

Glucose Determination by Biosensors

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Sugars constitute a class of biocomponents, which can reach in plants an amount of almost 50% of their dried weight. Glucose, fructose and saccharose are found in significant amounts in fruits and honey. Glucose or dextrose occurs naturally in fruits and vegetables as the primary product of photosynthesis. Most ingested carbohydrates (saccharose, lactose, starch) are converted to glucose during digestion, and this is the form of sugar that is transported in the bloodstream. Sugars in diet can be naturally occurring or added, and contribute largely to the nutritional value of the foodstuffs in which they are found or incorporated. Naturally occurring sugars are found in fruits and honey (glucose, fructose, saccharose) and milk (lactose). The major sources of added sugars are soft drinks, fruit juices, chocolate, candies, cakes, cookies, pies, dairy desserts and milk products (ice cream, sweetened yogurt and sweetened milk).

Glucose can be manufactured from starch by enzymatic or acidic hydrolysis. Glucose represents a key analyte in many fields, like food analysis or biomedical analysis. Its determination can rely on its reducing character, using the titrimetric methods Luff-Schoorl, Bertrand or Somogyi [1] or by colorimetric methods [2]. Nevertheless, these methods are characterized by reduced selectivity and necessitate previous precipitation of proteins, which could interfere in the analysis [2].

Under this circumstances, biosensors represent a viable alternative to laborious and time consuming conventional techniques. Biosensors are sophisticated analytical instruments which incorporate a biorecognition element (e.g. enzyme, nucleic acid, antibody, whole cell), in close contact to a transducer. The latter senses the changes that take place in the system, as a result of the substrate (analyte)-biocatalyst (enzyme) interaction. The transduced parameter can be electrical (current intensity, potential/pH difference), optical (absorbance, reflectance, fluorescence, luminiscence), thermal, piezoelectrical [3-6].

Among biosensors, enzyme electrode combine the specificity and selectivity imparted by the biomolecule, to the accuracy, sensitivity and rapidity of the physico-chemical transduction (e.g. electrochemical, optical) and necessitate minimum sample pre-treatment [3-6], thus being preferred to the previously mentioned classical techniques.

The enzyme electrodes, the most commonly used biosensor type, can use various detection techniques: electrochemical (potentiometric, amperometric, conductometric), optical, thermal, piezoelectrical, the first mentioned being the most widely employed [4].

Enzyme immobilization in biosensor construction provide the advantages of repetitive enzyme use, biocatalyst stabilization, cost diminution. Many techniques have been used for enzyme immobilization: physical adsorption, gel entrapment, covalent coupling with or without crosslinking, or the construction of chemically modified electrodes based on carbon paste, carbon nanotubes, composite materials, screen printing or sol-gel technique [3-5].

The reaction exploited in glucose potentiometric biosensors, is the oxidation of this analyte, catalysed by glucose oxidase, for which FAD functions as cofactor. This method relies on the pH diminution monitoring, due to gluconic acid generation, as a result of the enzyme reaction.

For potentiometric biosensing, nylon or cellophane semipermeable membranes with immobilized glucose oxidase, were fixed on the sensitive bulb of the pH-glass electrode [7,8]. Metal/metal oxide redox electrodes [9-11] were also used as transducers in the development of potentiometric glucose biosensors. Thus, a SnO₂ redox electrode functioned as transducer in potentiometric glucose biosensing [11]. Glucose oxidase, catalase and bovine serum albumin were coimmobilized on a Pt electrode, for potentiometric glucose assessment [10]. For potentiometric glucose sensing, glucose oxidase was also immobilized in a polypyrrole film, deposited on a Pt electrode. The external polypyrrole layer eliminated the interferences given by ascorbic acid [11]. A recent approach in glucose potentiometric biosensing [12] was based on the use of a polymeric membrane Ag⁺-selective electrode, fabricated from benzothiazole calix arene [4]. Silver nanoparticles were synthesized and demonstrated to be used as a potentiometric redox marker for glucose biosensor construction. The hydrogen peroxide generated in the glucose oxidase-catalysed reaction was able to oxidize silver nanoparticles to free silver cations. The amount of Ag⁺ ions can be related to glucose concentration, thus glucose could be determined by using the Ag⁺ ion selective electrode with a detection limit of 10⁻⁵ M [12].

Amperometric biosensors are based on redox reactions, namely the oxidation/reduction of electroactive species generated/consumed in an enzymic reaction. The role of the biocatalyst is to generate or consume an electroactive species (ex. O₂ or H₂O₂), whose amount can be stoichiometrically related to the concentration of the substrate (analyte). Thus, the measured current intensity, generated by the electrochemical reaction is proportional to the analyte concentration [3-5]. During an amperometric measurement, the working electrode or sensor, is held at a constant potential, while the current is monitored. The current is then related to the concentration of the analyte present [13].

The first amperometric biosensor was developed by glucose oxidase immobilization on an oxygen electrode [14]. The enzyme was trapped in a polyacrylamide gel, which was then fixed on the oxygen electrode. A glucose biosensors based on H₂O₂ determination at a Pt electrode, used glucose oxidase was immobilized on a cellulose acetate membrane, by crosslinking with a bifunctional reagent, glutaric dialdehyde. A silanized polycarbonate membrane covers the enzyme layer, extending the linear range of the biosensor, which allowed glucose determination in soft drinks [15]. The diminution of the working potential in amperometric

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biosensors based on H₂O₂ estimation was achieved by the use of mediators: benzoquinone, ferricyanide, ferrocene, tetrathiafulvalene, tetracyanoquinodimethane, methyl viologen, Meldola Blue, Prussian Blue etc [3-5]. For glucose amperometric biosensing glucose oxidase was incorporated in a polymeric film of polypyrrole or polyaniline [16,17]. Another approach uses screen-printed biosensors, based on horseradish peroxidase (HRP) and glucose oxidase (GOx) [18]. Potassium ferrocyanide was used as a mediator. Recent advances in biosensor development imply the use of composite electrodes: multiwall carbon nanotubes and the ionic liquid n-octylpyridinium hexafluorophosphate were used to develop a composite electrode with good activity towards hydrogen peroxide, with the possibility to incorporate glucose oxidase within the composite matrix and obtain a sensitive biosensor for glucose [19].

Glucose analysis by optical biosensors was performed with an oxygen optrode with immobilized glucose oxidase. The molecular oxygen consumption was monitored with a fluorescence indicator, decacylene [20]. The chemiluminescence of luminol, in the presence of H₂O₂ generated in the glucose-oxidase catalyzed reaction, can be exploited in glucose analysis [1,2]. H₂O₂ generated in the enzyme reaction quenched the photoluminescence of quantum dots. The photoluminescent QDs and glucose oxidase were integrated into the composite film by electrostatic layer-by-layer self-assembly technique [21]. Another chemiluminometric glucose flow biosensors was developed, using Mg-Al carbonate layered double hydroxides as catalysts. The silica sol-gel with glucose oxidase and horseradish peroxidase, was immobilized in the first half of the inside surface of a clear quartz tube, and luminol-hybrid carbonate layered double hydroxides were packed in the second half [22].

Calorimetric biosensors employ thermistors as transducers, the analytical signal being the result of all enthalpy variations in the reaction medium [3]. Glucose oxidase was co-immobilized with catalase, for improving the sensitivity [23].

A viscometric biosensor was developed, for continuous monitoring of glucose in biological fluids. The sensing principle of this chemo-mechanical sensor was based upon the viscosity variation of a sensitive fluid with glucose concentration. The device included both actuating and sensing piezoelectric diaphragms, as well as a flow-resistive microchannel [24].

Due to their advantages (selectivity, specificity, rapidity, minimum sample pre-treatment, possibility of automation, portability and miniaturization) versus conventional methods, glucose biosensors find their applications in fields like biomedical analysis (assessment of glucose in biological fluids, such as blood saliva, sweat, urine, and serum) [25], as well as food and beverage analysis [26].

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