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TUTORIAL

CO_2 Measurement in Microfluidic Devices

Facilitating Biological Applications Using a Flow-Through Cell

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The reliable, noninvasive measurement of dissolved gases in microfluidic devices is an important and difficult activity for biological applications. The **PreSens** pCO_2 mini and chemical optical CO_2 sensors have been designed to noninvasively measure carbon dioxide in the recirculating fluid in these devices.

The small CO_2 sensor spot placed in contact with the solution takes up a small volume and has a thin profile, at approximately 5 mm diameter and 0.1 mm thickness. Microfluidic devices for biological applications limit the culture media to small volumes and small flow rates, creating the advantage of minimizing the use of reagents.

However, the small volumes create challenges for measuring concentrations of various chemical species within the media. For microfluidic devices for biological applications, the response time and ability to detect small changes in gas concentration are very important.

The dissolved gas levels in the recirculating media must be within a physiological range to maintain the proper environment for the cellular components. A rapid response time is critical for the creation of a feedback mechanism to enable adjustment of gas concentrations quickly. The small volumes of media used in the microfluidic devices preclude the sampling of media for analysis, so the ability of the sensing system to monitor noninvasively is crucial. With the small volumes and low flow rates used in these microfluidic devices, the application limits the consumption rate of gases by the sensor to small rates.

The pCO_2 mini system was evaluated for its ability to monitor changing dissolved CO_2 concentrations inside a microfluidic device to be used in a microfluidic biological culture device. Two configurations were evaluated: a flow-through cell located external to the microfluidic device and a sensor spot housed inside the device. The repeatability and response time were measured and compared to a commercially available Severinghaus-type sensor (a generic expresson for a CO_2 measuring system based on a pH electrode in contact with a bicarbonate buffer).

Materials & Methods

The flow-through cell with integrated CO_2 sensor from PreSens was configured in series with the Severinghaus-type dissolved CO_2 sensor using 1/16” ID Viton® tubing, selected to minimize gas transport through the tubing. Two concentrations of bubbled water, 5% and 10% pCO_2, were pumped alternately via a peristaltic pump through the two flow-through cells with sensors at a flow rate of 900 µL/min. The two

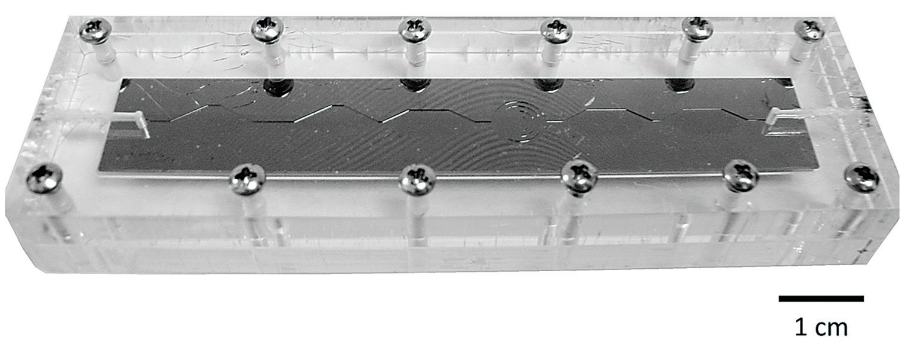


Figure 1. Microfluidic device used in the described experiments

Figure 2. Comparison of PreSens pCO_2 mini measurement system and Severinghaus-type carbon dioxide sensors in flow-through configuration. Gas bubbled liquid was flowing at 900 µL/min.

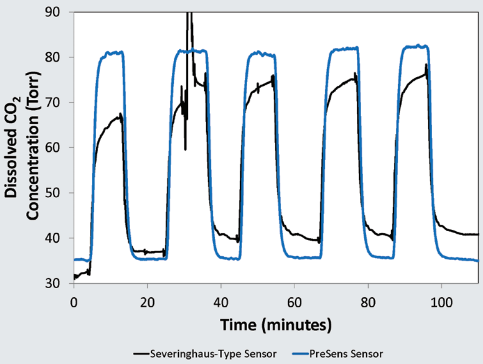
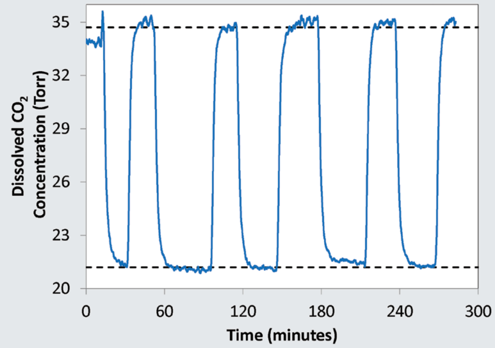


Figure 3. PreSens CO_2 sensor spot response in microfluidic device, at flow rate of 30 µL/min



sensors were calibrated using these two liquids.

The PreSens CO_2 sensor was calibrated with one of these liquids using the 1-point adjustment provided in the software after entering the inspection data from factory calibration. The pCO_2 mini transmitter recorded the measurements from the CO_2 flow-through cell to a computer, and a LabTrax data acquisition module recorded the measurements from the Severinghaus-type flow-through cell to a computer.

For evaluation of the PreSens CO_2 sensor spot performance inside a microfluidic device, the sensor spot was attached using Kwik-Sil™ to the acrylic surface of the microfluidic device, inside a chamber designed to hold the spot. An upstream chamber inside the device was used as a gas transfer chamber, in which CO_2 diffused from a gas-filled chamber through a PDMS membrane and into water being pumped through the device (*Figure 1*).

The gas inside the gas-filled chamber was alternated between 5% and 10% CO_2. A much smaller flow rate, 30 µL/min, was used to match flow rates appropriate for the microfluidic device. The configuration of the system produced dissolved CO_2 levels within the flowing liquid below the equilibrium concentrations, due to the limited residence time of a volume of flowing liquid in the gas transfer chamber.

Results

The repeatability was drastically better and the response time was much faster for the PreSens CO_2 flow-through cell at 900 µL/min versus the Severinghaus-type sensor flow-through cell (*Figure 2*). The response time for the PreSens sensor was about half that of the Severinghaus-type sensor, with the PreSens sensor reading 99% of the final value after 3.7 minutes, versus 7.8 minutes for the Severinghaus-type sensor.

'The Severinghaus-type sensor response also jumped, typically returning to the previous measurement within a few seconds, though in at least one case, the response remained unstable for several minutes. In contrast, the PreSens sensor spot response was smooth and continuous while responding faster than the Severinghaus-type sensor.

The Severinghaus-type sensor used was only available as a flow-through cell, so noninvasive monitoring inside the microfluidic device could not be performed with the Severinghaus-type sensor. The PreSens pCO_2 mini was easily incorporated into the housing of the device.

The response of the PreSens pCO_2 mini is shown in *Figure 3* as the gas concentration in the device was changed in a chamber designed to diffuse carbon dioxide into the recirculating liquid, thus changing the steady state concentration of dissolved gas.

In the microfluidic device, with a low flow rate of 30 µL/min, the PreSens CO_2 sensor spot response was repeatable and demonstrated full-scale response in about 10 minutes. With a change in dissolved gas concentration of 13.5 torr, the standard deviation of the steady state responses at the high and low concentrations was 0.4 and 0.2 torr, respectively.

The expected values, shown as dashed lines in *Figure 3*, are slightly different than the measured values from the PreSens sensor spots, likely due to performing the simplest type of calibration (1-point). The 10 minute full-scale response time includes not only the time for the sensor to respond, but also the length of time for the liquid to reach steady state in the flow system at this low flow rate after a switch in gas concentration.

Conclusion

The PreSens chemical optical CO_2 sensors were shown to outperform the Severinghaus-type dissolved carbon dioxide sensor in repeatability, stability, and response time for small volume, low flow-rate applications. Additionally, the PreSens pCO_2 mini system allowed for the noninvasive incorporation of a sensor spot into a microfluidic device, which was not possible with the commercial Severinghaus-type sensor.

The pCO_2 mini system effectively measured dissolved carbon dioxide concentrations in the microfluidic device rapidly and with repeatability, and is a viable option for measuring dissolved carbon dioxide concentrations for microfluidic applications. GEN

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