A Conductimetric Transducer for Biosensor Systems

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ABSTRACT- A conductimetric transducer, *i.e.* a conductivity meter, was developed to detect a small change in conductivity of solution. It consists of a signal generator with a fixed frequency where the amplitude of the input signal is adjusted to give the best output signal. Enzyme sensors for the determination of acetylcholine and urea based on the detection of the conductivity change of the solution were studied. In these systems acetylcholinesterase or urease was used to catalyze the hydrolysis of acetylcholine or urea into charged products, thus, increased the conductivity of the sample solution. The device was designed specifically to contain the offset and the amplification circuits that enable the small conductivity change due to the enzymatic reaction to be measured. A preliminary study of these biosensors indicated that this conductivity meter can be used as a conductimetric transducer in biosensor systems.

KEY WORDS - conductivity, urea, acetylecholine, enzyme, sensor, flow injection, immobilized

บทคัดย่อ - พัฒนาคอนดักทิเมทริกทรานสดิวเซอร์เป็นเครื่องวัดสภาพนำไฟฟ้าที่สามารถวัดการเปลี่ยนแปลงปริมาณน้อยในสารละลาย เครื่องดังกล่าวประกอบด้วยวงจรกำเนิดสัญญาณที่จ่ายไฟฟ้ากระแสสลับที่มีความถี่คงที่แต่ปรับขนาดของแอมพลิจูดได้เพื่อให้ได้สัญญาณเอาท์พุทที่ดีที่สุด ศึกษาเอนไซม์เซนเซอร์สำหรับหาปริมาณอเซทิลโคลีนหรือยูเรียโดยวัดการเปลี่ยนแปลงของสภาพนำไฟฟ้าของสารละลาย ระบบดังกล่าวใช้เอนไซม์อเซทิลโคลีนเอสเทอเรสหรือยูริเอสเร่งปฏิกิริยาไฮโดรไลซีสของอเซทิลโคลีนหรือยูเรีย ได้เป็นผลผลิตที่มีประจุซึ่งทำให้สภาพนำไฟฟ้าของสารละลายเพิ่มขึ้น ออกแบบอุปกรณ์ดังกล่าวให้มีคุณสมบัติเฉพาะเพื่อให้ใช้เป็นในระบบไบโอเซนเซอร์ได้อย่างมีประสิทธิภาพ ในตัวเครื่องจะมีออฟเซทและส่วนขยายสัญญาณที่ใช้ปรับให้สามารถวัดการเปลี่ยนแปลงปริมาณน้อยของสภาพนำไฟฟ้าที่เกิดจากปฏิกิริยาของเอนไซม์ จากการทดลองเบื้องต้นพบว่าเครื่องวัดสภาพนำไฟฟ้าที่พัฒนาขึ้นนี้สามารถใช้เป็นทรานสดิวเซอร์วัดสภาพนำไฟฟ้าในระบบไบโอเซนเซอร์ได้ดี

คำสำคัญ - การนำไฟฟ้า อเซทิลโคลีน ยูเรีย เอนไซม์ เซนเซอร์ โฟลอินเจกชัน สภาวะตรึง

1. Introduction

A biosensor is a device or a technique uses to determine analyte concentrations. Biosensors use biological materials in collaboration with appropriate transducers to determine the substrate in the samples. The biological sensing element responds to the analyte being measured and the transducer converts this observed change into a measurable signal that is proportional to the concentration of the analyte. Biosensor involves a wide range of analytes and transducers (see, *e.g.*, [1-3]).

The transducers, the detector devices, frequently employed are electrochemical, optical, piezoelectric and thermometric. The most applied detection principle is probably electrochemical which include, potentiometric, amperometric and conductimetric transducers.

Conductimetric transducer measure conductivity which is the measure of the ease of passage of electric current through a solution and this is directly proportional to the concentration of ions in the solution. This measurement can be used to follow any reactions that produces a change in the number of ions, the charge on the ions, the dissociation of ions or the mobility of ions [4]. Although this measuring technique is said to be non ion specific if used together with a biological element, which catalyses a specific biochemical reaction and

gives rise to conductivity change, this technique can become a very specific detection system.

Acetylcholine, a neurotransmitter, and urea, an end product of protein metabolism, are two important analytes in clinical analysis [5]. Their enzyme catalysis reactions involve changes of the number of ions in the solution. The hydrolysis reaction of acetylcholine by enzyme acetylcholinesterase (AChE) is [6]

$$acetylcholine + H_2O \xrightarrow{AChE} choline + CH_3COO^- + H^+ .$$

Urea is catalysed by enzyme urease as in the reaction [4]

$$urea + 2H_2O \xrightarrow{urease} 2NH_4^+ + HCO_3^- .$$

Therefore, it is possible to use a conductimetric transducer, *i.e.*, a conductivity meter, in biosensor systems for the analysis of these two compounds. However, some specific features have to be incorporated into this transducer since the conductivity change is quite small and the background solution would also give rise to a certain value of conductivity.

In this work we report the development of a conductimetric transducer, designed specifically to use in a biosensor system. A flow injection system using enzyme reactor column together with this transducer was developed. Preliminary study of the use of biosensor systems to determine acetylcholine and urea were investigated.

2. Proposed Techniques

2.1 Conductimetric Transducer

This conductrimetric transducer consists of a signal generator where the amplitude of the input signal can be adjusted to give the best output signal, an offset, to null the background solution conductivity, and the amplification circuits that enable the small conductivity change due to the enzymatic reaction to be measured.

Block diagram of the conductivity meter is shown in Figure 1. The AC current from the signal generator is passed through the first electrode into the solution and to a second electrode. The received current is then converted to voltage and amplified.

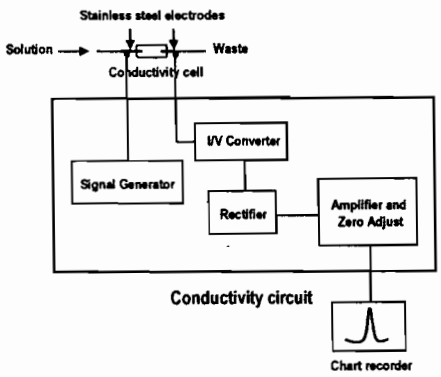

Figure 1. Block diagram of the conductimetric transducer.

Figures 2-5 show the circuit diagrams used in this transducer. The sine wave generator circuit (Figure 2) generated a 1.6 kHz sine wave with a steady amplitude. The size of the signal could also be adjusted from 0 to 10 V.

The current that passed through the solution was picked up by another electrode and the signal was fed the into the I/V converter circuit (Figure 3). There is a high pass filter at the output of the OP-AMP with a cutoff frequency of 100 Hz. The relationship between the input current (I_{in}) and the output voltage from this circuit (V_{out}) is linear, *i.e.*, $V_{out} = -I_{in} \times 1K$.

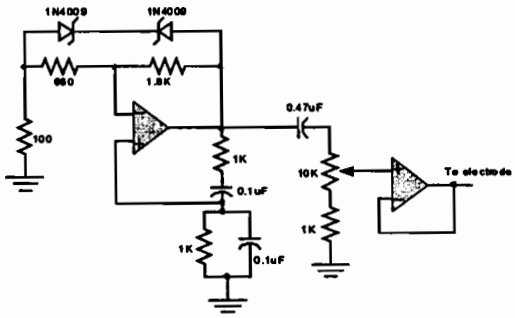

Figure 2. Circuit diagram: Sine wave generator.

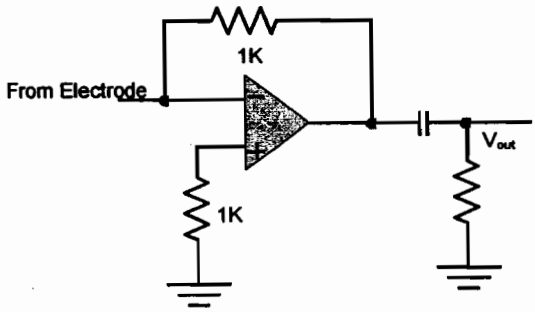

Figure 3. Circuit diagram: I/V converter.

The AC voltage output from the I/V converter was then changed to DC through the rectifier circuit (Figure 4). Since the voltage from the I/V converter is small both diodes and OP-AMP were employed in this circuit, which is a full-wave rectifier. The 0.47 μF capacitor is used to filter the voltage so the output DC voltage would have a mean-absolute-value closed to the rms.

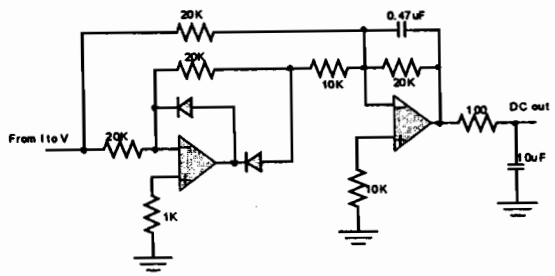

Figure 4. Circuit diagram: Rectifier.

The final circuit (Figure 5) is to amplify the signal from the rectifier. The maximum amplification is 50 times, adjusted through VR2. The zero offset was done by adjusting VR1.

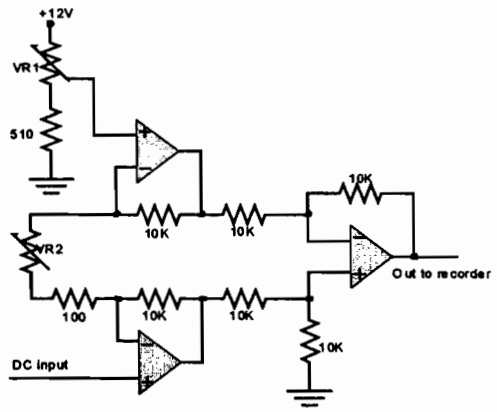

Figure 5. Circuit diagram: Amplifier and zero adjust.

2.2 Instrumentation

Figure 6 shows the principle of the flow injection conductimetric biosensor system. The sample is injected into the sample carrier buffer that is pumped through a dialyser before being sent to waste. The dialyser allows small molecules, including the analyte to pass through the membrane and these were collected in the buffer on the other side of the membrane. When the solution containing the analyte passed through the enzyme column, ions were generated by the hydrolysis reactions described earlier. The increase of charged products was measured as the change in the conductivity of the solution in the conductivity cell at the outlet of the enzyme reactor column.

The conductivity change within the conductivity cell is measured by a conductivity meter. In this system the background conductivity signal of the solution can be adjusted to zero allowing only the changes to be detected and amplified. The alternating current response is converted to a direct current voltage and is recorded on a chart recorder. This voltage signal is linearly related to the solution conductance as well as the concentration of the analyte.

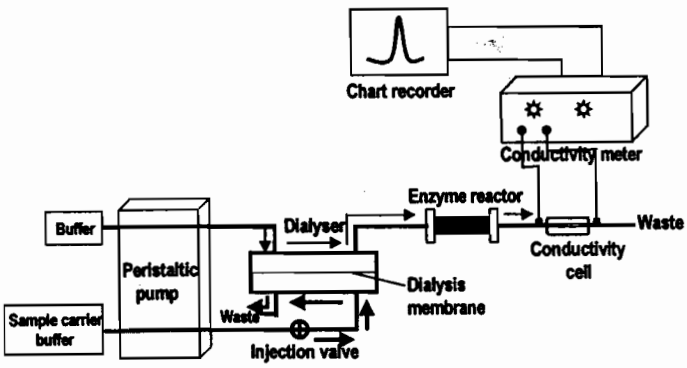

Figure 6. Schematic diagram showing the flow injection conductimetric biosensor system.

2.3 Immobilization of enzyme

Immobilization of urease and AChE on inorganic support material surface was by covalent binding followed the procedures described by Weetall [7].

Urease (amidohydrolase EC 3.5.1.5 Type IX: from jack beans, SIGMA, USA) or AChE (EC 3.1.1.7 Type VI-S from electric eel, SIGMA, USA) was dissolved in 5 ml of 0.05 M sodium phosphate buffer pH 7.0 and added to 1 ml of activated support materials. The mixture was tumbled at room temperature. After 4–5 h, 50 mg of sodium cyanoborohydride was added. The mixture was tumbled again for another 15 h and was then washed with 500 ml of buffer. After this 25 ml of 0.1 M ethanolamine pH 8.0 was added and the reaction was allowed for another 2 h. The preparation was then washed with 500 ml of buffer and was packed into a small column to be used in the analytical process.

2.4 Responses of the biosensors

Initially the conductimetric biosensors for acetylcholine and urea were investigated in a simple system without a dialyser. This was done by testing the conductivity change due to the catalysis reaction of the immobilized enzyme reactors. The system for urea was further tested in the system with dialyser (Figure 6) which would be used to filter off large molecules in real samples, such as human serum, from blocking the enzyme column.

Solutions of urea and acetylcholine were prepared in 0.05 M glycine-NaOH buffer pH 8.80. The sample solutions were introduced as pulses in the continuous flow of buffer. The responses were the measured peak heights.

3. Experimental Results

3.1 Conductimetric Transducer

To detect the small conductivity change in the solution due to the enzyme activity in a biosensor system a conductimetric transducer was designed such that the amplitude of the input signal could be adjusted to give the best output signal. The device also contains the offset and the amplification circuits that enable this small conductivity change due to the enzymatic reaction to be measured.

The circuits in Figures 2-5 are the final results of a number of modifications to obtain a transducer that would provide a very steady signal with minimal noise and drift. This enabled it to be applicable to the biosensor system which only measure the small change in the conductivity of the solution. Figure 7 shows the responses of the transducer when tested with different concentrations of urea. It is evident that this final conductimetric transducer provided very good signals with high precision.

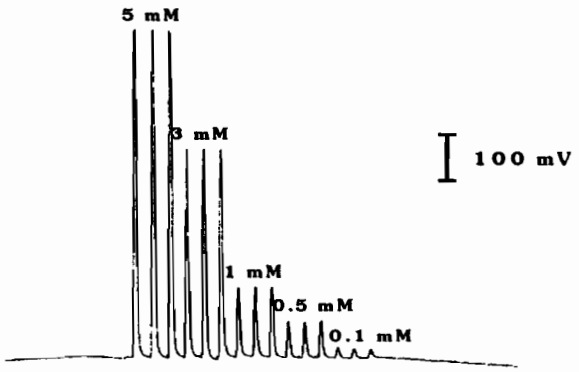

Figure 7. Example of the responses due to the change in conductivity in a conductimetric biosensor to determine urea.

3.2 Conductmetric responses of biosensors

For acetylcholine, parameters influencing the performance of the flow system, that is, flow rate, sample volume, concentration and pH of buffer were optimized to obtain responses with high sensitivity (slope of the linear range) while using a short period of analysis time. The responses of the system to acetylcholine were then tested at optimum conditions. Figure 8 shows the plot of responses from the conductimetric system *vs* acetylcholine concentrations. The linearity was in the range of 0.1-7.0 mM. Three replicates were done for each concentration and the relative standard deviations (RSD) of the response signals were less than 4%.

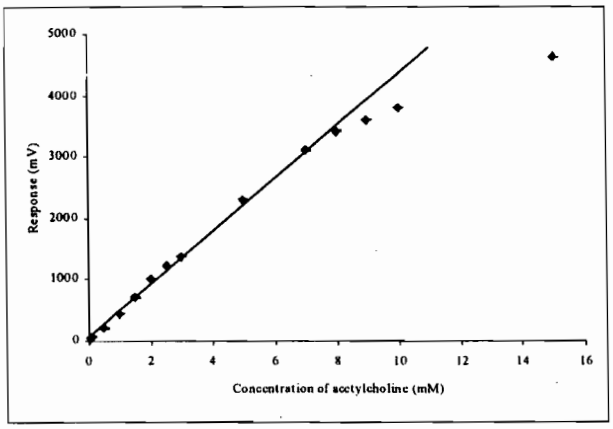

Figure 8. Responses of conductimetric biosensor to acetylcholine.

Preliminary test for urea, without system optimization, was also investigated. Good responses were also obtained. The system provided a linear range between 5-45 mM, also with an RSD of less than 4%.

To analyse real samples, such as serum, large molecules in the sample matrix must be dealt with. The analysis of urea was investigated further in a flow system containing a dialyser (Figure 6). This is to filter off large molecules, preventing them from blocking the enzyme reactor. Flow rates on both sides of the dialyser were optimized as well as buffer condition and sample volume.

Standard solutions of urea were analysed. At optimum conditions the detection limit was 0.5 mM and the response was still linear up to 150 mM (Figure 9) (RSD = 1-2%). The optimization helped to provide a lower detection limit than in the system without the dialyser, 0.5 mM *c.f.* 5 mM. This would be low enough to determine urea in human serum since the accepted reference interval for serum urea is 2.3-8.3 mM [8]. The extended linear range was due to the dilution of the analyte through the dialyser.

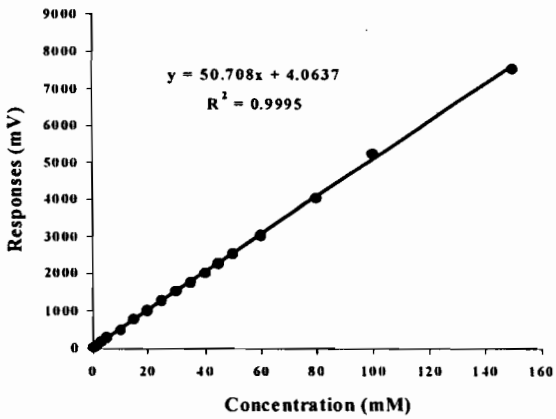

Figure 9. Responses of conductimetric biosensor to urea.

4. Conclusions

The experiments reported here show that the developed conductimetric transducer is suitable for the measurement of the change in conductivity in a biosensor system due to the change in the number of ions in the solution. However, the transducer can also be applied to follow the change in conductivity causes by the change of charge on the ions, the dissociation of ions or the mobility of ions.

The versatility of this proposed system was that different analytes can be determined by just changing the enzyme reactor column and this has been shown in this work. When used in collaboration with immobilized acetylcholinesterase enzyme reactor the system was analysing acetylcholine and when the immobilized enzyme was urease it can determine urea.

When used together with a dialyser, it provided a sample handling method to separate large particles from the analytes and, thus, preventing them from blocking the enzyme column. Therefore, sample preparation before analysis is not required. With further development this system could be used to analyse acetylcholine or urea in real samples, or any other biosensor systems that produce conductivity change.

Acknowledgements

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