# An integrated phenol ‘sensoremoval’ microfluidic nanostructured platform

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**ABSTRACT**: Phenol is a widely used chemical that for several reasons may be released into the environment and, consequently, its detection and subsequent destruction into the ground and surface waters are of special importance. Herein, a simple lab-on-a-chip (LOC) device based on biocompatible and biodegradable $CaCO_3$- poly(ethyleneimine) (PEI) nanostructured microparticles (MPs) to detect and remove phenolic wastes is proposed. The detection of phenol using a hybrid polydimethylsiloxane (PDMS)/glass chronoimpedimetric microchip and its removal in the same LOC system through the use of an extra $CaCO_3$-PEI MPs microcolumn is achieved. For the first time, the chronoimpedance technique is applied in a LOC system for phenol sensing in a range of 0.01 to 10 µM achieving the limit of detection limit of 4.64 nM. Moreover, this device shows a high repeatability with a relative standard deviation of 3 % which is almost 4 times lower than that for the chronoamperometry technique. This LOC system represents an integrated platform for phenol sensing and removal (sensoremoval) that can be easily fabricated and is of a low cost, disposable and amenable to mass production.

*Keywords*: $CaCO_3$-PEI microparticles, chrono-impedance technique, phenol sensoremoval.

## 1. INTRODUCTION

The detection and removing of phenol traces from industrial wastes (petrochemical, wood preservatives, textiles, plastics, dyes, paper, herbicides and pesticides) released into the ground and surface water is of special importance for maintaining the quality of water for human or agricultural use (Juang et al., 2006; Michalowicz et al., 2007; Materna et al.,2004). Many technologies based on physical and chemical processes for the removal / degradation of phenolic compounds in waste water have been investigated (Hannafi et al., 2008; Sanchez et al., 2007). These methods lead to secondary effluent problems due to formation of toxic compounds such as cyanates, chlorinated phenols, hydrocarbons, etc (Hannafi et al., 2008; Sanchez et al., 2007; Bandhyopadhyay et al., 2001; Popovtzer et al., 2005; Dimoglo et al., 2004 ). $CaCO_3$-poly(ethyleneimine) (PEI) nanostructured microparticles (MPs) with high functionalizing capacity for removing phenol compound without formation of toxic compounds has been reported (Lopez-Marzo et al., 2012). On the other hand, the use of nanomaterials (especially nanoparticles) in developing innovative sensing systems devices is getting an increasing attention for monitoring of phenolic compounds (Zhao et al. 2010), heavy metals (Aragay et al., 2011; Willner et al., 2010) and pesticides (Aragay et al., 2012). The tyrosinase (Tyr)-based biosensor is one of most important device for phenol quantification (Alegret, 1996). The integration of Tyr-based biosensors within lab-on-a-chip (LOC) technologies permits automation, integration and control of the reagents addition steps and consequently the production of reliable and low-cost microdevices at an industrial level.

The development of smart LOC systems able to achieve an in-situ and on-line sensing and destroying of pollutants (i.e. phenols compounds) can be considered an emerging area of research and development. We propose here for the first time a phenol sensing & removing

system integrated within a LOC device. This phenol sensing is *in-situ* achieved through a polydimethylsiloxane (PDMS)/glass fluidic microchip with an integrated SPE modified with $CaCO_3$-PEI MPs and Tyr as described before (Mayorga-Martinez et al., 2013). To achieve phenol removing in the same LOC system an extra $CaCO_3$-PEI MPs microcolumn is included. The contact/reaction time is an important parameter to consider for the chemical adsorption of phenol onto the $CaCO_3$-PEI MPs during the sample flowing within the LOC system. The chronoamperometry technique used in LOC system developed before (Mayorga-Martinez et al., 2013) for phenol sensing works with a fast flow rate (500 µL/min) which is not suitable to be implemented in a phenol senso/removal system. Given this drawback of the chronoamperometric system herein we develop for the first time a chronoimpedance technique in a LOC system that allows operation under lower flow rates suitable for both phenol detection and removal. This chronoimpedance technique was applied previously in batch system for glucose detection (Mayorga-Martinez et al, 2010; Mayorga-Martinez et al., 2011; Mayorga-Martinez et al., 2012). The range of phenol detection (0.01–10 µM) and the low LOD (4.64) in the current system are similar to those obtained with chronoamperometry technique used before (Mayorga-Martinez et al., 2013) but with the advantage of working at lower flow rate (50 µL/min) and higher reproducibility. This 'sensoremoval' microfluidic system is useful to give information on the quantity of the phenol present in a contaminated water sample and at the same time evaluate the efficiency of contaminant removal.

## 2. MATERIALS AND METHODS

All chemicals reagents were purchased from Sigma-Aldrich (Germany). The inks used for the fabrication of SPE were purchased from Electroday (Twintec S.L, Badalona-Spain) and used as received. For the preparation of stock solution of 0.1 M PBS pH 6.5 MilliQ water was used. The stock solution of 100 mM of phenol was prepared in 0.1 M PBS pH 6.5. 1 mg of tyrosinase enzyme from mushroom (≥1000 unit/mg) was dissolved in 50 μL of 0.1 M phosphate buffer at pH 6.5. 1 % glutaraldehyde solution was prepared daily in MilliQ water.

### 2.1 Microdevice fabrication

The LOC for phenol detection and removing was based on a PDMS/glass microchip with integrated electrochemical detector which consists of a set of three SPE. The fabrication of SPE is based on the sequential deposition of a graphite ink and Ag/AgCl ink onto a glass substrate. After the deposition of each layer a drying process is followed by keeping the glass substrate at 120 ºC for 45 min (graphite) and 30 min (Ag/AgCl). Then working electrode of SPE was successively modified with 5 μL $CaCO_3$-PEI MPs (1 mg/mL), 5 μL tyrosinase enzyme solution (0.2mg/ μL) and 5 μL of glutaraldehyde 1%. $CaCO_3$-PEI MPs and Tyr films were dried at room temperature for 20 min and 3 h respectively, while the glutaraldehyde layer was dried at 40 ºC for 30 min. The prepared SPE / $CaCO_3$-PEI /Tyr biosensor was kept at 4 ºC while not in use. The microchannel was fabricated in PDMS by soft lithography (Devaraju et al., 2012; Lalo et al., 2010). PDMS was poured onto an aluminum micromachined mold and cured at 65 ºC for 4 h. The channel was 5 mm wide by 100 μm depth and 5 cm long. Two reservoirs were punched at the inlet and the outlet of the channel. Finally, the PDMS and the glass substrate with SPE/ $CaCO_3$-PEI /Tyr biosensor integrated were aligned and irreversibly bonded using a 30 s oxygen plasma treatment. For phenol

absorption a silicon tube of 500 µm (in diametrer) was filled with cotton fibers, 5mg of $CaCO_3$ MPs and again with cotton to avoid leaking of the MPs. This tube is connected between pump and PDMS channel. For measurements in LOC microsystem, two syringe pumps (standard infusion-only pump 11 Elite, from Harvard apparatus) were used for injecting the phenol stock solution and buffer pH 6.5 solution respectively using the rate of 50 µL $min^{-1}$. The developed LOC given their low cost, are in principle previewed as disposable devices after being used in up to three sensing and removal operations within the same day.

For batch measurement SPE fabricated in polyester substrate (Mayorga-Martinez et al., 2013) was used. These electrodes were modified with $CaCO_3$, Tyr and gluraldehyde following the same protocol described before.

The synthesis of $CaCO_3$-PEI MPs was performed following the protocol was previously described (Lopez-Marzo et al., 2012). Equal volumes of the PEI (4 mg/mL) containing dissolutions of $CaCl_2$ (0.33 M) in water/ethanol (1:1, v/v) and $Na_2CO_3$ (0.33 M) in water were quickly mixed under sonication and during 45 min. at room temperature to obtain the nanostructured vaterite $CaCO_3$-PEI hybrid material. $CaCO_3$ precipitate was washed three times, air dried, collected and saved under room temperature conditions while not in use.

2.2 Electrochemical experiments.

In order to assess the performance of the biosensor at different phenol concentration, electrochemical impedance spectroscopy (EIS) studies were performed using an Autolab302 potentiostat/galvanostat/ frequency-response analyzer, controlled by GPES/FRA Version 4.9 sofware. The experiments were carried out at room temperature and in phosphate buffer solution at pH 6.5 with 0.1M KCl applying signal composed by -200 mV of DC potential and

100 mV of AC potential at 0.1 Hz to 100 kHz frequency range. Real time measurements of impedance changes (|Z| and Φ at different phenol concentrations) were carried out by applying a composed signal of -200 mV DC plus a 100 mV RMS AC at fixed frequency of 0.4 Hz, using potentiostatic method and time scan option using frecuency response analyzer (FRA) sofware. The data were presented in Bode plot as a funtion of time .

## 3. RESULTS AND DISCUSSION

Before implementing of this new biosensing transduction technique into the microfluidic system, its operation efficiency during phenol detection in a batch system was studied using EIS. The measurements were done by using a SPE fabricated in polyester substrate. Figure S1A shows the Nyquist plot obtained from the SPE modified with $CaCO_3$-PEI MPs and Tyr in a phosphate buffer solution (PBS) pH 6.5 with 0.1 M KCl in absence and the presence of different phenol concentrations. Changes of the electrode–electrolyte interface impedance (EEIZ), by increasing of the phenol concentration are evident at a composed signal of 100 mV AC plus -200 mV DC. In order to find the optimum working potential, measurements while adding phenol were carried out at different AC potentials (50, 100 and 150 mV). Reproducible and stable EEIZ changes caused by the increase of phenol concentration were evident at 100 mV AC potential (at 50 mV the response is smaller and at 150 mV it is not stable).

The variation of the impedance is due to the charge transfer which occurs during the electrochemical reaction of o-quinone product of phenol oxidation that is further electrochemically reduced to catechol at moderately negative potential (Mayorga-Martinez et al., 2013) at the interface between the electrode modified with $CaCO_3$-PEI/Tyr and the electrolyte.

The bioelectrocatalysis of phenol oxidation in this biosensor is observed in the low frequency range, where the Nyquist plots show the greatest separation for different concentrations. For this reason it is possible to perform the phenol detection via real time measurements of impedance at certain frequency. In order to verify this assertion, the real time determination of impedance module (|Z|) was carried out by using two frequencies (0.4 Hz and 10 Hz), in PBS after successive additions of 10 µM phenol. The high sensitive response obtained at 0.4 Hz is observed, while the response at 10 Hz is less appreciated (Figure S1B). The signal composed by -200 DC plus 100 AC at 0.4 Hz was used in the next experiments for the real time impedance measurements. Figure S2 shows the selectivity evaluation. To evaluate the selectivity of the biosensor, impedance responses toward aniline, benzaldehyde, benzylalcohol, $Mg^{+2}$ and $Cu^{+2}$ were measured in bath system. The selected species for interference studies seem to be the ones usually present with the phenolic compounds in contaminated samples (Mayorga et al., 2013). The developed biosensor combines the selectivity of the enzyme (Tyr) with the device operation at very low potential being the effects of the interfering species negligible.

In order to achieve a good performance of the biosensor in LOC system, different flow rates (50, 100 and 500 µL $min^{-1}$) were also tested. The highest changes of the impedance module and the phase by additions of different phenol amounts were found using the rate of 50 µL $min^{-1}$ (see Figure 1). At higher flow rates (100 and 500 µL $min^{-1}$), response time and the impedance peak decrease. The improved response achieved at low flow rate (50 µL $min^{-1}$) is probably related to the diffusion process as the response time of the biosensor is slow at the used frequency of 0.4 Hz. The plot of the impedance module and phase versus the phenol concentration in the range of 0.01–10 µM (insets of Figure 1) is shown. The obtained limit of

detection (LOD) of 4.64 nM (calculated considering the linear response vs. concentration dependence) is comparable with the LOD value obtained by chronoamperometric technique reported recently (Mayorga-Martinez et al., 2013). The results of the triplicate sets, indicated by error bars, reveal the reproducibility (inset Fig. 1) of the measurements with a relative standard deviation (RSD) of 3%.

Fig. 1

The chronoimpedance technique is a simplification of typical EIS method. In this case, a real time impedance measurement was performed using a single-frequency analysis while EIS requires multiple impedance measurement and elemental electrical circuit to calculate a single Rct value. It is well known that the EIS have the advantage of low error measurement and high signal to noise ratio. For these reasons chronoimpedance represents a robust electrochemical technique well fitted to such 'sensoremoval' applications.

Considering the high adsorption capability of the $CaCO_3$-PEI MPs toward organic compounds, a microcolumn with these MPs was integrated into the same LOC system before phenol sensing part (Figure 2 and Figure S3). Several parameters can affect the adsorption of phenol in a flow system. In this context the adsorption capacity of $CaCO_3$-PEI MPs for phenol solution at different concentrations were evaluated (data not shown). A quantity of 5 mg of $CaCO_3$-PEI MPs was found sufficient for the complete removing of phenol from a 20 µL of its solution of 2 µM. Another important parameter is the contact time of the phenol sample with the $CaCO_3$-PEI MPs. For an efficient removal of the phenol the LOC system requires the use of low flow rate. For this reason the chronoimpedance technique being able

to perform at a very low flow rate is much more convenient than the chronoamperometric one which usually operates at a 10 times higher flow rate.

Fig. 2

The phenol removal is based on its chemical adsorption onto the surface of $CaCO_3$-PEI MPs via hydrogen bond formation between acid hydrogen in the phenol molecule and PEI's nitrogen given the high affinity of $H^+$ proton of the OH groups in the phenol with nitrogen of the PEI amino (See inset in Figure 2). Additionally, this MPs were composed of highly regular and uniform nanoparticles (average diameter of around 50 nm) (see Figure S4) showing a large contact area that ensures a high efficiency of phenol removal.

In-LOC, the chronoimpedimetric measurements were also carried out to evaluate phenol adsorption by $CaCO_3$-PEI MPs (see Figure 3). Measurements were performed under different experimental conditions. The first (Figure 3A) and second (Figure 3B) LOC experiments were carried out without $CaCO_3$-PEI MPs and cotton matrix in the phenol adsorption part injecting 20 µL of PBS and phenol (2 µM), respectively. The third one (Figure 3C) corresponds to a tube containing only cotton, and the fourth setup (Figure 3D) uses a reactor (small tube) with $CaCO_3$-PEI MPs and cotton, both microsystems working with injections of 20 µL of phenol (2 µM). A decrease of |Z| of around 136 KΩ and 122 KΩ can be observed for the $2^d$ and $3^d$ system respectively (see Figures 3B and 3C) while, the $4^{th}$ microsystem (Figure 3D) does not show any response toward phenol injection. These experiments clearly show the capacity of $CaCO_3$-PEI MPs to adsorb phenol.

Fig. 3

Phenol has benzene rings, which present a strong inhibitive function for biological degradation and they are very difficult to degrade into small inorganic molecules by using

common methods (Shi et al., 2011). Different nanomaterials with highly efficiency for phenol degradation have been employed so far (i.e. phenol photodegradation using fly ash supported titanium dioxide and Fe-Grafene nanostructures for phenol treatment in wastewater based on their intrinsic peroxidase activity) (Shi et al., 2011; Peng et al, 2011). While these methods require long exposure times and complex procedures removing phenol by absorption on CaCO3-PEI, is a rapid, simple and highly efficient method. On the other hand, the rapid phenol detection after its *in-situ* removing allows evaluation of the efficiency of the whole process thereby simplifying it within a single integrated device.

## 4. CONCLUSIONS

In conclusion, we have successfully developed a new LOC microsystem that allows the *in-situ* and *on-line* phenol 'sensoremoval' (sensing and removal). The rapid phenol detection after its *in-situ* removing allows the evaluation of the efficiency of the whole process thereby simplifying it within a single integrated device. On the other hand, here we demonstrated that such interesting 'sensoremoval' capability of nanostructured $CaCO_3$-PEI MPs may open the way to several other applications with interest for environment, safety and security applications. In addition, to the best of our knowledge, for the first time the chronoimpedance technique is applied to phenol sensing in a LOC. The developed LOC microsystem exhibited good analytical performance to phenol detection in terms of reproducibility, sensitivity, selectivity and limit of detection. This 'sensoremoval' system is easy to be fabricated, inexpensive, disposable and enable to mass production showing good promises for further applications in automatic control systems with interest for the environment, food, safety and security. Its application to sensoremoval of phenol and other pollutants such as pesticides, heavy metals and other toxic species might be with interest in case of accidental contamination scenarios where a fast detection/control should be always accompanied by the application of an efficient remediation strategy whose *in-situ* / in-field evaluation and decision-taken should be urgent.

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REFERENCES

Aragay, G.; Pino F.; Merkoçi, A. 2012. Chem. Rev. 112, 5317- 5338.

Aragay, G.; Pons, J.; Merkoçi, A. 2011.Chem. Rev. 111, 3433-3458.

Bandhyopadhyay, K.; Dasb, D.; Bhattacharyya P.; Maiti, B.R. 2001. Biochem. Eng. J. 8, 179-186.

Devaraju, N. S. G. K.; Unger, M. A. 2012. Lab. Chip. 12, 4809-4815.

Dimoglo, A.; Akbulut, H. Y.; Cihan F.; Karpuzcu, M. 2004. Clean Techn. Environ. Policy 6, 288-295.

Hannafi, N. E.; Boumakhla, M.A.; Berrama, T.; Bendjama, Z. 2008. Desalination. 223, 264-268.

Juang, R.-S.; Tsai, S.-Y. 2006. Water research. 40, 3517-3526.

Lalo, H.; Ayela, C.; Dague, E.; Vieu, C.; Haupt, K. 2010. Lab. Chip. 10, 1316-1318.

Lopez-Marzo, A.; Pons, J.; Merkoçi, A. A. 2012. J. Mater. Chem. 22, 15326-15335.

Materna, K.; Goralska, E.; Sobczynska, A.; Szymanowski, J. 2004. Green Chem. 6, 176-182.

Mayorga Martinez, C. C.; Treo, E. F.; Madrid, R. E.; Felice, C. J. 2010. Biosens. Bioelectron. 26, 1239-1244.

Mayorga Martinez, C. C.; Treo, E. F.; Madrid, R. E.; Felice, C.J. 2011. Biosens. Bioelectron. 29, 200-203.

Mayorga-Martinez, C. C.; Cadevall, M.; Guix, M.; Ros J.; Merkoçi, A. 2013. Biosens. Bioelectron. 40, 57-62.

Mayorga-Martinez, C. C.; Hlavata, L.; Miserere, S.; López-Marzo, A.; Labuda, J.; Pons, J.; Merkoçi, A. 2013. Electrophoresis 34, 2011-2016.

Mayorga-Martinez, C.C.; Guix, M.; Madrid, R.E.; Merkoçi, A. 2012. Chem. Commun. 48, 1686-1688.

Michałowicz, J.; Duda, W. 2007. Polish J. of Environ. Stud., 16, 347-362.

Peng, C.; Jiang, B.; Liu, Q.; Guo, Z.; Xu, Z.; Huang, Q.; Xu, H.; Tai, R.; Fan, C. 2011. Energy Environ. Sci. 4, 2035-2040.

Popovtzer, R.; Neufeld, T.; Biran, D.; Ron, E. Z.; Rishpon, J.; Shacham-Diamand Y., 2005 Nano Lett. 5, 1023-1027.

Alegret, S. 1996. Analyst 121, 751- 758.

Sanchez, I.; Stüber, F.; Font, J.; Fortuny, A.; Fabregat, A.; Bengoa, C. 2007. Chemosphere. 68, 338-344.

Shi, Z.; Yao, S.; Sui, C. 2011. Catal. Sci. Technol., 1, 817-822.

Willner, I.; Willner, B. 2010. Nano Lett. 10, 3805-3815.

Zhao, Z.; Lei, W.; Zhang, W.; Wang, B.; Jiang, H. 2010. Sensor, 10, 1216-1231

Figure Captions

**Figure 1** Chronoimpedimetric detection of phenol at different concentration levels using LOC microsystem. Insets: biosensor calibration curve given as impedance module and phase versus phenol concentration introduced in the LOC microsystem.

**Figure 2** Schematic diagram of the integrated dual microfluidic system for phenol removing and sensing (A). The phenol adsorption (B) and detection (C) principles are described in the insets.

**Figure 3** Chronoimpedance response of the LOC microsystem with different injections and reactors. Injection of 20 µL buffer and phenol (2 µM) is used.