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Electrochemical/Enzymatic Amplification Schemes for the Sensitive Detection of Pathogens in Electrochemically Activated Capillaries

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Early detection of pathogens in clinical and food analysis applications is a critical tool for application of HACCP plans in the food industry and in combating antibiotic resistance development in hospital settings. The low limits of detection required (typically 1 colony forming unit (CFU) per 50 or 100 g of sample). For fast detection methods to be widely used, they have to achieve selectivity and remarkably low detection limits. Typical approaches use selective media to achieve selectivity (after a lengthy pre incubation) and molecular methods (PCR amplification) to achieve the detection limits.
We propose an alternative method based on a combination of electrochemical, immunoaffinity, and electro-enzymatic amplification "unit operations" to achieve the levels of selectivity and sensitivity required.

This method ascertains selectivity at two levels: firstly magnetic nanoparticles modified with antibodies or phages are used to extract from the sample the specific pathogens. With a combination of magnetic field application and electrophoretic deposition of the particles on electrodes, the pathogens are concentrated close to the detection surface. However, such affinity separation is not very efficient, especially in the presence of high concentrations of competing microorganisms. We use therefore the detection of metabolic products of the cells as a means of increasing both the selectivity of the method and achieving very low detection limits through electrochemical and enzymatic amplification techniques.

In general we use the enzyme alkaline phosphatase as a generic indicator of presence of cells and cell viability since most microorganisms express the enzyme in large quantities. A bienzymatic cascade (the alkaline phosphatase (AP) released from the cells and PQQ-glucose dehydrogenase (PQQGDH) immobilized on the electrode surface) permits an 1000-fold amplification of signal when the AP-produced p-aminophenol is used as a mediator for the immobilized PQQGDH in the presence of glucose. We present an electrochemical study of the amplification scheme and determine the most useful kinetic and mass transport parameters that allow to optimize the system. We also quantify the amount of AP released from the cells with different methods including electroporation. In general, up to 10^5-fold amplification can be achieved with judicious tuning of hydrodynamics, electrode materials, and immobilized biocatalyst.

Although the use of AP allows the sensitive detection of viable cells, it does not increase the specificity and selectivity of the detection scheme. To achieve this, we use pathogen-specific metabolic pathways and try to selectively amplify the electrochemical signal that might be produced from metabolites or enzymes specific for certain species. In the case of specific enzymes we use synthetic substrates that under the action of the enzymes produce electrochemical indicators that can be recycled with an electroenzymatic method. Such substrates have the general dimeric structure I-E. The enzymatic action of the cellular enzyme usually hydrolyses the substrate to two products: I that is specific for the action of the enzyme and E, a moiety that when released can be used by PQQGDH in a redox enzymatic cascade with the help of an electrode. We present the synthesis and characterization of such substrates and as before, an electrochemical study of the amplification scheme and determine the most useful kinetic and mass transport parameters that allow to optimize the system.

We show that when these basic principles are incorporated in easy to fabricate screen printed electrochemical capillaries, low cost (that can reach the market at less than $5 per analysis) devices can be realized. A positive result can be obtained in less than two hours while negative results would still need verification until the detection limit of the device reaches the 1 CFU per mL level.

We will present that currently 100 CFU/mL limits of detection can be reached in complex samples for *Salmonella* and *Pseudomonas* species.

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References:

Washe, A.P., Lozano, P., Bejarano, D., Katakis, I., ChemPhysChem, in press (2013) http://dx.doi.org/10.1002/cphc.201300042
Mata, D., Bejarano, D., Botero, M.L., Lozano, P., Constanti, M., Katakis, I., Electroch. Acta, 55(14):4261.6 (2010)