for these systems. Similar sensitivities have been obtained for other pesticides.

CONCLUSIONS

We have described the sonochemical fabrication of microarrays suitable for the detection of a variety of analytes. We have applied these arrays to the determination of a wide variety of analytes. Chlorine, both free and combined can be detected in solutions in the range of 0-20 ppm. Incorporation of enzymes within polyaniline microarrays has led to construction of sensors offering lower limits of detection for organophosphorus pesticides down to 10^{-17} M.

References

1. C. A. Galan-Vidal et al., *Trac-Trends In Anal. Chem.* **14**, 225-231 (1995).

2. R. M. Wightman, *Anal. Chem.* **53**, A1125-A1135 (1981).

3. S. Myler et al., *Anal. Chim. Acta*. **357**, 55-61 (1997).

4. Higson, S. P. J. (1996). Sensor. European Patent Number EP0823050.

5. G. Gordon et al.,.Disinfectant Residual Measurement Methods, 2nd edn. AWWA Research Foundation and American Water Works Association (1992).

6. A. N. Ivanov et al., *Anal. Chim. Acta*. **404**, 55–65 (2000).

7. K. S. Suslick, K.S., 1990. *Science* **247**, 1439–1445 (1990).

8. A. C. Barton et al., *Biosen. Bioelec.* **20**, 328-337 (2004).

9. O. Ordeig et al., *J. Electroanal. Chem.*, **592,** 126-130 (2006).

10. S. Ward-Jones et al., *Ultrasonics Sonochem.*, **13,** 261-270 (2006).

11. J. Pritchard et al., *Biosen. Bioelec.* **20**, 765-772 (2004).

12. K. A. Law, S. P. J. Higson, *Biosen. Bioelec.* **20**, 1914-1924 (2004).

List of figures.

Figure 1. Schematic of formation of microelectrode arrays (a) deposition of insulating polymer, (b) sonochemical ablation to form microelectrodes, (c) deposition of polyaniline containing entrapped biological moieties.

Figure 2. Scanning electron micrographs of (a) micropores formed by sonochemical ablation (b, c) polyaniline “mushrooms”.

Figure 3: (a) Schematic of final sensor construction; (b) final four-electrode assembly.

Figure 4: Combined chlorine calibration curve; ‘Low’ range from 0 to 0.5 ppm (mean RSD 4.1%), ‘High’ range from 0 to 20 ppm (mean RSD 4.3%)

Figure 5: Free chlorine calibration curve; ‘Low’ range 0 to 0.5 ppm (mean RSD 3.8%), ‘High’ range free chlorine calibration curve from 0 to 20 ppm (mean RSD 4.2%).

Figure 6. Calibration curve showing the effect of pesticide concentration on enzyme electrode performance; (a) Screen printed electrodes for the determination of organophosphate pestcides: Plots show a typical current transient response for an AChE-modified electrode to 2 mM acetylthiocholine chloride, before (b) and after (c) the addition of 1×10^{-17} M paraoxon.

Figure 1.

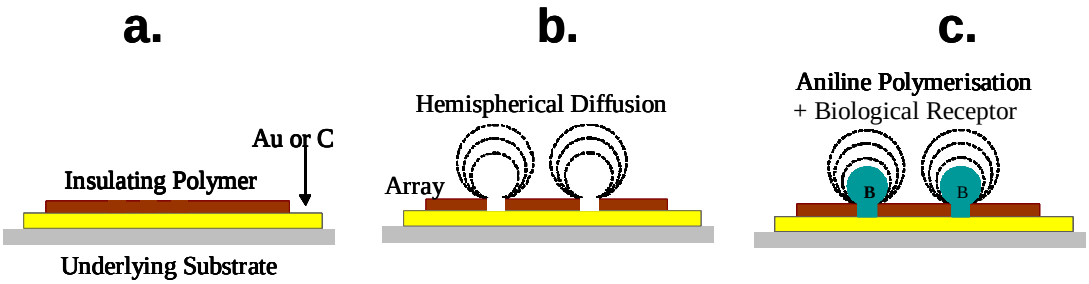
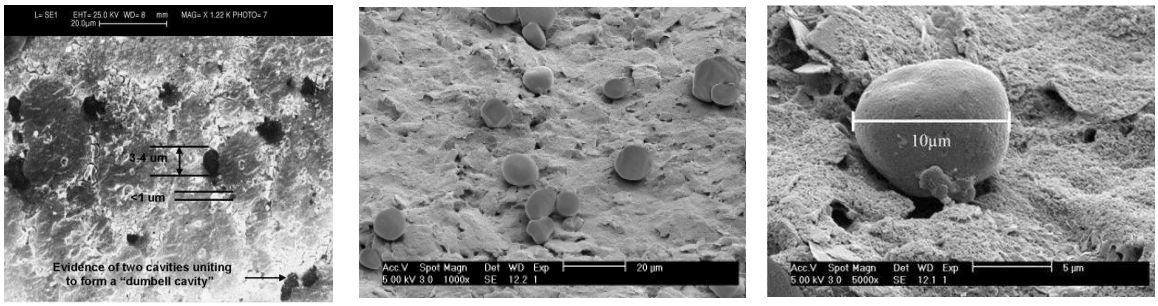

a.
b.
c.
Au or C
Insulating Polymer
Underlying Substrate
Hemispherical Diffusion
Array
Aniline Polymerisation
+ Biological Receptor
B
B

Figure 2.


3.4 um
<1 um
Evidence of two cavities uniting to form a "dumbell cavity"
10µm

a. **b.** **c.**

Figure 3.

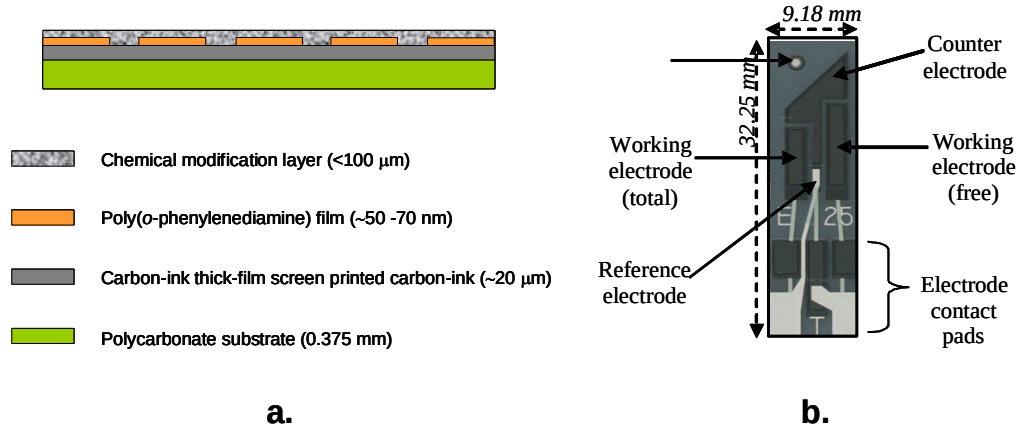

Chemical modification layer (<100 μm)
Poly(o-phenylenediamine) film (~50 -70 nm)
Carbon-ink thick-film screen printed carbon-ink (~20 μm)
Polycarbonate substrate (0.375 mm)
a.
9.18 mm
32.25 mm
Counter electrode
Working electrode (total)
Working electrode (free)
Reference electrode
Electrode contact pads
b.

Figure 4.

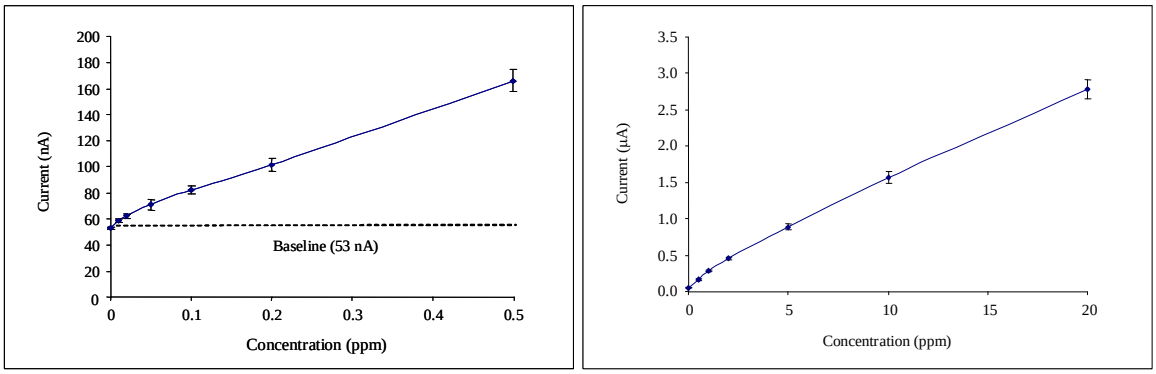

Current (nA)
Concentration (ppm)
Baseline (53 nA)
Current (μA)
Concentration (ppm)

Figure 5.

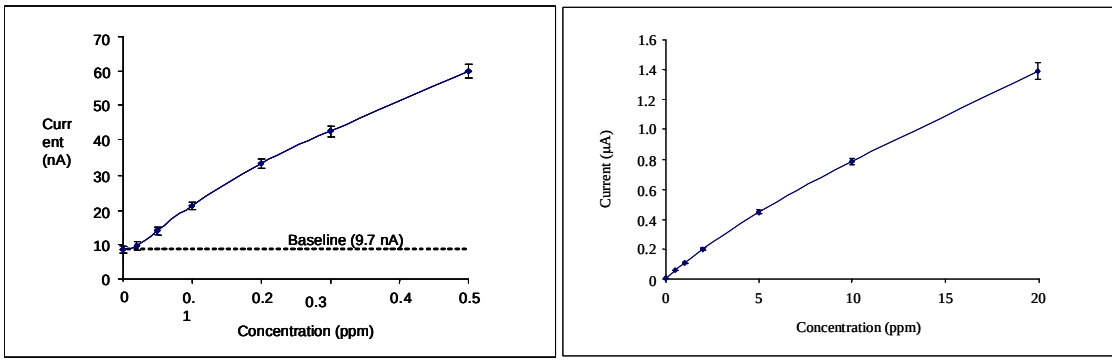
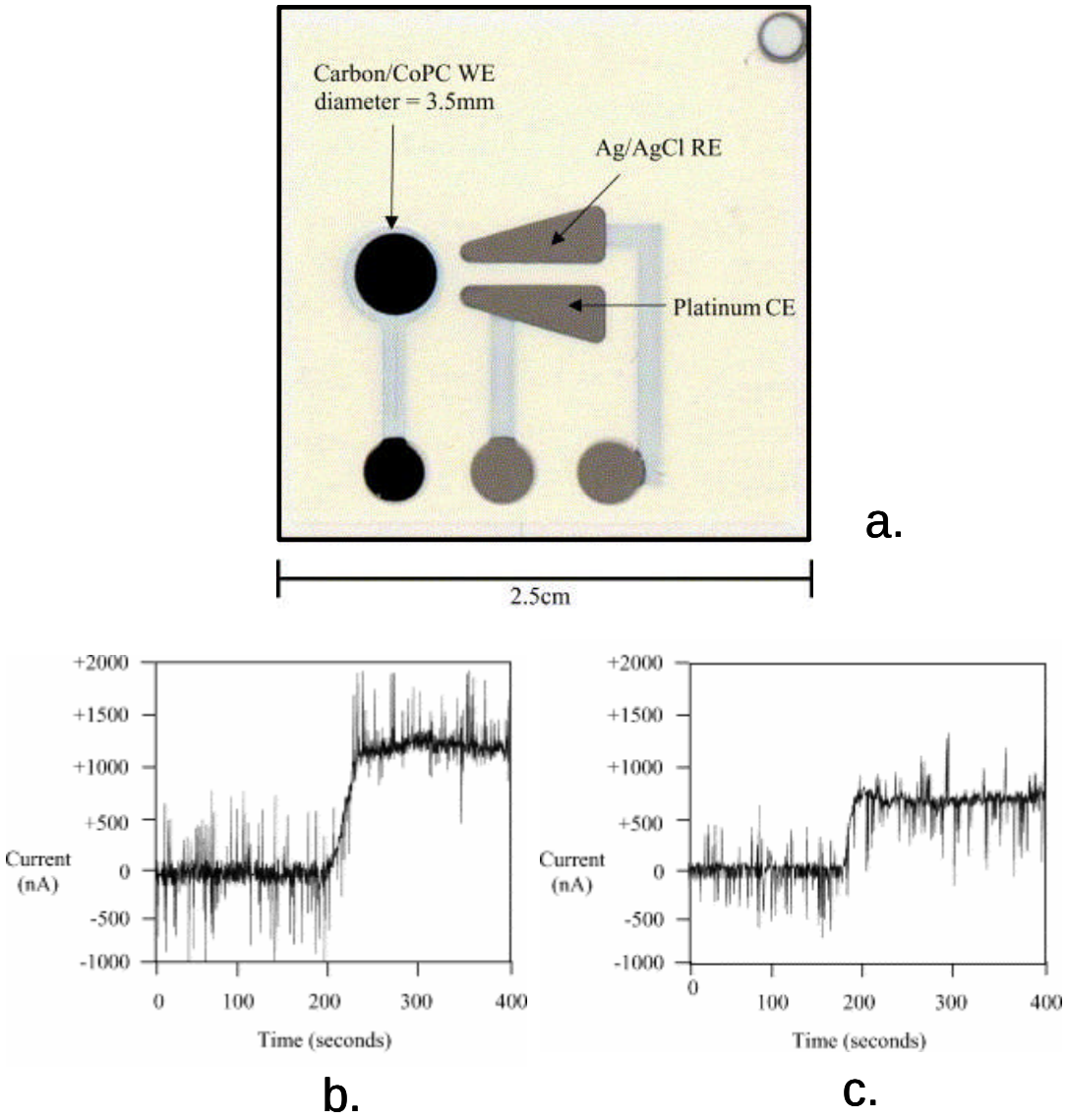

Curr
ent
(nA)
70
60
50
40
30
20
10
0
Baseline (9.7 nA)
0
0.
1
0.2
0.3
0.4
0.5
Concentration (ppm)
Current (μA)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
Concentration (ppm)

Figure 6.


Carbon/CoPC WE
diameter = 3.5mm
Ag/AgCl RE
Platinum CE
2.5cm
a.
Current (nA)
+2000
+1500
+1000
+500
0
-500
-1000
0
100
200
300
400
Time (seconds)
b.
Current (nA)
+2000
+1500
+1000
+500
0
-500
-1000
0
100
200
300
400
Time (seconds)
c.