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TECHNICAL COMMENT

Respirometry: Anhydrous Drierite Equilibrates with Carbon Dioxide and Increases Washout Times

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ABSTRACT

Respirometry is a standard technique in comparative physiology laboratories, with measurements of rates of O_2 consumption and CO_2 production available for hundreds of species. A common recommendation when carrying out respirometry is that water vapor be removed from the air stream before entering the analyzer. Often, this is accomplished with the use of chemical desiccants, such as Drierite. However, here we show that Drierite has an affinity for CO_2 when new and completely anhydrous, and therefore it has an adverse effect on the washout characteristics of this gas. Exposing the Drierite to room air reduces this CO_2 affinity, and a 2-min exposure at 20°C and 50% relative humidity is sufficient to reduce the 99% washout time by almost a factor of 4, at the cost of only a 5% reduction in water vapor capacity. When Drierite is exhausted and then recharged according to the manufacturer's instructions, the CO_2 affinity is further reduced, and washout times are less than 60% greater than when no desiccant is used.

Introduction

Respirometry (the measurement of rates of oxygen consumption, $\dot{V}_{O_2}$, and carbon dioxide production, $\dot{V}_{CO_2}$) has been a standard technique in comparative physiology laboratories for decades, with $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements now available for hundreds of species (e.g., White and Seymour 2005). Typically,

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the technique involves measurement of changes in partial pressure of O_2 and CO_2 as air is either passed through a chamber containing an animal or drawn through a mask into which an animal breathes. Details of the procedures necessary for successful respirometry measurements are available in a number of articles that describe the specifics of measurement techniques, chamber design, and the derivation of appropriate equations for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ calculation (Depocas and Hart 1957; Withers 1977, 2001; Fedak et al. 1981; Frappell et al. 1989; Koteja 1996). A common recommendation is that water vapor be scrubbed from the excurrent air stream before it enters the analyzer. This is necessary because water vapor dilutes O_2 and CO_2 and potentially interferes with CO_2 measurement with infrared analyzers, although technological advancements mean that this interference is now less of a problem than it has previously been.

A number of commercially available chemical desiccants can be used to remove water vapor, including silica gel, phosphorus pentoxide, fused potassium hydroxide, sulfuric acid, and fused sodium hydroxide. Alternatively, water vapor can be removed by condensation at low temperature; that is, liquid air would give a dew point of about −190°C and a dry ice/acetone mix one of about −78°C (Withers 2001). Possibly the most well-known and commonly used chemical desiccant in comparative physiology laboratories is Drierite (anhydrous calcium sulfate; Hammond Drierite, Xenia, OH). When CO_2 is measured, Drierite is used in preference to silica gel, another common laboratory desiccant, because silica gel is known to equilibrate with CO_2 (Withers 2001).

Drierite dries air by chemically binding water as the hemihydrate of calcium sulfate, and it leaves a residual water content of about 0.005 mg L^{-1}. It is often impregnated with a cobalt chloride indicator (marketed as self-indicating Drierite) so that a color change from blue to pink indicates when the Drierite is exhausted. As part of a study of the energetics of birds, we used new Drierite that had never been recharged to scrub water vapor from the excurrent air stream of a positive-pressure open-flow respirometry system and observed that the CO_2 washout time during automated baseline checks was poor compared with that of O_2 and that the situation improved when smaller quantities of Drierite were used. Despite having chosen Drierite in preference to silica gel to avoid interfering with CO_2 measurements, we nevertheless observed an affinity for CO_2. Here

Table 1: Calculated 99% equilibration times ($\pm$ SE) for drying columns of varying Drierite capacity

Capacity (g)	99% Equilibration Time (s)		
	Empty	Full (New)	Full (Recharged)
30	15.0 $\pm$ .0	188 $\pm$ 4	22 $\pm$ 1
50	16.8 $\pm$ .4	312 $\pm$ 36	26 $\pm$ 2
100	39 $\pm$ 1	456 $\pm$ 34	44.7 $\pm$ .6

Note. Columns are either empty or contain new, unused Drierite or Drierite recharged according to the manufacturer's instructions. The effects of column capacity (30, 50, or 100 g) and Drierite (empty, new, recharged) on log(equilibration time) are significant (ANOVA; $F_{1,21} = 200$, $P < 0.0001$ and $F_{2,21} = 1,101$, $P < 0.0001$, respectively); their interaction is not ($F_{2,21} = 2.0$, $P = 0.16$).

we report the nature of the affinity of Drierite for CO_2 and describe a simple procedure to minimize it.

Methods

Dry gases (100% N_2 or 1% CO_2 in N_2) were drawn through a drying column by the internal pump of an ML206 O_2 and CO_2 analyzer (ADInstruments) at 200 mL min^{-1}. The analog voltage outputs of the analyzer were connected to a PowerLab ML750 analog-to-digital converter (ADInstruments) and recorded with Chart software (ADInstruments) at 4 Hz. A solenoid valve (SMC EVT307, Radio Spares, Corby, UK) under computer control (PowerLab ML750, ADInstruments) was used to automate the transition between the gases, which were provided in excess at atmospheric pressure.

The responses of the analyzer to changes in CO_2 content were compared when gases were passed through three different lengths of drying column, each of which were tested both with and without the presence of new, unused Drierite, as well as with Drierite that had been exhausted during routine animal respirometry studies and then recharged according to the manufacturer's instructions. Drierite capacities of the tubes were 30 g (small), 50 g (medium), and 100 g (large). The medium and large columns were probably larger than those that would be typically used for most measurements but were used in this study to ensure that differences were noticeable. Three complete CO_2 loading/unloading cycles were completed for each of the nine Drierite/column combinations.

Washout constants (k) for each of the columns were determined for the 0%–1% CO_2 transition as the absolute value of the slope of the relationship between $\ln(Fco_{2,eq} - Fco_2)$ and time, where Fco_2 is the fractional CO_2 content of excurrent air at a given time and $Fco_{2,eq}$ is the equilibrium fractional CO_2 content (Bartholomew et al. 1981; Seymour et al. 1998). Washout times (Δt) to a fraction of equilibrium (z; 0.99) were then calculated as $\Delta t = \ln(1 - z)/ - k$ (Withers 2001).

To examine the effect of water vapor uptake on the CO_2 washout characteristics of Drierite, the washout of the full me-

dium column was examined with new, anhydrous Drierite and following three successive 1-min exposures of the Drierite to room air (ca. 20°C, 50% relative humidity [RH]). A quantity of Drierite equal to or greater than the amount required to fill the column was exposed to air in a layer one granule thick on a paper towel and immediately was used to fill the drying column. Any excess was allowed to remain on the paper towel until pink, at which time it was considered used and was recharged according to the manufacturer's instructions.

Results and Discussion

Drierite clearly has an affinity for CO_2. The presence of new, anhydrous Drierite in a column increases CO_2 99% washout times by more than an order of magnitude (Table 1), a tendency that has been noted previously for a range of desiccants, including Drierite (Elia et al. 1986). During steady state measurements, the affinity of Drierite for CO_2 is unlikely to have an impact on measurements because the Drierite will be in equilibrium with the gases passing through the desiccant column. However, whenever the CO_2 content of the gas passing
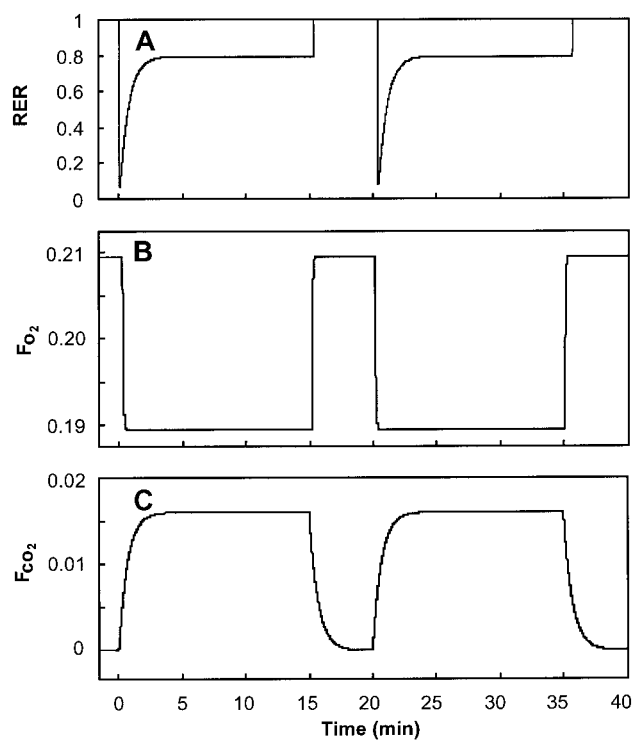

Figure 1. Model of the apparent changes in respiratory exchange ratio (RER; A) arising from differences in the washout characteristics of O_2 (B) and CO_2 (C). The system was modeled with a 30-g tube of new, anhydrous Drierite (Table 1); the O_2 washout was modeled as equivalent to that of CO_2 in the absence of Drierite. Fractional O_2 and CO_2 concentrations (Fo_2 and Fco_2, respectively) were calculated assuming an actual RER of 0.8, with an automated 5-min baseline ambient ($Fo_2 = 0.2095$, $Fco_2 = 0.0$) period every 20 min.
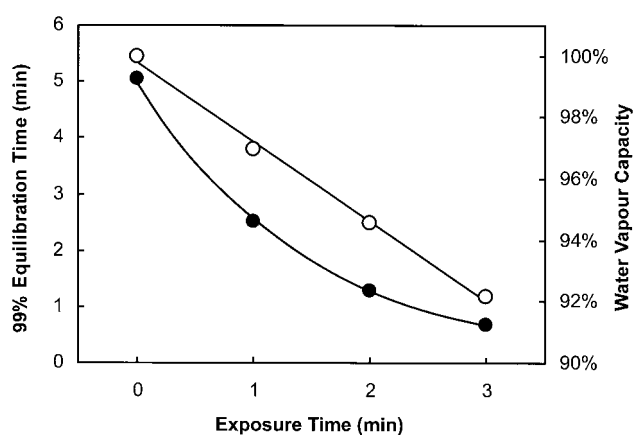
Figure 2. Relationship between time of air exposure and time to 99% equilibration (*left axis*, *filled circles*) and residual water vapor capacity of Drierite (*right axis*, *open circles*).

through the column changes, the slow washout of CO_2 is likely to be detrimental. Such problems will be apparent during non–steady state (instantaneous) measurements (Bartholomew et al. 1981; Seymour et al. 1998; Withers 2001) or when baseline (ambient) conditions are checked frequently to account for analyzer drift and barometric pressure changes. During instantaneous measurements, the slow washout of CO_2 through the column is likely to be a problem only when the washout of the respirometry chamber is faster than the washout of CO_2 through the column. When the washout of the chamber is slower than that of the drying column, as is often the case, the slow washout of CO_2 through the column will be largely masked by the slower washout of the chamber. However, when the drying column contributes most or all of the total washout, such as when air from a respirometry chamber is substituted with atmospheric air to check for baseline drift, the slow CO_2 washout will be a more serious problem.

Consider, for example, a positive-pressure open-flow system where a subsample of excurrent air is passed through a 30-g tube of new Drierite at a flow rate of 200 mL min^{-1} before entering the O_2 and CO_2 analyzer. Because of barometric pressure fluctuations, the temperature sensitivity of the analyzer, and electronic drift, a baseline check is required every 20 min, and the excurrent subsample and ambient air streams are switched by a solenoid valve located upstream of the drying column so that both air streams are dried by the same column. Such a system is in routine use in our laboratory, where we currently measure $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of great cormorants *Phalacrocorax carbo* and barnacle geese *Branta leucopsis*. Following a switch to ambient, the Drierite will require 3 min to equilibrate with the altered CO_2 level (Table 1), followed by 1 or 2 min of stable baseline measurement and a further 3 min to equilibrate after the switch to excurrent air from the respirometry chamber. Thus, 7 or 8 min are required for the complete

baseline measurement cycle, leaving only 12 or 13 min for measurement of excurrent air from the chamber. During the equilibration period following the switch from ambient air to excurrent air from the respirometry chamber, $\dot{V}_{CO_2}$ will be underestimated, and because CO_2 washes out of the desiccant column at a slower rate than O_2, the respiratory exchange ratio (ratio of $\dot{V}_{CO_2}$ to $\dot{V}_{O_2}$) will also be underestimated (Fig. 1). This observation illustrates the importance of examining raw data traces to ensure that systems are performing as expected and that the presence of chemical absorbents is not interfering with measurements.

Articles on standard respirometry (Depocas and Hart 1957; Withers 1977, 2001; Fedak et al. 1981; Frappell et al. 1989; Koteja 1996) make no mention of this problem and therefore provide no solution. Elia et al. (1986) reported that the affinity of Drierite for CO_2 decreased with increasing hydration, but they do not indicate the extent of hydration that is necessary to decrease affinity to an acceptable level. We found that exposing Drierite to room air (ca. 20°C, 50% RH) for only 2 min decreased 99% washout times from 5 to 1.3 min, at a cost of only a 5% reduction in water vapor capacity (Fig. 2). Further air exposure provides diminishing improvements in washout constant, with a linear decrease in water vapor capacity of ca. 2.6% min^{-1}. The CO_2 washout characteristics of Drierite can therefore be improved by exposing it to room air for a short period, but the duration of the period will be influenced by the temperature and humidity of the air to which it is exposed. A 2-min exposure at 20°C and 50% RH was sufficiently long for the indicating Drierite to begin to undergo a slight color change from blue to pink, so this change can be used as a rough indicator of the appropriate exposure time when ambient conditions differ substantially. Given that CO_2 washout time is related to Drierite quantity (Table 1), minimizing the size of the drying column also reduces washout times, and a respirometry system should be designed to incorporate the smallest quantity of Drierite that is compatible with the experimental duration required. Finally, Drierite that has been used and then recharged according to the manufacturer's instructions has a lower affinity for CO_2 and far shorter CO_2 washout times than does new Drierite (Table 1), so the use of recharged Drierite will largely circumvent CO_2 washout problems.

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