

USER MANUAL FOR GLUCOSE ASSAY KIT

Kit p/n: MF-8925
Manual p/n: MF-9050

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Introduction

The most common - and most sensitive - means of detecting glucose is by the coupling of the specific enzyme glucose oxidase to d.c. amperometry (1). Such a combination forms the basis for the blood glucose analyzer which has been used in clinical laboratories for over two decades. A platinum working electrode, poised at the appropriate potential to sense hydrogen peroxide, is covered with one or more membranes, one of which contains covalently bound glucose oxidase. The electrode is dipped into the stirred solution to be analyzed, and glucose and oxygen are brought to the electrode by a combination of convection and diffusion. H_2O_2 is produced, and its oxidation current is measured. The membrane containing glucose oxidase is periodically replaced.

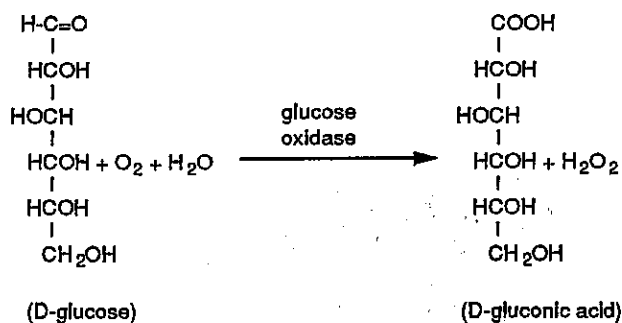


Figure 1. Conversion Scheme for Glucose to H_2O_2

Because of the importance of glucose determinations in body fluids and foods, a large number of other detection schemes have been devised including colorimetric (2), GC (3), UV (4), reflectometric (5) potentiometric (6) and fluorimetric (7). Several of these methods require derivatization or time-consuming sample preparation. Flow injection analysis (FIA) is widely used for glucose determinations (8-12). FIA has no separation function. Depending on the chemistry used, interference from reducing agents such as ascorbic acid or uric acid can be a problem for some biological samples. In the case of amperometric FIA, the problem can be minimized by imposing a membrane between the electrode and the flow chamber (13). The membrane can be either charge or size selective.

Many liquid chromatography methods have been reported for the determination of glucose and other sugars. Ion exchange chromatography with a pure aqueous mobile phase (14, 15), or reverse phase chromatography using an amine column with 65% acetonitrile (16), using an ODS column with water as mobile phase (17), or using a silica column with an aqueous acetonitrile mobile phase containing an amine modifier (18, 19) were reported using a refractive index detector. A pulse amperometric detector (PAD) with a gold electrode has been reported for determination of glucose (20). Glucose has a pK of 12.28, and thus can be converted to an anion at high pH. Using an ion exchange column and 15 mM NaOH solution as an eluant allows the isocratic separation of a number of sugars, which were detected with the PAD. Another LCEC method, first published by Watanabe, utilized the post-column reduction of $\text{Cu}(\text{phen})^{2+}$ by reducing sugars and the subsequent oxidation of the generated $\text{Cu}(\text{phen})^+$ at a glassy carbon electrode for the determination of a number of sugars (21).

In this BAS kit, modern liquid chromatography is coupled to the enzymatic determination of glucose. However, unlike previous glucose analyzers, the enzyme is immobilized in a small column rather than a membrane. This difference allows for the complete conversion of glucose, since more enzyme can be bound on a high surface area stationary phase than on a membrane.

Kit Principle

Two cartridge columns are coupled in series in the liquid chromatography system (Figure 2).

First in line, the analytical column separates glucose from ascorbate, uric acid, and other electrochemically active molecules in the sample. Retention is governed by mobile phase factors such as pH, ionic strength, and ion pairing agents. The second column, a ready-to-use immobilized enzyme reactor, is coupled to the outlet of the analytical column. 60 U of glucose oxidase is reacted with this support by BAS personnel. It is to be stored at 4 °C prior to or between uses.

Amperometric detection is used because the hydrogen peroxide is easily oxidized at a Pt electrode held at +0.5 V vs. Ag/AgCl. While H₂O₂ can also be detected on carbon electrodes, the kinetics of electron transfer are slow, and the overall selectivity of the method suffers.

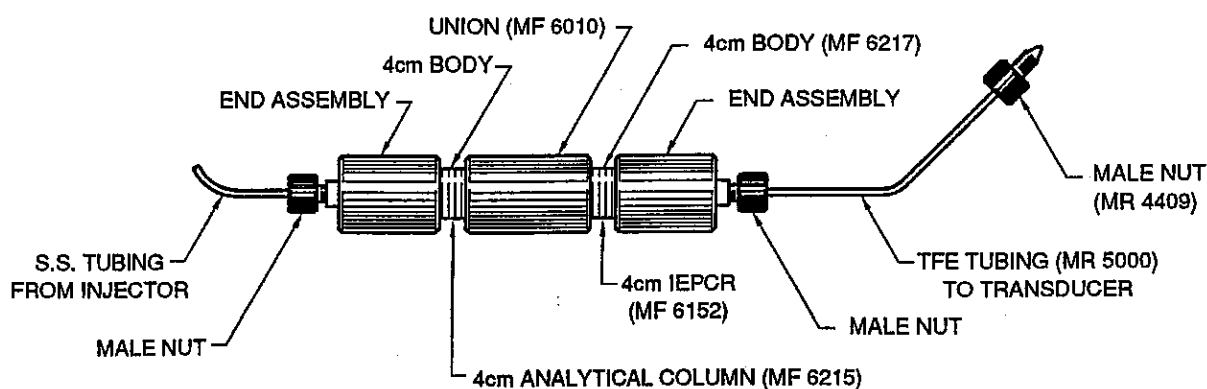


Figure 2. Column hardware for the kit

Advantages of this Kit

- The enzyme column is made from a polymer backbone rather than silica. Polymer packings are rugged from pH 2 to 13.
- The enzyme is covalently immobilized. This results in a more durable enzyme reactor.
- The system has been applied to the diverse analysis of microdialysates, foods and blood serum.
- The system follows the well-known acetylcholine/choline LCEC method (22).

Kit Inventory

Quantity	BAS P/N	Description
1 bottle	CF-2150	1% Kathon CG reagent, 100 mL
1 ea.	MF-6152	Glucose oxidase enzyme reactor, 40 x 2.1 mm
1 pk.	MF-6215	Phase II ODS 3µm column, 40 x 3.2 mm, pk. of 2
1 ea.	MF-6217	4 cm column holder body
1 ea.	MF-6010	Union
1 ea.	MF-6020	4 cm column holder
1 ea.	MF-1012	Dual platinum electrode bottom
1 ea.	MF-9050	Glucose kit manual
1 ea.	MR-4013	20 cm x 0.007" i.d. stainless steel tubing (column ↔ injector)
1 ft.	MR-5000	PTFE tubing (column ↔ detector)
1 ea.	MR-4409	Plastic nut and ferrule (use at detector)
1 ea.	MF-1046	Gaskets for electrode
1 ea.	--	Ice pack
1 ea.	--	Insulated shipping container
1 set	--	Material safety data sheets
1 ea.	CF-1049	Membrane application solution

Start Up Procedure

Summary

1. **Flush out the LC system.** This step will passivate all metallic surfaces and kill any resident bacteria. This step is mandatory.

Note: Bacteria will be present in most LC systems using the conditions below unless you take this necessary precaution. Bacteria are bad because they produce catalase, a very efficient enzyme scavenger of the hydrogen peroxide you wish to detect! The use of a bactericide is recommended in the mobile phase.

2. **Install the analytical column, the enzyme reactor, and Pt electrode.** The system is then equilibrated using the mobile phase provided.
3. **Make standard solutions.** Inject a test mixture.
4. **Fine tune the separation.** Variations in mobile phase ionic strength, solvent strength, and column temperature are used to provide optimal elution patterns. Charge or size-selective membranes may then be added to the working electrode, depending on the complexity of the sample.

STEP 1. Flush Out the LC System

1. Make three solutions:
 - a. H₂O. Filter 2 L of LC grade, deionized H₂O through a 0.2 μ m filter. 15-18 megohm-cm service is recommended.
 - b. Nitric acid solution. Add 100 mL of concentrated HNO₃ to 200 mL of H₂O. CAREFUL! Observe safety precautions. Use protective gear over face, arms, hands, etc. to avoid injury.
 - c. Acetonitrile/H₂O solution. Add 100 mL LC-grade acetonitrile to 900 mL LC-grade H₂O. Filter through 0.2 μ m membrane.
2. Disconnect the column inlet tube at the old column, if present, and divert all flow from the inlet tube to a waste receptacle.
3. To remove any previous mobile phases, especially buffers, pass through 50 mL of H₂O.
4. Pass through 50 mL of the nitric acid solution to passivate metal surfaces and kill bacteria. Flush both paths through the injection valve (LOAD and INJECT positions).
5. Repeat step 4, but use 50-100 mL H₂O. Keep pumping until the pH is 3 or greater. Test with pH paper. Remember to flush out both sides of the injection valve. If you are using the system immediately, skip to the next section.
6. Flush and store the system with 100 mL of the acetonitrile/H₂O solution.

STEP 2. Couple the Analytical Column, Enzyme Reactor, and Platinum Electrode

1. Make mobile phase as follows:
 - a. To approximately 900 mL LC-grade water (Millipore, Barnstead, etc.), add 2.76 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (20 mmoles), and stir to dissolve.
 - b. Add 750 μL of 1,5-dimethylhexylamine (Aldrich p/n: D16,129-2) and stir until completely mixed.
 - c. Calibrate your pH meter using pH 4 and pH 7 calibration buffers.
 - d. Adjust pH to 5.5 ± 0.05 with dilute phosphoric acid solution.
 - e. Add 5 mL of the 1% Kathon CG reagent supplied with the kit.

Note: 1% Kathon CG solution is a skin sensitizer; avoid skin contact. Wash with water immediately to prevent skin irritation. Further technical information is included in the Notes/Cautions section and on the Material Safety Data Sheet included with this kit.

- f. Pour the resulting solution into a 1.0 L volumetric flask and dilute to the mark with water.
- g. Filter through 0.2 μm membrane filter.
- h. Mix well, then cap to avoid evaporation.

Note: Kathon retards bacterial growth. The mobile phase should be made fresh weekly, if not more often. Designate an expiration date on the label in order to keep track of batches.

2. Flush out the system with the mobile phase. If the system has been stagnant in this mobile phase for more than 7 days, or if you are using mobile phase more than 7 days old, perform all steps in "Flush Out the LC System" above, before flushing with new mobile phase. To be sure all traces of acid are gone after flushing, test the pH of the effluent with pH paper before installing the enzyme column. Value should be in the 5-6 range.

- Couple the analytical column to the enzyme reactor using the union. The inlet of the analytical column is to the left as you read its label. The reactor's direction is not important. Connect the analytical column inlet to the injection valve with the steel tube provided. See Figure 2.

Note: the knurled cartridge end couplings should be only finger-tightened. Use the steel nuts and ferrules supplied with the cartridge holders to attach the tubes to the end couplings.

- Attach the detector to the enzyme reactor with the plastic outlet tubing and plastic fitting provided.
- Install the working and reference electrodes on the detector. See detector manual for details.
- Set flowrate to 0.8 mL/min and flush out the entire system with mobile phase.
- Set parameters:
 - range or gain = 500 nA f.s.
 - filter = 0.1 Hz or 2 sec. depending on model
 - potential = +700 mV vs. Ag/AgCl
 - recorder = 1 V full scale
 - chart speed = 2 min/cm
- Turn on the detector cell. The offset will initially surge offscale and then begin to subside.

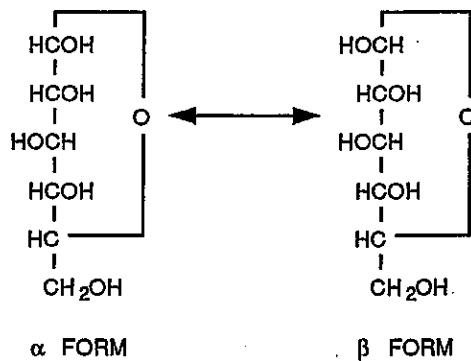
STEP 3. Make Standard Solutions

Glucose stock standard (2.0 mM)

Carefully weigh 36.0 mg of β -D(+)- glucose solid and transfer to a 100 mL volumetric flask. Dilute to the mark with mobile phase.

Note: Once in solution, D(+)- glucose will exist as an equilibrium mixture of two forms, α and β . These differ in their specific rotation, melting point, solubility, and rate of oxidation by glucose oxidase. In water, the equilibrium lies at about 1/3 α -D-glucose and 2/3 β -D-glucose.

Only the β form is oxidized significantly by the enzyme.



It will take approximately 2 hours for these forms to come to equilibrium once dissolved. Thus, either the α - or β -D(+)-glucose solid may be used to make the standard. If you start with the α form in solution and make injections immediately, the peak heights will be quite small (mostly due to a β impurity) and then reach a maximal response at equilibrium. Conversely, if you start with the β form of the same concentration, the peak heights will be higher initially, but fall to the same equilibrium value.

There is no need for a correction factor to get total glucose, because 20 μM represents total glucose at equilibrium, and all samples will be affected identically.

Glucose Working Standard (20 μM)

Pipette 1.0 mL of glucose stock standard to a 100 mL volumetric flask and dilute to the mark with mobile phase.

Hydrogen Peroxide Standard

In order to calibrate system response and debug problems, it is useful to compare injections of the substrate to injections of the product, at the same concentration.

Note: 30% reagent grade hydrogen peroxide is a very strong oxidizing agent. Be sure that all glassware is clean and dry. Avoid any bodily contact with this solution. Wear goggles and gloves.

Dilute as follows:

- Dilute 1.0 mL of 30% H_2O_2 to 100 mL with water (conc. = 88.8 mM).
- Dilute 1.0 mL of 88.8 mM solution to 100 mL with H_2O (conc. = 888 μM).
- Dilute 2.26 mL of 888 μM solution to 100 mL with mobile phase (20.0 μM).

The offset should now be below 20 nA. If not, assess the cause by referring to the Cautions section below. Inject 20 μL of the glucose working standard by overloading the loop of the injector, and compare your results to Figure 3.

Adjustments, if necessary, may be made in the mobile phase composition after referring to the next section.

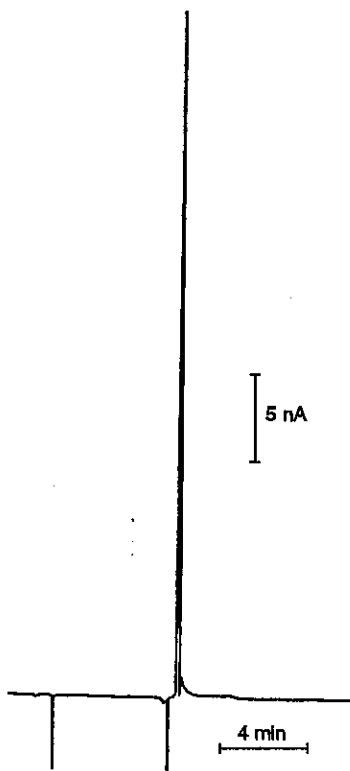


Figure 3. Left: mobile phase injection. Right: 400 pmole injection of glucose using 20 μM working standard

STEP 4. Fine Tune the Separation

The mobile phase is buffered at pH 5.5 in a range where enzyme activity is maximal (Figure 4), although it is certainly possible to work at more basic pH values up to 7 if desired. 1,5-Dimethylhexylamine acts as an ion pairing agent. At these pH values, it will be positively charged and enables longer retention of ascorbic acid and uric acid, both negatively charged.

You should allow 100-200 mL of mobile phase to pass through the system to waste before checking the retention of either ascorbate or urate, if these are potential interferences. You may increase their retention by using more amine in the mobile phase.

You can also fine tune the separation by simply preventing ascorbate and urate from reaching the electrode, through a perm-selective membrane applied to the surface. A vial of dissolved polymer is provided with your kit. The directions for applying the membrane are:

1. Pipette 2 μ L of the suspension over the clean, dry platinum electrodes and spread out the droplet over a diameter of about 8 mm. Use the side of a syringe needle.
2. Allow the electrode to sit in air at room temperature for 30 minutes. Make sure that the solvents are gone before using the block in a detector.

Do not inject pure solvents such as CH_3OH or acetonitrile onto the system. They will dissolve the film or damage the coating. The selectivity can be controlled by the thickness of the coating. A thicker coating will lower the response for ascorbic and uric acids. Simultaneously, the glucose response will decrease, but not to as great an extent.

The glucose response was linear up to 500 ng glucose injected when a bare Pt electrode was used (regression coefficient $r=0.9997$). The response was linear up to 400 ng injected when an anionic membrane-coated Pt electrode was used ($r=1.000$). Higher amounts were not tested. The detection limit and precision results are shown in Table 1. Ascorbic and uric acid are the most common compounds that would interfere with an amperometric glucose determination in serum, food, and other biological samples. In this study the ascorbic acid and uric acid peaks were well separated from the glucose peak. See, for example, Figure 10.

Other potential interfering substances were also tested. Hydrogen peroxide and sulfite, two common components in foods, eluted with glucose. However, unlike glucose, they are very unstable and easily converted *in situ* to electrochemically inactive compounds. Catecholamines had little response on the Pt electrode. For instance, 20 ng of norepinephrine, epinephrine and dopamine gave 0.5 to 3.0 nA responses. Since catecholamine concentrations in biofluids are low, their response would be negligible relative to glucose in diluted samples.

Coating the Pt electrode with an anionic membrane improved precision (Table 1). Although sensitivity towards glucose was reduced to 62% after coating, the selectivity was much improved (Table 2).

The effect of varying temperatures on system response was also tested between 25-35 $^{\circ}\text{C}$. Over this narrow range, each 5 $^{\circ}\text{C}$ increase in temperature resulted in an approximately 11% increase in the glucose response. Higher temperatures were not tested due to the enzyme.

Electrode Surface	Precision	Detection Limit (S/N=3)
Bare	2.2% (n=31)	0.3 ng injected
Coated	1.3% (n=27)	0.5 ng injected

Table 1. Comparison of coated and bare Pt electrodes

Analyte	Response
Glucose	62%
Ascorbic Acid	0.08%
Uric Acid	0.1%

Table 2. Selectivity of coated Pt electrode. Each result is relative to the same analyte's response on a bare electrode.

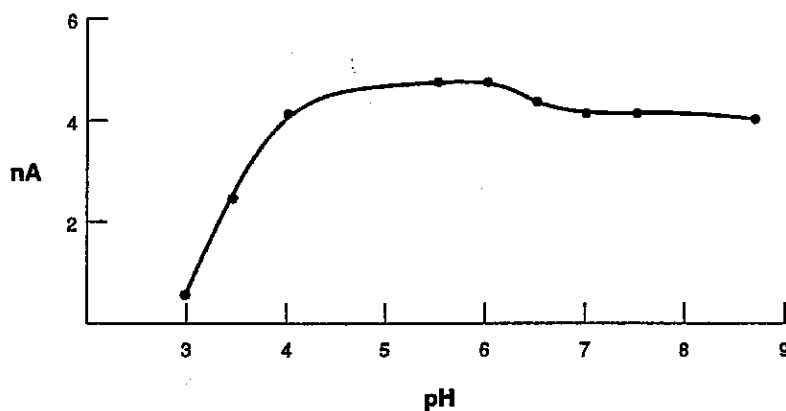


Figure 4. The relationship between mobile phase pH and glucose response. 5ng of glucose injected

Application to Microdialysates

For microdialysates, the Carnegie Medicin CMA/10 microdialysis probe (3 to 4 mm) was mounted on a CMA/130 *in vitro* test stand or a probe stereotaxic unit, and perfused with artificial CSF solution or Ringer's solution via a CMA/100 microinjection pump. The dialysate was collected in small Eppendorf tubes (CMA/140 fraction collector), diluted with mobile phase, and a 20 μL aliquot was injected into the analyzer. Microdialysis sampling of the extracellular space of the rat brain was accomplished using standard stereotaxic procedures.

Artificial CSF solution was prepared as follows: 126mM NaCl, 27.5 mM NaHCO_3 , 2.4 mM KCl, 0.5 mM KH_2PO_4 , 1.1 mM CaCl_2 , 0.85 mM MgCl_2 , 0.5 mM Na_2SO_4 , pH 7.5. Ringer's solution was: 147mM Na^+ , 2.3 mM Ca^{++} , 4 mM K^+ , 155 mM Cl^- , pH 6.0.

Glucose microdialysis was systematically studied. A microdialysis probe was inserted into standard glucose solutions. Flow rate and the glucose concentration were varied. Percentage recovery was calculated by comparison to the original glucose concentrations before microdialysis. Longer probes and solution stirring gave the highest concentration recovery at a given perfusion speed (Figure 5). Slower perfusion speeds also resulted in the expected higher relative recoveries. Perfusion rates of 1 to 2 $\mu\text{L}/\text{min}$ gave acceptable recoveries and convenient sample volumes.

Changes in concentration of the glucose solution (0.4-2.0 mg/ml) outside the probe did not influence the recovery (14% when a 3 mm long probe and perfusion speed of 2 $\mu\text{L}/\text{min}$ was used). However, a delay was found after each change in the sample solution because of the dead volume of the system. In order to see how fast the microdialysis procedure responded to changes in glucose concentration, five glucose solutions with different concentrations were sequentially microdialyzed, 5 minutes for each solution. The response obtained was delayed 2 minutes for all sample changes (Figure 6). The outlet tubing was 20 mm long, with a dead volume of 0.3 μL . If a sample solution was unstirred, a 10% lower recovery was found after 40 μL of dialysate collected. This resulted from reduction of the glucose concentration surrounding the probe surface by continuous microdialysis. When the solution was kept stirred, the recovery was independent of time.

The influence of solution pH on glucose recovery was studied in pH 3-8 phosphate buffer, artificial CSF (pH 7.5) and Ringer's solution (pH 6.0). A perfusion rate of 2 $\mu\text{L}/\text{min}$ was used. A constant recovery (20%) was obtained in all these solutions. Solution temperature affected recovery. The higher the temperature, the higher the percent recovery (Figure 7). Precision was routinely tested during microdialysis by inserting a probe (3 mm) in serum and perfusing with Ringer's solution at a speed of 2 $\mu\text{L}/\text{min}$. After equilibrium, seven 10 μL aliquots were collected, diluted and injected onto the analyzer. The CV obtained was less than 4%.

Since Ringer's solution can give a response at the same retention as glucose, a blank injection of perfusion fluid should be performed when rat brain dialysate is not sufficiently diluted with mobile phase. Increasing the retention of glucose under conditions compatible with glucose oxidase has been a difficult problem due to the very hydrophilic nature of the molecule.

In vivo brain microdialysis of an ether anesthetized rat indicated a glucose concentration of 0.35 mM. Postmortem, 35 minutes, the concentration was reduced to 0.03 mM in brain.

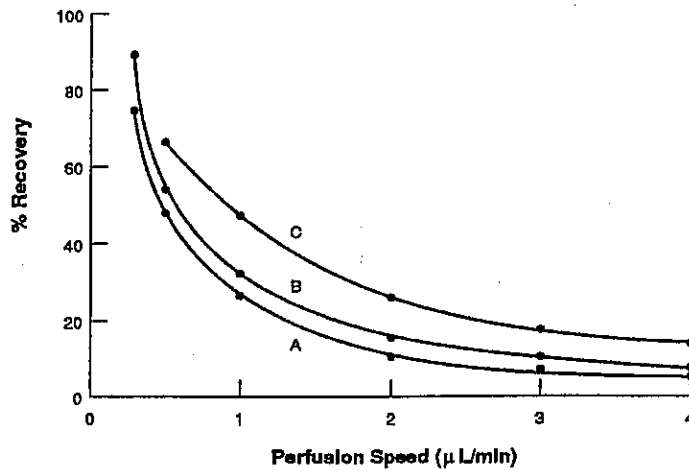


Figure 5. Recoveries at different perfusion speeds and stirring status. Ringer's solution was the perfusion buffer. The glucose sample was 1 mg/ml glucose in Ringer's solution. (A) 3 mm long probe, stirring, (B) 3 mm long probe without stirring, (C) 4 mm long probe without stirring.

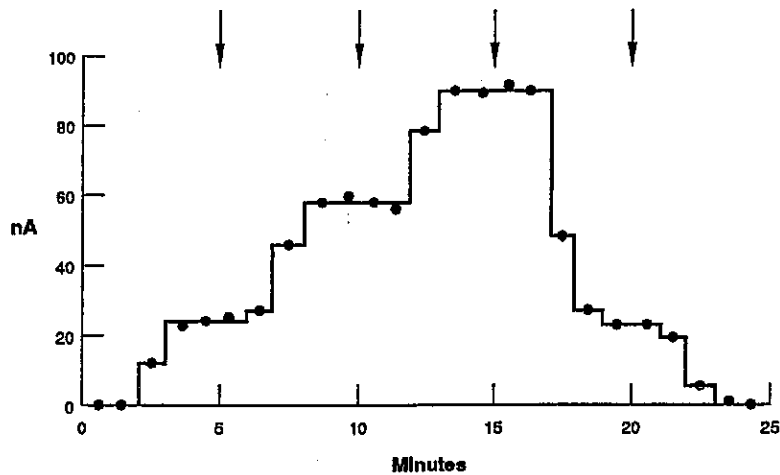


Figure 6. Delay associated with a microdialysis system. 4 mm long probe, 2 μL/min flow rate. The concentrations of four glucose solutions were 0.13%, 0.3%, 0.5%, and 0.13%. Each solution was dialyzed for 5 minutes, and 2 μL samples were collected.

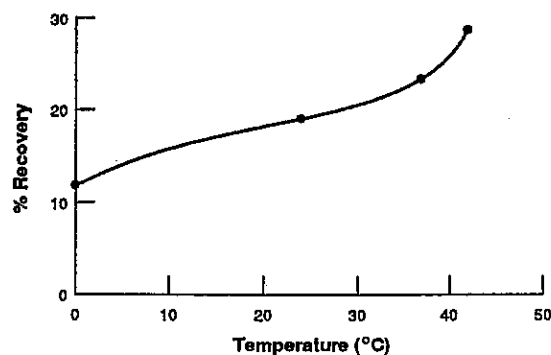


Figure 7. Effect of temperature on glucose dialysis recovery for 3 mm probe, 1.5 μL/min perfusion speed. Ringer's solution was perfusion buffer. The sample was not stirred.

Application to Foods

For all foods, a membrane coating is recommended over the electrode in order to increase selectivity.

For liquid samples: dilute sample with 200 - 2000 volumes of mobile phase (minimal sample size was 1 μL). Centrifuge if necessary and then directly inject 20 μL .

For solid samples: accurately weigh appropriate size sample (0.1 - 5 g) into sealable vial. Add 100 mL of hot water (80 - 90 $^{\circ}\text{C}$ for high fat samples) and shake vigorously. Let samples sit 3-10 min to allow insoluble material to settle or rise. Cool to room temperature in the case of high fat samples. Centrifuge if necessary; dilute 100 μL of supernatant to 10.0 mL with mobile phase. Inject 20 μL of the sample solution into the analyzer.

Note: since the analyzer is very sensitive, samples are normally diluted hundreds of times and sample-filtering is not necessary, but proceed at your own risk. The column can be protected with an inline filter or precolumn as an extra precaution.

The following food sample types are representative of those which have been studied with this analyzer:

white wine	apple juice	pineapple juice	instant banana
red wine	spiced cider	lemon juice	rice cereal
cola drink	potato powder	lime juice	chocolate

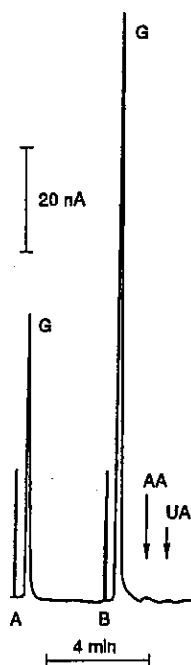


Figure 8. Glucose in lemon juice using a coated Pt electrode.

A. Glucose (G), ascorbic acid (AA), uric acid (UA) standards, 50 ng of each injected.

B. Glucose in lemon juice. 200-fold dilution, 20 μL injected.

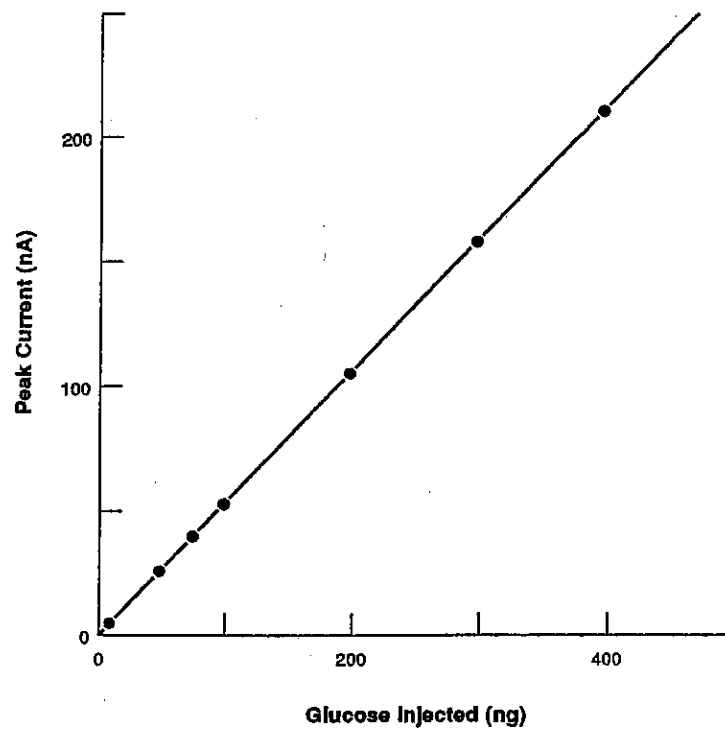


Figure 9. *Glucose calibration curve*

<u>Sample</u>	<u>Glucose (%)</u>
White wine	0.69
Red wine	3.6
Diet cola	0.013
Pineapple orange juice	8.9
Lemon juice	1.1
Lime juice	0.25
Apple juice	2.6
Spiced cider	1.8
Potato bud	0.88
Instant banana	15.3
Rice cereal	12.1
Cola	6.9
Chocolate 1	0.09

Table 3. *Glucose content on a weight basis*

Application to Blood Serum

Dilute 1 μL or more of serum with 200-1000 volumes of mobile phase. Directly inject 20 μL , or load the CMA/200 autosampler tray for autoinjection.

A standard addition calibration scheme was used for serum microdialysis. The regression coefficient of the calibration curve was 0.999.

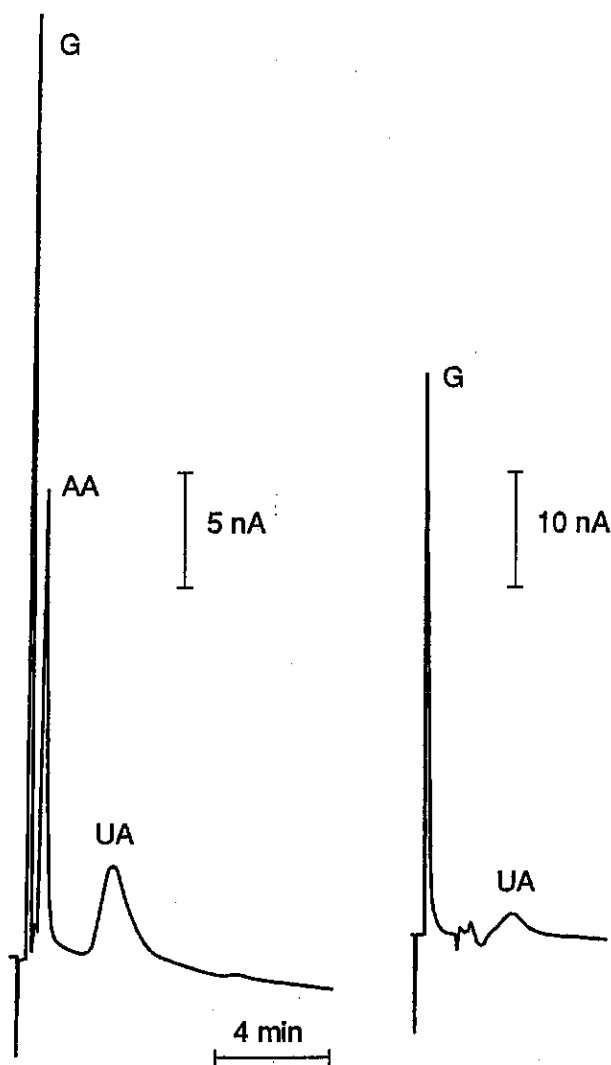
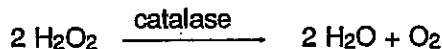


Figure 10. Chromatograms of glucose standards and samples. Left: Glucose (G), ascorbic acid (AA), and uric acid (UA) standards using a bare Pt electrode, 40 ng of each injected. Right: Glucose in serum using a bare Pt electrode, 500-fold dilution, 20 μL injected.

Notes/Cautions

1. Both the enzyme and analytical columns should not remain in stagnant mobile phase at room temperature. Keep the flow at 0.1 mL/min when not in active use, or store at 4 °C.
2. If the system will not be in use on a daily basis, return both the enzyme column and analytical column to storage at 4 °C. Both should be stored immersed in freshly made mobile phase containing Kathon, in the shipping tubes provided. If Kathon is not present or available, add 2 drops of toluene to each tube for anti-microbial control. You should clean the shipping tubes between uses with soapy water and rinse thoroughly with distilled water.
3. Enzyme activity is a function of use. The 60 units of glucose oxidase is more than sufficient to insure complete conversion of the substrates to H₂O₂. Due to this excess loading, you will not notice a lapse in conversion efficiency until the number of units drops below a certain value, even though some activity has been lost each day of use.
4. Don't underestimate the possibility of bacterial contamination in your system. The problem is due to the catalase produced by bacteria:



Turnover can be so high that virtually all peroxide is scavenged. You can test for gross bacterial contamination of your mobile phase by using special kits (e.g. Millipore #MSPC 000 25) or by following the flowchart in note 5.

5. A lack of method sensitivity can be attributed to several causes. These are:
 - a. Negligible enzyme activity remaining in the reactor
 - b. Inhibition of the enzyme
 - c. Bacteria
 - d. Membrane on electrode too thick
 - e. Passivation of platinum electrode or shift of reference electrode potential
 - f. No oxygen in the mobile phase - oxidase needs oxygen to function

The best way to determine cause is to compare the system's response to stoichiometrically-related equivalents of glucose and H₂O₂. Due to the stoichiometry of Figure 1, 20 μM solutions of hydrogen peroxide and β-D(+)-glucose should both yield the same peak area. However, due to equilibrium of the α and β anomers, only about 60% -70% of the H₂O₂ response should be seen for glucose.

The diagnosis can be made after two short experiments. The first checks the recovery of hydrogen peroxide injected through the system. You need to be sure that the enzymatic product can pass through the columns unscathed. If catalase is present, the recovery will be very low. The second experiment determines if the enzyme is functional.

Experiment 1

Remove the analytical column and the enzyme reactor. Connect a plastic 1/16" tube from the injector to the detector.

You will be measuring areas. A simple convenient way to do this is to use the highest chart speed on a strip chart recorder and then "cut and weigh" the peaks as a measure of area.

Inject the 20 μM peroxide solution and measure the peak area. Be sure it is made as described in Step 3, Make Standard Solutions. Also inject the mobile phase and subtract its area, if not negligible. Let A = net area due to peroxide. Do this in triplicate and take an average.

Now reinstall the analytical and enzyme columns and make the injections again, in triplicate. Let B = net average area with the columns in place. Calculate:

$$\% \text{ recovery} = 100 \times B/A$$

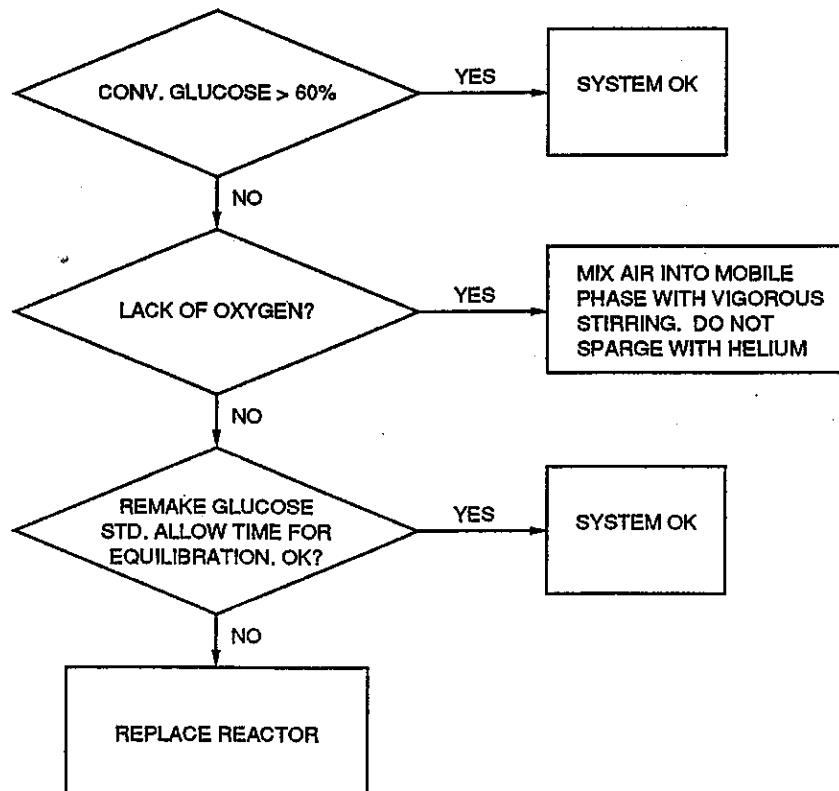
The recovery should be $100 \pm 20\%$. Low recovery of H_2O_2 is most likely due to bacteria or catalase.

Experiment 2

With both columns in-line, inject 20 μM peroxide and 20 μM glucose. Measure areas. Calculate:

$$\% \text{ conversion} = 100 \times \text{glucose area}/\text{peroxide area}$$

Use this data and the flowchart below to identify the problem.



6. With membrane-covered electrodes, there is an interesting hysteresis when you inject large amounts of hydrogen peroxide (>500 nA signals). To observe this, make several injections spaced 2 minutes apart. The peak height will drop as if the electrode is being passivated. However, if you wait 1 hour, the response will return to normal.

Recall that oxidation of hydrogen peroxide yields oxygen, a relatively insoluble gas. We speculate that within the pores of the membrane, the local oxygen concentrations become so high that bubbles may form and momentarily block out electrode area for subsequent injections. We do not see this with bare surfaces.

7. On the use of Kathon CG: A recent paper by Tyrefors and Carlsson in the Journal of Chromatography, 502 (1990) 337-350 suggests the use of Kathon CG, a cosmetic grade preservative, as an effective agent for preventing bacterial growth. We have evaluated this over a limited time period and believe it to be useful. A 1% solution in acetate buffer is now included in your kit along with the appropriate Material Safety Data Sheet. We are supplying this bactericide in diluted form since the original concentration is corrosive and capable of causing chemical burns. At 1:100 dilution, the dilution is classified as a "skin sensitizer".

Mix 5 mL of 1% Kathon CG (as supplied) with 1 L of mobile phase. Larger concentrations are no better effective and may cause cloudiness or precipitation in the mobile phase.

We find that a 1 L batch of mobile phase, suitably covered from dust, is good for at least 1 week. You may recycle the mobile phase if the tube from the detector back to the mobile phase vessel is first flushed with the nitric acid solution and then with water.

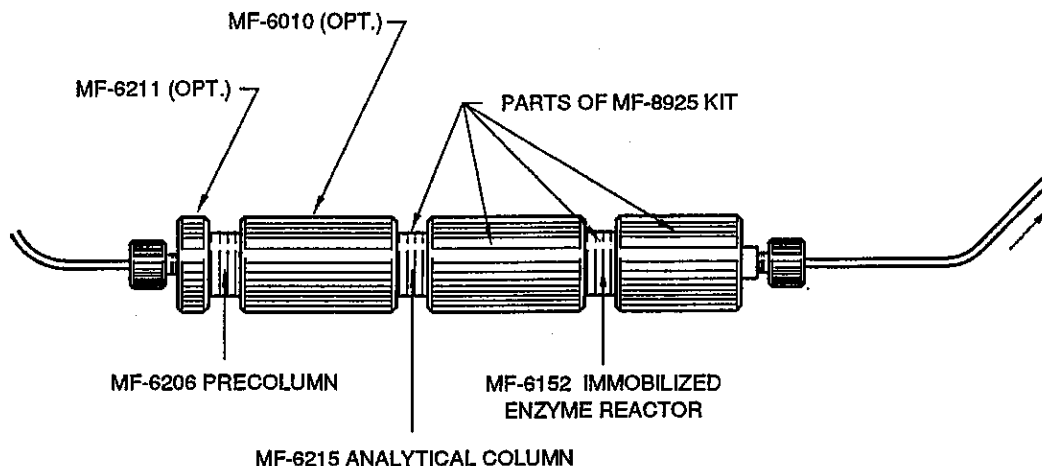
8. The analytical column may be flushed with the usual cleaning solvents: methanol, acetonitrile, etc.

These solvents should not enter the enzyme reactor. Simply store the reactor in fresh mobile phase at 4 °C between uses, in the shipping container.

9. We suggest that a precolumn be used if columns start showing increased backpressures. The additional components to order are:

MF-6206 precolumn, ODS 7 μm , pk. of 3
MF-6010 union
MF-6211 precolumn holder

These would be assembled as follows:



10. A freshly polished or cleaned platinum working electrode will exhibit more drift in its response than a glassy carbon electrode. Initially, it will be very sensitive, but after 6-8 hours it will be about one-half as responsive. The decline is characteristic of a platinum electrode held at fixed potential.

Allow time in your experiment schedule for the electrode response to stabilize before making critical injections. An overnight wait is recommended. Of course you can still assess retention times, sample characteristics, etc., with preliminary injections during this period of decline.

11. Eventually the electrode response will be too small for use. Two approaches may be used:
- Wipe the electrode with lab tissue soaked in methanol; use light pressure.
 - Keep the electrode in place and simply alternate the operating potential between +700 mV and -700 mV. Stay for 2-10 seconds at each potential and do about 10 cycles. You are restoring the surface to contain those catalytic sites responsible for peroxide detection.

Polishing by abrasive slurries is not necessary.

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