Amperometric microsensor for measurement of gaseous and dissolved CO₂

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ABSTRACT

A simple method to measure dissolved CO₂ at a microscale would be beneficial for many scientific and medical applications. A simple amperometric microsensor for CO₂ with a 20–100 μm wide tip was developed by placing a layer of acidic O₂ trap solution containing Cr²⁺ in front of a Clark-type electrochemical sensor. The Clark-type sensor contains a Ag cathode in an ionic liquid, and also a Ag guard cathode behind the sensing cathode to prevent interference from reducible contaminants in the ionic liquid. The constructed sensors exhibited linear response over relatively large intervals of CO₂ partial pressure, but for CO₂ partial pressures of <20 Pa the response was only about 60% of that observed at 20–1000 Pa. The slope of the calibration curve at 2–6 kPa was about 80% of the slope from 0 to 1 kPa. A high baseline signal caused by water diffusing through the membrane into the ionic liquid was avoided by making very conical sensors so that the water concentration around the cathode was kept low by diffusional transport into the bulk reservoir of ionic liquid, but elevated zero currents by a factor of about two was often observed for sensors more than 1 month old. The lifetime of the sensor by continuous operation can be more than 4 months, but with a slow decrease in sensitivity that may be caused by lower membrane permeability. Use of the sensor was demonstrated by measuring CO₂ dynamics in the thallus of a red algae along with variations in O₂ and pH.

1. Introduction

All living organisms convert carbon dioxide, by autotrophic consumption or heterotrophic production, and measurement of CO₂ concentration changes are thus essential in many scientific and medical studies. Very large investments are currently made to lower the anthropogenic emission of CO₂, and even to transform the CO₂ now being emitted into organic compounds by chemical processes [1]. Several methods can be used to measure CO₂. The strong absorption of CO₂ in the infrared region thus makes it simple to determine CO₂ in gaseous samples [2]. Determination of dissolved CO₂ in liquid samples is more difficult, but several methods have been developed that are based on diffusion of dissolved CO₂ through a gas permeable membrane and resulting change in pH or conductivity in a medium behind this membrane [2]. The simplest CO₂ measuring equipment based on gas permeable membranes are sensors where CO₂ diffuses through the membrane and into a stagnant layer of buffer where the change in pH is measured by an electrochemical sensor (Severinghaus sensors) [3], or the change in pH results in protonation or deprotonation of an organic buffer that can be determined by optical means (CO₂ optodes [4,5]). The Severinghaus sensors have, however, problems with sensitivity and response time, especially at low CO₂ partial pressures such as the 40 Pa found in air. One reason for a relatively slow response is the rather slow hydration of CO₂ to form H₂CO₃, and the enzyme carbonic acid anhydrase may therefore be added to the buffer solution within Severinghaus sensors to speed up the hydration/dehydration [6]. Addition of an easily degradable protein as carbonic anhydrase may, however, lead to a short lifetime of the sensor.

The concentration of dissolved CO₂ may be determined by measuring the concentration of CO₂²⁻ with an ion selective electrode and then at known pH calculate the CO₂ concentration from pKₐ values. An alternative to this is to record the potential between the CO₂²⁻ electrode and a pH electrode, resulting in a log-linear signal for CO₂ [7]. Such an approach is limited to relatively high pH environments and assumes equilibrium between CO₂ and H₂CO₃.

Attempts have been made to construct sensors containing non-protic electrolyte in which CO₂ can be reduced at a cathode charged at a very negative potential, and these attempts and have recently been reviewed...
The primary reduction product of CO$_2$ in an aprotic electrolyte is the CO$_2$$^-$ radical, and two CO$_2$ radicals will subsequently combine to form oxalate, C$_2$O$_4$$^2-$, or disproportionation may lead to CO + CO$_3$$^2-$ [10]. Oxygen is reduced (to superoxide [8]) at much less negative potentials than CO$_2$, and any protic chemical species entering through the membrane, such as water, may also be reduced. A large variety of CO$_2$ reduction products may be formed in the presence of water or any other reactive chemical species such as alkenes and alkynes [1], and even trace amounts of water contamination in an amperometric electrolyte may affect the outcome of the cathodic reduction [11]. A negatively charged gold-plated front membrane has been used to reduce O$_2$ before it entered the cathodic chamber of a CO$_2$ sensor through a second membrane, but a reasonable response was only obtained above 2% CO$_2$, and the response to changes in CO$_2$ concentration was very slow [12]. Alternative means to compensate for the O$_2$ interference (voltage step chronoamperometry), or determination of less net superoxide formation by cathodic reduction of O$_2$ in the presence of CO$_2$ [9] do not seem suited for routine monitoring.

For many applications it could be beneficial to determine the microscale distribution of CO$_2$. Microscale CO$_2$ sensors based on the Sveringhaus principle have been constructed and used for environmental analyses [6,13], but they were characterized by lifetimes of a few days and unstable calibration curves. A microscale CO$_2$ optode has been described [14] that exhibited excellent response characteristics, but there were problems with long term stability and poisoning by acidic gasses. Planar optodes may offer an alternative to show the microscale distribution of CO$_2$ [15], and planar optodes for CO$_2$ are commercially available from Presens GMBH. The experimental procedure by insertion of an optode sheet into a sample is, however, far from being non-invasive. In the following we describe the construction and application of an amperometric microsensor for CO$_2$ in which O$_2$ interference is avoided by removal of O$_2$ in a thin layer of reductant placed at the sensor tip. The sensor has been used in studies of plant physiology [16].

2. Materials and methods

2.1. Sensor construction

The CO$_2$ microsensor (Fig. 1) is almost identical to the N$_2$O sensor previously described [17], but with different electrolyte, and different O$_2$ trap solution in the front compartment. The general construction procedure for Clark-type [18] microsensors with silicone membranes is described in detail elsewhere [19,20], and will here not be repeated in detail. Two different types of cathodes were constructed, one with a platinum core and one with a tungsten core. The 50 μm platinum (Goodfellow) and tungsten (50 μm “rods” from Science Products GmbH) wires were etched in saturated aqueous KCN solution with 2–5 V AC applied to obtain suitable tip diameters and shapes [20]. The glass used for construction of the sensors was ordinary soda-lime glass (Schott AR glass) except for the glass coating of the platinum or tungsten wire making contact with the cathode material, which was made from the highly insulating Schott 8512 glass for platinum and aluminum silicate glass (Schott 8252) for tungsten. These insulating glasses were fused with an AR glass shaft by melting (Schott 8512) or for the aluminum silicate glass by gluing with UV curing cement (Loctite 3494). The glass was removed from the tip ~30 μm of the metal wires by pinching with tweezers under the microscope. After exposing the metal wire the tips were electroplated with a thin (2–3 μm) layer of gold (see below), and subsequently the tips were heated with a hot nichrome heating loop under the microscope to close glass cracks and for the tungsten-based cathode also to ensure sufficient adhesion of the gold. After heating, the tips were electro-plated with metal using various electroplating solutions contained in a glass capillary while the process was followed in the microscope at 100x magnification. For gold plating we used a 5% (wt/wt) HAuCl$_4$ solution at -0.8 to -1.2 V, for silver plating a Ag(CN)$_2$$^{-}$ containing solution [21] and -1.2 to -1.6 V, and for indium plating a 20% (wt/wt) InCl$_3$ in 1 M HCl and -1.3 to -1.6 V. A platinum wire served as anode for all platings. The initial plating to fabricate a cathode of suitable diameter was always with gold, and a thin layer (1–2 μm) of silver or indium was then subsequently added. The guard cathodes were made from 0.25 mm (incl. insulation) Teflon-insulated silver wire (Goodfellow), from which the insulation was removed from the tip 2–3 mm. The exposed wire was subsequently brought down to 10–20 μm thickness by etching in 2 mol L$^{-1}$ KCN at 2–5 V AC. The casing around the cathode was made rather conical to facilitate the positioning of the guard cathode tip close to the sensing cathode and to facilitate diffusional exchange of electrolyte constituents. The best results were obtained with casings around the cathode that were relatively parallel sided with a diameter of 30–50 μm for distant ~2 mm, but then had a shoulder with an increase in diameter to 200–300 μm. Such a shape to facilitates the positioning of the guard cathode tip close to the sensing cathode and also facilitates diffusional exchange of electrolyte constituents, including the transport of water away from the cathode. The front chamber containing the O$_2$ reducing solution had to be very conical to allow for an efficient diffusional supply of the reductant to the tip region. The sensor photographed in Fig. 1 thus had an increase in diameter from 60 μm at the tip to 230 μm at a distance of 500 μm behind the tip. It was constructed and glued onto the tip as described previously [17] and had a volume of about 0.2 mL. The silicone membranes were made by inserting the tips into Dow Corning 734 Sealant with subsequent curing at room temperature for 24 h. The membrane in the inner capillary should be at least 40 μm thick to create some diffusion resistance against the entry of water into the electrolyte around the cathode.

The electrolyte for the electrochemical sensor was deoxygenated by Ar bubbling before ~0.1 mL was injected into the sensor. The sensor was subsequently placed in a desiccator and exposed to low vacuum to
ensure filling to the very tip. After filling the electrolyte chamber (∼1 mL) almost completely to the top, the chamber was sealed with UV curing cement (Loctite 3494). Several electrolytes were tested, as described in the “results” section. The outer chamber was glued onto the electrochemical sensor with UV curing cement. A little water (∼20 μL) was introduced into the outer chamber and the tip was filled by vacuum treatment as described above. The water was then replaced with 0.1 mL 1 mol L⁻¹ CrCl₃ (99.99%, beads, Sigma Aldrich) solution in 0.1 M HCl, and the chamber was sealed immediately. Alternatively the tip was filled with propylene carbonate (Chromosolv, Sigma Aldrich) before vacuum treatment, and this was subsequently replaced with 1 mol L⁻¹ diphenylphosphine (Sigma Aldrich) in propylene carbonate where O₂ and CO₂ had been removed by Ar bubbling.

2.2. Measuring circuit

The current from the sensors was measured with a 4-channel Unisense multimeter connected to a portable PC equipped with the program Sensor Trace Basic (Unisense A/S). If not otherwise stated a polarization of -1.2 V between Ag pseudo-reference and cathodes was applied.

2.3. Calibration and testing

Calibrations were conducted either in the gas phase using compressed gasses and a Brooks 0260 gas mixer, or in acidified (0.1 M HCl) demineralized water supplemented with small volumes of saturated water or bicarbonate solution. Calibrations points were read after sufficient time (5–10 min) to allow near complete sensor response and also dehydration of the formed carbonic acid when bicarbonate was used for calibration. The concentration of CO₂ at a partial pressure of 101 kPa was calculated according to Carroll et al. [22]. Calibrations were done both under fully oxic conditions (∼21 kPa O₂) and under anoxic conditions. For sensors with a non-aqueous O₂ trap they were also tested in dry gas and in water saturated gas. The sensitivity to stirring was determined as the difference between the signals in water vigorously bubbled with 1% CO₂ (∼1 kPa partial pressure) and 5 min after bubbling was terminated. If not otherwise stated the experiments were conducted at a room temperature of 20–22 °C.

2.4. Demonstration in algal thallus

Microscale sensors are especially suited for measurement inside stagnant matrices such as plant or animal tissue, and we demonstrated use of the sensor by measuring CO₂ dynamics inside the thallus of the red algae Gracilaria vermiculophylla. During analysis at a room temperature of 21 °C it was covered by 1 cm of seawater (24% salinity). Turbulent mixing of the seawater was ensured by continuous bubbling with air. Values of O₂ and pH were measured simultaneously with the CO₂ measurements using O₂ and pH microsensors [20]. Light was provided by a 20 W halogen lamp (Schott KL200) illuminating the thallus at an irradiance (400–700 nm region) of 240 μmol photons m⁻² s⁻¹. The sensors were held and inserted by a computer controlled micromanipulator (Unisense A/S) attached to a heavy stand. The position of the microsensor tips could be controlled by the program Sensor Trace Pro (Unisense A/S), that was also used to collect the data.

3. Results and discussion

3.1. Cathode material

Sensors with Au, Ag, and In cathodes were tested. The best performing sensors (i.e., high sensitivity and linearity of calibration curves) had Ag cathodes, and only data obtained with this cathode material are shown here. However, sensors with Au cathodes also functioned, whereas sensors with In cathodes exhibited a poor sensitivity. The best performance was obtained with cathodes that almost filled the inner diameter of the tip. The best compromise between signal, zero current, electrical noise, and response time was obtained with 20–80 μm diameter cathodes.

3.2. Electrolyte

The following electrolytes including several types of Ionic Liquid (IL) were tested: 1) Tetrabutylammonium iodide (0.3 mol L⁻¹) in propylene carbonate, 2) Tetrabutylammonium tetrafluoroborate (0.3 mol L⁻¹) in propylene carbonate, 3) Tetrabutylammonium iodide (0.3 mol L⁻¹) in acetonitrile (very aggressive to membrane), 4) Diethylmethylimidazolium bis(trifluoromethylsulfonyl)imide (IL), 5) Triethylsulfonium bis(trifluoromethylsulfonyl)imide (IL), 6) 1-Butyl-3-methylimidazolium hexafluoroantimonate (IL), 7) 11-Methyl-3-octylimidazolium tetrafluoroborate (IL), and 8) 1-Ethyl-3-methylimidazolium dicyanamide (IL).

When tested in dry N₂ and without O₂ trap compartment, electrolytes 1, 7 and 8 gave the overall best performance. No. 8 resulted in the lowest zero signals, but the response to changes in CO₂ partial pressure was slower than for no. 1 and 7.

3.3. Oxygen trap solutions

Early in our work it was observed that exposure of CO₂ sensors without O₂ trap compartment and based on electrolytes 1 and 7 to protic solvents like H₂O, methanol and ethanol led to extremely high and drifting zero signals. It was therefore decided to search for a non-aqueous O₂ trap solution as electrolytes 1 and 7 were evaluated as resulting in the overall best CO₂ response. It turned out that the solubility of suitable inorganic O₂ scavengers in membrane compatible aprotic solvents were too low, but organic phosphines were promising. After testing several phosphines it was found that diphenyl phosphine (DPP) was the best choice [23], and a 0.5 mol L⁻¹ DPP was used in many subsequent sensor tests. It was early recognized that DPP is aggressive towards polymer membranes, and that the permeability of membranes exposed to DPP decreases over days to weeks. DPP is very efficient as oxygen trap, and DPP based CO₂ sensors exhibited no interference on the zero signal from O₂. The interference from H₂O was also negligible. Very unexpected it turned out, however, that the presence of O₂ led to less sensitivity towards CO₂. Near-linear calibration curves were found under both oxic and anoxic conditions, but the slopes of the curves obtained under oxic conditions were lower (about 30% lower at atmospheric levels of O₂).

We had observed that sensors based on electrolyte no. 8, 1-ethyl-3-methylimidazolium dicyanamide, did not exhibit any response to H₂O, and it was therefore decided to test an aqueous trap of 1 mol L⁻¹ of CrCl₃ in 0.1 M HCl on such sensors. Sensors based on this aqueous O₂ trap solution exhibit low zero signals of 10–50 pA (corresponding to the signal for about 20 – 100 Pa CO₂ partial pressure). For some sensors the zero signal did, however, increase over time, possibly due to increasing water concentration in the IL. Acidic Cr²⁺ solutions are very good O₂ scavengers, and the distance between the two tip membranes can therefore be down to 40 μm.

Construction at a microscale makes it possible to efficiently supply reagents or remove products in the tip of a sensor. Diffusion alone can thus supply sufficient Cr²⁺ to the tip region of a sensor as the one photographed in Fig. 1, so that it remains O₂ insensitive for months. It is exactly the same principle as used in the N₂O sensor [17] where alkaline ascorbate is used to remove O₂. The “chemical trap” compartment used in our sensors for H₂ [24], N₂O and CO₂ does, however, result in relatively long sensor response times.

3.4. Sensor performance

Calibration of sensors based on a CrCl₃ solution for trapping of O₂
often resulted in almost linear calibration curves from 0 to 6 kPa CO₂, but with a tendency to lower sensitivity at high concentrations (Fig. 2A). For the calibration curve shown in Fig. 2B the sensitivity in the 0–0.5 kPa (or 0–175 μM) range was thus 145 pA kPa⁻¹ and decreased to 120 pA kPa⁻¹ in the 1–6 kPa (or 3.5–21 mM) range. When calibrated at very low concentrations (< ~0.02 kPa, or < 7 μM) the sensitivity was often considerably lower (~60%) than at slightly higher concentrations (Fig. S1). Calibration curves are thus usually slightly sigmoidal, but the degree of sigmoidal shape is variable. It should be noted that the calibration curve shown in Fig. 2B still has a linear regression coefficient of 0.999 despite the slightly sigmoidal response. The sensors exhibit excellent stability over hours, but with a decrease in sensitivity over days and weeks. The sensor calibrated in Fig. 2 decreased its sensitivity (in the 0.1–5 kPa range) from 210 pA kPa⁻¹ on day 1 to 120 pA kPa⁻¹ on day 49, but then with only a minor decrease for the next 2-month period. A decrease in sensitivity over time is observed for all similarly constructed amperometric gas sensors from our laboratory (O₂ [19], N₂O [17], H₂ [24]) and must be due to decreasing silicone membrane permeability. Sensors with larger diameter also produce higher signals. A sensor with a tip diameter of 50 μm and a distance of 140 μm from exterior to cathode (the one photographed in Fig. 1) thus exhibited a sensitivity of 1080 pA kPa⁻¹ in the 20–80 Pa range and had a baseline current of 22 pA at 21 °C (Fig. S1). That sensor gave a signal of 60 pA for the 0.04% atmospheric CO₂ level. The limit of detection calculated as three times the standard deviation of the zero determination [25] divided by the sensitivity was 0.2 Pa. Three consecutively made sensors (nos. 316–318) with tip diameters of 34–51 μm were analyzed for detection limit with an identical procedure, determining the standard deviation of 20 data points (200 s) prior to CO₂ addition, and detection limits of 0.9, 1.4, and 0.6 Pa were calculated.

About 80% of the sensors that were successfully assembled functioned, but there were variations in lifetime and degree of calibration curve non-linearity. Substantial differences were found between sensors made with different batch numbers of IL, pointing to a need of purification.

Carbon dioxide reacts very quickly with superoxide [8] formed by O₂ reduction at the cathode, and it is therefore essential to remove O₂ from the electrolyte before the sensors is assembled. Even when the electrolyte is carefully deoxygenated a lower sensitivity is, however, observed at concentrations below about 20–100 Pa CO₂ partial pressure (Fig. S1).

The response time of the sensors depends on the geometry of the whole sensor tip. A long distance between guard tip and cathode leads to a slow response, but a large gap between the inner tip and the guard casing also leads to a slow 90% response, as some of the CO₂ diffusing into the guard will pass the inner tip and slowly build up behind this tip. A slow return to low zero current is observed when the sensor has been exposed to very high CO₂ levels, as some of the CO₂ will accumulate in the CrCl₂ solution behind the inner tip, and then slowly diffuse back to the tip region. We therefore did not calibrate the sensors to concentrations above 6 kPa CO₂. Raw data from calibration of a sensor are shown in Fig. 2A. The 90% response time to a change in partial pressure from 0 to 1 kPa by this specific sensor was 200 s (tested by injection of CO₂ saturated water into stirred water), and even slower at lower partial pressures. The signal change during exposure to 1 kPa CO₂ and subsequent return to zero CO₂ for the sensor depicted in Fig. 1 is shown in Fig. S2. A tight fit between the two capillaries at the tip results in faster 90% response time, as only little CO₂ will pass into the reservoir of Cr²⁺ solution behind the inner silicone membrane. Other tested ILs resulted in much faster response (data not shown) but did not allow for aqueous O₂ trap solution and also exhibited less stable baseline currents.

The response time of the microsensor is longer than for interfering N₂O (see below). The solubility of CO₂ in the cyanamide IL is similar to the solubility in other ILs [26], and cannot explain why the response is much slower than in for example 11-methyl-3-octylimidazolium tetrafluoroborate. It does not seem that the reaction rate at the cathode surface is the limiting factor, as cathodes with very large surface areas did not improve the response time (data not shown) but resulted in an increased zero current.

Several sensors with diameters varying from 20 to 70 μm were tested, and they all exhibited a sensitivity difference between vigorously stirred and stagnant water of 2–5%, which is acceptable for most purposes [19].

A polarization of -1.2 V against the internal Ag pseudo-reference and counter electrode was applied as a standard for the cyanamide-based sensors, but the effect of polarization voltage on zero current, response time, and sensitivity was investigated for all cathode types and electrolytes. Calibration curves for polarizations of -1.1 to -1.3 V for two cyanamide-based sensor are shown in Fig. 3. One sensor (Fig. 3, left) exhibited almost identical sensitivity at polarizations from -1.15 to -1.3 V, and the zero current was not significantly affected by the
changes in polarization. The sensitivity of the other sensor (Fig. 3 right) increased with more negative polarizations, although the difference between -1.25 and -1.3 V was small. The zero current of this sensor also increased at more negative potentials. It is noteworthy that a polarization of -1.3 versus -1.2 V did not result in faster response or better sensitivity at low CO₂ concentrations. Many cyanamide-based sensors (>50) were tested for effect of polarization, and the examples shown in Fig. 3 represent the extremes. All sensors exhibited a transient increase in zero current immediately after change to more negative polarizations, and it was thus necessary to wait for a minimum of 2 h after each change in polarization before a recording of a calibration curve. Most sensors did not show major increases in sensitivity at polarizations more negative than -1.20 V, and this voltage was therefore routinely used. Sensors based on a platinum core often exhibited very high zero currents and even formed H₂ bubbles at the cathode when exposed to polarizations more negative than -1.3 V, probably due to electrolyte (containing some H₂O) access to the platinum at the glass-gold interface. Tungsten should therefore be the preferred material, and the combination of tungsten and aluminum silicate glass also resulted in the lowest baseline currents at more moderate polarizations. There have been many studies of potential catalysts for electrochemical CO₂ reduction [27], but most of such catalysts are for reduction of CO₂ to products more reduced than oxalate and thus require a source of protons.

All electrochemical sensor signals are affected by changes in temperature. The effect of temperature on zero current and sensitivity to CO₂ of three sensors is shown in Fig. 4. The effect is shown in terms of both dissolved CO₂ concentration and partial pressure. The temperature effect on the sensitivity is much larger than seen for similar sensors with two silicone membranes and front trap compartment [28]. It is evident that it is necessary to compensate for these effects by even small changes in temperature of the analyzed media.

3.5. Interferences

Interference on CO₂ sensors based on a Cr²⁺ oxygen trap can be expected from any reducible uncharged substance that can pass a silicone membrane and avoid reduction in the Cr²⁺ solution. Potential interfering agents thus include protic small molecules like ethanol, methanol and acetic acid. Addition of 1% (vol/vol) of the alcohols to the calibration vessel did, however, not result in any change in signal. Addition of acetic acid (100 µmol L⁻¹ final concentration) to the acidic solution (pH 2) in the calibration vessel also did not produce any signal. A high interference was, however, seen from N₂O, and the sensitivity to N₂O was about 10 times higher than for CO₂ (on a molar basis). The 90% response to changes in N₂O concentration was <1 min for all investigated sensors, and thus much faster than for CO₂. The relatively slow response for CO₂ as compared to N₂O may be due to a combination of very high CO₂ solubility in the IL and possible complex formation between CO₂ and the IL [8]. The solubility of N₂O in the IL was determined to be 61 mM at 21 °C, which is about 30% of the CO₂ solubility (see supplementary information). Hydrogen sulfide caused inactivation of the sensor. We have measured CO₂ gradients in liquid pig manure (K. Maegaard et al. unpublished results) which must be about the most hostile environments for a chemical sensor, but this was only possible after addition of FeCl₂ to precipitate sulfide.

3.6. Effect of water

The CO₂ microsensor may function for several months, but it is then crucial to avoid excessive hydration of the ionic liquid around the cathode, as hydration results in elevated zero currents. The chosen IL, 1-ethyl-3-methylimidazolium dicyanamide, is rather hygroscopic [29], and it is possible that a comparably low chemical activity at a given water concentration contributes to a low water interference. The concentration of water near the cathode can be minimized in two ways: 1) By use of very conical glass capillaries around the cathode as in this study (Fig. 1) so that any water penetrating into the ionic liquid is diluted away by diffusion into the bulk reservoir of ionic liquid. 2) By use of membranes with a higher CO₂/H₂O permeability factor than silicone rubber in front of the cathode. Teflon AF 1600 or 2400 membranes have very favorable CO₂/H₂O permeability quotients [30,31] and Teflon AF2400 actually has a CO₂ permeability similar to silicone rubber (polydimethylsiloxane). The adhesion of Teflon AF to glass is unfortunately poor, even when the glass is coated with fluorosilane (S. Borisov, pers. comm.).

3.7. Measurement in thallus of Gracilaria vermiculophylla

The low effect of stirring on the sensor response and thus also a low
effect of variable diffusive properties in a stagnant matrix makes the CO2 microsensors well suited for measuring inside matrices producing or consuming CO2, such as animal and plant tissue. The data shown in Fig. 5 from illuminated and dark-incubated *Gracilaria vermiculophylla* thallus illustrate the type of investigations enabled by the new sensor. The concentration of CO2 0.5 mm inside the thallus was measured along with values for O2 and pH during light-dark cycles (Fig. 5). During illumination CO2 was consumed by CO2 fixation, while O2 was being produced. The consumption of CO2 resulted in elevated pH. During darkness respiration consumed O2 and produced CO2, and pH decreased. All kinds of life is characterized by the production or consumption of CO2, and with the new sensor we now have a direct way of following the changes, even within microenvironments.

4. Conclusions

The CO2 microsensor described here has superior characteristics as compared to earlier described amperometric CO2 sensors (8, 10), and it allows for routine measurement of microscale CO2 distribution. The addition of an aquatic G22+ - based O2 trap resulted in sensors that did not exhibit any interference from O2, H2O and low concentrations of alcohols and acetic acid. The sensors can be made very sensitive so that they can record less than 2% changes from atmospheric concentration. Future minor changes in design, cathode surface, electrolyte, and membrane composition will no doubt result in even better performance in terms of resolution, response time and long term stability. The only relevant interfering agents found were H2S, which inactivates the sensor, and N2O. The sensor may thus not only find use for fine-scale measurements in the life sciences as demonstrated here, but may be used also for bulk monitoring.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2018.12.038.

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