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Development of Borate Electrode for the Measurement of Glycosylated Proteins

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Abstract

Diabetes is a metabolic disease which requires a regular glisemic control in order to eliminate undesirable complications to improve and continue. Glisemic control can be executed from fasting blood glucose level measurement or measurement of glycated protein levels [14],[15]. There were many improvements made on measurement techniques of glycated proteins over the past 30 years. However these techniques require professional staff, some preliminary procedures and they are expensive to execute. This project's aim is to produce a highly borate selective electrode that can be used as a detector in the glycated protein measurement which will be used in diabetes diagnosis and monitoring. A micro scaled borate electrode was produced. This working electrode will be silverborate based composite solid electrode. Borate can make complex structures with glycated proteins therefore this compound can be used as a measurement technique. Developed borate selective electrodes were tested by a potentiometric reading in standing solutions varying from 1,0*10-1M and 1,0*10-5M. Developed borate selective electrode had a stable, linear and replicable potentiometric behaviour between 1,0*10-1M and 1,0*10-5M borate ion concentration. Developed electrode had selectivity to borate ions over sulfate, chloride, nitrate and some organic acid ions. Potentiometric readings of the electrode were consistent when pH was between 4 and 8 values. Developed electrode gave consistent readings through almost 3 months. Response time of the electrode was determined as 10 to 12 seconds. Response time being this low enhances this electrode's reliability on fluid stream systems. Developed borate selective electrode is expected to be used in flow injections systems to help monitoring glycated protein levels in blood streams.

Keywords: Borate electrode, glycated protein detection

INTRODUCTION

When glycated protein term is being used, it refers to glycated hemoglobin (HbA1c) and glycated albumin(HSA). Hemoglobin is one of the many protein that undergoes non-enzymatic glicosylation. HbA1c is one of the most abundant hemoglobin kind. The results provide 3 month average values. This is desired since red blood cells have a lifespan around 120 days. HbA1c formation depends on the encountering of red blood cells with high sugar levels. The more high sugar meets red blood cells, the more HbA1c forms. HbA1c level increasing in the blood refers to the diabetic not being in regulation [1],[8]. Other protein that is being used for glisemic control is serum albümin. Serum albumin is widely being used when anaemia or kidney failure present in the patient or when the HbA1c measurements are effected through hemoglobin alternatives [14]. Human serum albümin has a half life or 12-21 days therefore HSA measurements can give precise results upon the previous 2-3 weeks.[14].

There are many HbA1c measurement methods developed in the last 30 years [9].

These are: immunochemical tests depending on the relationship between antigen and antibodies, chromatographic methods depending on the relationship between phenylboronic acid and HbA1c and their structural differences, chromatographic methods that include HPLC and depending on the charge diference between HbA0 and HbA1c. Basic methods that are being used for glycated HSA measurements are: boronate affinity chromatography, immunoassay methods and enzymatic methods [2],[10]. Studies are generally focused on glycated albumin and fructosamine measurements that gives 2-4 week span results.

Chromatographic methods are relatively expensive methods that require lots of procedures and professional handling [3],[9]. Immunochemical tests create some non-linear calibration curves and involves easy-to-break materials inside kits [8],[11].

In this study, it was aimed to develop a micro-sized borate-selective electrode in the form of a silver borate-based composite that provides simultaneous (short, medium, long term) glucose measurements in a flow injection analysis system using the ability to bind to glycated proteins.

MATERIALS AND METHODS

Material

Chemicals used inside the composite electrode:

Silver borate: 0,1 M sodium tetraborate and 0,1 M silver nitrate (Emir Chemical) got mixed 1:1. Solutions got stirred with ultrasonic cell crusher and centrifuge. After silver nitrate collapsed to the bottom of the beaker, solutions left to settle for 3 days.

Silver sulphide: Produced by collapsing of 0,1 M sodium sulphide and 0,1 M silver nitrate. It also got commercially bought from Alfa Aesar. Copper Sulphide: Produced by collapsing of 0,1 M copper(1) chloride and 0,1 M soduim sulphide. It also got commercially bought from Alfa Aesar. Solid contact material: %50 grafit(Alfa Aesar), %35 epoxy (%15 hardener) and THF/Acrylic (Merck) are the content for the solid contact material.

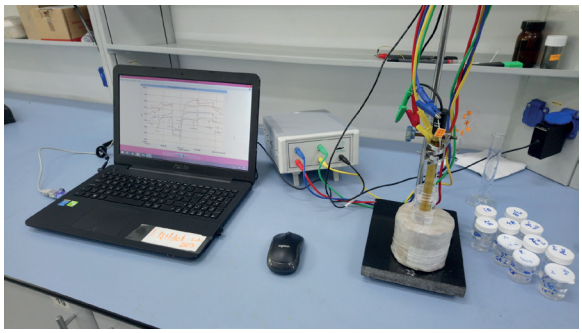


Figure 1. Potentiometric Measurement System

Potentiometric measurements require a reference electrode, an indicator electrode, and a potential measuring device. In process, electrochemical cells are potentially measured.[12],[13].

Potentiometry is gathered from MEDISEN R&D Company and the reference Ag/Agcl electrode is bought from HANNA Company.(Figure 1).

Preparation of Electrodes

All materials that are used in this study was washed with acetone-alcohol(70%)-ultra pure water and left to dry before use. Table 1 below shows the content of the composite electrode and their respective amounts.

Table 1. Substances in the composite electrode and their respective amounts.

SUBSTANCE	AMOUNT (g)
CNT- Ag3BO3	0,010
CNT	0,100
Ag2S	0,080
Cu2S	0,010
Epoxy	0,800

Content shown in Table 1 was weighed and crushed into smaller particles in the mortar. Crushed content got mixed again with the help of a micro sized mixer. Homogeneous solution gathered on top of a watch glass. After the addition of epoxy and its solvent acrylic, content constantly stirred under laminar cabin. Electrode content filled into the catheter pipe. Material inside the catheter got constantly pushed to the center. Drying process took approximately 2 weeks. Depending on how much material left inside the catheter pipe, small cylinder sections get separated, then fastened to a copper wire through a liquid contact that dries on top of the connecting part and solidifies the area.

Outside Shell (except for membrane and solid contact) of the electrode got isolated by liquid glue patex in order to avoid any leaks. (Figure 1).

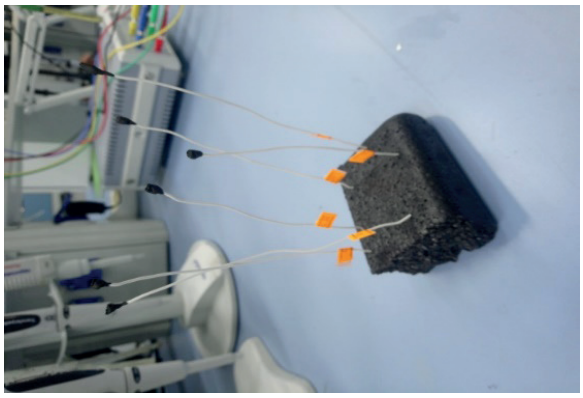


Figure 2. Electrode Being Fastened On The Copper Wire.

Dried electrode left to stay in a $1,0*10^{-1}$ M borate solution so that the electrode gets saturated with borate ions.

RESULTS AND DISCUSSION

Developed borate selective electrode got tested inside a stagnant environment in 5 different solutions that varies from $1,0*10^{-1}$M and $1,0*10^{-5}$M borate concentration. Gathered results are shown in Figure 4-6.

Developed borate selective electrode provided stable and linear results through 10^{-5} M and 10^{-1} M borate concentration solutions and showed repeatability(Figure 4). Electrode showed selective behaviour for not only borate ion but also for chloride, sulphate, nitrate, phospate, chlorate and some organic acid ions(Figure 5-6). Borate selective electrode gave similar results within the pH range of 4-8.

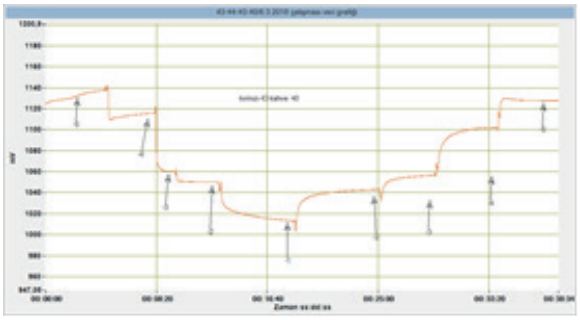


Figure 4. Stagnant environment measurements of the electrode numbered as 40 in $1,0*10^{-1}$M and $1,0*10^{-5}$M concentrations of NaCl and borate solutions.

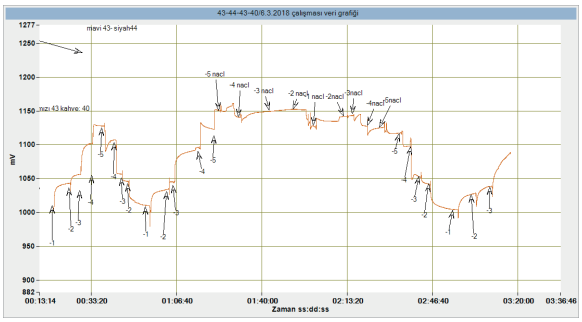
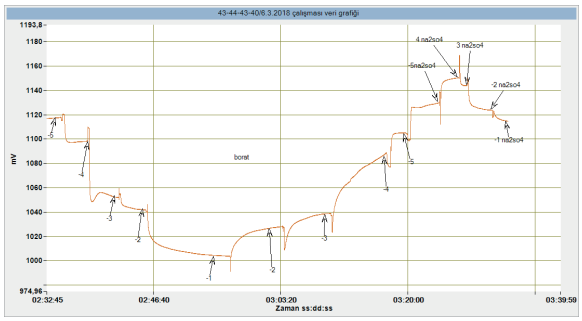


Figure 5. Stagnant environment measurements of the electrode in $1,0*10^{-1}$M and $1,0*10^{-5}$M concentrations of NaCl and borate solutions.



Şekil 6. Stagnant environment measurements of the electrode in $1,0*10^{-1}$M and $1,0*10^{-5}$M concentrations of NaCl and Na2So4 solutions.

Borate selective composite electrode gave very similar results for 2 months. Response time of the electrode was between 10-12 seconds. Response time being low is a good sign of an electrode to function as a reliable detector in not only stagnant environment but also for flow injection environment.

Presumably, this developed micro sized composite borate selective electrode will be able to function as a glycated protein detector in blood. As mentioned above, it is also presumed that the electrode will function inside the flow injection analysis as well.

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