Chapter 27

Glucose Monitoring via Reverse Iontophoresis

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Background

Frequent glucose monitoring is essential for people with diabetes to manage their blood glucose levels effectively. Present procedures for obtaining such information, however, are invasive and painful. Development of a painless approach would represent a significant improvement in the quality of life for people with diabetes. In addition, results from the DCCT [1], UKPDS [2] and Kumamoto Trials [3] showed that a tight-control regimen, which uses aggressive therapy with frequent glucose measurements to guide the administration of insulin and oral agents, leads to a substantial decrease in the long-term effects of diabetes. Nevertheless, even as many as seven measurements per day were not sufficient to prevent an increase in hypoglycemic events in those patients who followed this aggressive therapy [1]. The GlucoWatch® biographer provides a means to obtain painless and non-invasive measurements for up to 12 hours using a single blood measurement for calibration. A monitoring system that provides automatic and frequent measurement of glucose could provide a warning of impending hypoglycemia, potentially making aggressive diabetes management safer.

Each of the current techniques for measuring blood glucose concentrations has drawbacks. The most generally accepted method relies on extraction of small

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aliquots of blood, obtained via a fingerstick. It is painful and invasive however, often resulting in poor patient compliance to a glucose-monitoring program. The measurement frequency of a typical user is not sufficient to achieve tight control. Implantation of biosensors or microdialysis tubing to sample from the subcutaneous tissue or peritoneal cavity is capable of providing frequent measurements [4,5]. However, poor biocompatibility, due to protein deposition, for example, limits the life of these devices, and the invasiveness of the method prevents wide acceptance. Near infrared spectroscopy, a non-invasive technique for blood glucose measurement [6,y], is not commercially established and requires large, expensive equipment. As such, there is no commercial device that allows non-invasive, frequent measurement of blood glucose.

Iontophoretic Glucose Extraction

The non-invasive method described here extracts glucose through the skin using an applied potential (a process known as reverse iontophoresis), and measures the extracted sample using an electrochemical/enzymatic sensor. Iontophoresis is a technique whereby a constant, low-level electrical current (0.3mA/cm^2 in these studies) is conducted through the skin between an anode and cathode. Due to the applied potential, sodium and chloride ions (from beneath the skin) migrate towards the cathode and anode, respectively [8,9]. Uncharged molecules (e.g., glucose) are also carried along with the ions by convective (electroosmotic) transport. It is this convective flow that causes interstitial glucose to be transported across the skin [10]. The skin has a negative charge at neutral pH, and hence, there is greater net transport to the cathode. As a consequence, glucose is preferentially extracted at the cathode. Over the typical range of iontophoretic current densities (0–0.5 mA/cm^2), glucose extraction is linear with current density and duration of iontophoretic current [8,9].

The feasibility of iontophoretic glucose extraction has been demonstrated both *in vitro* [10] and in human subjects [11]. In the studies with human subjects, glucose extraction was measured by HPLC analysis. Changes in blood glucose levels correlated with glucose extracted into a buffer receiver solution over a 15 minute period of iontophoretic current application (current density of 0.3 mA/cm^2). Calibration of the system was performed to account for possible biological variability in skin permeability. A single point calibration was found to compensate for this variability. The calibration was performed by taking a reading using a traditional blood glucose measurement method, and using this reading to calibrate all subsequent extraction readings. It was found that glucose transport correlates well with blood glucose in a linear fashion, however the sensitivity (i.e. the amount of glucose extracted compared to the blood glucose) varied among individuals and skin sites. The results of this feasibility study showed a mean correlation coefficient of 0.92, and a mean absolute relative error of 13% for the comparison of extracted to blood glucose values. The extraction process, however, yields a glucose concentration which is about 0.1% of that found in blood. Therefore, in contrast to the sensitivity

for glucose detection required for conventional fingerstick measurements, increased sensitivity is required to measure iontophoretically-extracted glucose.

Glucose Detection of the Iontophoretic Extraction

In the GlucoWatch® biographer the concentration of extracted glucose is measured by a biosensor. An amperometric, electrochemical-sensing chemistry was chosen as the most suitable for this application. The biological selectivity element in this biosensor is the enzyme glucose oxidase (GOx), which catalyzes the oxidation of glucose to gluconic acid. This enzyme is extremely selective towards glucose. To obtain a signal from this enzyme reaction, it must be coupled to the sensing electrodes. This is achieved by the direct detection of glucose oxidase-generated, hydrogen peroxide (H_2O_2).

$$\text{glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2$$

The H_2O_2 is detected via an electrocatalytic oxidation reaction at a Pt-containing working electrode in the sensor, producing an electric current, and regenerating O_2.

$$H_2O_2 \rightarrow O_2 + 2\,H^+ + 2\,e^-$$

Thus, for every glucose molecule extracted, two electrons are transferred to the measurement circuit. The magnitude of the resulting electric current is correlated to the amount of glucose collected through the skin.

The main challenge in developing the biosensor for measuring iontophoretically-extracted glucose is the small amount of glucose transported through the skin, and the resulting low concentration that must be accurately quantified. For example, at blood glucose level of 50 mg/dL, approximately 50 picomoles of glucose are extracted through the skin during three minutes of iontophoresis, resulting in a concentration at the biosensor of about 4 μM. This concentration is almost three orders of magnitude lower than the blood glucose concentration measured by typical fingerstick blood glucose monitors. The biosensor that has been developed for the GlucoWatch biographer has high sensitivity and low noise, resulting in an extremely low limit of detection for glucose. The operating principles of the electrochemical/enzymatic sensor are described in detail elsewhere [12].

Operation of the GlucoWatch Biographer

A miniaturized device for the combined extraction and detection of glucose (the GlucoWatch biographer) is shown schematically in Figure 1. The extraction and detection is achieved using two hydrogel pads placed against the skin. The side of each pad away from the skin is in contact with separate iontophoretic and sensing electrodes. Two such electrode assemblies are required to complete the iontophoretic

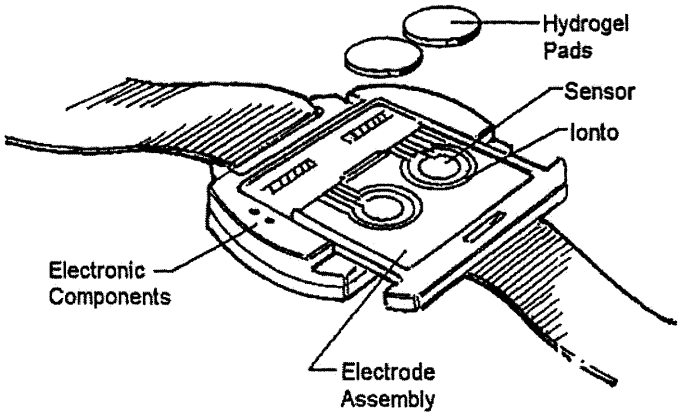

Figure 1. A schematic diagram of the GlucoWatch® biographer.

circuit. During operation, one iontophoretic electrode is cathodic (negatively charged) and the other anodic (positively charged), enabling the passage of current through the skin. As a consequence, glucose is collected in the hydrogel during the iontophoretic extraction period. The iontophoretic time interval is adjusted to minimize skin irritation and power requirements, yet extract sufficient glucose for subsequent detection. It has been found that an optimal time for extraction of glucose is about three minutes, under the conditions described here.

The hydrogel is composed of an aqueous salt solution in a crosslinked polymer containing the enzyme, glucose oxidase. As described above, this enzyme catalyzes the conversion of the glucose (in the presence of oxygen) to hydrogen peroxide and gluconic acid. The peroxide is subsequently detected at an electrochemical sensor.

Glucose exists in two forms: α-glucose and β-glucose, which differ only in the position of the hydroxyl group at the C-1 position in the six membered ring [13,14]. These two forms (called anomers) are in a proportion of 37% and 63% for α and β forms at equilibrium, respectively. The same proportion of α- and β-glucose is also found in blood and interstitial fluid. As glucose enters the hydrogel, it diffuses throughout, but only the β-form of glucose reacts with the GOx enzyme. As the β-form is depleted, the α-form then converts (mutarotates) to the β-form to re-establish the equilibrium. The products of the GOx reaction (H_2O_2 and gluconic acid) also diffuse throughout the hydrogel.

On the side of the hydrogel away from the skin, and adjacent to the annular iontophoretic electrode, is the sensing electrode (see Figure 1). A sensing electrode is found at both the iontophoretic anode and cathode. Thus, there are two sensing electrodes, noted as sensor A and B. These circular sensing electrodes are composed of a platinum composite, and are activated by applying a potential of 0.3-0.8 V (relative to a Ag/AgCl reference electrode). At these applied potentials, a current is then generated from the reaction of H_2O_2 (generated from extracted glucose) which has diffused to the platinum sensor electrode. The measured current is proportional to the amount of H_2O_2, and hence, extracted glucose.

The current (mA) utilized in iontophoresis potentially interferes with detection of the low current (nA) generated at each electrochemical sensor. Consequently, the iontophoretic and sensing electrodes are not activated at the same time. Instead, a typical situation is to have the iontophoresis proceed for about three minutes to collect an adequate amount of glucose. During this period about 10ng of glucose is typically extracted at the cathode. Iontophoresis is then stopped and the sensing electrodes are activated for typically seven minutes. This period of seven minutes is chosen so that all of the glucose (both α and β) has been converted to H_2O_2, and that all of the hydrogen peroxide has diffused to the platinum electrode, and subsequently oxidized, to generate a current. Thus, all extracted glucose and H_2O_2 are consumed during this cycle. The integrated current (or charge) over this seven minute interval is then proportional to the total amount of glucose that entered the hydrogel during the iontophoresis interval. The iontophoresis polarity is reversed and cycle is then repeated [15]. Thus, if sensor A is at the cathode during the first cycle, sensor B is the cathode during the second cycle. The combined cycle requires 20 minutes, and the

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