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# Biosensor for Biomedical Applications

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**Abstract**: In this paper, A total internal reflectance fluorescence (TIRF)-based biosensor for progesterone in bovine milk was developed and tested by measuring the progesterone level in daily milk samples for 25 days, covering a whole estrus cycle. The detection is based on total internal reflectance fluorescence. An immunobiosensor assay was developed for the multi-residue screening of a range of nitrofuran compounds in avian eyes. A polyclonal antibody which binds at least 5 of the major parent nitrofurans was raised in a rabbit after inoculation with a nitrofuran mimic-protein conjugate. Sample homogenates were extracted into 0.1 M hydrochloric acid and subjected to clean-up by solid phase extraction and micro-centrifugation prior to biosensor analysis.

**Keywords**: Biosensor, applications, glucose, pregnancy.

## I. INTRODUCTION

Businesses are analytical tools for the analysis of bio-material samples to gain an understanding of their bio-composition, structure and function by converting a biological response into an electrical signal.

The analytical devices composed of a biological recognition element directly interfaced to a signal transducer which together relate the concentration of an analyte (or group of related analytes) to a measurable response. The analyte: Molecule which do you want to detect such as protien,toxin, peptide, vitamin,suger, metal ion. Bioreceptor: The sensitive biological element-a biologically derived material or biomimetic component that interacts(bind or recognize)the analyte under study [1]. Transducer: is more generally defined as device which convert energy from one to another.

Electrical interface: Works in physiochemical way; optical, piezoelectric, electrochemical, etc. that the signal resulting from the interaction of the analyte with the biological elements into electrical signal form. The blood glucose biosensor–developed by father of biosensors Leland C. Clark in 1962 who has phd of university of rochester in biochemestry and physiology. Biosensors are molecular sensors that combine a biological recognition mechanism with a physical transduction technique.

They provide a new class of inexpensive, portable instrument that permit sophisticated analytical measurements to be undertaken rapidly at decentralized locations . However, the adoption of biosensors for practical applications other than the measurement of blood glucose is currently limited by the expense, insensitivity and in¯exibility of the available transduction methods. Here we describe the development of a biosensing technique in which the conductance of a population of molecular ion channels is switched by the recognition event. The approach mimics biological sensory functions and can be used with most types of receptor, including antibodies and nucleotides. The technique is very ¯exible and even in its simplest form it is sensitive to picomolar concentrations of proteins [2]. The sensor is essentially an impedance element whose dimensions can readily be reduced to become an integral component of a microelectronic circuit. It may be used in a wide range of applications and in complex media, including blood. These uses might include cell typing, the detection of large proteins, viruses, antibodies, DNA, electrolytes, drugs, pesticides and other low-molecular-weight compounds.

The molecular structure of the tethered membrane 5±14 results in an ionic reservoir being formed between the gold electrode and the membrane. The ionic reservoir can be accessed electrically through connection to the gold electrode. In the presence of an applied potential, ions ¯ow between the reservoir and the external solution when the channels are conductive. The ion current is switched off when mobile channels diffusing within the outer half of the membrane become crosslinked to antibodies immobilized at the membrane surface. This prevents them forming dimers with channels immobilized within the inner half of the membrane. The number of dimers is measured from the electrical conduction of the membrane. The switch has a high gain; a single channel facilitates the ¯ux of up to a million ions per second. A quantitative model of the biosensor has been veri®ed experimentally; see Supplementary Information.

While commercial biosensors (BIACORE and IAsys) are simple to operate, accurately interpreting binding reactions is not always straightforward. Since the majority of published biosensor data do not fit a simple bimolecular interaction model (A ‡ B = AB), many investigators are concerned about the validity of biosensor analysis.

However, the inability to fit data to a simple model is often a result of how the experiments are run and not a flaw in the technology. Many investigators collect data under conditions that are not suitable for measuring binding kinetics. There are a number of experimental artifacts that can complicate biosensor analysis, including surface-imposed heterogeneity, mass transport, aggregation, avidity, crowding, matrix effects and nonspecific binding (Myszka, 1997; Morton and Myszka, 1998). Improving the design of biosensor experiments, as well as improving the way binding data are collected and processed, can eliminate most of these artifacts (Myszka, 2000). By improving the quality of the sensor data, we have

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described a number of systems with simple interaction models (Myszka et al., 1996; Roden and Myszka, 1996; Myszka et al., 1997; Stuart et al., 1998). For example, Fig. 1 shows a data set for an IL- 2–receptor interaction globally fit with a simple interaction model (Myszka, 2000). Note that the association and dissociation phases responses for each IL-2 concentration are described very well by this simple model. This article highlights the key steps required to improve the quality of data when the goal is to interpret the binding kinetics recorded on biosensors [3].

## II. ELECTROCHEMICAL GLUCOSE BIOSENSORS

Different nanomaterials were investigated to determine their properties and possible applications in biosensors. Most of studies on metal (gold, silver), carbon and conducting polymers that used to prepare nanomaterials such as nanoparticles [1,2], nanotubes [3,4] and nanowires The intrinsic properties of nanoparticles are mainly determined by size, shape, composition, crystallinity, and structure. The synthesis of nanoparticles with desired size/shape/composition/crystallinity/structure has, therefore, enormous importance, especially in the compelling of nanotechnology. In this case, fabrication of electrochemical biosensors for directly determination of glucose in whole blood by help of advanced nanotechnology is our research target As been well known, in spite of the many impressive advances in the design and use of glucose biosensors, the promise of tight diabetes management has not been fulfilled. Until now, primary methods of dectecting blood glucose concentration are performed by biochemical analyzer and glucose meter. However, the accurate results of glucose concentration cannot be provided by the two biomedical instruments above. Most hospitals now measure and report the serum glucose levels, from which the serum samples are isolated from whole blood by centrifugation process, by biochemical analyzer. The test results are influenced by the different model numbers of test instruments and detection reagents, treatment processes of blood samples, factitious operations, especially additional centrifuge and too long measure time from collecting specimen of blood to examination. As for commercial glucose meter, there are some defects that cannot be ignored during its operation. For example, the blood samples are obtained from fingertip peripheral but not vein, and doped easily with tissue fluid.

The accurate measurement of glucose is extremely important in the diagnosis of diabetes and pre-diabetes. Despite the many technological advances in biosensor research and development and the introduction of many different products, glucose biosensors were still performed in serum. At present, it is very difficult to design and prepare a electrochemical biosensor that can be used in whole blood because the biofouling of electrode surface can be developed by platelet, fibrin and blood cell adhesion in the complex environment of whole blood media. And the biofouling of eletrode surface will bring catastrophic damage to the electron transfer between enzyme and electrode redox center. So the development of novel glucose biosensors for antifouling, rapid, highly sensitive, and selective detection is of paramount importance for blood glucose concentration monitoring in whole blood samples [4].

Our group have designed and explored the hydrophilic polymer coating as antibiofouling surface for preparing for electrochemical glucose biosensor that can be used directly for whole blood samples. Based on previous works, the novel polyurethane-heparin nanoparticles (PU-Hep NPs) were synthesized and used to establish an amperometric glucose biosensor that can be applied in whole blood directly, which was attributed to the antibiofouling property of PU-Hep NPs. The PU-Hep NPs were found to have special performance relative to conventional nanomaterials due to their better hemocompatibility and antibiofouling property. More details of preparation of glucose oxidase (GOx) modified (PU-Hep)/glass carbon electrode (GCE) biosensor and its electrochemical behavior in whole blood were presented figure 1.

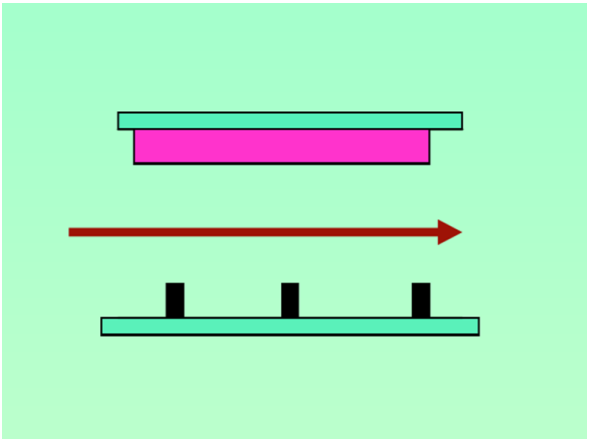

Figure1: Glucose Biosensors

## III. PREGNANCY BIOSENSOR

The described biosensor uses the binding-inhibition test (Fig. 3) in heterogeneous phase as assay principle. Therefore, a high-affinity antibody directed against a specific analyte and an analyte derivative that can be covalently bound to the sensor surface is required. To reduce non-specific binding to the surface an immobilized biopolymer (aminodextran) layer is used. The antibody is first labeled with a dye (CyDye™ Cy5.5), then the measurement routine starts with the incubation of the sample using the specific antibody. For that purpose, 100 µL of the antibody stock solution (30 ng mL−1 in each sample) are mixed with 900 µL of the sample. The antibody binds the analyte during the incubation step until a well-defined condition of the reaction is reached. Afterwards, when the sample is pumped over the sensor surface, only the antibody molecules with free binding sites can bind to the surface. The binding of the antibody to the surface has to be mass-transport-limited to yield quantitative results with the binding-inhibition assay. To ensure these mass-transport-limited conditions, the number of binding sites on the surface must be much higher than the amount of antibody used in the

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measurement [5]. Thus, only a small amount of antibody is used in the measurements and a large number of potential binding sites on the surface are provided by using the dextrane surface with the immobilized antigen derivatives.

This has been demonstrated by additional reflectometric interference spectroscopy measurements as already described in literature (Glaser, 1993) and surface evaluation experiments (Kröger et al., 2002 and Jung et al., 2001). We observed linear correlations between the increasing fluorescence signal and the antibody concentration used in TIRF experiments. Even at highest antibody concentrations, no saturation effects can be observed. Thus, the much higher excess of immobilized antigen derivatives versus the amount of antibody used could be verified. Active esters were prepared with the derivatives, which are analyte molecules modified using a spacer containing a carboxyl group. Approximately, 5.0 mg of the derivative were dissolved in 100 µL of dry N,N-dimethylformamide (DMF).

N-hydroxysuccinimide (NHS) and N,N′-dicyclohexylcarbodiimide (DCC), each in 1.1-fold molar excess (referring to the amount of analyte derivative) were added to the solution. After stirring for several minutes, the solution was kept at room temperature over night. Finally, the solution was centrifuged (12,000 rpm) at approximately 4 °C, and the supernatant was stored under refrigeration. The 50 mg aminodextran were dissolved in a mixture of 500 µL Milli-Q water and 500 µL DMF. The active ester solution was added, mixed thoroughly and again kept at room temperature over night. A 10-fold volume excess of methanol precipitated the aminodextran conjugate. The supernatant was removed and the conjugate freeze-dried.

The glass transducers were cleaned using a freshly prepared mixture of hydrogen peroxide and concentrated sulfuric acid (ratio 2:3) for up to 30 min and rinsed with Milli-Q water. After drying under a nitrogen flow, 25 µL of (3-glycidyloxypropyl)trimethoxysilane (GOPTS) were applied to the surface and reacted for up to 60 min. The silanized surface was rinsed with dry acetone and dried under a flow of nitrogen. Subsequently, the aminodextran conjugates were dissolved in Milli-Q water at a concentration of 2.0 mg mL−1 and applied for immobilization using an ink-jet dispenser. The remaining area between the detection spots was covered with non-conjugated aminodextran to prevent non-specific antibody binding ( Tschmelak et al., 2004 and Tschmelak et al., 2005b).

Figure2: PREGNANCY BIOSENSOR

## IV. OPTICAL BIOSENSOR

The nitrofuran group of compounds are antibacterial agents which have been used in animal feed as growth promoters and as both prophylactic and therapeutic treatments for gastrointestinal infections caused by Escherichia coli and Salmonella spp. in livestock including cattle, pigs and poultry. They have also been used for the treatment of bacterial and protozoan infections in the aquaculture and apiculture industries [1] and [2]. They are still being used in both veterinary and human medicine, variously, as topical applications for skin burns and infections (NFZ), and for the treatments of cholera (FZD) and urinary infections (NFA) [3], [4] and [5].

The use of FZD and, subsequently, all nitrofuran antibiotics in animals producing food for human consumption has been banned within the European Union (EU) since 1993 and 1995 respectively, as evidence was produced to show that they have potential risks for causing both mutagenic and carcinogenic effects in humans and . From 2002 to date, import restrictions have been imposed on a number of countries following the detection of nitrofuran residues in food exports bound for the EU market. Since 2003 there have been more than 450 Rapid Alerts issued by EU Member States in relation to the finding of nitrofuran residues in food originating from more than 30 countries.

The nitrofuran parent compounds, containing a characteristic 5-nitrofuran ring structure, are unstable in vivo and are rapidly metabolised following ingestion into their respective tissue bound side-chain metabolites. This inherent instability in animal tissues has made the monitoring of nitrofuran abuse through the targeting of the parent compounds problematic. Their metabolites, however, have been shown to persist in animal tissues for an extended period and it has been suggested that the metabolic mechanism for this occurs via cleavage of the nitrofuran ring leaving the specific tail group covalently bound to tissue proteins. These can then be subsequently released by the action of mild acids, at concentrations similar to those found in the human stomach. The residues so released are, in turn, bioavailable to a second species eating contaminated meat.

The prolonged persistence of residues in tissues makes the nitrofuran metabolites the most suitable marker residues for regulatory monitoring programmes. The most commonly detected nitrofuran metabolite has been the controversial marker residue semicarbazide (SEM), which is the metabolite of NFZ. Any confirmed detection of this metabolite following analysis was originally taken as proof of the illegal use of the parent compound.

The reliability of this method of testing for nitrofuran abuse was later questioned, however, as SEM contamination of foodstuffs was shown to have been caused by exposure to azodicarbonamide, which is used both as a flour treatment agent in bread baking and and as a blowing agent in the manufacture of lid gaskets used in the production of, for example, jars of baby food figure 3.

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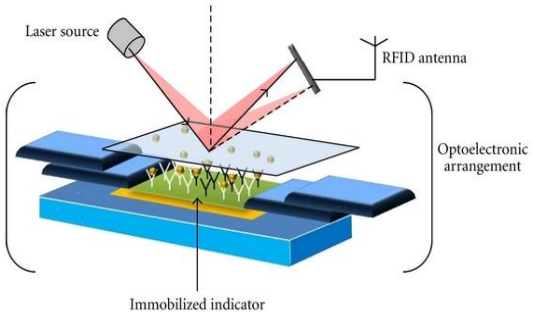


Figure 3: Optical Biosensor

## V. CONCLUSION

Considering the significance of blood glucose in the area of biomedical and health for life, this study has practical importance. The novel electrochemical biosensors we prepared can be conveniently applied to veracious evaluate the level of blood glucose with the help of antibiofouling technology. The result of CD spectra indicated that the protein on PU-Hep NPs retained near-native secondary structures. The SEM results showed that the blood cells and platelets adhering were remarkably suppressed on the surface of substrate modified with GOx/(PU-Hep). The developed assay for progesterone using only commercially available compounds demonstrates good results that compare to the previously described assay. As the reduced assay time of about 5 min has only little effects on the assay performance, it is possible to determine the progesterone concentration of a cow during the milking procedure. According to the characteristics of an ideal system for detecting the estrus cycle of a cow mentioned in the introduction, continuous monitoring is possible by obtaining a progesterone value of the cow twice a day.

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