REVIEW ON THE APPLICATION OF NANOBIOSENSORS IN FOOD ANALYSIS

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ABSTRACT

Nano-biosensors could be defined as biosensors, which are combined with nanotechnology by using several techniques. This strategy could be seen as a key to yielding device which exhibits rapid responses combined with very high sensitivities. In recent years as consumer demand traceability and legislators and account-ability in the food chain distribution has increased, the need for rapid and verifiable methods of food quality assurance has grown rapidly. Sensing technologies for food analysis including optical, chromatographic, colorimetric, etc. are employed. Biosensors allow the detection of analyte's wide spectrum in complex sample matrices, and have shown great promise in areas such as food analysis, environmental monitoring and bioprocess. Biosensors can be divided into six groups which depend on the method of signal transduction: magnetic, optical, electrochemical, mass, thermal and micromechanical sensors. The aim of this paper is to present the directions of the development of nano-biosensors and their useability to detect a range of biological and chemical compounds in the food industry market.

Key words: nano-biosensors, biosensors, food, components, analysis

INTRODUCTION

In the food sector, the nanotechnology usage derived food ingredients, supplements, additives and contact materials are expected to grow rapidly. According to the conducted research, companies are interested in the nanotechnology usage in engineering, agriculture, processing, delivery or packaging of nutritional supplements and food. Food safety would also potentially benefit from the introduction of nano-based detectors, sensors and labelling. In some countries, nanomaterials are already used in food packaging and supplements, with nano-silver as antimicrobial agent and nanoclays as diffusion barriers [Tiede et al. 2008]. Sensors were generally prepared by drop casting the formulation directly onto a polyethylene terephthalate (PET) substrate. Alternatively, the sensors were screen printed onto the PET substrate using a model 247 screen printer (DEK International) [Crowley et al. 2005].

From high cost techniques, high performance such as GC-MS (Gas chromatography-mass spectrometry), through electronic noses and solid-state detectors, there are lots of methods for gas-phase analysis to the simplest sensors and low cost, such as those consisting of chemically reactive dyes immobilised in polymer or sol-gel matrices [Crowley et al. 2005]. The analytical methods used for quantification and separation of biogenic amines are usually based on reversed phase high performance liquid chromatography (HPLC) or gas chromatography with post-column, pre-column or on-column derivatization techniques, coupled

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to fluorescence or UV-DAD detectors [Carelli et al. 2007]. Alternative approaches are based on HPLC tandem ion-exchange chromatography with pulsed conductivity or amperometry detection and mass spectrometry. Moreover, these methods require expensive equipment and extensive sample pretreatments [Carelli et al. 2007].

The food quality is essentially based on biochemical composition of food. Biosensors have been designed for the measurement of various components in the food samples. Optical, electrochemical, calorimetric, immunosensors to screen-printed three electrode systems are various types of biosensors. Monitoring of the fruit quality is one of the main concerns in the food industry. Particularly, there is a progressive need to develop analytical tools which could provide monitoring of the quality for the entire food processing operation, including starting materials and final products [Rana et al. 2010].

Biosensors are highly selective analytical tools, for the high selectivity of the biological recognition elements used, which have been applied in a sequence of disciplines which include industry, medicine, food technology, environmental analysis, and military. Recently the fruit production economics permits a produce in one country and then shipping it to different countries in the world [Rana et al. 2010]. Fruit quality should be carefully monitored through all the stages of production, transport and storage. Particularly the foodstuff selection by a consumer is largely based on sensory taste perception, which is influenced by various factors including sweetness, saltiness, acidity and bitterness as perhaps the most important factor. Texture is also an important parameter and is influenced by many factors including fat and moisture content, protein and carbohydrate levels. The shape, colour and aroma of the foodstuff are the other important sensory factors. Hence; portable, rapid and accurate methods are required for the assessment of physiological state and fruit quality. In consequence of their various attributes, biosensors potentially submit a fast, stable, relatively cheap, accurate, user-friendly and portable tool for in situ monitoring of fruit quality and maturity [Rana et al. 2010].

BIOSENSOR

Sensor systems are analytical tools combining a chemical, biochemical or biological recognition component with a transducer. The recognition component is capable of selectively interacting with an analyte directly or indirectly, emitting a signal through the transducer [Mansouri et al. 2008]. A biosensor could be defined as a compact analytical tool incorporating a biological or biologically derived sensing element either integrated or associated in a physicochemical transducer (Fig. 1). The usual aim of such a tool is
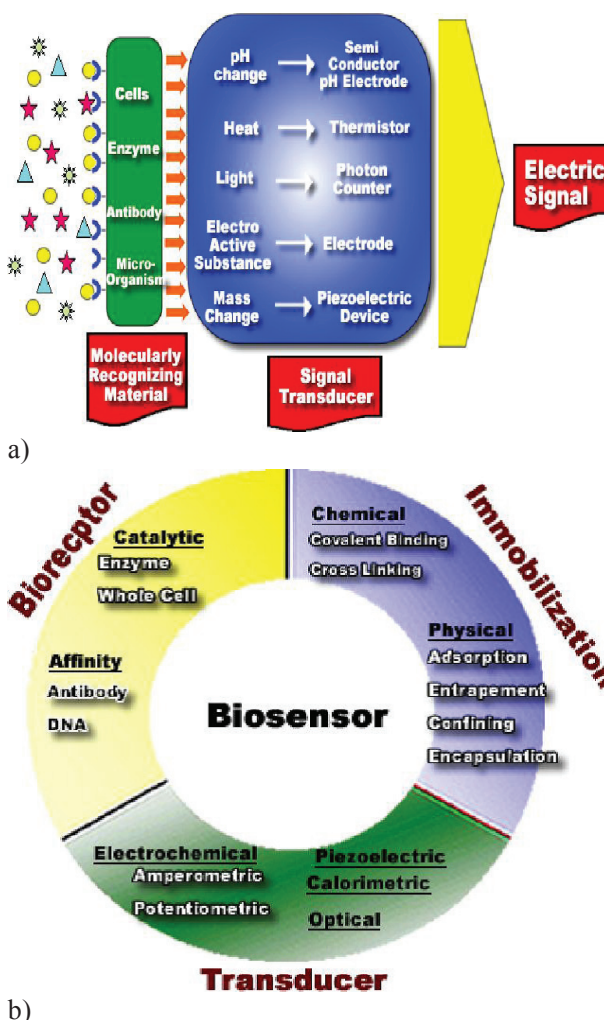

a)

b)

Fig. 1. a) Scheme of a typical biosensor, b) Biosensor Components [Rana et al. 2010]

to produce either a continuous or discrete digital electronic signal that is proportional to a related group of analytes or a single analyte present in a sample [Rana et al. 2010].

Principle of biosensor

In food quality parameters, analytical chemistry plays an important role, because nearly every sector of public service and industry resis on quality control. A food quality biosensor is a tool, which could transform the response into a detectable signal, often an electric signal and respond to properties of food. This signal might have a known relation to the quality factor or might provide direct information about the quality factors to be measured. There are numerous types of biosensors which could be classified as; Enzyme-based biosensors, Electrode based biosensors, Whole-cell-based biosensors, Tissue/whole organism-based biosensors (Antibody and receptor-based biosensors), Amperometric Biosensors, Immunosensors, Acoustic Biosensors, Electrochemical Biosensors, Potentiometric Biosensors, Optical Biosensors and Calorimetric Biosensors.

Enzyme-based biosensors. In food analysis, the freshness-test based on biogenic amines is prevalent. There are several articles published based on multi-enzyme or single systems. This type of test is generally applied to analysis of sauerkraut, fish etc. [Baeumner et al. 2003]. For the simultaneous determination of the three biogenic amines (putrescine, histamine and tyramine), an enzyme sensor array by model recognition using an artificial neural network and its application to different food samples is defined. A monoamine oxidase, a diamine oxidase (with specific activities adequate for rapid detection) and a tyramine oxidase combination are immobilised each on a separate screen printed thick film electrode on glutaraldehyde and transglutaminase to compare these cross linking reagents with regard to their suitability. To calculate a specific biogenic amine amount, the raw data from multichannel software were transferred to a neural network. The sensor array takes 20 min except statistical data analysis with only subsequent neutralisation step and one extraction required prior to sensor measurement. The enzyme sensor's lower detection limits were 5 mg/kg for putrescine and 10 mg/kg for histamine and tyramine with a linear range up to

100 mg/kg for putrescine and 200 mg/kg for histamine and tyramine. The enzyme sensor array's application area was tested from meat to fish products, beer, sauerkraut, dairy products, wine and further fermented foods and then compared with the conventional LC (Liquid Chromatography) analyses data (mean correlation coefficient: 0.854) [Lange and Wittman 2002].

Another study on detection of the freshness; based on L-lactate detection in tomato paste and infant food or by using a continuous measurement format of enzyme flow reactors [Baeumner et al. 2003]. A graphite-Teflon-tyrosinase composite biosensor was developed for the benzoic acid quantification in soda drinks and mayonnaise [Baeumner et al. 2003]. Glycoalkaloids were detected using a butyrylcholinesterase as the biorecognition element and a field-effect transistor as the transducer [Baeumner et al. 2003]. Phytotoxins, especially those produced by algae and found in seafood were of interest [Baeumner et al. 2003].

Electrode based biosensors. A research on the activity on electrode based biosensors (especially screen-printed) is discussed with respect to the different procedures for immobilization of the biorecognition component and basic configurations of biosensors based on screen-printing technology. A large variety of poisoning by terrestrial based food and by seafood is caused by low-molecular-weight marine toxins' ingestion. This kind of toxins includes domoic acid, okadaic acid, tetrotoxin and brevetoxin. These toxins' investigation has been performed by immunological analysis in an enterprise to develop generic disposable immunosensors, based on a screen-printed transducer, which could efficiently detect with high accuracy, traces of marine toxins, such as detection limits in the ppb range in food samples [Tudorache and Bala 2007].

Mycotoxins, such as aflatoxin B_1, have been currently used as targets for analysis performed with screen-printed immunosensor devices. Such immunosensors have been developed in an array configuration, suitable for use in conjunction with 96-well micro titer plates, and in the disposable screen-printed biosensor's classical configuration, respectively. Both configurations allow the determination of aflatoxin M_1 and aflatoxin B_1 at ppb concentration range. L-lactate, acetaldehyde and D-lactate are also analytes of interest intensively monitored by use of screen-printing tools in foodstuffs. A researcher group used screen-printed

biosensors with many configurations for lactates and acetaldehydes' quantitative determination in wine. An example is the monoenzymatic amperometric biosensor based on a screen-printed carbon electrode combination with aldehyde dehydrogenase immobilized by a sol-gel entrapment method. Highly sensitive such as, 10-260 µmol·L^{-1} dynamic range and free from NADH (reduced form of (NAD) Nicotinamide adenine dinucleotide) interference, the method was validated by commercially available enzyme kit usage and finally used for analysing French wine. Many kinds of these biosensors were developed in collaboration, and were executed for food and environmental analysis. L-lactate biosensor has also been reported recently. Lactate dehydrogenase (LDH) immobilisation on electrochemically polymerized Meldola blue films has been accomplished by monomer solution's electrode position containing the enzyme. This kind of biosensor was characterised by two-weeks storage stability, a response time of 100 s and a detection limit of 0.1 mmol·L^{-1}. The sensor allowed rapid, sensitive and easy analysis of L-lactate [Tudorache and Bala 2007].

To monitor the quality and fermentation processes of foodstuffs, ethanol biosensors have been constructed. In beverages (beer, wine and spirits) and food, ethanol determination is also of general importance. Screen-printed electrodes, which contain immobilised enzyme alcohol oxidase have been reported. A disposable ethanol sensor based on a cobalt-modified screen-printed carbon electrode has been developed and it is successfully used for beer analysis [Tudorache and Bala 2007]. Low cost, screen-printed electrode involved a three-electrode system (Ag/AgCl pseudo-reference electrode, carbon counter electrode and platinum working electrode) and a polyester substrate with the enzyme immobilised in a poly(carbamoyl) sulfonate hydrogel by use of PEGDGE (polyethylene-glycol diglycidylether) has also been fabricated. An ethanol biosensor's optimum configurations have also been reported. Combination of a cross-linker, a redox polymer and an enzyme on a conventional graphite screen-printed surface resulted in an electrode allowing ethanol flow-injection analysis at concentrations as low as 1 µmol·L^{-1}. The biosensor has been used for ethanol on-line monitoring during wine fermentation. A screen-printed electrode-based biosensor has also

been used to screen wines for contaminants such as, biogenic amines, 2,4,6-trichloroanisole, mycotoxins and biosensors have also been used to screen food for insecticides such as, organophosphorus and carbamate pesticides in milk and other foods, herbicides such as, acetochlor in milk and drinking water, and other target analytes such as, vitamin B$_1$, dichlorophenoxyacetic acid and glycerol [Tudorache and Bala 2007].

Whole-cell-based biosensors. Genetically engineered yeast or bacteria cells to bear the luc or lux gene operon, expressing luminescence proteins as the green fluorescence protein (GFP), and similar techniques have been investigated for years. Review of articles giving an overview of this technology in recent years can be found, which focus on hydrocarbon stress, pollutants or include descriptions of other signal reporter systems as alkaline phosphatase, insect luciferase, bacterial luciferase, β-galactosidase, green fluorescent protein etc. Depending on the cell type chosen and the genetic engineering approach, biosensors specific for one analyte could be constructed, or those that exhibit general stress response as genotoxicity and toxicity sensors. Some examples are given concerning biosensors. Those sensors based on either the fusion of the lux operon with fatty acid synthesis, with respect to toxicity sensors, those based on strains sensitive to a variety of stresses as membrane, DNA or oxidative damage have been reported [Baeumner et al. 2003].

Some scientists have used a biosensing system for single chemicals or mixtures detection and suggest that it could find application in the classification of toxic chemicals in wastewater [Baeumner et al. 2003]. Some scientists described an on-line microbial sensor for the control of water quality. Plant and microbial cell biosensors based on respiratory detection are suggested especially for wastewater analysis. In recent publications, scientists have suggested the use of *Pseudomonas* sp. isolated from corroded metal surfaces for the detection of microbiologically influenced metal corrosion and developed an amperometric biosensor. Some scientists developed a toxicity sensor for estuarine water monitoring based on *Cyanobacteria* and an amperometric transducer [Baeumner et al. 2003]. To food analysis, *Saccharomyces cerevisiae* immobilised on an oxygen electrode was used for the cyanide detection in fruit brandies [Baeumner et al. 2003].

Recently, surface display techniques have found application in whole-cell biosensors. Novel surface proteins are introduced into the outer bacteria membrane generating harmless bacteria with catalytic functionalities or new binding on their cell surface. These are used as a biosorbent material or biorecognition element in bioremediation processes and are envisioned to be excellent biorecognition elements for biosensors in various application areas including food and environmental analysis [Baeumner et al. 2003]. Excellent review articles on the technology and earlier publications are available. Examples include the use of metal-binding polyhistidyl peptides or inserting the fungal cellulose-binding domain into a Staphylococcus for the binding of divalent metal ions as Cd^{2+} and Ni^{2+} [Baeumner et al. 2003].

Tissue/whole organism-based biosensors. *Antibody and receptor-based biosensors.* As a model analyte, 2,4-Dinitrophenol (2,4-DNP) was detected for dioxin with a detection limit of 0.01 ngmL^{-1} by using a quartz crystal microbalance as transducer. Some scientists investigated the organic solvents effect on a similar system. A potentiometric biosensor for simazine was developed and applied to the analysis of foodstuff as milk, cucumbers, tomatoes and meat extracts. A different format was developed as a flow-through immunosensor for isoproturon which was applied to agricultural foodstuff analysis and well water [Baeumner et al. 2003].

Amperometric biosensors. Amperometric biosensors usage in signal transduction has proved to be the most widely reported using an electrochemical approach. Both on-line (multi measurement) and "one-shot" (disposable) sensors tools are commercially available, monitoring a wide range of target analytes [Rana et al. 2010].

In contrast with potentiometric devices, the amperometric biosensors principle operation could be defined by a constant potential applied between a reference and a working electrode. Application of potential results in redox reactions, causing a net current to flow. This current magnitude is proportional to the electro active species concentration present in test solution and both anodic (oxidizing) and cathodic (reducing) reactions could be monitored amperometrically. Most of these biosensors defined enzymes usage as the biorecognition element. Dehydrogenase

and oxidase enzymes generally have been the most frequently exploited catalysts used for this biosensor formats [Rana et al. 2010]. According to a research the development and application of an amperometric biosensor is reported for the determination of total biogenic amines content by using the commercial diamino oxidase (DAO from Porcine kidney E.C. 1.4.3.6) as the biocomponent, entrapped by glutaraldehyde onto an electrosynthesized bilayer film. Despite of a very low activity of the commercial diamino oxidase, the biosensor displayed high response sensitivity in low detection limits, short response time, flow experiments and a good linear response. The excellent anti-interference characteristics enabled the use of the biosensor in screening analysis of food products [Carelli et al. 2007].

Biogenic amines, low molecular weight's organic bases, are used as quality markers because of their impact on human health if present in food at high concentration levels. These compounds are not only biosynthesized in plant and animal cells but also produced by microbial aminoacids' decarboxylation. Type and amount of biogenic amines comprised is strongly influenced by the microbial flora, food composition and other parameters, which elevate bacterial growth during food storage, since the biogenic amines' concentration could change during food storage and processing. The most frequent intoxication involves histamine. Histamine poisoning is referred to as "scombroid fish poisoning" because it is generally associated with the scombroid fish: sardines, mackerel and tuna consumption. Hence, the maximum permitted histamine level in fish samples illustrated by Italian law is 100 mg·kg^{-1}, and similar limits are adopted by European Commission regulation [Carelli et al. 2007].

Recently, electrochemical bioreactors or biosensors for the biogenic amines detection based on home or commercial-purified enzymes have been proposed in the literature. Diamine oxidase (DAO) used in the fabrication of these tools has been obtained from various sources such as; microorganism, animal and plants tissue with very different enzymatic activities. Commercial DAO by "Porcine kidney" shows a very low activity ($\geq$ 0.00083 U·mg^{-1} solid). Hence, in order to immobilize a large amount of this enzyme, small reactors have been assembled, requiring a more complex preparation procedure [Carelli et al. 2007].

In spite of the specificity of these biocomponents, the tools sometimes suffer from some limitations as poor reproducibility, short stability, long response time, and inefficient elimination of interferences. As well known, one of the problems to overcome in the development of amperometric biosensor for food analysis is denoted by electroactive interferents, normally present in these matrices. A valid approach to accomplish the interference problem is denoted by the development of non-conducting polymeric films with built-in perm selectivity, which have been used successfully for an efficient elimination of general interfering species such as; urate, cysteine, ascorbate and paracetamol during lactate and glucose determination in biological or food matrices. This biosensor showed a good stability, sensitivity and a complete suppression of electroactive interferences even in flow injection systems. Biosensor performances were tested by the biogenic amines determination in some food products [Carelli et al. 2007].

Acoustic biosensors. Piezoelectric quartz crystals could be influenced by a mass change at the crystal surface; this phenomenon has been successfully exploited and used to develop acoustic biosensors. For practical applications, the crystal surface could be modified with recognition elements such as antibodies that could bind specifically to a target analyte [Rana et al. 2010].

Immunosensors. These sensors are based on exploiting the specific antibodies interaction with antigens. Immunoassays (as the enzyme-linked immunosorbent assay technique) usually employ a label such as antibody, fluorescent marker, enzyme to detect the immunological reaction. Biosensor platforms usage, linked to an immunoassay format, submits a route to accurate and rapid quantitative measurements of target analytes [Rana et al. 2010].

Electrochemical biosensors. These biosensors are based on monitoring electroactive species that are either consumed or produced by the biological components such as, cells and enzymes action. Produced signal transduction could be performed using one of several methods under two broad headings: Amperometric Biosensors and Potentiometric Biosensors [Rana et al. 2010].

Potentiometric biosensors. These types of biosensors are based on monitoring the potential of a system at a working electrode, with respect to an accurate reference electrode, under conditions of essentially zero current flow. In progress, potentiometric measurements are related to the target species analyte activity in the test sample. Potentiometric biosensors could operate over a wide range of concentrations. The potentiometric biosensors usage for food quality analysis has not been as widely reported as for amperometric sensors. Besides, determining urea levels in milk, estimating monophenolase activity in apple juice, measuring concentrations of isocitrate in fruit juices and determining the sucrose concentration in soft drinks are some samples which are used this approach for food quality analysis [Rana et al. 2010].

Optical biosensors. These types of sensors are based on measuring responses to light emission or to illumination. Optical biosensors are based on well-founded methods including fluorescence, phosphoresence, light absorbance, photothermal techniques, chemiluminescence, surface plasmon resonance (SPR), total internal reflectance, light rotation and polarization and could employ a number of techniques to detect the presence of a target analyte. As an example, these technical usages have been demonstrated to detect the presence of allergens, particularly peanuts, during food production [Rana et al. 2010].

Calorimetric biosensors. Most of the biochemical reactions are accompanied by either heat production or absorption. Calorimetric transduction based sensors are designed to detect heat consumed or generated during a biological reaction; by using sensitive heat detection tools. Numerous biosensors for specific target analytes have been constructed. In the food quality analysis field, such biosensors usages to detect metabolites have been defined [Rana et al. 2010].

CARBON NANOTUBES

Carbon nanotubes (CNT) have become the intense investigation subject since their discovery. This kind of interest reflects the unique behaviour of CNT, including their remarkable chemical, structural, electrical and mechanical properties. CNT could display superconducting, metallic and semiconducting electron transport, possess a hollow core suitable for storing guest molecules and have the largest elastic modulus

of any known material. CNT could be made by carbon arc methods, laser evaporation or chemical vapor deposition and could be divided into multi wall carbon nanotubes (MWCNT) and single wall carbon nanotubes (SWCNT). MWCNT comprise of several layers of grapheme cylinders which are concentrically nested like rings of a tree trunk. SWCNT possess a cylindrical nanostructure with a high aspect ratio, formed by rolling up a single graphite sheet into a tube (Fig. 2) [Wang 2005].
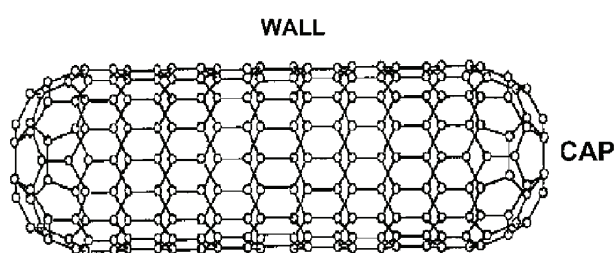

Fig. 2. Structure of SWCNT [Wang 2005]

In general the unique properties of CNT make them extremely attractive for the task of chemical sensors and in particularly, electrochemical detection. Recent studies have demonstrated, CNT could promote the electron-transfer reactions of proteins (including those where the redox center is embedded deep within the glycoprotein shell) and could enhance the electrochemical reactivity of important biomolecules. Additionally, to enhanced electrochemical reactivity, CNT-modified electrodes have been shown useful to decrease surface fouling effects such as those involved in the NADH oxidation process and to accumulate important biomolecules such as nucleic acids [Wang 2005].

The significant sensitivity of CNT conductivity to the surface adsorbates allows the use of CNT as highly sensitive nanoscale sensors. These kinds of properties make CNT extremely attractive for a wide range of electrochemical biosensors ranging from DNA hybridization biosensors to amperometric enzyme electrodes. To take advantages of the significant properties of these unique nanomaterials in such electrochemical sensing applications, the CNT need to be properly immobilised and functionalised [Wang 2005].

Other CNT-oxidase biosensors

For food analysis, biotechnology, clinical diagnostics or sport medicine reliable lactate monitoring is essential. For amperometric monitoring of lactate, some researchers described a CNT/mineral-oil paste, containing lactate oxidase. The accelerated electron transfer reaction of hydrogen peroxide at the CNT-based paste electrode offered a rapid low-potential (0.10 V) detection of the substrate. For amperometric monitoring of polyphenolic compounds, a similar CNT/mineral-oil paste configuration containing polyphenol oxidase was employed. High sensitivity was observed, reflecting the CNT-induced electrocatalytic detection of the enzymatically generated catechol-quinone [Wang 2005].

CNT-based dehydrogenase biosensors

Reagentless amperometric devices based on the dehydrogenase enzymes coimmobilisation and their nicotinamide adenine dinucleotide (NAD$^+$) cofactor to various solid electrodes are generally employed for the important substrates biosensing as glucose, lactate or alcohol. The NADH product oxidation serves as the anodic signal and regenerates the NAD$^+$ cofactor. Problems inherent to such anodic detection are surface fouling associated with the accumulation of reaction products and the large overvoltage encountered for NADH oxidation at ordinary electrodes. CNT-modified electrodes are especially useful for addressing these problems as they submit an accelerated electron transfer of NADH along with minimization of surface fouling. Scientists reported on a CNT-based ethanol amperometric biosensor based on the coimmobilisation of ADH (ethanol dehydrogenase) and its NAD$^+$ cofactor within the CNT/Teflon matrix. In the overvoltage, the marked decrease for the oxidation of the liberated NADH facilitated convenient low-potential stable detection of the ethanol. Respectively, corresponding advantages are expected in connection to the biosensing of glucose or lactate in connection with glucose or lactate dehydrogenases [Wang 2005].

Highly sensitive bioelectronic protocols for detecting of proteins and DNA have been described recently based on the coupling of several CNT-derived amplification processes. In these procedures CNT played a dual amplification role in both the recognition and transduction events, namely as carriers for numerous

enzyme tags and for accumulating the α-naphthol product of the enzymatic reaction (Fig. 3). Coverage of around 9600 enzyme molecules per a CNT such as, binding event was estimated. Such CNT-derived double-step amplification pathway (of both the transduction and recognition events) allows the detection of DNA and proteins down to 1.3 and 160 zmol, respectively, in 25-50 μL samples and indicates great promise for PCR-free DNA analysis [Wang 2005].

Nanoparticles could facilitate electron transfer and could be easily modified using a wide range of chemical and biomolecules ligands. For their applications and properties, carbon nanotubes (CNTs) have been widely studied. CNTs' unique electrical properties have generated a huge amount of research in nanosensors and nanoelectronic devices. The first CNT-based chemical sensor build for detecting NH_3 and NH_2 gases. On the sidewall of CNTs, proteins immobilized through a linking molecule. The use of CNTs as biological sensors for detecting glucose is demonstrated.

CNTs' unique electric properties together with significant surface enlargement; make them important component in sensing applications. Peptide nanotubes have potential, well-ordered, self-assembled and discrete application for electrochemical monitoring formed by the diphenylalanine peptide. These kinds of tubular structures were discovered during the search for the minimal amyloidogenic self-assembled fragment of the β-amyloid polypeptide which related to Alzheimer's disease. This polypeptides' diphenylalanine core recognition may self-assemble into discrete and well-ordered tubular structures. The suggestion could be that geometrically restricted aromatic interactions contribute directionality and order – mediate these well-ordered nanostructures formation. For various nanotechnological applications the diphenylalanine-based peptide nanoassemblies have many attractive properties, e.g. they can be easily modified with biological and chemical elements, show a notable similarity to carbon nanotubes in their aspect and
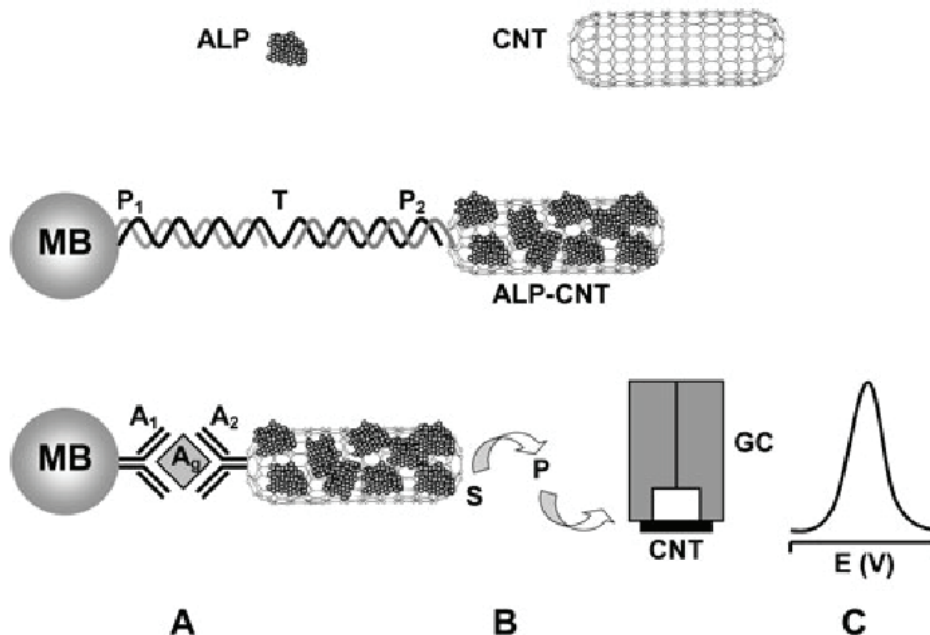

Fig. 3. CNT-derived amplification of the transduction and recognition events. A. Alkaline-phosphatase capture (ALP)-loaded CNT tags to the streptavidin-modified magnetic beads by the antibody or DNA recognition events; B. addition of the substrate and enzymatic reaction; C. electrochemical detection of the product of the enzymatic reaction at CNT-modified glassy carbon electrode [Wang 2005]

morphology ratio, are biocompatible and readily self-assembled in soluble nanostructures. A present work which has an aim to build and design a highly sensitive amperometric enzyme biosensor based on immobilised self-assembled peptide nanotubes attached to a gold electrode surface. Ethanol dehydrogenase (ADH) and glucose oxidase (GOx) were used as model enzyme systems to demonstrate the sensor's advantages [Yemini et al. 2005].

SOME INDUSTRIAL SAMPLES

Testing for arsenic pollution is generally performed with chemical test kits of unsatisfying accuracy. Bacterial biosensors are a challenging alternative as they are simple, easily produced, and highly accurate devices. A set of bacterial biosensors are described, based on a nonpathogenic laboratory strain of *Escherichia coli*, the natural resistance mechanism of *E. coli* against arsenite and arsenate, and three reporter proteins: bacterial luciferase, Green Fluorescent Protein (GFP) and β-galactosidase. To reduce background expression, the biosensors were genetically optimized in the absence of arsenic. In calibration experiments with the biosensors and arsenite amended potable water, arsenite concentrations at 4 μg of As/L (0.05 μM) were accurately and routinely measured. The presently most quantitative system expressed the bacterial luciferase such as reporter protein, responding proportional with a concentration range between 8 and 80 μg of As/L. Sensor cells might be stored such as frozen batches, resuspended in plain media, and exposed to the aqueous test sample, and light emission was measured after 30 min incubation. For arsenite, field testing was achieved with a system which contained β-galactosidase, producing a visible blue color at arsenite concentrations above 8 μg/L. For this kind of sensor, a protocol was developed in which the sensor cells were dried on a paper strip and then placed in the aqueous test solution for 30 min after which time color development was allowed to take place. The GFP sensor showed good potential for continuous rather than end point measurements. In all situations, production of the strip test and growth of the biosensors was achieved by very simple means with general growth media, and quality control of the sensors was performed by isolating the respective plasmids with the genetic constructs according to simple standard genetic technologies. Thus, the biosensor cells and protocols might submit a realistic alternative for measuring arsenic contamination in potable water [Stocker et al. 2003].

Another nanobiosensor is nano-sized bacterial pores were inserted into a lipid membrane for the detection of single peptide molecules. The analysis of both the blockage duration and current is able to provide specific structural information and allows specific peptides' detection in bulk mixtures. In biosensor research single molecule detection (SMD) is currently a major focus due to not only the increasing sensitivity, but also the ability to ensure useful information normally concealed in data averaging during bulk sampling. As an example, the kinetics and thermodynamics of the protein unfolding process could be easily obtained with SMD. The nanometer-scaled bacterial pore, that is able to self-assemble in a planar lipid bilayer, could be utilized through SMD, and has already been successfully used as a biosensor to detect specific DNA sequences. According to this study, the ability to differentiate single peptide molecules with the collagen-like sequence (Gly-Pro-Pro)n (n = 1, P1; n = 2, P2; n = 3, P3) in an equal mol mixture using nanopores is demonstrated. In Figure 4, a schematic diagram of the nanopore (α-hemolysin pore) is shown. The α-hemolysin' crystal structure exhibit a heptameric protein with a 3-4 nm vestibule leading into the "stem" with a limiting aperture whose diameter is approximately 1.5 nm, allowing the relatively large linear polymers' transportation through it. Nanopore has a role which is a conductance channel for electrolyte ions [YiTao and MengNi 2009].

As a result, these three-peptides' mixture was tested to find out whether the bacterial nanopore has sufficient selectivity to distinguish the peptides with tiny differences as it was used as a biosensor component. In order to obtain more precise information, a 2-D binning contour map of blockage duration versus blockage current was made by analysing the original data of blockage events. This map is shown in Figure 5. In this figure there are three well-defined peaks with little overlapping area representing the three different kinds of peptides. The colours' variation shown in Figure 5 indicates the population of current blockade events. The darkest area has the largest population, while the lightest area has the smallest population.
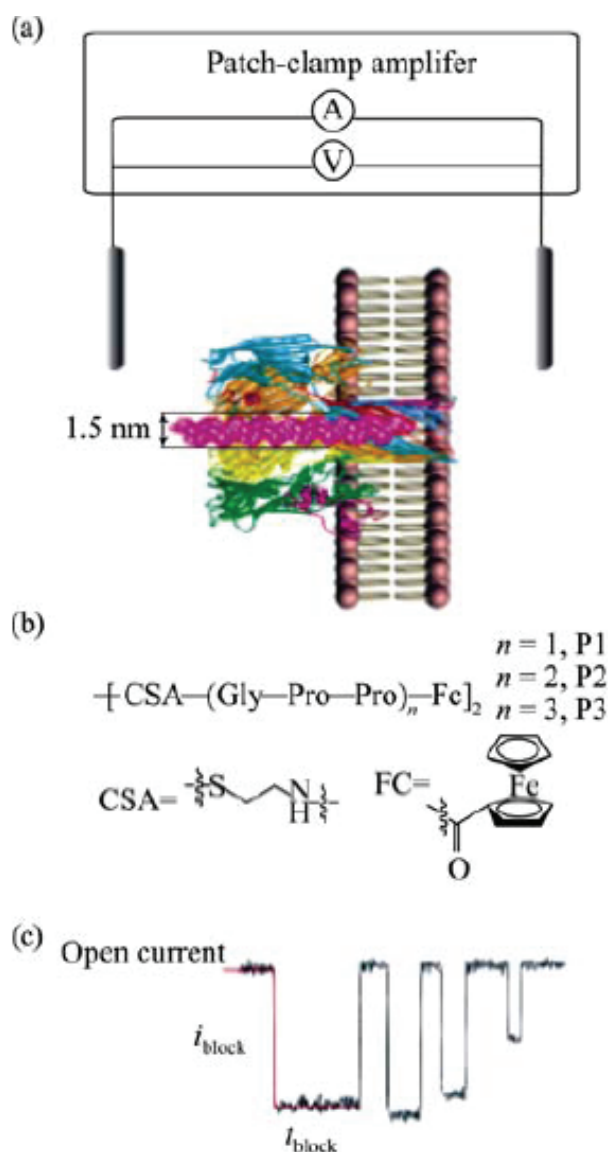
Fig. 5. Current transient of the mixture of peptides' contour plots P1, P2 and P3 [YiTao and MengNi 2009]

Fig. 4. (a) Schematic of a SMD experimental setup using a α-hemolysin nanopore. A α-hemolysin nanopore with a limiting aperture of 1.5 nm is shown embedded in a lipid bilayer with a peptide molecule transporting through the pore under an applied potential. (b) The structural formula of the peptides used in this research. (c) The representation of signals obtained in a single molecule detection experiment. The red line represents the idealized current and iblock is equal to the difference between the open current and the average amplitude of the blockage current [YiTao and MengNi 2009]
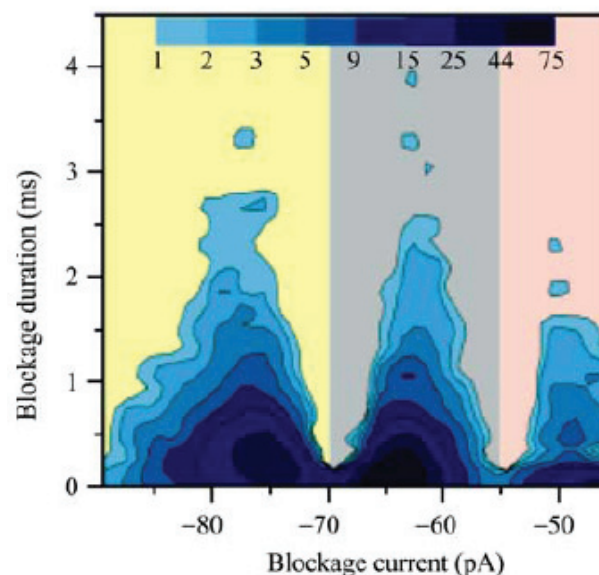
According to the result of the study the bacterial nanopore is able to simultaneously analyse peptide molecules with similar sequences, different lengths; indicating the nanopore potential to be used as a component of a nano-biosensor for single peptide molecule detection [YiTao and MengNi 2009].

According to a study; a gas-phase sensor for the detection of basic amine spoilage products in packaged fish is described. The change of the sensor is visual and could be measured against a reference colour around the sensor, though if quantization is required a colorimeter or imaging system could be employed. Sensor performance is currently being assessed through packaging trials. The solid-state dedectors are best suited to smart packaging applications produced cheaply and rapidly in addition to being easily deployed within the pack during processing. The type of dye incorporated within the sensor depends on the nature of the foodstuff spoilage process. In the case of packaged white fish, lots of volatile compounds are released as the flesh degrades, specifically the volatile amines: ammonia (NH_3, pKa = 9.25), trimethylamine (TMA, pKa = 9.81), dimethylamine (DMA, pKa = 10.73). Then these compounds are released, the package headspace

becomes increasingly basic. This change in pH might be monitored with a suitable indicator dye – immobilised within a gas-permeable polymer membrane [Crowley et al. 2005].

CONCLUSIONS

Nano-sized bacterial pores could be used as a fast, simple, selective and effective way to detect the structure of peptides at the single molecule level. It is a promising candidate as a nano-biosensors' component designed to detect single molecules. Aerolysin also showed nice performance according to its usage on single peptide molecule detection. According to this point, the foreground of bacterial nanopores in nano-biosensor scope is quite broad with the possibility of finding some other kinds of bacteria that could play a role as an appropriate component of a nano-biosensor [YiTao and MengNi 2009].

A biosensor is an inexpensive, portable and suitable analytical tool, but a required sample preparation step prevents its use as a field device. One solution to this challenge is the bioanalytical microsystems' development, in which biosensing systems and sample preparation modules could be integrated on the same platform. They therefore allow the design of easy-to-use and portable analytical tools. Additionally, the combination of research in microfabrication, material science and nanofabrication technology will become an excellent resource for the development of suitable sample preparation steps, as concentration, isolation and extraction. It could also play a major role in the improvement of the transducer biorecognition element interface. Under environmental conditions, biosensors repeated use with complex sample matrices and the long-term storage is a remaining challenge. Maybe the solution to this problem would be found with inexpensive microbiosensors designed for single use in order to avoid deterioration of the biosensor elements in complex matrices. Moreover, novel material research might help to ameliorate the situation. According to the future use of biosensors in food contaminant and environmental analysis, the sensors would need to meet monitoring demands by being integrated as sensor networks, providing multi-analyte detection, and being combined with wireless signal transmitters for remote sensing. Biosensors in air ventilation systems,

and biosensing food packaging material might become feasible [Baeumner et al. 2003].

CNT attractive properties have paved the way for a wide range of electrochemical biosensors construction exhibiting an attractive analytical behaviour. The marked electro-catalytic activity towards NADH and hydrogen peroxide allows effective low-potential amperometric biosensing of numerous important substrates. The enhanced electrochemical reactivity is coupled to resistance to surface fouling and also to high stability. The CNT molecular wires usage submits great promise for achieving efficient electron transfer from electrode surfaces to the enzymes redox sites. Better control of the CNT's physical and chemical properties and understanding of CNT's usage as molecular wires should lead to more efficient electrical sensing devices. Electrochemical DNA biosensors could greatly benefit from the CNT usage support platforms and from the enhanced detection of the product of the enzyme label or the target guanine. These kinds of developments offer future interdisciplinary efforts might yield new CNT-based biosensors generations for a wide range of applications [Wang 2005].

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MOŻLIWOŚCI ZASTOSOWANIA NANOBIOSENSORÓW W ANALIZIE ŻYWNOŚCI

STRESZCZENIE

Biosensory stworzone z użyciem nanotechnologii określamy jako nanobiosensory. Dzięki wspomnianej technologii możemy otrzymać czujniki o bardzo dużej czułości i dużej szybkości reakcji. W ostatnich latach, wraz z upowszechnieniem śledzenia pełnego procesu produkcji żywności, zaistniało zapotrzebowanie na urządzenia pozwalające na bardzo szybką identyfikację badanych próbek. Nanobiosensory mogą być istotnym ich elementem. Do wykonania niezbędnych oznaczeń można wykorzystać na przykład metody optyczne czy chromatograficzne. W zależności od techniki wzmacniania sygnału wejściowego biosensory mogą być podzielone na kilka grup (6). Urządzenia z nanobiosensorami można wykorzystać w analityce chemicznej, ochronie środowiska i biotechnologii. Celem pracy jest przedstawienie kierunków rozwoju nanobiosensorów oraz ich przydatności w analizie różnych substancji występujących w żywności.

Słowa kluczowe: nanobiosensory, biosensory, żywność, składniki, analiza