Ozone Monitoring Based on a Biosensor Concept Utilizing a Reagentless Alcohol Oxidase Electrode

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An electrochemical method based on the concept of a biosensor for the monitoring of ozone is described for first time. The proposed method includes two parts: a selective sorbent for ozone, that is, eugenol, and a formaldehyde amperometric biosensor mounted into a flow-through cell. Ozone adds rapidly to the double bond of the allyl group of eugenol, which has been immobilized onto a hydrophobic C-18 reactor and the so produced formaldehyde is collected into the working buffer solution (sampler) and pumped to the detector. A multimembrane assembly consisting of an alcohol oxidase-modified nylon membrane sandwiched between an outer polycarbonate and an inner cellulose acetate membrane was fitted onto a Pt electrode and the enzymatically produced H₂O₂ was monitored at +0.65 V (vs Ag/AgCl/KCl 3 M). Under optimum conditions, a linear calibration curve over the concentration range 3–200 μg·mL⁻¹ ozone was constructed. The detection limit (S/N = 3) was calculated at 1.1 μg·mL⁻¹ ozone. The proposed method is interference-free from other gases such as O₂, Ar, N₂, N₂O, NOCl, SO₂, NH₃, and CO₂, which were tested at concentrations >200-fold higher than that of 100 μg·mL⁻¹ ozone used for comparison. Besides selectivity, the method is easy to perform and reproducible; its applicability in synthetic gaseous samples is also demonstrated.

Ozone is a strong oxidant and is widely used to supplement or replace other sterilization agents in a variety of processes including sterilization of drinking water, medical tools, and so on. Another large application of ozone is the treatment of air and the purification of gases. It is also used to increase the biodegradability of organic compounds, to destroy taste and odor control in medicinal treatment, and several experimental applications. However, ozone as a strong oxidant molecule is toxic for all living organisms including humans.

Surface ozone background has several sources, both natural and anthropogenic, which include the following: (a) downward transport of stratospheric ozone through the free troposphere to near-ground level, (b) in situ ozone production from methane emitted from swamps and wetlands reacting with natural NOₓ, (c) in situ production of ozone from reactions of biogenic VOCs with natural NOₓ, and (d) long-range transport of ozone from distant pollutant sources.²³

As ozone plays a double role, either as a useful and essential-for-life agent or as a toxic pollutant, the legislations of most countries have established threshold values for long- and short-term exposure periods, whose infringement may lead to measures to reduce its formation, such as traffic restrictions. These limits are in the range of 50–200 ppb(v), 98–392 μg·m⁻³, while 30 ppb(v), 59 μg·m⁻³, is the background level.⁴

Several methods have been developed so far for the determination of ozone. Most of the chemical methods are based on redox reactions including iodometric procedures⁵–⁸ and gas-phase titration.⁹,¹⁰ Various spectrophotometric¹¹–¹⁵ methods have also been used for the determination of ozone in aqueous solutions. Gas-phase and liquid-phase chemiluminescence¹⁶–²¹ and fluorescence methods²² have also been reported.

Electrochemical methods for the determination of ozone relied on the direct electrochemical reduction of ozone at a solid electrode. Various electrode assemblies based on two matched gold electrodes connected in series, with an indigo filter between them,²³ gold electrodes in combination with gas-permeable²⁴ or gas-permeable²⁵ or

(3) Environmental Protection Agency. Air Quality Criteria for Ozone and Related Photochemical Oxidants; EPA-600/P-93-004aF; 1993; pp 3–6.

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ion-exchange (Nafion)\textsuperscript{26–28} membranes, a pressed pellet of Agl with a Ag mesh and a Pt mesh on each side,\textsuperscript{29} and a large specific area carbon electrode with a PTFE membrane,\textsuperscript{30} have also been proposed.

Other measuring principles based on the change of the open circuit potential of the indicator electrode as a result of the reaction of the ozone with iodide ions\textsuperscript{31} or on the measure of the thermal decomposition heat\textsuperscript{32} have also been published. The direct determination of ozone in gas phase in the higher ppb and ppm range has also been well documented with sensors based on semiconducting oxide films.\textsuperscript{33–36}

All the above-mentioned methods have certain advantages and disadvantages. Titrimetric and spectrophotometric methods are very simple; however, they suffer from continuous consumption of reagents and thus are not indicated for routine analysis. Chemiluminescence methods, even though the most popular for the detection of ozone, use a rather elaborate instrumentation. Gas-phase chemiluminescence measurement of ozone is also very sensitive but requires accurate control of various experimental parameters thus making calibration quite complex. Electrochemical techniques provide a unique possibility for in situ, automated, and cost-effective measurement of ozone. A serious drawback of these approaches is the lack of inherent selectivity to ozone and the requirement for electrode maintenance. Metal oxides-based conductivity sensors provide sufficient performance simplicity; however, these devices operate at high temperatures requiring a large power consumption. As a general conclusion, the majority of the existing methods are not specific for ozone and they are large power consumption. As a general conclusion, the majority of the existing methods are not specific for ozone and they are

In this paper, we demonstrate an electrochemical method based on the concept of biosensors, for the monitoring of ozone in synthetic gaseous samples. Our approach combines the analytical simplifications of biosensors, namely, reagentless measurements, selectivity, and reusability, with that of the specific reaction of ozone with with formaldehyde, which is produced by the ozonolysis reaction of the allyl group of Eugenol (4-allyl-2-methoxyphenol), using an alcohol oxidase biosensor mounted in a flow injection manifold.

The method was designed to provide reliable and reproducible measurements under a reagentless mode. Details regarding the experimental setup, the performance, and the analytical utility of the method were studied and discussed.\textsuperscript{37}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Method & M. E. & R. E. \\
\hline
Titrimetric & 0.05 & 0.003 \\
Spectrophotometric & 0.1 & 0.005 \\
Chemiluminescence & 0.01 & 0.0005 \\
Electrochemical & 0.005 & 0.0002 \\
Biosensor & 0.0005 & 0.00002 \\
\hline
\end{tabular}
\caption{Comparison of various detection methods for ozone.}
\end{table}

**EXPERIMENTAL SECTION**

**Chemicals and Membranes.** Alcohol oxidase (AOX, EC 1.1.3.13 from *Pichia pastoris* in 30% sucrose, 30 units/mg of protein) and eugenol (GC grade) were purchased from Sigma and Aldrich, respectively. Sodium dihydrogen phosphate and cellulose acetate (40% acetyl) were purchased from Fluka. Oxygen (99.99%), CO\textsubscript{2} (99.99%), and N\textsubscript{2}O (99.9%) were supplied from Air Liquide. Argon (99.98%), NO\textsubscript{2} (99.98%), SO\textsubscript{2} (99.98%), and NH\textsubscript{3} (99.8%) were supplied from MG, Matheson, Matheson, and Merck, respectively. Note: NO\textsubscript{2}, SO\textsubscript{2}, and NH\textsubscript{3} must be handled wearing a mask and within a hood. Do not inhale vapors! The Sep-Pak TC-18 cartridges were supplied by Waters. Double-distilled water (DDW) was used throughout.

Paraformaldehyde (tablets) was obtained from Mallinckrodt. Formaldehyde stock standard solution was made by dissolving 15.0 g of paraformaldehyde in 1.0 L of DDW according to a previously reported method.\textsuperscript{38} The standardization of the solution was achieved by a titrimetric method as follows: 5.00 mL of the solution along with 100 mL of H\textsubscript{2}O and 5.0 mL of hydroxylamine hydrochloride (10% in DDW) were left to react for 20 min according to the reaction scheme

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\text{HCHO} + \text{NH}_2\text{OH-HCl} \rightarrow \text{HCH} - \text{NOH} + \text{H}_2\text{O} + \text{HCl}
\]

and then titrated with a standard solution of 0.1000 M NaOH. Bromophenol blue (Aldrich, 0.05% in DDW) was used as indicator for the end point observation from yellow to blue. An iodometric method in a neutral potassium iodide buffer solution and the Gastec detector tubes (18L, Gastec) mounted in a Gastec stroke pump (model GV-100S) were used as reference methods for the determination of ozone in liquid and gas phase.

**Membranes.** HA membranes (mixed esters of cellulose, 0.45-\mu m pore size) were purchased from Millipore. Biodyne B (modified with quaternary ammonium groups, positively charged nylon 66 membrane, 0.45-\mu m pore size) and Immunodyne ABC (preactivated nylon 66 membrane for covalent bonding, 0.45-\mu m pore size) were a kind gift of the Pall Corp. Polycarbonate membranes (thickness 10 \mu m, 0.03-\mu m pore size) were supplied from Nuclearopore. A wet film applicator (Urai) was used for casting the cellulose acetate membrane.

**Assembly of the Biosensor.** The construction of the biosensor was based on a multimembrane architecture according to the experimental details given in our previous work.\textsuperscript{40} AOX was immobilized onto the above-mentioned membranes by spotting 5 \mu L (6.75 units) of an enzyme stock solution (135 units) in 50 mM phosphate, pH 7 buffer solution onto each side of the dry membrane. Enzyme spots were left to air-dry, and the unattached protein was removed by washing the membranes (3 x 10 mL) with the same buffer solution. The enzyme membrane was sandwiched between an outer polycarbonate membrane, which prevents microbial attack and also leaching of the enzyme and an inner cellulose acetate membrane (20 \mu m thick, 100-Da nominal MW cutoff), which prevents fouling phenomena and also eliminates interference from electroactive species. The whole mem-
Figure 1. Schematic representation of the apparatus used for ozone monitoring. Inset scheme: multimembrane assembly used for the construction of the AOX amperometric biosensor. AN, analyte; IN, interferences; PC, polycarbonate membrane; EM, enzyme membrane; CA, cellulose acetate membrane.

brane assembly was placed with the aid of a 0-ring onto the working electrode (Figure 1, inset scheme).

Eugenol-Modified C-18 Reactor. C-18 reactors were cleaned with copious amounts of acetonitrile using a 5-mL syringe. Prior to its use, eugenol was pretreated with anhydrous Na$_2$SO$_3$ and anhydrous Na$_2$SO$_4$ in order to remove the already contained formaldehyde, due to the oxidation of eugenol from the atmospheric ozone, and the humidity, respectively. An aliquot of 2 mL of eugenol was then left to pass slowly (30 min), and the reactor was totally drained under a gentle stream of argon.

Apparatus. All electrochemical experiments were conducted with a computer-controlled potentiostat (Autolab PSTAT10, Eco-Chemie). The flow injection experiments were carried out, by using an in-house fully automated flow injection (FI) manifold. A detailed description of the FI manifold and the electrochemical detector has been reported earlier. The working electrode was a platinum electrode (2-mm diameter, IJ Cambria Scientific). All measurements were conducted at +0.65 V versus a Ag/AgCl/3 M KCl reference electrode, at room temperature. Ozone production was obtained by using the Medozons BM ozone generator (Medozons Ltd.).

Procedures. The experimental setup used for the determination of ozone is illustrated in Figure 1. The carrier stream (0.05 M phosphate buffer, pH 5.5–8.5) was continuously pumped at a flow rate of 0.31 mL min$^{-1}$ toward the detector, and the background current was allowed to decay to a stable value. The method was calibrated by passing ozone streams of different concentrations, 60–4000 µgL$^{-1}$ ozone, at a flow rate of 0.25 L min$^{-1}$ for 1 min (route A) through the eugenol-modified C-18 reactor. Different combinations of ozone generator output/flow rate/sampling time were also tested (see below). The so produced formaldehyde was collected in a high-neck reservoir containing 5.0 mL of the working buffer solution (sampler), and then it was driven to the detector. Steady-state current outputs (route A) or FI peaks (250-µL sample size through route B) were used as a measure of the ozone concentration. In a similar way, according to route C, or through the injection valve, gas-phase synthetic samples were measured.

The optimization of the system as a formaldehyde biosensor was performed as follows: standard solutions of HCHO (route B) were introduced as short pulses of 250 µL via the loop injection valve, and peak heights of the current response were taken as a measure of the formaldehyde concentrations.

RESULTS AND DISCUSSION

Chemical Reactions and Enzyme Pathway. The mechanism of the oxidative cleavage of C=C double bonds (ozonolysis) has not been fully elucidated. According to a widely accepted theory (syn=anti zwitterion mechanism), ozone reacts with a C=C double bond via two successive 1,3-dipolar cycloadditions to form an intermediate ozonide, such as the one illustrated in Figure 2A. These ozonides are not isolated but converted into cleavage products, aldehydes, or ketones or both. Decomposition of the ozonide with water liberates the third atom of oxygen in part as hydrogen peroxide and in part as peroxidic derivatives of the carbonyl compound formed. The formation of formaldehyde upon the action of ozone to the allylic C=C double bond of eugenol can be represented with the simplified ozonolysis reaction illustrated in Figure 2A.

Formaldehyde, in aqueous solutions, is all practically hydrated to methylene glycol according to the reaction shown in Figure 2D. The catalytic action of AOX onto formaldehyde, in the form of methylene glycol, is described by the enzymic pathway shown in Figure 2D. It is well known that AOX catalyses the oxidation of short-chain primary alcohols (C$_1$–C$_4$), substituted primary alcohols, and formaldehyde. The proposed enzyme scheme has already been used for the development of a colorimetric method for the determination of formaldehyde. Moreover, the construction

The selection we made between AOX and formaldehyde dehydrogenase (FDH) as biochemical transducers of formaldehyde was based on the fact that AOX is superior in terms of working and storage stability. Moreover, AOX needs no extra coenzyme or activator to operate, thus providing reagentless measurements. On the other hand, FDH needs NAD$^+$ to operate, and the use of the latter is associated with fouling phenomena and high overpotentials. Under these conditions, the system is subjected to a gradually loss of its original activity and is more susceptible to interference from various reducing agents. In general, oxidase-based biosensors provide more rigid and reliable biosensors compared with those based on NAD$^+$-dependent dehydrogenases.$^{49}$ Especially, multimembrane architectures have been successfully used so far in various commercially available analyzers.$^{49}$

It should be mentioned, however, that this selection was made at the cost of a lower sensitivity. Biosensors based on FDH are quite sensitive and LOD values as low as 1 $\mu$M have been reported.$^{50}$ On the other hand, AOX-based electrodes suffer from modest sensitivity, since the methods that have been proposed so far detect formaldehyde in the sub- to millimolar range.$^{46-48}$

It is clear that the selection of the biochemical transducer imposes a compromise between sensitivity and simplicity. The latter feature, along with the necessity to design a cost-effective method able to operate in a reagentless mode has driven us to proceed further employing the multimembrane AOX-modified.

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electrode. However, the consequent low sample throughput, especially in the case of samples with low concentration of ozone, is a certain drawback of this choice.

**Optimization Studies and Analytical Characteristics of the AOX-Based Biosensor.** Different types of membranes have been used for the immobilization of AOX. The difference lies in the type of immobilization due to (a) physical adsorption (HA, Millipore), (b) ionic affinity (Biodyne B), and (c) chemical binding of the enzyme to aldehyde preactivated membranes (Immunodyne ABC). The efficiency of each membrane was tested by taking into account the percent relative response of the biosensor with respect to the most active one and the reproducibility of the system toward successive injections of 0.25 mM HCHO.

The results showed that Biodyne B was the most efficient type of membrane while HA and Immunodyne ABC achieved only 60 and 22% of the relative response, respectively, all compared with the response received by the Biodyne B, which is taken as 100%. Lower relative efficiency in the case of HA was somehow expected, since physical adsorption-based protocols usually result in low enzyme loadings. On the other hand, chemical bonding of AOX in the Immunodyne ABC membrane seems to affect the conformation of the active site of the enzyme, since this membrane it claimed to have the highest protein capacity (135 μg of IgG/cm²). Among the tested membranes.

The pH dependence of the biosensor response was also tested in the pH range 5.5–8.5 using a 0.05 M phosphate buffer solution. The optimum pH was found to be 7.0 (data not shown); thus, all the subsequent experiments were performed at this pH value. Other buffering systems were not tested.

The calibration of the method for the monitoring of ozone involves enrichment with oxygen (Figure 1), while the unknown samples contain only the naturally occurring oxygen in the atmosphere. Since AOX uses oxygen as an electron donor, the effect of oxygen concentration on the performance of the system was investigated. Standard solutions of 0.5 mM formaldehyde were treated either by bubbling with oxygen (oxygen rich) or by purging with argon (oxygen poor) for 1 min, then they were introduced in the FI manifold, and the corresponding FIgrams were recorded (data not shown). A slightly increase of the signal in the case of “oxygen-rich” samples and a decrease of the signal in the case of “oxygen-poor” samples, both compared with the response recorded for the untreated samples, was observed. To eliminate the dependence of the oxygen concentration on the sensitivity of the system, unknown samples were oxygenated (0.25 L·min⁻¹ for 1 min) before entering the FI manifold.

Under optimized conditions, a linear calibration curve $I/IA = f([\text{formaldehyde/mM}])$ over the concentration range 0.01–1.0 mM HCHO was constructed. Data fit the equation, $y = (142.1 ± 1.9) [\text{HCHO, mM}] + (1.9 ± 0.9), r^2 = 0.9996$. The detection limit for a signal-to-noise ratio of 3 ($S/N = 3$) and the relative standard deviation (RSD) of the method for a 0.5 mM formaldehyde standard solution were calculated to be 0.02 mM formaldehyde and 2.3% ($n = 9$), respectively.

The stability of the biosensor is satisfactory, and the system can be successfully used to measure more than 100 samples of 0.5 mM HCHO, without need of intermediate recalibrations. The proposed biosensors also displayed very good storage stability when stored in the working buffer solution at +4 °C, if not in use. They retain their initial activity for more than 2 months.

**Ozone Monitoring.** Eugenol-modified C-18 reactor was found to be quite effective in the heterogeneous ozonolysis reaction used in the present work. To evaluate its performance, an air stream including 4 mg·L⁻¹ ozone, at a flow rate of 0.25 L·min⁻¹, for 10 min, viz. 10 mg of O₃, was driven through the euganol reactor and the output was collected in a neutral iodide buffer solution trap. The quantitative decomposition of ozone passing through the C-18 euganol reactor was verified, since no color change of the iodide solution was observed in the presence of starch as an indicator. The reactor can be used for more than 50 runs without any loss of its original activity. When it was not used, it was stored dry at +4 °C for more than one month; however, it should daily be treated with argon to remove the amount of formaldehyde produced due to the oxidation of euganol from the atmosphere.

The conversion ratio of ozone to formaldehyde was calculated at three different quantities of ozone (1, 2, and 4 mg) and different flow rates, that is, the three user-selectable flow rates provided by the ozone generator. To achieve these concentrations, ozone outputs of 200, 400, and 800 μg·mL⁻¹ ozone were provided for 20, 10, and 5 min at each of the provided flow rates (0.25, 0.5, and 1.0 L·min⁻¹), respectively. For each combination of ozone generator output/flow rate/sampling time, two experiments were performed. One for the determination of ozone, produced by the ozone generator, and one for the determination of formaldehyde, produced by the heterogeneous ozonolysis reaction, when the ozone stream is passing through the C-18 euganol reactor. The amounts of the so-produced ozone and formaldehyde were separately collected in neutral iodide and hydroxylamine hydrochloride traps, determined with the corresponding titrimetric methods, respectively, and mean (at the three different flow rates) conversion ratios of 81.5 ± 3, 76.5 ± 2, and 74.4 ± 1 for concentrations of 1, 2, and 4 mg of O₃, respectively, were calculated. Conversion ratios at lower quantities of ozone are not provided, since the titrimetric methods used for the determination of the concentrations of ozone and formaldehyde failed to provide accurate and reproducible results at quantities lower than 1 mg of ozone.

To explain the values of the calculated conversion ratios and any deviation of them from the theoretical value of 1, we assume a nonstoichiometric reaction between euganol and ozone, according to the tentative mechanism illustrated in Figure 2. Besides the allylic ozonolysis reaction (Figure 2A), a part of the ozone is also consumed for the oxidation of (I) to (II) (Figure 2B). The presence of (I) and (II) along with the dimmer products of (I) and (II) and that between (I) and (II) was supported by mass spectroscopy data (data not shown).

Moreover, ozone is also consumed through the aromatic ozonolysis reaction illustrated in Figure 2C. According to this reaction scheme, ozone can also cleave the C=C bonds of the aromatic rings but usually at a slower rate than that observed for olefinic substrates, and although less is known about such reactions, the current consensus is that aromatic ozonolysis proceeds via a 1,3-dipolar cycloaddition mechanism similar to that proposed for olefinic ozonolysis. Previous studies have indicated...

that electron-releasing substituents tend to promote aromatic ozonolysis, and preferential cleavage between the ring carbons bearing the hydroxyl and methoxyl substituents can proceed simultaneously oxidative ring opening, which is consistent with a 1,3-dipolar cycloaddition mechanism.53 Electron-releasing substituents tend to promote aromatic ozonolysis, and preferential cleavage between the ring carbons bearing the hydroxyl and methoxyl substituents can proceed simultaneously oxidative ring opening, which is consistent with a 1,3-dipolar cycloaddition mechanism.53

Interference Study. Interference from various gases, such as O₂, Ar, N₂, SO₂, CO₂, NH₃, N₂O, and NOCl, was investigated according to the route (D) illustrated in Figure 1. Interferrences were injected with a syringe into a continuous nitrogen stream, at concentrations in the range of 20–180 mg·mL⁻¹ gas. These concentrations are 200–1800-fold higher compared with that of 100 µg·mL⁻¹ O₃ used for comparison. The quantity of each gas was calculated by weighing the syringe empty and full of each gas. As can be seen from the FI-grams in Figure 3, the interference effect of the majority of the tested gases is negligible. Slight changes in both the baseline level and the current output of the control sample of ozone are attributable to changes of pH of the carrier. The results revealed that the proposed method can be potentially used for the determination of ozone in real samples. A problem is expected to be raised due to the formaldehyde present in ambient atmosphere.54 A blank measurement, obtained through bypassing the eugenol reactor, is necessary for applications in real samples. In the presence of water (or humidity in gas samples), hydrogen peroxide should be found among the others products of the ozonolysis reaction4 (Figure 2A). To avoid the production of hydrogen peroxide, which is the measuring species in the proposed method, an air filter (Acro50, Pall) containing 1.0 g of anhydrous Na₂SO₄ was inserted before the C-18 reactors (Figure 1).

Ozone Monitoring and Application in Synthetic Gaseous Samples. Under optimum conditions, two calibration curves, I/nA = f (µg·mL⁻¹ ozone), one based on peak current signals of the FI-grams (route B) and the other based on the steady-state current outputs (route A) were constructed, applying the least-squares method (Figure 4). The equation for the FI-grams-based straight line is $y = (1.42 ± 0.01) \mu g·mL^{-1} \text{ozone} + (5.66 ± 0.99)$, with a correlation coefficient $r^2 = 0.9998$. The detection limit for a signal-to-noise ratio of 3 was 2.1 µg·mL⁻¹ ozone, and the RSD of the method was calculated as 0.74% ($n = 8$, 100 µg·mL⁻¹ ozone). Correspondingly, the equation for the steady-state-based straight line is $y = (2.23 ± 0.01) \mu g·mL^{-1} \text{ozone} + (0.92 ± 0.78)$, with a correlation coefficient $r^2 = 0.9998$. The detection limit for $S/N = 3$ was 1.1 µg·mL⁻¹ ozone, and the RSD of the method was calculated as 0.69% ($n = 5$, 100 µg·mL⁻¹ ozone). It should be mentioned that identical calibration curves were obtained, when the standard solutions used as calibrators were prepared by using different combinations of ozone generator output/flow rate/sampling time, showing that at this concentration range of ozone and under the tested flow rates the conversion ratio of ozone to formaldehyde remains constant. This fact is also supported by the good linearity achieved all over the tested concentration range and the absence of any hysteresis effect in the low concentration part of the calibration curve. Using the above calibration curves, standard solutions of ozone (20, 50, and 100 µg·mL⁻¹) and formaldehyde (20, 50, and 100 µg·mL⁻¹) were measured, and a mean conversion ratio of 33 ± 1% was calculated.

The analytical applicability of the proposed method was evaluated by determining the ozone concentration in synthetic gaseous samples. A stainless steel thermostated chamber (14.22 m³), being equipped with a ventilation system, fan, and proper in/out-puts for enrichment, sampling, or probing purposes, was conditioned with atmospheric air for 2 h, sealed, and then enriched with two different concentrations of ozone using the ozone generator. The resulting synthetic atmosphere was homogenized for 15 min, and a volume of 51 L was sampled and equally divided into two channels: one bearing a eugenol-modified C-18 reactor (working) and the other bearing a plain C-18 reactor, used as blank. Sampling was made with a pump (MCS10, SKC), and gaseous samples were collected in two high-neck vials containing 2.0 mL of the working buffer solution. The volume of the liquid samples was corrected to its original volume, and then they were introduced in the FI manifold as short pulses of 250 µL via the injection valve. The concentration of formaldehyde in the blank sample was corrected with the calculated conversion ratio of 0.33, and the corresponding concentration of ozone was calculated in micrograms per cubic meter (Table 1). Similarly to the results obtained with the proposed method, measurements made with the Gastec system showed that the concentration of the ozone is depleted with time, due to its interaction with other species present in the chamber.
Conclusions. Results demonstrate the validity of the proposed concept for the monitoring of ozone using a reagentless alcohol oxidase biosensor. The proposed method offers all the advantages of biosensors and, moreover, is applicable to synthetic gaseous samples.

Even though our experiments were primarily aimed at proving this concept and not optimized for a specific analytical application, the proposed approach fulfills the analytical requirements for the monitoring of ozone in industrial workplaces or as a process gas in various technical and industrial applications.

Given that the experimental setup can be easily integrated into a portable device (sampling pump, samplers, peristaltic pump, detector, and potentiostat), the opportunity for in-field measurements is attainable if proper instrumentation studies are made. Toward this direction, the adopted philosophy regarding the construction of the biosensor acts epicurean, since multimembrane architecture has found wide use in most of the commercially available analyzers based on the biosensor’s concept.

ACKNOWLEDGMENT

This research was cofunded by the European Union in the framework of the program “Pythagoras II” of the “Operational Program for Education and Initial Vocational Training” of the 3rd Community Support Framework of the Hellenic Ministry of Education, funded by 25% from national sources and by 75% from the European Social Fund (ESF). The authors thank Dr. S. Karkabunas and Nephrotech company for the provision of the ozone generator.

Received for review February 3, 2006. Accepted May 15, 2006.

AC060230H

Table 1. Ozone Concentration in Synthetic Gaseous Samples*

<table>
<thead>
<tr>
<th>sampling no.</th>
<th>theoretical value, O₃ (µg·m⁻³)</th>
<th>experimental value, O₃ (µg·m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>787.68</td>
<td>349.41</td>
</tr>
<tr>
<td>2</td>
<td>1575.36</td>
<td>480.00</td>
</tr>
</tbody>
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*Conditions: temperature 20 °C, flow rate 0.85 L·min⁻¹, sampling time 30 min, and sample volume 2.0 mL