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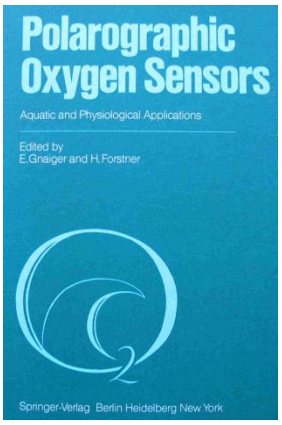
Oxygen Calibration and Solubility in Experimental Media



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Summary: High-resolution respirometry critically depends on accurate calibration of the polarographic oxygen sensors which respond to partial oxygen pressure. Expressing the oxygen signal in terms of partial pressure has several advantages, but respiration is expressed in molar units related to biochemical stoichiometries. Conversion of oxygen partial pressure to oxygen concentration requires accurate information on oxygen solubilities in experimental incubation media. Absolute errors up to 10% are commonly found in the established literature. The oxygen solubility of mitochondrial respiration medium MiR06 relative to pure water (oxygen solubility factor, F_M) is 0.92, accurately determined (for MiR05, i.e. MiR06 without catalase); [MiPNet14.13] at 37 °C and 30 °C. At air saturation at standard barometric pressure (100 kPa) and 37 °C, the partial oxygen pressure is 19.63 kPa, and the oxygen concentration is 190.7 µM in MiR06. A clean and ready-to-use Oxygraph chamber is a requirement for saving time and quickly calibrating the polarographic oxygen sensors.

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Oxygen calibration in high-resolution respirometry requires a fundamental analysis of oxygen solubilities based on a compilation of the pertinent physicochemical background. Consideration of this thermodynamic review helps to express instrumental specifications of the Oxygraph in generally valid units, e.g. using oxygen concentrations at standard rather than local barometric pressure as a reference.

1 O2k-Chamber: Cleaning and Storage with 70% Ethanol

1.1 Cleaning the Chamber

1. Clean the chamber before an experiment: Chemical sterilization is achieved by filling the measuring chamber, including the stirrer, and stopper cannula, with 70% ethanol. This is best performed without time delay by maintaining 70% ethanol in the chamber overnight (even over weeks if no experiments are performed). Ethanol should be filled up to the top of the chamber holder above the glass chamber, to include the thread in the cleaning. The stopper then slides into a loosely inserted position. Fill the cups at the top of the stoppers and close them with the Perspex cover slips. The ethanol will spill over and can be easily siphoned off with the integrated suction system (ISS). After such storage, washing with distilled water can be done immediately after switching on the Oxygraph-2k. In specific cases (Steininger et al., 2002), 3% formaldehyde may be applied for 10 min.
2. Clean the chamber after an experiment involving lipid-soluble inhibitors (such as oligomycin, rotenone, or antimycin A): The chamber must be cleaned rigorously with ethanol, since such inhibitor(s) are difficult to be washed out from the chamber and may inhibit mitochondrial respiration in subsequent experiments. Siphon off the cell/mitochondrial suspension at the end of the experiment and rinse the chamber with distilled water three times, by filling the chamber up to the rim. Also rinse the surface and cannula of the stopper with distilled water properly. Fill the water-cleaned chamber with 70% ethanol and replace the stopper making sure that the ethanol fills up the receptacle, and leave for 5 min. Remove the stopper and siphon off the ethanol to empty the chamber. Repeat these cleaning steps with 70% ethanol three times. Then fill the chamber with absolute ethanol (99.6%) and insert the stopper making sure that the ethanol fills up the cup at the top

of the stopper. Replace the perspex cover on top of the stopper and leave for 15-20 min. At the end of this rigorous cleaning procedure remove the stopper and place it inverted (with the receptacle at the bottom) on a clean paper towel. Siphon off the ethanol from the chamber and rinse it with distilled water three times. Rinse the stopper by holding it at the receptacle, not at the shaft that fits into the chamber to avoid contamination.

1.2 Storage in 70% Ethanol

An improved economical strategy is described for saving time and chemicals, and for improving wash-out of rotenone and other ethanol-soluble inhibitors from the Oxygraph-2k chamber. Experimental tests were performed on storage of the polarographic oxygen sensors (POS) in chambers of the Oxygraph-2k filled with 70% ethanol over periods extended up to two weeks. Based on these results, we recommend to fill the O2k chambers with 70% ethanol for storage over night and over extended periods of time, instead of using distilled water. Storage with ethanol thus replaces the time-consuming procedure described previously, and improves experimental reliability in high-resolution respirometry.

Until August 2006: 70% ethanol for 20 min: For many years, we recommended to maintain distilled or deionized water in the O2k chamber during short-term storage over a few days or weeks. This made it necessary to fill the chamber with 70% ethanol for a minimum of 20 min, to obtain a chemically sterilized system.

New tests and recommendations: Extended storage with 70% ethanol: Intensive tests were carried out which show that the oxygen sensor remains fully functional after storage for several days (weeks) in 70% ethanol. The following considerations led to a new recommendation on using 70% ethanol for short-term storage (20 days) and washing five times with distilled water immediately before addition of mitochondrial respiration medium (experimental salt solution). The test runs have been performed with our PEEK stirrer bars and with new PVDF stirrer bars, and with our titanium stoppers and with the new PVDF stoppers.

1. Time saving: At the end of an experimental day, the chambers are washed with water and then filled

completely with 70% ethanol, which remains in the chamber until the next experiment. Then it is not necessary to (1) wait for 20 min upon addition of ethanol, (2) wash the chambers with water, and (3) repeat the 20-min ethanol incubation at the subsequent experimental day. Before the next experiment, the ethanol is simply siphoned from the chamber (ISS), and a chemically sterilized chamber is available.

2. Ethanol saving: Instead of washing with EtOH in the evening and before the next experiment, a single filling of the chamber is sufficient for the O2k chambers and stoppers.
3. Washout of ethanol-soluble inhibitors and uncouplers: Long-term storage with 70% ethanol ensures an extensive solution of trace amounts of inhibitors from the materials of the chamber and stopper into the large volume of EtOH (>5 ml). The stopper is loosely inserted into the chamber, then the receptacle of the stopper is filled up completely and is sealed with the Perspex cover slips put on top of the stoppers.

The following observations provide a firm basis for the new recommendation on ethanol storage:

1. Over a period of 20 days, the calibration factor of the polarographic oxygen sensors (POS) changed by <2% when measured intermittently in salt solution after storage in 70% ethanol.
2. The POS signal stability at air calibration with salt solution corresponded to a slope of 0.1 ± 0.3 pmol·s^{-1}·ml^{-1} (mean ± SD) in 18 test runs with 6 different sensors over a 20 day period of ethanol storage.
3. Oxygen consumption by the POS at air saturation in salt solution was 2.0 ± 0.2 pmol·s^{-1}·ml^{-1} (mean ± SD) in 18 test runs (25 °C; 2 ml chamber volume; 6 different chambers) over a 20 day period of ethanol storage.
4. Air equilibration for estimation of the calibration factor was equally rapid after storage in 70% ethanol or in distilled water.
5. The exponential time constant of the POS remained constant over a 20 day period of ethanol storage.
6. The zero current of the POS remained stable over a 20 day period of ethanol storage, when measured in salt solution after oxygen depletion by isolated mitochondria.

2 Short Two-Point Calibration

1. Add 2.1 to 2.5 ml of experimental medium. Before addition, this medium should be at or slightly above experimental temperature, to avoid the formation of gas bubbles and minimize the temperature disturbance of the chamber.
2. With the stirrer on, insert the stopper fully into the volume-calibrated chamber. Siphon off excess medium from the top of the stopper.
3. Push up the stopper to insert the stopper-spacer tool, allowing air to equilibrate with the medium. Equilibrium (in terms of temperature and oxygen pressure) is achieved when the oxygen signal reaches a constant level.
4. Prepare a stock of bakers yeast, with 200 mg dry yeast in 2 ml physiological salt solution. Stirr heavily to obtain a homogenous suspension of yeast cells.
5. When apparent oxygen flux is zero (<± 0.5 pmol·s^{-1}·ml^{-1}) for c. 10 min, set a mark for air calibration, R1 [V].
6. Close the chamber by fully inserting the stopper.
7. After stirringing the yeast suspension, add 50 µl yeast suspension into the 2 ml chamber through the cannula of the stopper, using a Hamilton syringe. Oxygen depletion is very rapid, zero oxygen is reached within a few minutes, but a few more minutes may be required until a stable "zero" signal is obtained, R_0 [V].
8. Set a mark over the stable "zero" signal (R_0), to complete the two-point oxygen calibration [F5]. Select Mark R1 and Mark R0 for R_1 and R_0 (or select the appropriate marks, if they were not renamed or named differently). The same zero signal is obtained with highly dilute yeast cells, at respiratory oxygen fluxes >10 pmol·s^{-1}·ml^{-1}. This is due to the high oxygen affinity of yeast cells. Internal zero calibration in studies of oxygen kinetics extends the accuracy of zero calibrations (Gnaiger et al 1995; Gnaiger 2001).
9. Rinse the chamber and removed stopper with water several times, while the stirrer is left on. Rinse with 70% ethanol and fill the chamber fully with 70% ethanol. The stoppers may be inserted loosely, to maintain chamber and stopper in 70% ethanol for 20 min. Rinse with water several times.
10. Add 2.1 to 2.5 ml of experimental medium, and perform another air calibration as described above. R_1 will be replaced by a new calibration value which should be very close to the previous R_1, which provides a

stability control for the POS. R_0 does not have to be determined again, and the previously measured R_0 is maintained during the two-point calibration.

3 Equations for Oxygen Calibration

3.1 Oxygen Concentration and Recorded Signal

The recorded oxygen signal, R_t, at experimental time t, is calibrated in terms of oxygen concentration at time t, $c_{O_2}(t)$,

$$c_{O_2}(t) = (R_t - a_c) \cdot F_c \quad (1)$$

where F_c is the calibration factor based on concentration (Eq. 2),

$$F_c = \frac{c_1 - c_0}{R_1 - R_0} \quad (2)$$

and a_c is the POS signal at zero oxygen concentration,

$$a_c = \frac{c_1 \cdot R_0 - c_0 \cdot R_1}{c_1 - c_0} \quad (3)$$

$c_1 = c_{O_2}^*$ is the oxygen concentration at equilibrium with air. Typically, R_1 and R_0 are the calibration recordings at air saturation and zero oxygen (if c_0 = 0 µM, then $a_c = R_0$.

3.2 Oxygen Pressure and POS Current

In the more general case, the oxygen sensor responds to partial oxygen pressure, and a linear oxygen calibration can be performed at any two calibration pressures of oxygen, p_1 and p_0. The corresponding oxygen signals in terms of current [µA] are I_1 and I_0. A sensor current of 1 µA yields a raw signal of 1 V at a gain setting of 1 V/µA. G is 2 or 4 V/µA in most O2k applications, and can be changed in the O2k Setup window [F7] to 1, 2, 4 or 8 V/µA. The sensor current, I_t, at any time t, therefore, is related to the recorded signal, R_t [V], according to the gain setting,

$$I_t = R_t/G \quad (4)$$

The zero current or offset, a [µA], is

$$a = \frac{p_1 \cdot I_0 - p_0 \cdot I_1}{p_1 - p_0} \quad (5)$$

If the calibration point p_0 is chosen at zero oxygen concentration, then $a = I_0$. The corresponding calibration factor, related to partial pressure and current, is F_p [kPa/µA],

$$F_p = \frac{p_1 - p_0}{I_1 - I_0} \quad (6)$$

After calibration, comparable to Eq.(1), the partial oxygen pressure, $p_{O_2}(t)$, can be calculated from the POS signal current,

$$p_{O_2}(t) = (I_t - a) \cdot F_p \quad (7)$$

3.3 Oxygen Concentration and Oxygen Pressure

The oxygen partial pressure is related to the oxygen concentration, $c_{O_2}(t)$ [µM=nmol/ml], by the oxygen solubility, S_{O2} [µM/kPA], which is calculated by DatLab on the basis of experimental temperature and the oxygen solubility factor of the experimental medium, F_M.

$$c_{O_2}(t) = p_{O_2}(t) \cdot S_{O_2} \quad (8)$$

3.4 Oxygen Signal and Background Oxygen Consumption

The oxygen-related POS current, I_t-a [µA] (Eq. 7), results from the steady-state oxygen diffusion from the medium across the membrane and oxygen consumption at the cathode of the POS. Based on the stoichiometry of 4 electrons per molecule O_2 reduced at the cathode and the Faraday constant (96,485 C/mol), oxygen consumption is expected at 2.591 pmol $O_2 \cdot s^{-1} \cdot \mu A^{-1}$. The oxygen consumption by the POS, per volume of the O2k chamber, V [ml], is $J°_{O_2,POS}$ [pmol·s^{-1}·ml^{-1}], calculated as

$$J°_{O_2,POS} = 2.591 \cdot (I_t - a_p) / V \quad (9)$$

When the O2k-chamber is closed after equilibration at air saturation, the measured instrumental background oxygen consumption, $J°_{O_2}$, can be compared with this theoretical value. Considering the POS signal at gain 2 and 37 °C to be around 4 V (at gain 4: around 8 V), then $I_t - a$ is about 2 µA (Eq. 4). At a volume of 2 ml, therefore, the expected instrumental background at air saturation is 2.6 pmol $O_2 \cdot s^{-1} \cdot ml^{-1}$ (Eq. 9).

4 O_2 Solubility and Concentration at Air Saturation

4.1 Oxygen Pressure and Concentration

It is practical to calculate the saturation concentration for pure water, which then is corrected by the solubility factor of the medium, F_M, to account for the reduced O_2 solubility in salt media. Owing to the salting-out effect, F_M must be <1.0 in salt media used for respiratory studies of mitochondria, cells and tissues.

F_M is typically near 0.9 for Oxygraph media (0.92 for MiR06 and MiR05); [MiPNet14.13]. Several oxygen solubilities reported in the literature must be critized on the basis of physicochemical considerations.

Water in equilibrium with air contains an oxygen concentration proportional to the oxygen solubility and the partial oxygen pressure of air. In the gas-liquid boundary, air is saturated with water vapor at the partial pressure of $p_{H_2O}^*$. The water vapor pressure is subtracted from the total barometric pressure, p_b, to obtain the partial pressure of dry air, p_b-$p_{H_2O}^*$. The volume fraction of dry air is constant at $\Phi_{O_2} = 0.20946$. Therefore, the partial oxygen pressure at air saturation is, for any temperature and barometric pressure,

$$p_{O_2}^* = (p_b - p_{H_2O}^*) \cdot 0.20946 \quad (10)$$

The saturation O_2 concentration depends on the O_2 solubility, S_{O_2} [µmol·dm^{-3}·kPa^{-1}],

$$c_{O_2}^* = p_{O_2}^* \cdot S_{O_2} \quad (11)$$

Oxygen solubility is a function of temperature and composition of the medium. In other words, oxygen solubility, S_{O_2}, is defined as the ratio of partial oxygen pressure and concentration,

$$S_{O_2} = c_{O_2}^*/p_{O_2}^* \quad (12)$$

4.2 Temperature Effect on Saturation O_2 Concentration

$p_{H_2O}^*$ (Eq. 10) is the saturation water vapor pressure at experimental temperature. $p_{H_2O}^*$ is a function of absolute temperature, T [K], obtained from the experimental temperature, θ, recorded in units °C,

$$T = \theta + 273.15^{*} \quad (13)$$

The saturation water vapor pressure [kPa] is (Table 1),

$$p_{H_2O}^{*} = \exp[(-216961 \cdot T^{-1} - 3840.7) \cdot T^{-1} + 16.4754] \quad (14)$$

Until recently, the atm-standard pressure has been used: 1 atm = 760 mmHg = 101.325 kPa. For pure water in equilibrium with air at this atm-standard pressure, the 'unit standard concentration' of oxygen, C^{*}, is calculated by the polynomial expression,

$$C^{*} = \exp<\{[(-8.621949 \cdot 10^{11} \cdot T^{-1} + 1.243800 \cdot 10^{10}) \cdot T^{-1} - 6.642308 \cdot 10^{7}] \cdot T^{-1} + 1.575701 \cdot 10^{5}\} \cdot T^{-1} - 135.90202> \quad (15)$$

Table 1. Saturation water vapor pressure, $p_{H_2O}^{*}$, oxygen pressure, p_{O_2}*, and oxygen concentration, $c_{O_2}^{*}$, at air saturation and standard barometric pressure, p_b^o = 100 kPa, in pure water as a function of temperature. S_{O_2} is the oxygen solubility, independent of choice of standard pressure. f^o is the multiplication factor to convert partial O_2 pressures and concentrations given at atm-standard pressure (1 atm = 101.325 kPa) to the *IUPAC* standard pressure of 100 kPa (compare Eq. 15),

$$f^o = (100 - p_{H_2O}^{*}) / (101.325 - p_{H_2O}^{*})$$

θ	T	$p_{H_2O}^{*}$	$p_{O_2}^{*}$	$c_{O_2}^{*}$	f^o	S_{O_2}
°C	K	kPa	kPa	µmol·dm^{-3}		µmol·dm^{-3}·kPa^{-1}
40	313.15	7.38	19.40	197.6	0.9859	10.18
37	310.15	6.27	19.63	207.3	0.9861	10.56
35	308.15	5.62	19.77	214.2	0.9862	10.83
30	303.15	4.24	20.06	233.0	0.9864	11.62
25	298.15	3.17	20.28	254.8	0.9865	12.56
20	293.15	2.34	20.46	280.4	0.9866	13.70
15	288.15	1.70	20.59	310.9	0.9867	15.10
10	283.15	1.23	20.69	348.1	0.9868	16.83
5	278.15	0.87	20.76	393.9	0.9868	18.97
4	277.15	0.81	20.78	404.3	0.9868	19.46

4.3 Barometric Pressure Effect on Saturation O_2 Concentration

The unit standard concentration and the oxygen concentration at air saturation and actual barometric pressure are related by (compare f^o in Table 1),

$$c_{O_2}^* = C^* \cdot p_{O_2}^*/[(101.325\text{-}p_{H_2O}^*)\cdot 0.20946] \cdot F_M$$

$$= C^* \cdot (p_b\text{-}p_{H_2O}^*)/(101.325\text{-}p_{H_2O}^*) \cdot F_M \qquad (16)$$

Values for characteristic temperatures are given in Table 1.

4.4 The O_2 Solubility Factor in Salt Solutions

The salting out effect is responsible for the reduced oxygen solubility in aqueous solutions compared to pure water (Fig. 1). Detailed equations are available for calculating the oxygen solubility of sea water at different salinities (Forstner and Gnaiger 1983).

Physiological solutions commonly used in Oxygraph studies (Rasmussen, Rasmussen 2003; Reynafarje, Costa, Lehninger 1985) are compared with pure water and 20‰ sea water in Fig. 1. The corresponding polynomial equations are summarized in Table 2 for calculating the oxygen saturation concentration in equilibrium with air at various temperatures and standard pressure (Table 3). Characteristic temperatures are commonly used in experimental studies. Under these conditions it is convenient to use oxygen solubility factors for the medium, F_M (Fig. 1). This factor is independent of barometric pressure, but F_M changes with temperature (compare Fig. 1). The solubility factors are compiled in Table 4 for different salinities of sea water (Forstner and Gnaiger 1983) and two typical media used with isolated mitochondria (Reynafarje, Costa, Lehninger 1985). The latter values have been criticized on methodological grounds by Rasmussen and Rasmussen (2003), and the complex temperature dependence of F_M compared to sea water is doubtful from a thermodynamic perspective Fig. 1).

The oxygen solubility factor of MiR05 (MiR06) is 0.92, at 30 °C and 37 °C (Rasmussen, Rasmussen 2003), corresponding to an oxygen concentration in equilibrium with air under standard conditions ($c_{O_2}^*$) of 214.4 and 190.7 µM, respectively. The oxygen solubility

of serum is 9.4 nmol $O_2 \cdot cm^{-3} \cdot kPa^{-1}$ at 37 °C (Baumgärtl and Lübbers 1983). In comparison to the oxygen solubility in pure water (10.56 nmol $O_2 \cdot cm^{-3} \cdot kPa^{-1}$ at 37 °C; Table 1), this corresponds to a solubility factor for serum of F_M = 0.89 (Fig. 1) and $c_{O_2}^*$ of 184.5 µM.

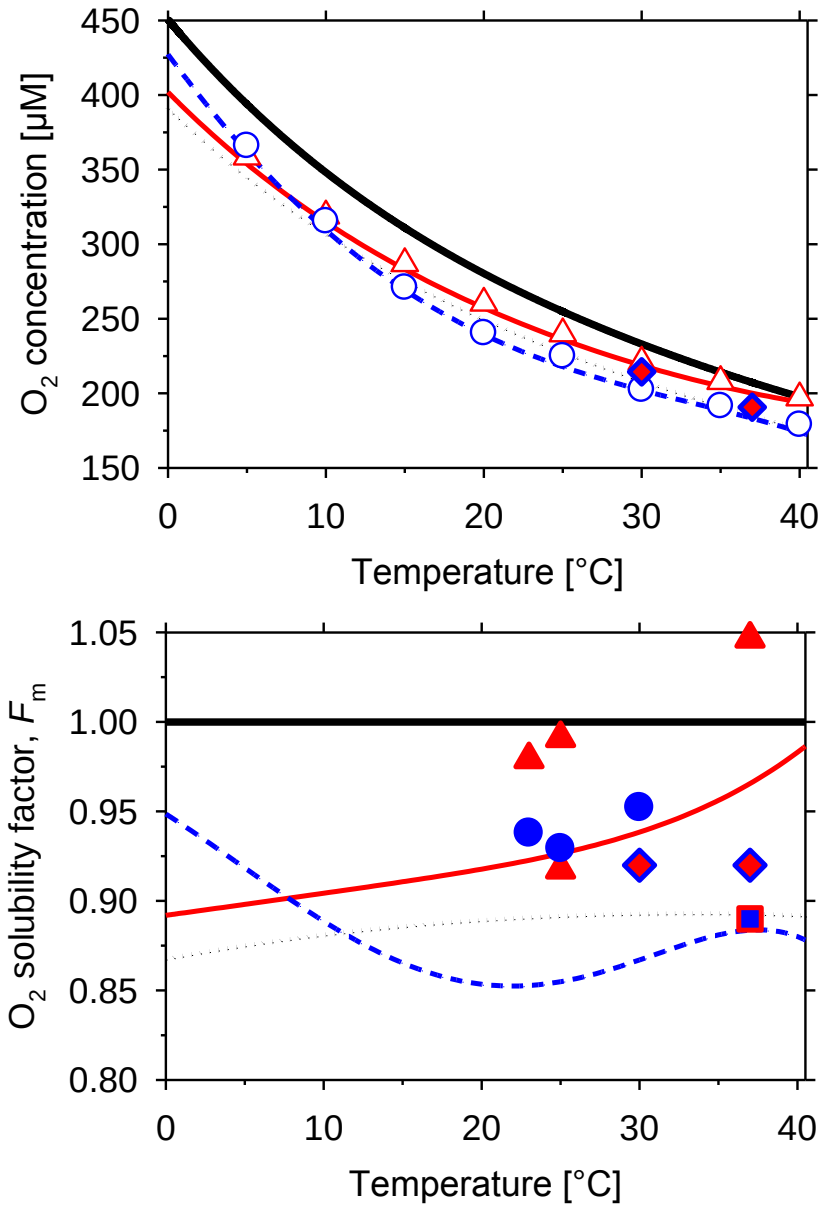

Figure 1. Oxygen concentration at air saturation and standard barometric pressure (100 kPa; top) and oxygen solubility factor (bottom) in MiR05 (diamonds), KCl medium (open trianlges, full line; 150 $mmol \cdot dm^{-3}$ KCl) and sucrose medium (open circles, dashed line; 250 $mmol \cdot dm^{-3}$ sucrose; data for both media from Reynafarje et al 1985), compared to pure water (upper full line) and 20‰ sea water (lower dotted line). For the parameters of the polynomials see Table 2. The solubility factor for serum is shown by the full square (bottom). Literature data (bottom) on KCl media (closed triangles) and sucrose media (closed circles) show (i) the wide scatter of solubility data, (ii) the erroneous use of values even higher than solubility established for pure water, and (iii) a trend to higher values, particularly in sucrose medium, compared to Reynafarje et al 1985.

Table 2. Parameters of the polynomial fits of oxygen saturation concentration in equilibrium with air at p_b^o = 100 kPa, for sea water (0‰ and 20‰) and typical Oxygraph incubation media, in the range of θ from 5 to 40 °C. Instead of the theoretically based plot of $\ln(S_{O_2})$ versus T^{-1}, the fits were performed on the untransformed data, with temperature, θ, in units of °C ($r^2 \geq 0.999$ in all cases). The equation in nested form is,

$$c_{O_2}^* = \{[(b_4 \cdot \theta + b_3) \cdot \theta + b_2] \cdot \theta + b_1\} \cdot \theta + a$$

Medium	A	B_1	b_2	b_3	b_4
0‰	450.5946	-12.60381	0.2712233	-0.003808	$2.379 \cdot 10^{-5}$
20‰	390.8769	-10.2165	0.2051415	-0.002746	$1.621 \cdot 10^{-5}$
KCl	401.9152	-10.70002	0.2291496	-0.003283	$2.492 \cdot 10^{-5}$
Sucrose	427.411	-14.4983	0.2762108	-0.0003628	$-3.606 \cdot 10^{-5}$

5 References

Baumgärtl H, Lübbers DW (1983) Microaxial needle sensor for polarographic measurement of local O_2 pressure in the cellular range of living tissue. Its construction and properties. In: *Polarographic Oxygen Sensors*, Gnaiger E, Forstner H (eds) Springer, Berlin, Heidelberg, New York: 37-65.

Beavis AD, Lehninger AL (1986) Eur J Biochem 158: 315-322.

Brand MD; Harper ME, Taylor HC (1993) Control of the effective P/O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes. Biochem J 291: 739-748.

Chapell JB (1964) Biochem J 90: 225-237.

Forstner H, Gnaiger E (1983) Calculation of equilibrium oxygen concentration. In: *Polarographic Oxygen Sensors. Aquatic and Physiological Applications.* Gnaiger E, Forstner H (eds) Springer, Berlin, Heidelberg, New York: 321-333.

Gnaiger E, Steinlechner-Maran R, Méndez G, Eberl T, Margreiter R (1995) Control of mitochondrial and cellular respiration by oxygen. J Bioenerg Biomembr 27: 583-596.

Hinkle PC, Yu ML (1979) J Biol Chem 254: 2450-2455.

Lemasters JJ (1984) J Biol Chem 259: 13123-13130.

Rasmussen HN, Rasmussen UF (2003) Oxygen solubilities of media used in electrochemical respiration measurements. Analyt Biochem 319: 105-113.

Rettich TR, Battiono R, Wilhelm E (2000) Solubility of gases in liquids. 22. High-precision determination of Henry's law constants of oxygen in liquid water from T=274 K to T=328 K. J Chem Thermodynam 32: 1145-1156.

Reynafarje B, Costa LE, Lehninger AL (1985) O_2 solubility in aqueous media determined by a kinetic method. Analyt Biochem 145: 406-418

Stoner CD (1987) J Biol Chem 262: 11445-11453.

Wilhelm E, Battino R, Wilcock RJ (1977) Low-pressure solubility of gases in liquid water. Chem Rev 77: 219-262.

Appendix

Table 3. Oxygen solubility, S_{O_2} [µM.kPa^{-1}], for seawater at various salinities (10‰, 20‰, 30‰ and 36‰), and for two typical Oxygraph media (concentrations given in mmol·dm^{-3}); "Sucrose": 250 sucrose, 5 KCl, 3 K-Hepes, pH 7.05; "KCl": 150 KCl, 3 K-Hepes, pH 7.05.

θ	S_{O_2} for sea water				S_{O_2} for exp. Medium	
°C	10‰	20‰	30‰	36‰	Sucrose	KCl
40	9.62	9.08	8.58	8.29	8.96	10.01
37	9.98	9.43	8.90	8.61	9.33	10.19
35	10.24	9.67	9.14	8.83	9.54	10.36
30	10.98	10.37	9.80	9.47	10.07	10.90
25	11.86	11.20	10.57	10.21	10.74	11.64
20	12.92	12.19	11.49	11.09	11.70	12.58
15	14.21	13.38	12.59	12.14	13.07	13.75
10	15.79	14.82	13.91	13.39	14.95	15.22
5	17.75	16.60	15.53	14.92	17.42	17.04
4	18.19	17.00	15.89	15.26	17.99	17.45

Table 4. Oxygen solubility factor of the medium, F_M, for seawater at various salinities (10‰, 20‰, 30‰ and 36‰), and for two typical Oxygraph media (concentrations given in mmol·dm^{-3}); "Sucrose": 250 sucrose, 5 KCl, 3 K-Hepes, pH 7.05; "KCl": 150 KCl, 3 K-Hepes, pH 7.05.

θ	F_M for sea water				F_M for exp. Medium	
°C	10‰	20‰	30‰	36‰	Sucrose	KCl
40	0.945	0.892	0.842	0.814	0.880	0.983
37	0.945	0.893	0.843	0.815	0.884	0.966
35	0.945	0.893	0.844	0.815	0.881	0.956
30	0.945	0.893	0.843	0.815	0.867	0.938
25	0.944	0.892	0.842	0.813	0.855	0.926
20	0.943	0.889	0.838	0.809	0.853	0.918
15	0.941	0.886	0.833	0.804	0.865	0.911
10	0.939	0.881	0.827	0.796	0.889	0.904
5	0.936	0.875	0.819	0.786	0.918	0.898
4	0.935	0.881	0.817	0.784	0.925	0.897