Electrochemical and Spectroscopic Sensor Injection: Methodology and Application

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ABSTRACT

Investigations towards the development of methodologies to enhance the usefulness of chemical sensors are reported. Operation of chemical sensors in the Sensor Injection mode provides for the identification of sensor malfunction and drift, a way of compensating for the deviation, a mechanism to recondition a sensor on-line, and a method of prolonging the sensor lifetime. A conventional flow injection configuration is compared with a sequential injection methodology. Results are reported for an enzyme based amperometric glucose sensor, a potentiometric pH sensor, and a spectrophotometric pH sensor. Standard Error of Prediction (SEP) of 0.16 for the potentiometric sensor and 0.02 for the spectrophotometric sensor were obtained. Monitoring of glucose levels in a yeast fermentation is reported. Both filtered and unfiltered samples were examined. Reconditioning of the glucose electrode's enzymatic membrane is examined and discussed. Glucose determinations yielded a linear dynamic range from 0.5-200 mM and RSD of 0.67% at 10 mM glucose.

INTRODUCTION

Investigations into the development of new chemical sensors are at the forefront of chemical research. Among the areas of interest are the development of methods to reduce or eliminate problems of surface fouling, signal drift, and limited sensor lifetime. To date, frequent recalibration of sensors is the most common method used to identify and correct for these inherent problems.

Recent investigations in our laboratory utilizing flow injection methodologies for the minimization or elimination of these problems has led to the development of a novel technique, Sensor Injection (SI) (1). The central idea in sensor injection is to operate a sensor in an impulse/response mode, rather than in a steady state mode in which the sensor is in contact with the monitored solution at all times. In the SI mode, a valve is used in conjunction with a pump to provide a preprogrammed flow profile which reproducibly delivers a finite sample zone to the sensor cavity. This is followed by the refilling of the sensor cavity with a wash or carrier solution which yields a well defined subsequent readout, such as a baseline or signal for a known analyte concentration, and is at the same time beneficial to the sensor. Utilizing this methodology, the lifetime of the sensor is prolonged by maintaining the sensor in a suitable environment during most of the sensor's operational lifetime, thus reducing the fouling of the sensing surface due to the reduced contact time of the sensor surface with the sample matrix. Thus, sensor injection allows conditioning and frequent recalibration of the sensor response.

The features of flow injection analysis (reproducible injection of well defined zones into a flowing carrier stream, return to baseline between samples, continuous flushing of the detector cell with carrier) are beneficial to most sensors from the standpoint of reducing contact time with the sample matrices. A number of electrochemical flow injection techniques which have been used over the years to accomplish the same tasks as sensor injection (2-5). The work reported here is a further refinement of these earlier attempts and an extension to spectroscopic sensors.

The present studies deal with two ways in which sensor injection can be executed: 1) conventional flow injection (Fig. 1A) and 2) sequential injection (Fig. 1B, 1C) methodologies. Conventional flow injection uses an injection valve furnished with a sample loop of a fixed volume. A two-channel piston pump (6) is used here to simultaneously aspirate the sample solution into the sample loop (bottom piston in Fig. 1A) and to charge the second piston with a carrier solution (top piston Fig. 1A). When the valve is switched, the forward motion of the second piston discharges the sample zone into the sensor cavity and out to waste, while the excess sample is discharged to waste.

Sequential injection uses a multiposition selection valve and a single channel piston pump, which is preprogrammed in such a way that after charging the syringe with carrier a selected sample volume is aspirated into the

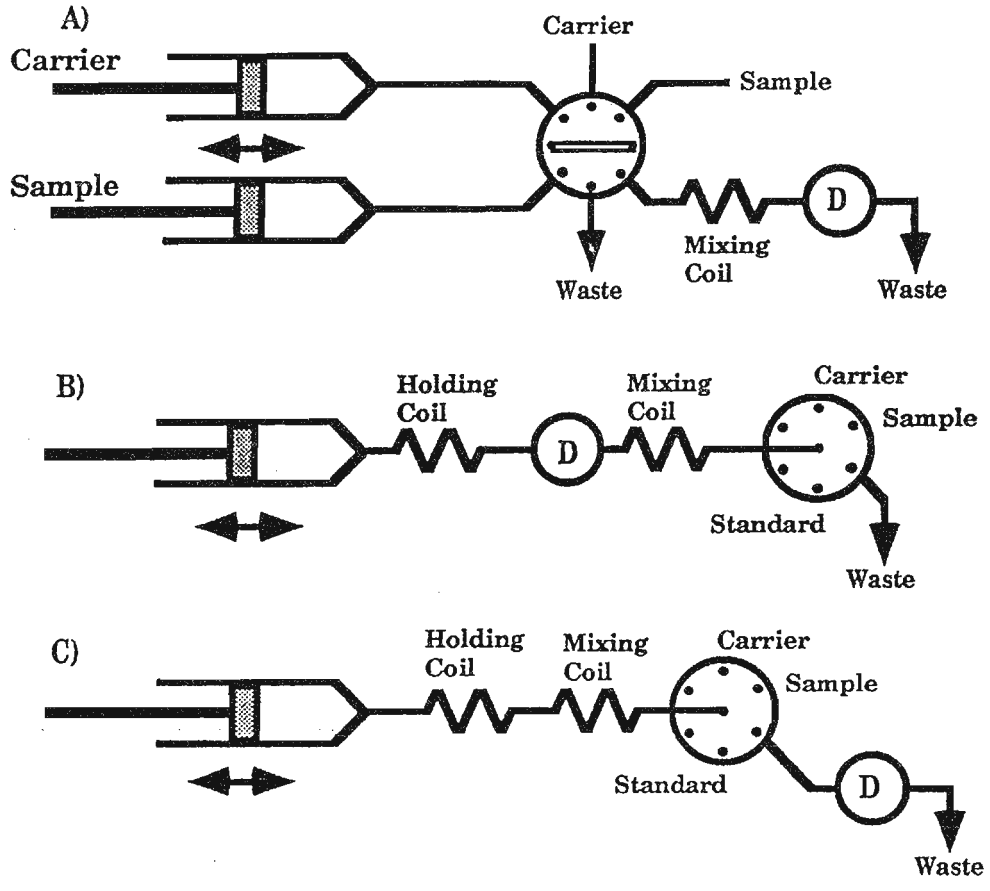

Figure 1. Flow manifolds for various systems used. A) Conventional flow injection configuration where during the load cycle the carrier fills the top syringe and sample flows through the sample loop into the sample syringe. Upon injection, the valve switches and the carrier transports the sample contained in the loop through the sensor flow cell to waste. B) and C) Sequential injection configurations where carrier is aspirated into the mixing coil, holding coil, and syringe. The valve is switched to the sample port after an appropriate amount of carrier has been aspirated, and a well defined zone of sample is then aspirated into the mixing coil. Once the sample zone has been stacked into the mixing coil, the valve is switched to a port leading to waste and the syringe is fully discharged. The bi-directional arrows indicate that the pump syringe moves in both directions.

tubular conduit situated between the valve and the pump. The valve is then switched to a port leading to waste and the sample zone is discharged from the system. The sensor can either be placed between the valve and the pump (Fig. 1B) or downstream from the valve (Fig. 1C). The relative merits and drawbacks of these configurations as well as applications of SI to electrochemical and spectroscopic sensors are discussed below. Potentiometric and spectroscopic measurements of pH are compared, and the use of sensor injection to study and eliminate sensor fouling is investigated for the measurement of glucose in a fermentation broth.

EXPERIMENTAL

Apparatus and Manifold

Sequential Injection System

The manifold configuration consisted of a cam driven syringe pump and an electrically actuated 6-port selection valve and has been described previously (6). The valve and pump were connected together in such a way that the valve position was incremented at every 30 degree rotation of the cam, by a signal generated at a microswitch mounted on the pump. The sequential flow manifold designs used are shown in Fig 1B and 1C.

Flow Injection System

The same pump that was used in the sequential system was used in this design. The valve used was a 10-port electrically actuated injection valve (Valco Instruments, Houston, TX). In this system, two microswitches were mounted on the pump and could be triggered by the rotation of the cam. The switches were placed such that the valve was switched at the points where flow from the pump was zero (i.e., at cam angles of 0 and 180 degrees). A 1 ml. syringe was used for the filling of the sample loop for 490 µl samples and a 3 ml syringe for 700 µl samples with a 5 ml. syringe used for the aspiration and delivery of the carrier solution in both cases. Fig. 1A shows the flow system for these experiments. Table I details the tubing lengths used in these studies. All tubing was 0.8 mm I.D. PTFE tubing.

Table I: Comparison of system parameters for the different manifolds.

	Sequential Injection		Flow Injection		
	Single Zone	Dual Zone	Electrochemical pH	Spectroscopic pH	Glucose
Wash Volume	2.7 ml	1.9 ml	3.5 ml	3.5 ml	3.5 ml
Sample Volume			490 µl	700 µl	20 µl
Zone 1	800 µl	800 µl			
Zone 2		800 µl			
Holding Coil	2.5 m	2.5			
Mixing Coil	5 cm	5 cm	5 cm	5 cm	60 cm
Dispersion			1.05	1.05	6.3
Zone 1	1.04	1.04			
Zone 2		1.56			

Sensors

Electrochemical

The measurements of pH by a potentiometric sensor were conducted with a Lazar flat glass combination electrode (Lazar Research Labs, Mfr. Number 1113, Los Angeles, CA) utilizing the flow cell shown (Fig. 2A). The electrode was connected to a Corning Model 145 pH meter and the signals were recorded on an REC80 Servograph (Radiometer, Copenhagen, Denmark) equipped with an REA112 High Sensitivity unit.

The glucose measurements were performed with a Yellow Springs Instruments (YSI) Model 18283 glucose electrode equipped with a Lucite flow cell (Fig. 2B) and a battery powered potentiostat. The sensor was operated using a YSI 2365 glucose oxidase (GOD) enzyme membrane as recommended by the manufacturer. The sensor response was also recorded by chart recorder output from the sensor's potentiostat.

Spectroscopic

The flow cell and sensor design for the spectroscopic pH studies have been previously described and the apparatus was used without further modification (Fig. 2C) (1). The spectrometer used for this work was a

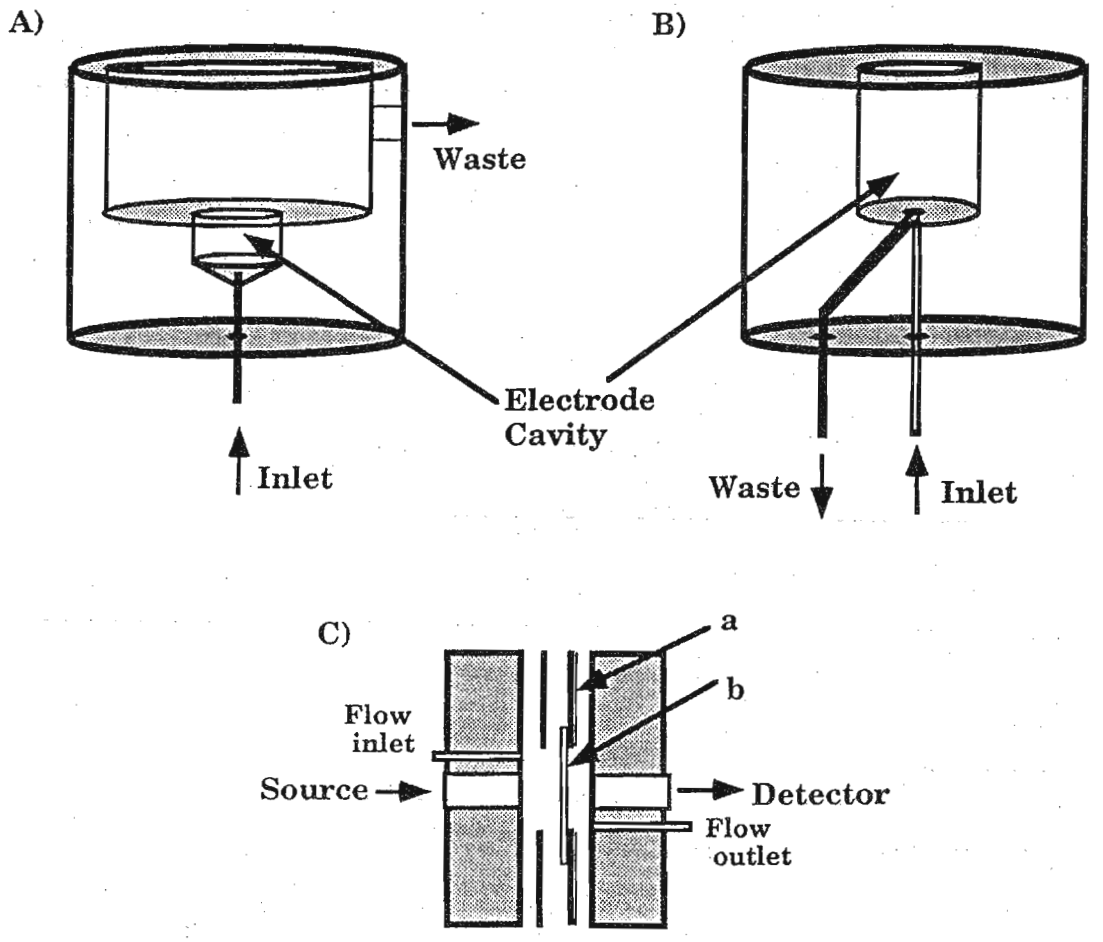

Figure 2. Schematics of flow cells. A) Flow cell for the measurement of pH by a potentiometric sensor. The electrode is held within the electrode cavity by three equally spaced set screws. The flow of the carrier and sample zones is directed across the sensing surface of the electrode by a small length of stainless steel tubing. B) Flow cell for the determination of glucose by GOD membrane electrode provided by Yellow Springs Instruments. The electrode is held within the electrode cavity by a spring loaded mechanism. The carrier and sample are directed across the face of the electrode as shown in the figure. C) Flow cell used in pH measurements by spectroscopy. The light source and signal are coupled into and out of the flow cell by fiber optic bundles. a) is a PVC spacer with the immobilized cellulose pad and b) is a PTFE spacer to provide the desired volume of the flow cell.

Brinkmann PC701 Colorimeter equipped with fiber optic cables. The pH pads used were ColorpHast pH indicator strips (EM Science, Gibbstown, NJ). The wavelengths used to monitor the pH of the samples were dependent on the particular pH pad being used. The three pads investigated were for pH ranges between 1-3, 6-9, and 9-12, with corresponding wavelengths of 520, 620, and 520 nm. Sensor response was recorded by chart recorder output from a laboratory-made logarithmic converter used to provide absorbance readings.

Reagents

All pH buffers were prepared following the procedures outlined in the CRC Handbook of Chemistry and Physics (7), except for the buffers at pH 6.88 and 9.22, which were prepared as described by NBS (8). All chemicals were used as obtained from J.T. Baker without further purification.

The glucose standards were prepared in YSI buffer 2357 solution as directed by the manufacturer. The carrier solution used in the flow system was also composed of the YSI buffer solution.

Procedure

Sequential Injection System

Sequential injection methodologies were used for electrochemical pH studies utilizing a 5 ml. syringe and examined the injection of both single and dual sample zones. The volumes of carrier and sample(s) aspirated are shown in Table I. The measurement cycle consisted of aspirating a volume of carrier solution followed by the aspiration of the sample(s). Once the cam had rotated to 180 degrees, the flow direction reversed and the sample was injected into the sensor flow cell. Following the measurement of the sample, the sensor cavity was flushed clean with carrier solution. In studies using a flow manifold as shown in Fig. 1B to measure pH by spectroscopy, the measurement of the sample was performed during the aspiration part of the cycle. A second more dispersed measurement was seen during the expulsion of the sample from the flow cell.

Flow Injection System

The sample volumes used in the injection valve studies were 490 µl for electrochemical pH measurements, 700 µl for spectroscopic pH measurements, and 20 µl for the glucose studies.

During a measurement cycle, a continuous flow of sensor-compatible carrier solution was maintained through the sensor cavity except during sample injections and refilling of the carrier syringe, when the forward flow of the carrier was temporarily stopped to allow the valve to switch without substantial pressure buildup. Following sample injection, the sensor cavity was flushed with the sensor compatible solution used as the carrier stream.

For spectroscopic pH studies, the carrier was a buffer solution which generated a fixed response from the pH indicator immobilized on the cellulose pad. For the pH pads responsive to pH between 1-3 and 9-12, a buffer at pH 6.88 was used as the carrier, and for the pH pad used in the range of 6-9, a buffer at pH 4.7 was used as the carrier. The potentiometric pH studies were performed with a pH 6.88 buffer solution as the carrier.

RESULTS AND DISCUSSION

Two different approaches were used in this work to measure the pH of samples. Each of these methods, electrochemical and spectroscopic, are discussed separately.

pH Measurements by Electrochemical Sensor

The electrochemical sensor utilized was a Lazar flat glass combination pH electrode which was commercially available. The electrode was held within a laboratory designed flow-through cell (Fig. 2A). The placement of the sensor flow cell within the flow manifold in the sequential system was critical for the proper operation of the electrode, due to the fact that during the aspiration and injection portions of the measurement cycle different forces act upon the sensor. If the sensor was placed between the pump and the valve, then during the aspiration period the sensor was subjected to reduced pressures, which caused the inner filling solution of the electrode to be aspirated through the junction, resulting in sensor failure. If the sensor was placed downstream from the valve,

then only positive pressures were experienced by the sensor during the measurement cycle. Hence, the sensor was placed downstream from the valve in all potentiometric experiments.

As the sample zone was injected into the flow cell, it was directed onto the electrode sensing surface by a small length of steel tubing, taking advantage of the wall jet effect. It passed across the sensing surface toward the exhaust port, resulting in a response signal from the pH meter output to the chart recorder. The baseline of the recorder was set at 50% of full scale to allow the recording of both positive and negative deflections. The baseline corresponded to a pH of 6.88, which was the pH of the carrier stream.

The first system used to evaluate the methodology consisted of a two-position injection valve and a two-channel syringe pump (Fig. 1A), configured in a conventional flow injection orientation. By utilizing both of the pistons available on the pump, it was possible to ensure that only uncontaminated sample was injected for analysis during each measurement cycle. While carrier was aspirated into the top syringe, the sample flowed through the sample loop and into the bottom syringe, and only the last portion of aspirated sample was within the sample loop at the time of injection. This allows the cleansing of the sample line during each measurement to prevent cross contamination. During the injection portion of the measurement cycle, the carrier transported the sample within the sample loop through the flow cell and to waste, while the sample syringe expelled the excess aspirated sample to waste.

The calibration curves obtained in this configuration yielded a response slope of 56.0 mV/decade and a correlation coefficient of 1.000 . An evaluation of the predictability of pH under this methodology yielded a standard error of prediction (SEP) of 0.16 pH units. The relative standard deviation (RSD) of measurements (n=21) at pH 9 was excellent (e.g., 0.3% for the recorded signal).

A second system that was tested utilized sequential injection methodology. These studies involved the injection of both single and dual sample zones, where in dual zone injections the first zone injected through the sensor was the sample of interest and the second zone was a standard to monitor the performance of the sensor over time.

Sequential injection methodology has been described previously (1). The basic principles involve aspirating a defined volume of wash solution through a holding coil into a carrier reservoir contained in one of the syringes.

The multiposition selection valve is then switched to the port containing standard buffer solution for a dual zone method. The desired volume of standard is then aspirated into the holding coil. The valve is then switched to the sample port, where the desired volume of sample is aspirated into the holding coil. The zones which are currently present in the system are stacked within the holding/mixing coil (Fig. 3). Finally the valve is switched to the port leading to the detector and the entire contents of the syringe and holding coil are expelled, carrying the sample and standard (if present) through the detector flow cell to waste. To ensure adequate cleansing of the system before the next cycle, it is necessary that the volume of wash solution used be four to five times the volume of the system downstream from the valve. The holding coil serves to prevent contamination of the contents of the syringe by any samples or reagents aspirated, which could lead to cross contamination.

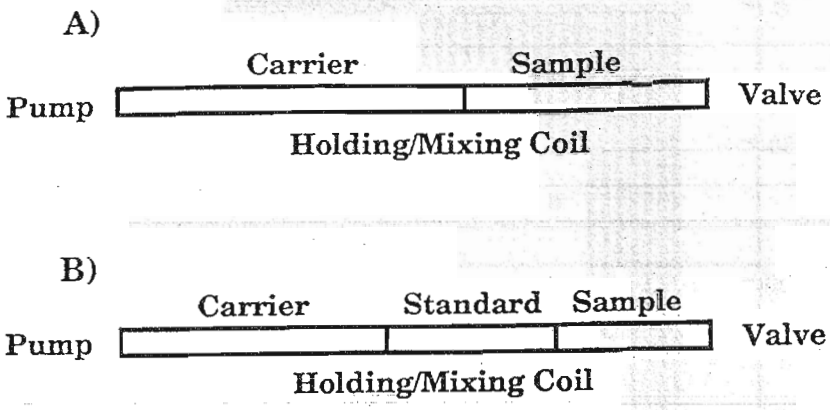

Figure 3. Representation of zones present in the holding/mixing coil just prior to injection cycle, for the sequential manifold Fig. 1C, using a A) single and B) dual zone method.

A typical set of recordings for multiple injections of various pH buffers utilizing single zone methodology is shown in Figure 4. The calibration curve obtained for the sequential method yielded a response slope of 57.0 mV/decade and a correlation coefficient of 0.995. An evaluation of the predictability of pH with this methodology yielded a standard error of prediction (SEP) of 0.16 pH units. The standard deviation of measurements was excellent and is illustrated for pH 9 in Figure 5.

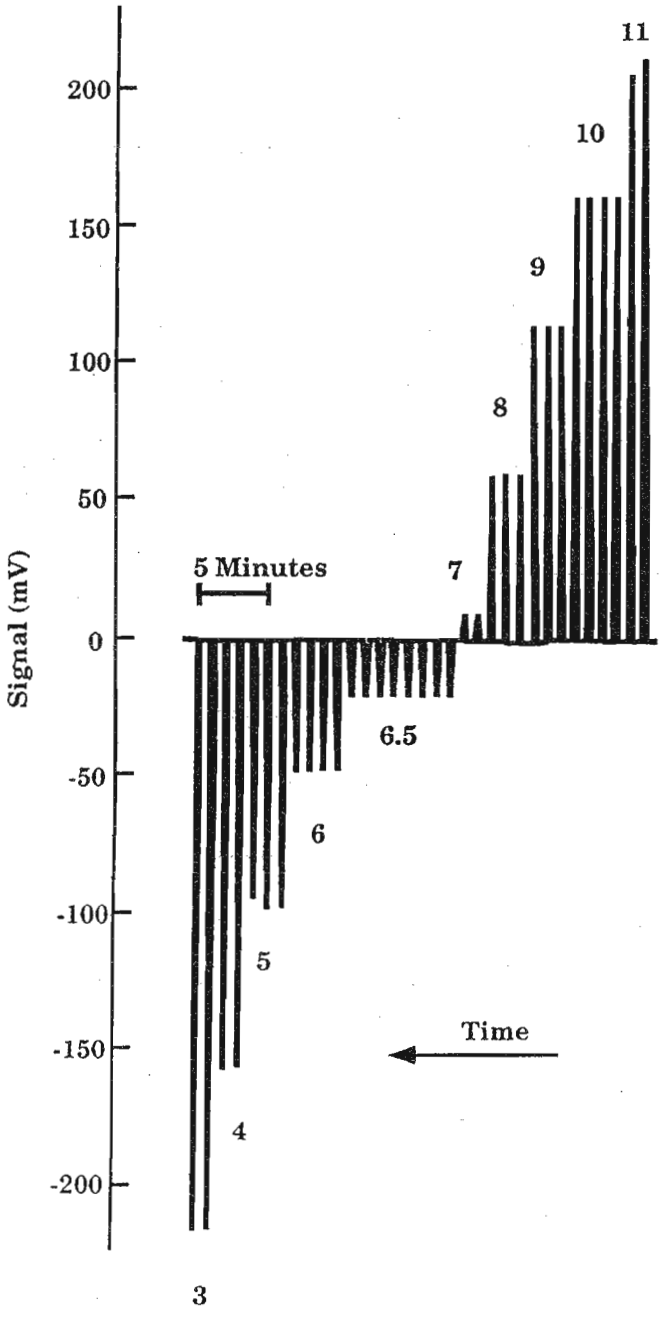

Figure 4. Replicate injections of various buffer standards using single zone sequential injection methodology. The electrode responses are shown with a baseline pH of 6.88. The pH values of the buffer standards are shown for each set of injections.

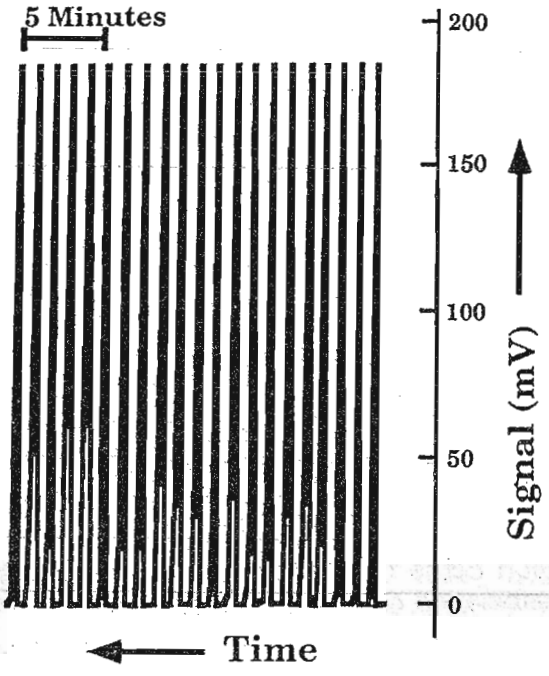

Figure 5. Replicate injections of pH 9 buffer using single zone sequential injection methodology to determine precision. (n=21)

An orthogonal full design (9) was used for the evaluation of the series of investigations utilizing a dual zone method. For the calibration set, pH 4, 7, and 10 standards were used, thus yielding nine calibration measurements. The sample set consisted of 13 combinations which covered the range from pH 2 to pH 12. The SEP for this method was 0.31 pH units with a regression coefficient for the calibration set of 0.996. The response of the electrode in these experiments was less Nernstian, at 51.0 mV/decade (Fig. 6).

The increase in prediction error in the method where dual standard/sample zones were injected and the increase in variance of the calibration measurements was most likely due to the increased dispersion of the calibration standard which traveled further into the holding coil. The sampling frequency was determined by the speed setting of the pump (100 rpm) which allowed 45 samples/hr.

Spectroscopic pH

The spectrophotometric determination of pH was performed in the identical manifold systems as the electrochemical determination of pH, with the

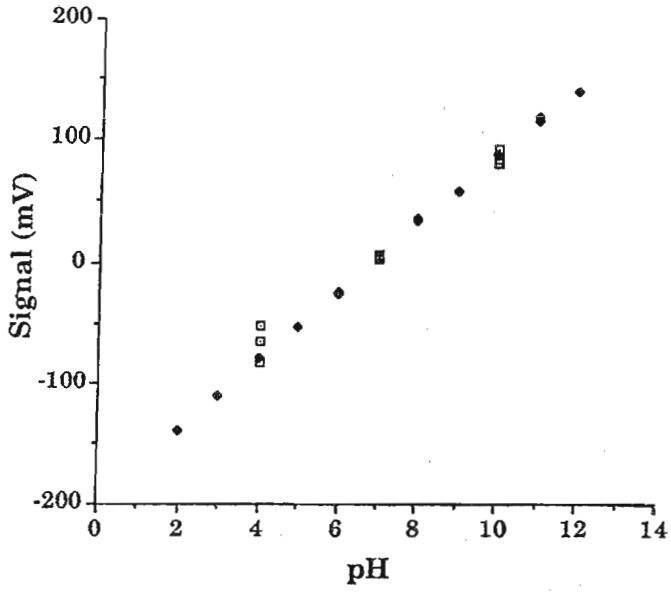

Figure 6. Calibration curve for electrochemical pH measurements with the Lazar flat glass combination electrode and dual zone injections by sequential injection methodology. Calibration sample set shown as open squares and sample set as solid diamonds.

exception that the sample volume was 700 µl. As can be seen by the response curves obtained for the injection of various pH standards with the different indicator pads used, the response is that expected for an acid-base indicator over its responsive pH range. A sigmoid curve centered about the pKa of the indicator is obtained (Fig. 7).

The conventional flow injection manifold design shown in Fig. 1A yielded a calibration curve with the three pads investigated which had a regression coefficient of 0.991 and an SEP of 0.14 pH units. The RSD (n=10) for this system was 0.02 pH units. The sequential injection method yielded comparable results: regression coefficient of 0.994, utilizing the region between pH 6 and pH 10.

Glucose Determination

A conventional flow injection system was used for the determination of glucose (Fig. 1A). The performance of the sensor was evaluated for glucose standards prepared according to the manufacturer's instructions and yielded a calibration curve with a regression coefficient of 0.999 and an RSD (n=10) of 0.67% at 10 mM. The linear dynamic range of the sensor was found to be 0.5 to 200 mM.

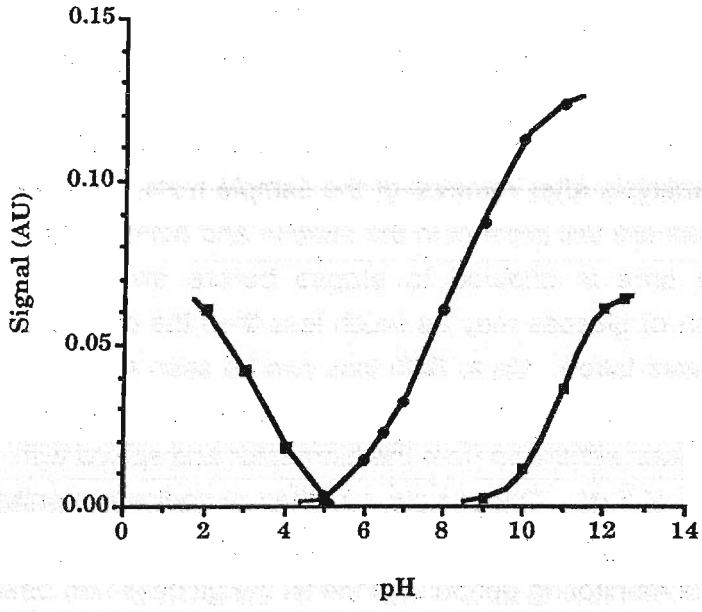

Figure 7. Response curves for the spectroscopic measurements of pH. The three cellulose pads used are shown as: 1) pad 1 sensitive to pH 1-3 (solid squares), 2) pad 2 sensitive to pH 6-9 (solid circles), and 3) pad 3 sensitive to pH 9-12 (solid diamonds). The response observed is consistent with the titration of an acid-base indicator over its sensitive range.

A small bench scale bioprocess reactor is in operation at the Center for Process Analytical Chemistry serving as a test bed for sensor development. The bioprocess produces ethanol by yeast fermentation utilizing ammonia and glucose as nutrients. The monitoring of glucose levels during the process is of importance to optimize yield. This system provided an evaluation of sensor fouling and cleanup. Since all current methods of analysis require the filtering of samples to remove the yeast cells prior to the actual determination of glucose, it was desired to use the sensor injection system for the monitoring of both filtered and unfiltered samples withdrawn from the bioreactor and to compare the results.

The results obtained for filtered samples follow the expected trend for the glucose fermentation. The fermentation being run at the time this data was taken involved beginning the run with a high glucose concentration. The batch was inoculated with the yeast cells and allowed to progress for ten hours. At this point, glucose was fed to the reactor every hour for the remainder of the experiment. The experiment ran 95 hours. The glucose profile expected for this experiment was an initially high glucose level (5 g/l), followed by a gradual decrease over the initial 10 hours, and the high concentration spikes every hour

for the remainder of the experiment. The lifetime of a spike was typically 10-15 minutes.

The monitoring of unfiltered samples is difficult since it requires immediate analysis after removal of the sample from the bioreactor, because the yeast cells are still present in the sample and actively consume glucose. If appreciable time is allowed to elapse before analysis, the measured concentration of glucose may be much less than the concentration at the time the sample was taken. Up to 50% loss can be seen to occur within the first 5 minutes after sampling (Fig. 8). This is demonstrated by an experiment where a 5 ml sample was withdrawn from the bioreactor and spiked with glucose up to a concentration of 5 g/l. The sample was then periodically monitored by glucose sensor injection and showed a steady decrease in glucose concentration over the 10 minute monitoring period until the all the glucose had been consumed by the yeast cells. The negative signal observed at baseline is a capacitance spike due to the change in ionic strength between the sample and the carrier.

Over the course of the experiment monitoring the glucose levels in filtered samples withdrawn from the bioreactor, it was observed that the slope of the calibration curve decreased with time (Fig. 9). This could have been due to

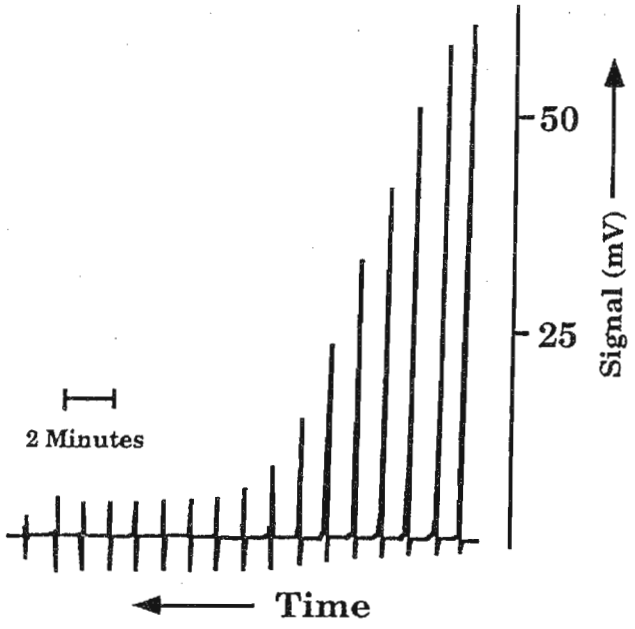

Figure 8. Monitoring of the glucose content in an unfiltered fermentation broth sample after spiking with glucose to a level of 5 g/l. Decrease in glucose signal with time is due to the consumption of the glucose by active yeast cells which are present in the sample.

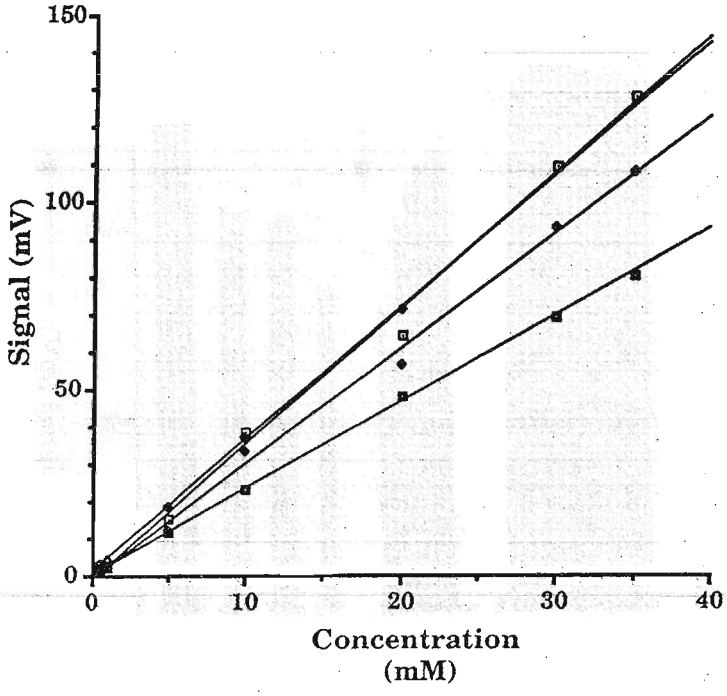

Figure 9. Changes in calibration curve of the glucose sensor as time progressed during the fermentation experiment. The initial calibration run before beginning to monitor the bioprocess is shown by the open squares. As monitoring continued over the period of three days, the calibrations were repeated daily and are shown as solid diamonds (day 2) and solid squares (day 3). The calibration curve obtained after treatment of the electrode with protease wash for 200 injections is indicated by open diamonds.

the loss of enzymatic activity caused by surface fouling of the membrane by the sample. By treating the membrane with a solution of the carrier to which was added 0.1% by weight of a cleaning enzyme mixture over a period of two days (approximately 200 injections), the slope of the calibration curve was restored close to the original slope obtained at the time when fermentation monitoring began (Fig.10). The enzyme used was obtained from Novo-Nordisk and was a mixture of a protease, a lipase, and an amylase. This would indicate that the surface of the membrane must have been fouled to some degree.

CONCLUSIONS

It can be seen that the two methods of performing sensor injection differ in their applicability. The use of flow injection configurations (Fig. 1A) is simple, robust, stable, and universally applicable. These may be used with much of the

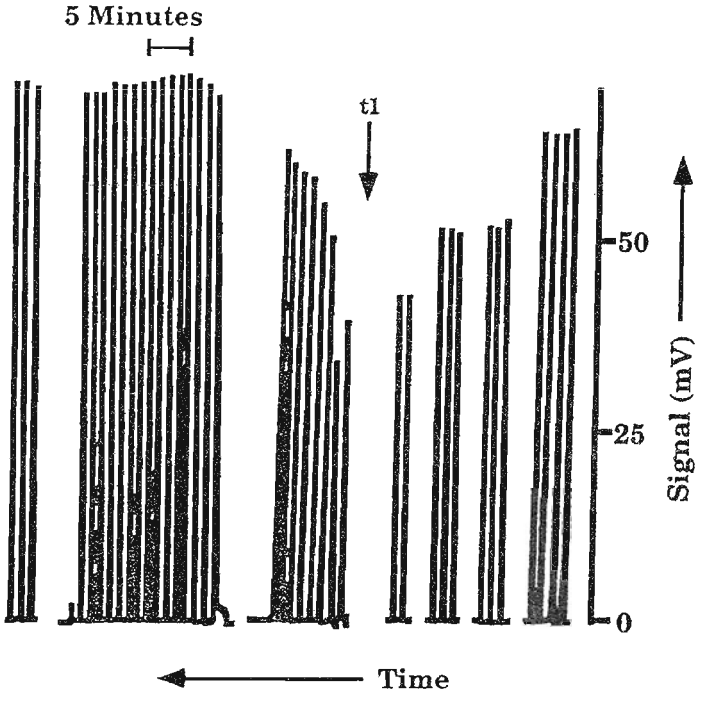

Figure 10. Response of the glucose sensor over the one week period of monitoring the fermentation and regeneration of sensor response during enzymatic treatment. All injections shown are for a 10 mM standard. A carrier stream contained 0.1% by weight of enzyme dissolved in the manufacturer's buffer solution was introduced beginning at point, **t1**. As the washing of the membrane with this enzyme solution continued, the response of the electrode to injections of 10 mM standard gradually increased until the response returned to close to the level exhibited at the beginning of the fermentation experiment.

equipment currently available, require no computer or custom software to run, and may be set to continuously run unattended. Sensor injection with the sequential injection technique, however, requires the use of a computer and custom software to exploit its flexibility.

The position of the sensor and the internal volume of the flow cell have been shown to be the most important variables in the choice of manifold design. For sensors which require undiluted sample to be measured, such as a conventional pH glass electrode measurement, the placement of the sensor is most critical. It was originally suggested by Ruzicka and Marshall (1) that sensor injection should be performed with the sensor between the pump and the valve (Fig. 1B). Although this provides a sample with the least amount of dispersion entering the sensor cavity, it was found here to be unsuitable for such sensors which do not tolerate an underpressure caused by flow reversal,

such as the junction of a glass electrode/reference pair. It also yields a more complex readout of the sensor's response, since the sensor response is seen for the analyte passing through the cavity twice. Therefore configurations of the type shown in Figures 1A or 1C are preferable for sensors similar to the glass combination electrode, whereas, for sensors similar to the spectroscopic pH sensor, all configurations will provide satisfactory performance.

It has been shown in these studies that this methodology may be used to recalibrate and recondition a sensor periodically during operation. By monitoring a sensor's performance by the periodic injection of a standard, it is possible to compensate for sensor drift and to indicate possible sensor failure. If a mechanism for sensor regeneration is available on-line, this may be initiated automatically once sensor performance drops below a specified level.

Equally important is the observation that the glucose content of the unfiltered fermentation broth can be successfully monitored by the Yellow Springs glucose sensor, and that the slow decrease in response with time may be reversed by injection of a proteolytic enzyme.

Since the simplicity of sensor injection methodology lends itself quite well to further miniaturization, it is worth considering the incorporation of flow injection or sensor injection methodology as an integral part of future technology for chemical sensors. The developments currently being made in the fields of micromachining and the production of microvalves and micropumps on silicon wafers allows us to visualize a sensor injection device on a single silicon chip(10).

Also recent progress in utilizing a single standard injection for the multipoint calibration of a flow injection system hold great promise for application to sensor injection (11,12), since it allows periodic recalibration of the sensor over the entire range of analyte concentrations with a single injection.

Finally, sensor injection methodology lends itself to the design of a small and portable chemical analyzer. The ability to take the chemical instrumentation to the sample site rather than taking the sample to the instrumentation will allow better utilization of diagnostic techniques and more efficient evaluation of potential problem sites.

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