About food and biosensors

New electrochemical platforms for genosensing, immunosensing and enzymatic sensing

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INTRODUCTION

Accelerated detection procedures would allow the food industry to reduce warehousing costs and permit increased testing of both food ingredients and final products. Food chemists, regulatory agencies and quality control laboratories thus require faster, more powerful, cleaner and cheaper analytical procedures.

The biologically modified electrodes, also known as biosensors, can meet these demands. Biosensing, thus, represent one of the most promising research areas within the field of food analysis. Biosensors are devices combining a biological recognition element (the bioreceptor) with a transducer. The former provides selectivity while the latter provides sensitivity converting the biological recognition event into a measurable electronic signal. However, among different kind of biosensors (electrochemical, optical, piezoelectric) those based on electrochemical transduction show advantages such as fast response, low cost, high sensitivity and robustness.

Three aspects need to be considered prior to the design of an electrochemical biosensor: i) selection of a biological molecular recognition element (the bioreceptor), ii) selection of an electrochemical transducing element (the transducer), and finally iii) the integration of both elements.

Traditionally, the biological recognition element has been immobilised onto the transducer through surface-modification procedures, either directly (adsorption, cross-linking, covalent bonding, etc) or through a gel or membrane. Taking into account the construction process, these immobilisation procedures require meticulous control of the variables affecting the immobilisation such as temperature, pH, ionic strength, reagent purity, concentration, time, etc. As such, these surface modification procedures can not be easily transferred on a large-scale production. Additionally, as most biological reactions can be considered irreversible, the surface-modified biosensors require a new modification of the surface or the replacement of the whole device after used. Except adsorption, these immobilisation procedures have proven to be tedious, expensive and time-consuming.

The development of new transducing materials, whose preparation is simple and suitable for mass fabrication, with a higher sensitivity and lower detection limits is a key issue

in the research of electrochemical biosensing, specially in DNA and immunological analysis for food industry applications.

Recent developments in the field of conducting composites have opened a new range of possibilities for the construction of electrochemical biosensors.

A composite results from the combination of two or more different materials. Each individual component maintains its original characteristics while giving the composite distinctive chemical, mechanical and physical qualities [1].

Rigid conducting graphite-polymer composites, especially graphite-epoxy composites (GEC) have been extensively used in our laboratories [1]. These materials are made by combining the non-conducting epoxy polymer with graphite powder that acts as a conducting phase. Epoxy resins are a family of polymers widely used for their excellent chemical properties, their good adhesion to other materials and their excellent insulating characteristics. Furthermore, they are easily prepared, inexpensive and widely available. GEC materials are highly mouldable before curing, permitting ease of construction of sensors of various shapes and sizes including flow cells. After curing, GEC is very stable from a mechanical point of view, and sustains moderate temperatures and washing steps. As GEC is made of small conductive particles dispersed in a polymer matrix, this material acts as a microelectrode array showing a higher signal-to-noise ratio and lower detection limits compared with their pure conductor counterparts.

When a biological component is added into GEC, a graphite-epoxy-biocomposite (GEB) is generated. In this case, the bioreceptor is bulk-modified into the electrochemical transducer, stating a clear example of integrated analytical system [1].

Biocomposite approaches present clear advantages over biological surface-modified devices. The conducting biocomposites act not only as transducer, but also as reservoir for the biomaterial. Biocomposite surfaces can be smoothed to provide fresh active biomaterial ready to be used in a new assay. Each new surface yields reproducible results because all individual compounds are homogeneously dispersed in the bulk of the biocomposite. The preparation of these devices involves `dry chemistry´, stating a clear advantage over traditional surface-modified biosensors. Their ease of preparation does not require specialised personnel, increasing the commercial potential of biocomposite materials. As such, their preparation can be easily transferred to large-scale production.

Various approaches for electrochemical genosensing and immunosensing were designed in our laboratories, in which the common element is the use of rigid graphite-epoxy composite and biocomposite (GEC and GEB) as transducers.

Firstly, we developed surface DNA-modified GEC transducer for electrochemical genosensing [2]. GEC have an uneven surface allowing DNA, oligonucleotides and free DNA bases to be immobilised using a simple and fast adsorption procedure. DNA target can thus be detected using both (i) a label-free electrochemical procedure based on its intrinsic guanine oxidation peak by DPV (differential pulse voltammetry) [2,3], as well as (ii) an enzyme labelling procedure based on HRP-streptavidin conjugate by amperometry [2,4-6]

Finally, we developed bulk-modified GEC transducer –i.e. graphite-epoxy biocomposite–. For this purpose, we have selected two-affinity bioreceptors for immunosensing and genosensing. The first bioreceptor selected was strep(avidin). It is known that avidin, but also its non-glycosylated analogous streptavidin, forms a complex with biotin at room temperature with an association constant in the range of 10^{15} M^{-1}. This tetrameric molecule can easily react with biotin modified DNAs and antibodies with high specificity. The second bioreceptor selected was Protein A, produced by *Staphylococcus aureus*. Protein A is a highly stable receptor able to bind the Fc portion of immunoglobulins, especially IgG from a large number of species.

Strept(avidin) and Protein A were thus used for constructing graphite-epoxy biocomposite [strept(avidin)-GEB and ProtA-GEB], serving as affinity matrix during immobilisation. These two molecules have universal affinity binding characteristics, generating transducer extremely flexible in their applications. These developed transducers can be thus considered as "universal" affinity platforms for electrochemical genosensing and immunosensing.

The first approach relies on strept(avidin)-graphite-epoxy biocomposite (strept(avidin)-GEB transducer), as an universal platform whereon biotinylated DNAs, antibodies, or enzymes can be captured by means of streptavidin-biotin reaction 2,77. Figure 1 shows the utility of the strept(avidin)-GEB transducer in electrochemical genosensing. The strong interaction between biotin–strept(avidin) allows immobilisation of a biotin labelled capture probe, containing the DNA sequence complementary to the target. Hybridisation of the target DNA to the capture probe and a digoxigenin signaling DNA probe is achieved simultaneously 7. Enzyme labelling is achieved with an anti-digoxigenin enzyme-labelled antibody.

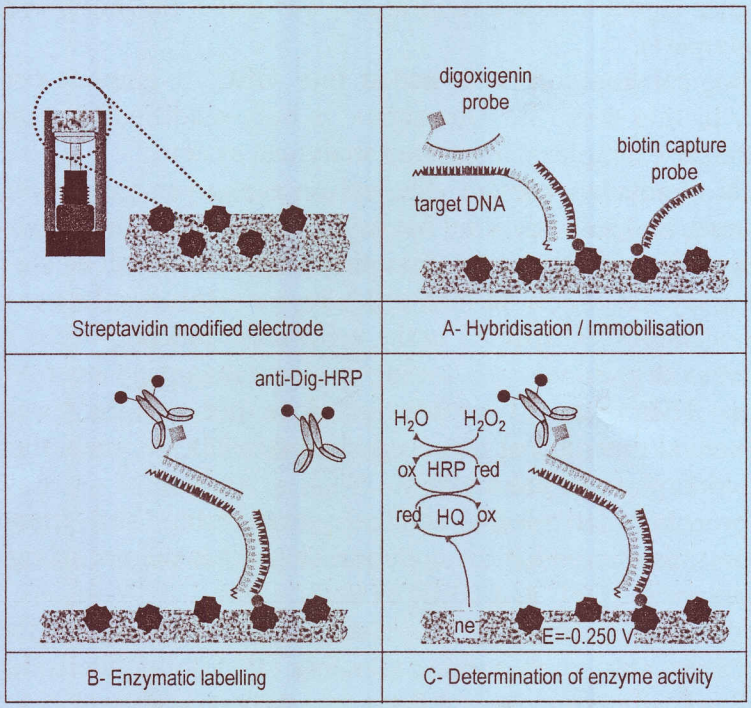

Figure 1. Schematic representation of the DNA analysis based on an electrochemical strept(avidin)-GEB transducer. (A) Streptavidin modified composite electrode (strept(avidin)-GEB transducer). (B) One step immobilisation/hybridisation procedure: Target DNA is hybridised with the biotinylated capture probe and with the digoxigenin modified probe. The complex biotin-dsDNA-Dig is immobilised on the electrode surface by linking the biotinylated capture probe with the streptavidin present in the biocomposite. (C) Enzyme labelling based on the immunological reaction between the immobilised dsDNA-Dig with anti-Dig-HRP. (D) Electrochemical detection of the enzyme (HRP) labelled dsDNA.

The second approach is based on Protein A- graphite-epoxy biocomposite (ProtA-GEB transducer) [8]. Protein A is able to bind the Fc region of antibodies serving as generic affinity matrix for immuno-immobilization onto the transducer (Figure 2).

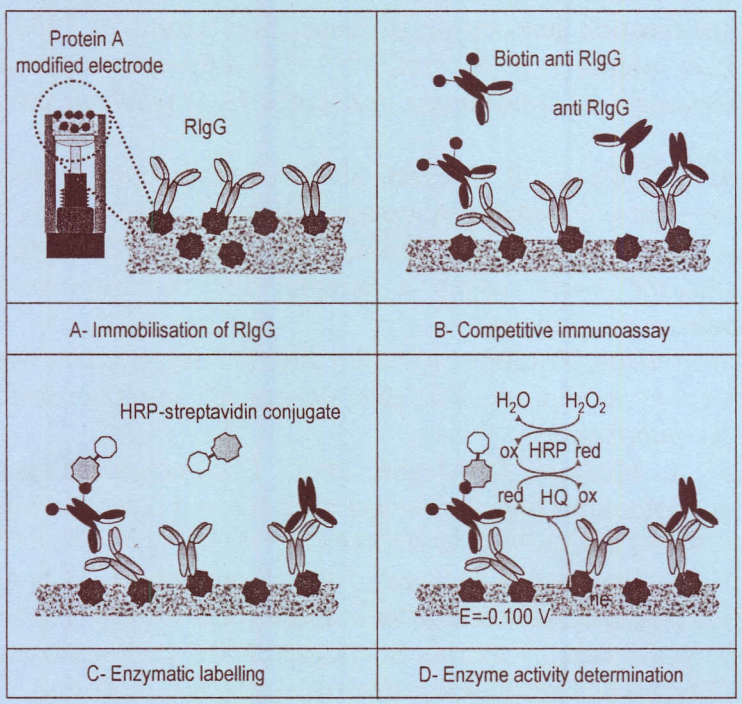

Figure 2. Schematic representation of the immunoassay based on ProtA-GEB transducer. (A) RIgG immobilisation on the surface of the electrode based on its interaction with Protein A. (B) Competitive immunoassay, using anti RIgG and biotinylated anti RIgG. (C) Enzyme labelling using HRP-streptavidin. (D) Electrochemical enzyme activity determination.

These "universal" affinity platforms offer many potentials advantages compared with classical assays or surface-modified biosensors [9]. These materials can be easily modified through `dry chemistry` using procedures that can be transferred to mass fabrication. As bulk-biological modified materials, the conducting biocomposites act not only as transducer, but also as reservoir for the biomaterial. After its use, the electrode surface can be renewed by a simple polishing procedure, stating a clear advantage of these approaches.

From an electrochemical point of view, GEC and GEB materials show higher signal-to-noise ratio compared to the corresponding pure conductors, thus improving sensitivity.

The utility of GEC and GEB transducers was demonstrated for the specific detection of *S. aureus* [5,6,10] and *Salmonella* spp [2,3,10,11].

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