

Continuous monitoring of gas-phase species at trace levels with electrochemical detectors

Part 1. Direct amperometric measurement of hydrogen peroxide and enzyme-based detection of alcohols and sulfur dioxide

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Abstract

A simple detection system suitable for the continuous measurement of atmospheric hydrogen peroxide in the parts per trillion (10^{12}) by volume (pptv) range is described. The proposed method involves the use of a flowing recipient buffer pumped through a given length of narrow-bore (0.2 mm) microporous polyethylene tubing and then on to a platinum electrode detector polarized at +0.4 V vs. SCE. Hydrogen peroxide in the gas phase surrounding the tube enters through the pores and is carried to the platinum electrode detector where it is oxidized, resulting in an anodic current directly proportional to the concentration of hydrogen peroxide in the gas phase. In addition, when appropriate immobilized enzymes are incorporated into the flow arrangement prior to the amperometric detector (e.g., sulfite oxidase and alcohol oxidase), the same measurement scheme can be used to monitor volatile substrates of these enzymes continuously at parts per billion (10^9) by volume (ppbv) levels (sulfur dioxide and low-molecular-weight alcohols). In this case, substrate gases enter the recipient buffer stream through the walls of the sampling device and are converted to products plus electrochemically detectable hydrogen peroxide by the immobilized enzymes. The sensitivity and selectivity of each of these detection systems is examined in detail, as are the prospects of enhancing sensitivity by using a novel stopped-flow/flow-injection analysis operating mode.

Keywords. Flow system, Amperometry; Air; Alcohols, Enzyme reactor; Gas detection, Hydrogen peroxide, Sulphur dioxide

The development of analytical methods that are suitable for continuous real-time monitoring of gas-phase species at trace ambient levels remains a formidable challenge. Although a variety of specialized monitors based on electrochemical or

spectrophotometric detectors are available from several manufacturers, most can only sense gas-phase molecules on a continuous basis when these species approach toxic levels (e.g., alarm-type systems). Measurements of low-level ambient gases and/or trace levels of gas-phase species associated with specific industrial processes must often be made by classical wet chemical approaches after passing known volumes of air through appropriate

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solid- or liquid-phase scrubbers. Unfortunately, these methods provide only periodic time-averaged values rather than desired real-time readings.

Previous efforts in this laboratory have demonstrated the practical advantages of utilizing a small-diameter microporous polyethylene collection tube in conjunction with sensitive electrochemical detection for the continuous monitoring of ambient ammonia at levels ranging from 0.4 to 260 parts per billion (10^9) by volume (ppbv) [1]. Similar tubular collection devices also have been utilized by others in conjunction with reagent-based spectrophotometric and fluorescence detection for real-time monitoring of several gases [2–5]. The fundamental parameters that influence the gas collection efficiency and thus the ultimate sensitivity achievable with systems based on these so-called “sniffer” tubes [1] or “diffusion scrubbers” [2–4] have been described [2,6]. The purpose of this series of papers is to demonstrate how this type of continuous sampling scheme can be coupled with appropriate flow-through electrochemical detectors and immobilized enzymes for the selective and continuous measurement of a variety of gas-phase species at trace levels. Specifically, this first paper describes the analytical performance of relatively simple systems suitable for the direct detection hydrogen peroxide at parts per trillion (10^{12}) by volume (pptv) levels and volatile enzyme substrates (e.g., sulfur dioxide and alcohols) at ppbv levels.

The measurement of atmospheric hydrogen peroxide has received considerable attention recently owing to its oxidant role in the conversion of sulfur dioxide to sulfuric acid within liquid phases of the atmosphere [7–9]. Several methods have been proposed for the quantitative detection of this species on a continuous and non-continuous (i.e., sampling) basis. The most common sampling procedure relies on the trapping of hydrogen peroxide for a period of time in aqueous solution and/or condensing atmospheric water vapor. In either instance, subsequent measurement of the collected hydrogen peroxide is accomplished via a luminol chemiluminescence reaction [10] or a fluorimetric method involving peroxidase enzyme [11,12]. The latter reaction method has been adapted for continuous detection of ambient hy-

drogen peroxide through the use of a continuous-flow liquid-phase scrubber in which the air sample is mixed directly with a flowing stripper solution [5], or the use of a microporous diffusion scrubber tube through which the scrubbing solution flows before combination downstream with enzyme and substrate reagents [2].

In the direct hydrogen peroxide detection system described here, gas-phase hydrogen peroxide readily dissolves in a recipient buffer solution as this solution is pumped through a 200-cm length of microporous polypropylene tubing incorporated as the sample loop of a standard flow-injection analysis valve (see Fig. 1A). The solution then passes on to a platinum electrode polarized at +0.4 V vs. SCE, where the hydrogen peroxide is oxidized to oxygen ($\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$). The current associated with this reaction is directly proportional to the concentration of the hydrogen peroxide in the gas phase surrounding

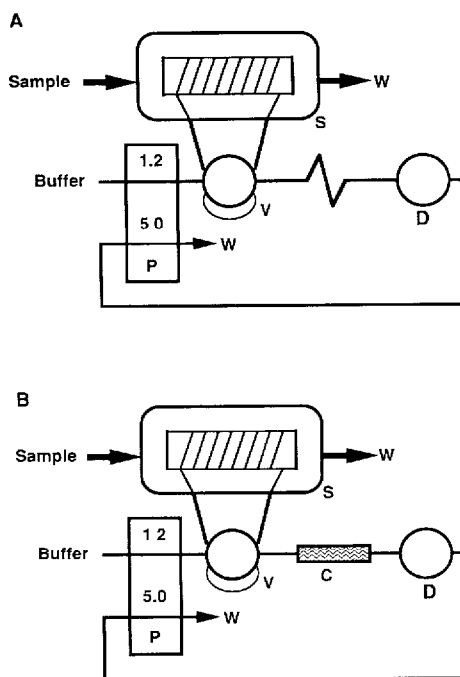
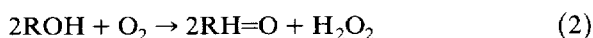
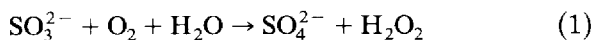


Fig. 1 Manifold arrangements used for detection of gas-phase (A) hydrogen peroxide and (B) sulfur dioxide and alcohols. S = glass chamber with coiled Celgard tubing; W = waste; P = peristaltic pump, V = six-port rotary injection valve; D = amperometric detector, C = controlled-pore glass enzyme reactor. Flow-rates are in ml min^{-1} .

the polypropylene tubing. In contrast to earlier continuous monitoring systems [2,3,5], no additional reagents are required.

If immobilized forms of specific oxidoreductase enzymes are incorporated into the recipient buffer stream prior to the platinum electrode (see Fig. 1B), the same basic hydrogen peroxide measurement arrangement can be adapted to monitor volatile substrates of these enzymes continuously. For example, sulfite oxidase and alcohol oxidase catalyze the following reactions, respectively:



where $\text{RH}=\text{O}$ represents the aldehyde form of an oxidized primary alcohol (ROH). The hydrogen peroxide liberated from these reactions can be detected amperometrically with the same polarized platinum electrode as used for direct detection of hydrogen peroxide. Although several different enzyme immobilization schemes and arrangements can be envisioned (e.g., enzyme immobilized on the inner walls of microporous tubing or immobilized on a platinum electrode detector), the use of a packed-bed enzyme reactor in an arrangement such as that shown in Fig. 1B proved to be the most effective for gas-phase monitoring of both sulfur dioxide and ethanol. It will be shown that a significant enhancement in sensitivity for hydrogen peroxide, sulfur dioxide and alcohols can be achieved by operating these various systems in a novel stopped-flow manner (rather than using a continuous flow of recipient buffer). In this mode, the flow of recipient buffer within the microporous collection tube is stopped for a fixed time period effectively to preconcentrate the analyte gas, with subsequent flow injection of this sample slug into the enzyme and/or electrochemical detector portion of the manifolds.

EXPERIMENTAL

Apparatus

The manifold arrangement used for gas-phase hydrogen peroxide detection is shown in Fig. 1A. A three-electrode, large-volume type wall-jet

amperometric detector configuration was employed [13,14]. The working electrode was a 6 mm diameter platinum disk (sealed in glass) (Corning, New York). This electrode together with a saturated calomel reference electrode (SCE) and a ring-shaped platinum counter electrode were connected to a Princeton Applied Research (Princeton, NJ) Model 264A potentiostat. For most experiments, a constant potential of +0.4 V vs. SCE was applied to the working electrode. Cell currents were recorded on a Fisher Recordall Series 5000 strip-chart recorder. When detecting sulfur dioxide or alcohols, the manifold was modified by incorporating an enzyme reactor containing either sulfite oxidase or alcohol oxidase immobilized on controlled-pore glass (see Fig. 1B).

The recipient buffer solution for hydrogen peroxide detection (0.1 M phosphate buffer, pH 7.2) was pumped through the system via a Rainin (Woburn, MA) Rabbit variable-speed peristaltic pump. Gas-phase alcohol detection was effected using the same recipient solution while more alkaline 0.1 M phosphate buffer (pH 8.5) was used for sulfur dioxide detection. The sample collection tube for all gaseous measurements consisted of a 200-cm length of Celgard microporous polypropylene (0.2 mm i.d.) (Celanese Separation Products, Charlotte, NC) connected as the sample loop of a standard low-pressure Rheodyne six-port rotary injection valve. This tube was coiled around a glass frame and was inserted into a glass chamber [1] which was purged with a calibrant or sample gas phase.

Reagents

All chemicals were of analytical-reagent grade. Solutions and buffers were prepared with deionized water. Hydrogen peroxide gas-phase standards were prepared using a permeation tube arrangement (see below). Sulfur dioxide and nitrogen dioxide permeation devices from Vici Metronix (Santa Clara, CA) were used to generate standards of these gases for testing/selectivity studies. Tanks of hydrogen chloride (107 ppmv), chlorine (52 ppmv) and carbon dioxide (9.2%) in nitrogen (Matheson, Chicago, IL) were employed for determining the potential interference induced by these species. These various standard sources

were diluted volumetrically with appropriate flow-rates of air to achieve the desired concentrations. Elevated air humidity was achieved by passing the stream of air through a bubbler containing deionized water.

Alcohol oxidase (E.C. 1.1.1.13) from *Pichia pastoris* (33 units mg^{-1} protein) and sulfite oxidase from chicken livers (32 units mg^{-1} protein) were obtained from Sigma (St. Louis, MO). These enzymes were immobilized as described previously [15] on alkylaminated controlled-pore glass (pore size 700 Å) obtained as a gift from M. Skłodowska-Curie University (Lublin, Poland). For sulfite oxidase, 160 units of enzyme were immobilized on 200 mg of glass beads, whereas for alcohol oxidase, 200 units were reacted with a similar mass of porous glass. After washing, the glass beads were packed in 8 or 3.5 cm long glass columns (3 mm i.d.) by pumping suspensions of the beads through tubes that contained a small piece of glass-wool at one end. All reactors were refrigerated at 4°C when not in use.

Generation of hydrogen peroxide, alcohol and sulfur dioxide standards for calibration of the systems

The arrangement shown in Fig. 2 was employed to generate gas-phase hydrogen peroxide standards. Laboratory compressed air was utilized as the main carrier gas source. This air was passed through a charcoal column and flow meter (0–36 l min^{-1}). This gas flow was merged with a second air stream emanating from the hydrogen peroxide diffusion tube. This diffusion device was prepared by incorporating a 15 cm length of microporous Gore-Tex tubing (W.L. Gore, Elkton, MD) concentrically within a similar length of glass tubing (see Fig. 2). A peristaltic pump (Ismatec, Zurich) was utilized to deliver recipient air through the center of the inner porous tubing while a second peristaltic pump delivered a 6% (w/w) hydrogen peroxide solution (in water) through the spacing between the two concentric tubes. The concentration of hydrogen peroxide in the recipient air phase as a function of the air and hydrogen peroxide solution flow-rates was determined by bubbling (through a fritted-glass bubbler) this gas phase into 0.1 M phosphate buffer (pH 7.2) for a

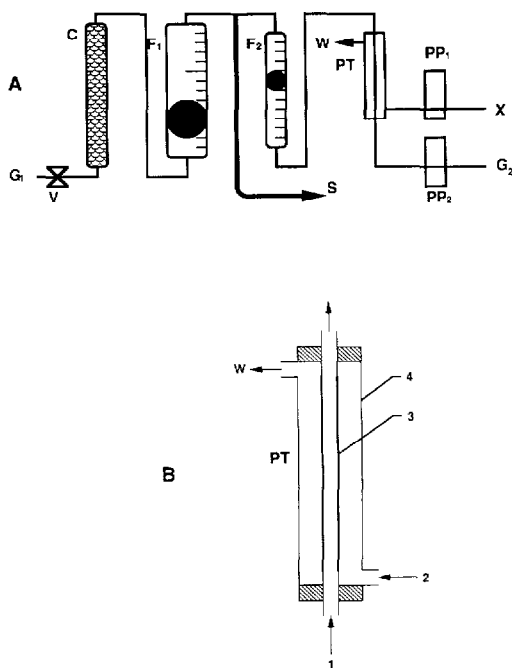


Fig. 2 Arrangement used to generate gas-phase hydrogen peroxide standards (A) Schematic diagram of entire gas flow system G = diluent air; V = control valve, C = charcoal column, F = flow meters, PT = permeation tube, PP = peristaltic pumps; X = hydrogen peroxide solution; G₂ = recipient air or nitrogen gas, W = waster, S = final gas phase standard (B) Expanded view of permeation tube arrangement 1 = Nitrogen or air recipient stream; 2 = hydrogen peroxide solution; 3 = Gore-Tex microporous PTFE tubing; 4 = glass tube.

fixed period of time and then injecting aliquots of this solution into a flow-injection system equipped with the electrochemical peroxide detector. The concentration of hydrogen peroxide in the scrubbing solution was determined by comparing peak heights with those obtained from injection of known and fresh hydrogen peroxide aqueous standards.

The concentrations of hydrogen peroxide in the combined air phase being passed by the Celgard collection tube (point S in Fig. 2) was varied by changing the flow-rate of the recipient air stream through the diffusion tube via valve F₂ and pump PP₂ so that the total flow-rate of gas phase did not change appreciably (i.e., the flow of diluent air from F₁ was almost 500 times greater than that originating from the hydrogen peroxide diffusion tube). It is important to keep the flow of gas past

the Celgard collection tube relatively constant, as the rate of mass transfer of analyte into the recipient buffer flowing through the tube is dependent on this gas flow-rate (see below and Part 2 [16]).

For generation of ethanol vapor, a 5% or 1% (v/v) solution of ethanol was passed through a permeation tube arrangement analogous to that used to generate hydrogen peroxide. Nitrogen gas was used as the recipient stream. The level of ethanol in the gas phase for given flow-rates of the donor and gas recipient streams was determined by trapping the ethanol gas stream in buffer for a fixed period of time and then injecting aliquots of this sample into a flow system containing immobilized alcohol oxidase. Final ppmv and ppbv levels of gas-phase ethanol were obtained by diluting the gas stream emanating from the permeation tube as described above for hydrogen peroxide.

Sulfur dioxide standards were generated from a commercially available gas emitter tube (standard type from Vici Metronics, Santa Clara, CA). The emission rate was $3.28 \mu\text{g min}^{-1}$ at 30°C . A glass U-tube containing the emitter and glass beads to enhance thermal equilibration of the recipient gas flow (nitrogen) was placed in a thermostated water-bath at 30°C . Final ppmv or ppbv levels of sulfur dioxide were obtained by varying the fraction of the total recipient nitrogen gas flow containing the sulfur dioxide that was allowed to dilute with a much higher volumetric flow of air.

RESULTS AND DISCUSSION

Direct hydrogen peroxide detection

Figure 3 illustrates the hydrodynamic voltammograms obtained for blank phosphate buffer (pH 7.2) and 1 mM solutions of hydrogen peroxide, sodium sulfite and formaldehyde, each prepared in the phosphate buffer. The voltammograms were obtained by continuously flowing these solutions through the three-electrode detector with different voltages applied to the working platinum electrode. The current response for hydrogen peroxide shows a well defined oxidation wave, beginning at $+0.2 \text{ V vs. SCE}$ and leveling at about $+0.6 \text{ V vs. SCE}$. Little or no signal is observed for formaldehyde in this same potential range. The re-

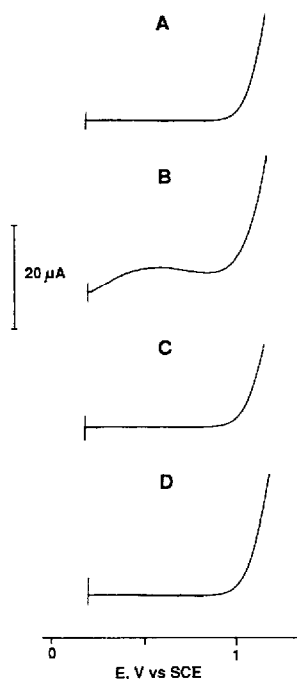


Fig. 3 Hydrodynamic voltammograms obtained for (A) background phosphate buffer and 1 mM (B) hydrogen peroxide, (C) sodium sulfite and (D) formaldehyde in phosphate buffer.

sponse to sulfite is about 1% of that found for hydrogen peroxide. On closer examination of the electrochemistry, however, the oxidation wave for sulfite appeared to be shifted to slightly more positive potentials than hydrogen peroxide (not shown). Thus, to achieve the optimum direct selectivity over sulfur dioxide, a working electrode potential of $+0.4 \text{ V}$ was employed for all subsequent gas-phase experiments. Under these conditions, the response to hydrogen peroxide was about 75% of the maximum found at 0.6 V .

In the continuous gas-phase hydrogen peroxide sensing mode, the flow-rate of the recipient buffer through the microporous Celgard tubing influences the amount of hydrogen peroxide captured into this solution in addition to the dynamic response time of the system toward this gas. Indeed, increasing the flow-rate decreases the signal observed for a constant sample gas flow containing 1.2 ppbv hydrogen peroxide (see Fig. 4). Increasing the flow-rate, however, does improve the response time of the system for step changes in the

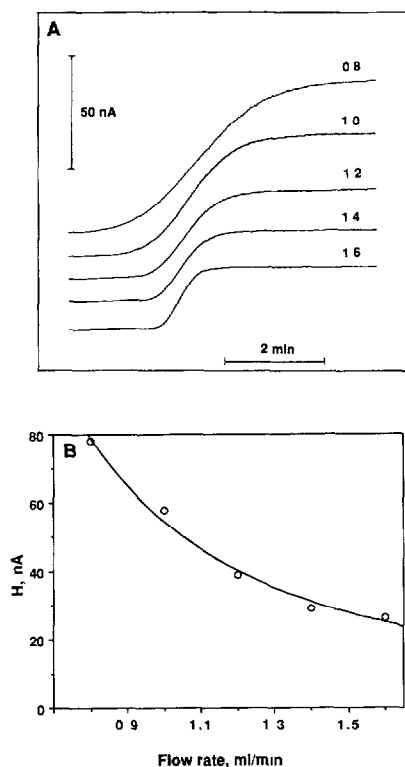


Fig. 4 Effect of recipient buffer stream flow-rate on response to gas-phase hydrogen peroxide (A) Typical strip-chart recording of response to 1.2 ppbv at flow-rates indicated; (B) plot of steady-state response for 1.2 ppbv standard as a function of flow-rate.

analyte gas (see Fig. 4A). A flow-rate of 1.2 ml min^{-1} was chosen as a compromise to give reasonably rapid response times for the system (ca. 2 min to reach 95% of the steady-state signal), while yielding signals for hydrogen peroxide that were still ca. 70% of that observed at lower recipient flow-rates.

The rate of total sample gas flow (in l min^{-1}) through the glass calibration chamber also influences the magnitude of the amperometric signal observed for a given level of hydrogen peroxide (this is also true for the sulfur dioxide and alcohol detectors described below). In general, higher flow-rates yield an increase in signal. This effect appears to be due to a change in the mass-transfer properties of the Celgard tubing as a function of gas flow. Subsequent experiments (see Part 2 [16]) point to a variation in the thickness of an un-

stirred recipient liquid layer within the pores of the polypropylene tubing as the cause of this effect. For further evaluation of the hydrogen peroxide detection system, the total sample gas flow was maintained at 7.65 l min^{-1} to minimize such sample flow-rate effects.

A typical calibration graph for the detection of hydrogen peroxide under continuous-flow steady-state conditions is shown in Fig. 5. Using a signal-to-noise ratio of 3, detection of hydrogen peroxide at levels as low as 25 pptv is possible under such conditions. Figure 5 also illustrates that ca. five-fold enhancement in sensitivity and detection limits can be achieved by operating the system in a 2-min stopped-flow measurement mode. In this instance, the recipient buffer is stopped for 2 min within the Celgard tubing using the rotary injection valve (V) in Fig. 1A. In this by-pass arrangement, recipient buffer continues to flow to the amperometric detector but without passing through the Celgard tube. After the 2-min period, the rotary valve is turned and the entire slug of sample in the Celgard tube moves on to the detector. The typical recorder output of such a stopped-flow measurement is shown in Fig. 6. Note that the initial signal appears as a large transient signal [i.e., analogous to flow-injection analysis (FIA)], followed by a steady-state plateau. This plateau corresponds to the signal obtained

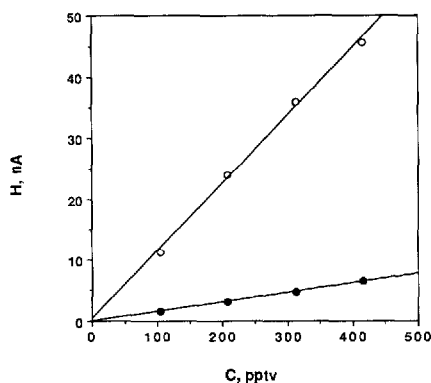


Fig. 5 Typical calibration graphs for detection of hydrogen peroxide under (●) continuous-flow and (○) 2-min stopped-flow conditions. For continuous flow, $\text{nA} = 0.0154 [\text{H}_2\text{O}_2] + 0.0002$, $r^2 = 0.999$, $\text{s.d.}_y = 0.13 \text{ nA}$, $\text{s.d.}_x = 1.2 \text{ pptv}$, for flow injection, $\text{nA} = 0.111 [\text{H}_2\text{O}_2] + 0.390$, $r^2 = 0.998$, $\text{s.d.}_y = 0.86 \text{ nA}$, $\text{s.d.}_x = 2.4 \text{ pptv}$.

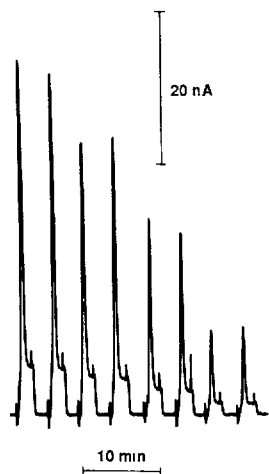


Fig 6. Typical amperometric detector response to 0.10, 0.21, 0.31 and 0.42 ppbv of hydrogen peroxide using 2-min stopped-flow measurement scheme.

for the continuous-flow measurements mode. Naturally, longer stopped-flow periods further enhance the sensitivity of the system, but this occurs at the expense of diminishing the real-time measurement capabilities of the gas-phase detection arrangement.

The reproducibility of the amperometric signals observed for low levels of hydrogen peroxide in either the continuous-flow or stopped-flow measurement modes was fairly good. At 210 pptv hydrogen peroxide, the relative standard deviation (r.s.d.) of the signals was 0.30% and 2.86% ($n = 10$) for the continuous-flow and stopped-flow (2 min) modes, respectively. At 350 pptv, the r.s.d.s were 0.17% and 2.01% ($n = 11$), respectively. It should be noted the poorer reproducibility of the flow-injection measurement mode is probably due to manual operation of the stopped-flow timing and valve injections.

The effect of other gases on the continuous-flow response of the system toward hydrogen peroxide was evaluated. Table 1 summarizes the results. Hydrogen chloride, carbon dioxide, nitrogen dioxide and oxygen have no observable influence on the amperometric signal for 895 pptv hydrogen peroxide, even when these other gases are present at levels $\gg 100$ times that of hydrogen peroxide. Greater than a 100-fold excess of chlorine gas decreases the response observed for peroxide, but

only very slightly, probably owing to its oxidation of the hydrogen peroxide. Sulfur dioxide is a positive interferent, with an almost 100-fold excess of this gas causing a +71% change in the peroxide signal. This response is due to the electrochemical oxidation of sulfite ions formed in the recipient buffer. As these ions are negatively charged, it should be possible to modify the surface of the platinum electrode to decrease the relative response of the detector to this species (relative to neutral hydrogen peroxide). Indeed, several groups have demonstrated that ionomer membrane coatings can be utilized to enhance electrochemical selectivity [17,18]. Thus, in preliminary experiments to enhance the selectivity over sulfur dioxide, a platinum electrode detector coated with Nafion [17] was employed as the working electrode. In separate flow-injection measurements, the response toward sulfite relative to hydrogen peroxide at +0.4 V was decreased approximately fivefold using the Nafion coated working electrode. As shown in Table 1, when this same Nafion-modified electrode was used in the gas-phase measurement mode, the influence of excess of sulfur dioxide on the hydrogen peroxide signal was decreased significantly. Clearly, further improvements in selectivity may be possible by varying the thickness and the exact nature of the materials used to coat the hydrogen peroxide detector (e.g., a low-molecular-weight cut-off cellulose acetate membrane could also be used, as is standard practice in certain glucose enzyme electrodes [19,20]).

TABLE 1

Effect of various gases on the continuous-flow steady-state response of proposed system to 895 pptv hydrogen peroxide ^a

Gas	Concentration (ppbv)	Signal change (%)
Cl ₂	112	-8.3
HCl	220	nc ^b
CO ₂	0.38%	nc ^b
NO ₂	138	nc ^b
SO ₂	70	+71 (+15.7) ^c
O ₂	21%	nc ^b

^a In a nitrogen stream at a total flow-rate of 15.3 l min⁻¹

^b nc = No change. ^c For Nafion-modified Pt disk electrode as detector

As expected, the humidity of the sample or standard gas phase passing by the Celgard tube influences the magnitude of the hydrogen peroxide response. This is due to variations in the evaporation rate of the recipient buffer solution passing through the tube. In fact, for the continuous detection mode, the response to 895 pptv hydrogen peroxide in a gas stream at 85% relative humidity decreases by ca. 15% compared with that observed when the gas phase is at <7% relative humidity. This is in good agreement with the humidity effects observed previously in the case of the ammonia-sensing system that employed the Celgard tube as a continuous sampler [1]. Clearly, at low humidity, the evaporation rate of the recipient is much higher (given the large surface area to volume ratio of the Celgard sampling tube) and the concentration of hydrogen peroxide is increased as the recipient solution passes on to the amperometric detector. This effect of relative humidity would be much less if the recipient buffer stream and the surrounding sample gas phase were in complete equilibrium (with respect to hydrogen peroxide) after the given residence time of the buffer in the collection tubing. However, as shown in Figs. 4 and 6, this is clearly not the case, as longer residence times result in an increase in the concentration of hydrogen peroxide in the recipient buffer, as evidenced by larger amperometric signals.

The system described here appears to have adequate sensitivity to detect hydrogen peroxide in ambient air. Figure 7 illustrates the responses observed for two peroxide standards and two types of air sample. The "tap" air refers to the labora-

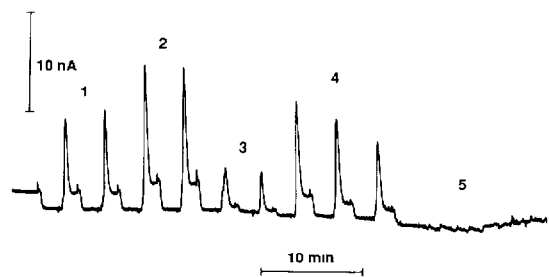


Fig. 7 2-min stopped-flow response of hydrogen peroxide system to (1) 49 pptv, (2) 98 pptv, (3) diluent air, (4) room air and (5) diluent air with catalase reactor in recipient stream flow line

tory compressed air that was first passed through a charcoal filter. Indeed, this is the same air used to dilute the hydrogen peroxide standards. Thus, as shown, the 2-min stopped-flow response and also the subsequent continuous-flow signal observed for this air sample are above the baseline signals observed when the Celgard sampling tube is bypassed. This implies that the gas standards are actually higher in concentration than indicated as a result of this background hydrogen peroxide in the diluent gas (i.e., essentially the standards of 49 and 98 pptv represent standard additions of these levels of peroxide to this background source of hydrogen peroxide). Using a standard addition approach, the level of hydrogen peroxide in this air was calculated to be 26 pptv (note that this background hydrogen peroxide also explains the slight positive y -offset in the stopped-flow calibration graph shown in Fig. 5). Proof that the signal is actually due to hydrogen peroxide can be obtained by adding a small enzyme reactor column containing immobilized catalase in the flow manifold prior to the amperometric detector. The catalase selectively decomposes the hydrogen peroxide to molecular oxygen and protons. As shown in Fig. 7, the presence of such a column completely eliminates the signal observed for the compressed air sample. The response to laboratory air pumped through the glass chamber at the same rate as the calibrating gases was significantly greater and more unstable when compared with the compressed air (see 4 in Fig. 7). Indeed, the average level of hydrogen peroxide determined from the stopped-flow signals was calculated to be 74 pptv (with correction for background level in calibrants). Similar experiments were performed on a number of days, and values determined for laboratory air consistently fell within the range 15–90 pptv. These values are in good agreement with those reported for normal room air [2,21]. Clearly, the proposed method has adequate sensitivity to detect even these trace ambient levels of hydrogen peroxide.

Sulfur dioxide and alcohol detection with immobilized oxidases

As described above, gas-phase hydrogen peroxide can be detected at trace levels via electro-

chemical oxidation at a platinum working electrode poised at +0.4 V vs. SCE. At this same potential, sulfite–hydrogensulfite and most primary alcohols display little or no anodic response. Thus, via the use of immobilized forms of sulfite oxidase and alcohol oxidase, electrochemically detectable hydrogen peroxide can be generated from both sulfur dioxide and ethanol. In the case of sulfur dioxide, these species can be effectively trapped in the form of hydrogensulfite and sulfite ions through the walls of the microporous Celgard sampling tube with a flowing alkaline recipient buffer. The optimum pH for sulfite oxidase, 8.5, limits the upper pH value of this buffer, as at higher pH, although the trapping efficiency may increase, the enzyme will lose activity [22]. For detection of alcohols, the efficiency of gas capture is dependent more on the solubility (e.g., Henry's constant) and diffusion coefficient (gas phase and solution phase) of the alcohol species. Thus, as alcohol oxidase displays an optimum activity at pH 7.5, a phosphate buffer at this pH was chosen as the recipient stream for all alcohol measurements.

Preliminary studies to evaluate the potential selectivity, sensitivity and stability of the flow-through enzyme-based detection arrangements for sulfite and alcohols under various conditions were carried out by replacing the microporous Celgard sampling tube in Fig. 1b with a conventional 50- μ l FIA sample loop, and injecting sulfite and alcohol standards in a conventional FIA manner. As with any non-equilibrium enzyme-based method, detection limits are dictated by the degree of substrate conversion to product within the enzyme reactor. By injecting standards of hydrogen peroxide, these values can be readily determined. Using a 3.5-cm sulfite oxidase reactor and a diluent buffer flow-rate of 1.0 ml min⁻¹, conversion efficiencies for sulfite–hydrogensulfite were 38% over the sulfite–hydrogensulfite concentration range 0.2–1.0 mM. Surprisingly, larger enzyme reactors (e.g., 8 cm) actually yielded lower conversion efficiencies (e.g., 23%), probably owing to a competing reaction involving the direct oxidation of the sulfite by the higher concentrations of hydrogen peroxide generated in the reactor. For alcohol oxidase, conversion efficiencies of about 46% were typically ob-

served for ethanol (using an 8-cm reactor). Activity toward long-chain aliphatic alcohols decreases with chain length, which results in lower conversion efficiencies and thus decreased sensitivity toward these species (i.e., most sensitive toward methanol). The sulfite oxidase reactor lost most (> 50%) of its activity over a 2-week period while the immobilized alcohol oxidase remained highly active for at least 1 month.

For selective gas sensing using the manifold arrangement shown in Fig. 1B, the enzyme-based flow systems can be operated in either the continuous-flow or stopped-flow mode. Figure 8 illustrates the typical amperometric responses of the sulfite oxidase-based system toward sulfur dioxide in both modes. Calibration graphs for these data are linear in the range 0–40 ppbv: for continuous flow, $nA = 5.60 [SO_2] + 0.083$, $r^2 = 0.999$, $s.d._y = 1.3$ nA, $s.d._x = 0.15$ ppbv; for flow injection, $nA = 17.9 [SO_2] + 20.0$, $r^2 = 0.995$, $s.d._y = 4.9$ nA, $s.d._x = 0.72$ ppbv. Using a recipient stream flow-rate of 1.2 ml min⁻¹, detection limits toward sulfur dioxide in the continuous-flow mode are 0.50 ppbv (at a signal-to-noise ratio of 3). The 2-min stopped-flow approach improves the sensitivity, yielding a detection limit of 0.15 ppbv. Also shown in Fig. 8 is the direct 2-min stopped-flow response of the electrode system to the same levels of sulfur dioxide but without the enzyme reactor in place.

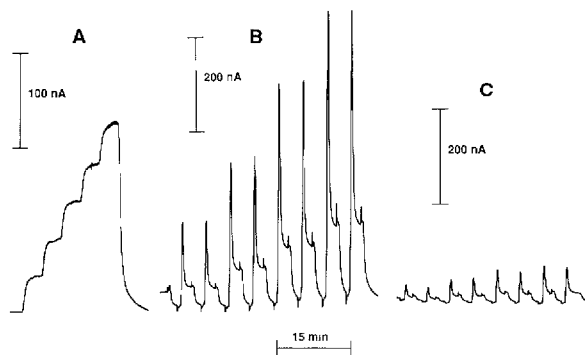


Fig. 8. Typical strip-chart recorder tracings for detection of gaseous sulfur dioxide using arrangement shown in Fig. 1B. (A) Continuous-flow response with 1.2 ml min⁻¹ flowing recipient buffer and immobilized sulfite oxidase; (B) 2-min stopped-flow mode with immobilized sulfite oxidase; (C) 2-min stopped-flow mode without enzyme reactor. Sulfur dioxide concentrations 7.5, 15.0, 22.5, 30.0 and 37.5 ppbv.

Note that the sulfite–hydrogensulfite formed in the recipient stream can be oxidized directly at the platinum working electrode (no Nafion coating), but the magnitude of the response is far less than that observed for the hydrogen peroxide generated from the oxidation of the sulfite–hydrogensulfite via the enzymatic reaction. This is in good agreement with the selectivity data presented above for the direct detection of gas-phase hydrogen peroxide.

The performance of the alcohol detection system was evaluated using ethanol as the analyte. As shown in Fig. 9, the response in both the continuous-flow and 2-min stopped-flow modes was similar to that found for sulfur dioxide. Indeed, the calibration graphs were linear in the range of gas levels shown in Fig. 9; for continuous flow, $nA = 1.19 [EtOH] + 0.024$, $r^2 = 0.999$, $s.d._y = 11.2$ nA, $s.d._x = 5.4$ ppbv; for flow injection, $nA = 2.85 [EtOH] + 4.50$, $r^2 = 0.999$, $s.d._y = 5.1$ nA, $s.d._x = 3.9$ ppbv. The detection limits were 1.0 and 0.5 ppbv, respectively. Under identical flow conditions, the upper limit of detection is ca. 4 ppmv (not shown). Above this level, the response becomes non-linear, probably owing to lack of molecular oxygen in the enzyme reactor. Other gas-phase primary alcohols can be sensed by the

same system but the relative sensitivities toward those with longer carbon chains will be less than that for ethanol.

With regard to selectivity, as both the alcohol and sulfur dioxide enzyme-based detection systems rely on the amperometric measurement of hydrogen peroxide, no response is observed toward nitrogen dioxide, carbon dioxide and hydrogen chloride with either enzyme-based system. The level of oxygen in the sample gas will affect the upper level of analyte that can be detected, as a stoichiometric amount is required for the enzymatic reactions. Very low levels of oxygen will therefore cause negative errors for the measurement of high concentrations of both sulfur dioxide and ethanol. Naturally, the presence of unusually high levels of hydrogen peroxide itself in the sampled gas phase would cause a significant positive interference; however, as shown above, the response toward endogenous levels of this electroactive species can be readily eliminated by incorporating a second enzyme column containing immobilized catalase prior to the alcohol or sulfite oxidase reactors. Sulfur dioxide interference in the alcohol detection scheme, arising from the direct electrochemical oxidation of sulfite–hydrogensulfite, can be reduced by using a platinum working electrode coated with Nafion [17]. Indeed, while the sensitivity toward analyte SO_2 is reduced ca. twofold (i.e., the coated detector exhibits less response to hydrogen peroxide), the relative selectivity for alcohol over sulfur dioxide is enhanced threefold using such an approach.

Conclusion

A gas-phase hydrogen peroxide detection system has been developed and characterized that combines a microporous collection tube in conjunction with direct amperometric hydrogen peroxide detection. No additional reagents are required to achieve detection of hydrogen peroxide at trace ambient pptv levels. The same basic peroxide detection system can be modified to sense other gas-phase species by incorporating appropriate immobilized enzyme reactors in the flow manifold. As model systems, sulfur dioxide and ethanol were detected at ppbv levels using immobilized sulfite oxidase and alcohol oxidase, re-

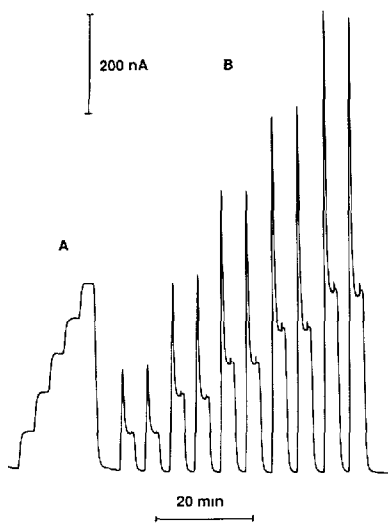


Fig. 9 Response output for detection of gaseous ethanol standards with immobilized alcohol oxidase in (A) continuous-flow and (B) 2-min stopped-flow modes

spectively. It has been shown that enhanced gas sensitivity for each system can be achieved by using a novel stopped-flow/flow-injection measurement mode. Given the simplicity of the equipment involved, these new electrochemical/enzyme detection schemes may prove to be attractive alternatives to existing gas-phase measurement methods.

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