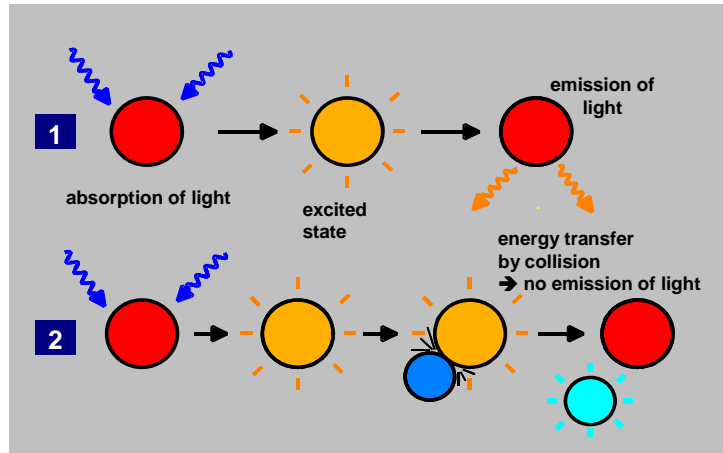# 1 Appendix

1.1 Basics in Optical Sensing of Oxygen

1.1.1 Dynamic Quenching of Luminescence

The principle of measurement is based on the effect of dynamic luminescence quenching by molecular oxygen. The following scheme explains the principle of dynamic luminescence quenching by oxygen.

Figure 13.1 *Principle of dynamic quenching of luminescence by molecular oxygen*

  (1) Luminescence process in absence of oxygen

  (2) Deactivation of the luminescent indicator molecule by molecular oxygen

The collision between the luminophore in its excited state and the quencher (oxygen) results in radiationless deactivation and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable luminescence signal decreases.

A relation exists between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime which is described in the Stern-Volmer-equation (**1**). Here, τ_0 and τ are the luminescence decay times in absence and presence of oxygen (I_0 and I are the respective luminescence intensities), $[O_2]$ the oxygen concentration and K_{SV} the overall quenching constant.

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2]$$
$$I = f([O_2])$$
$$\tau = f([O_2])$$

(**1**)

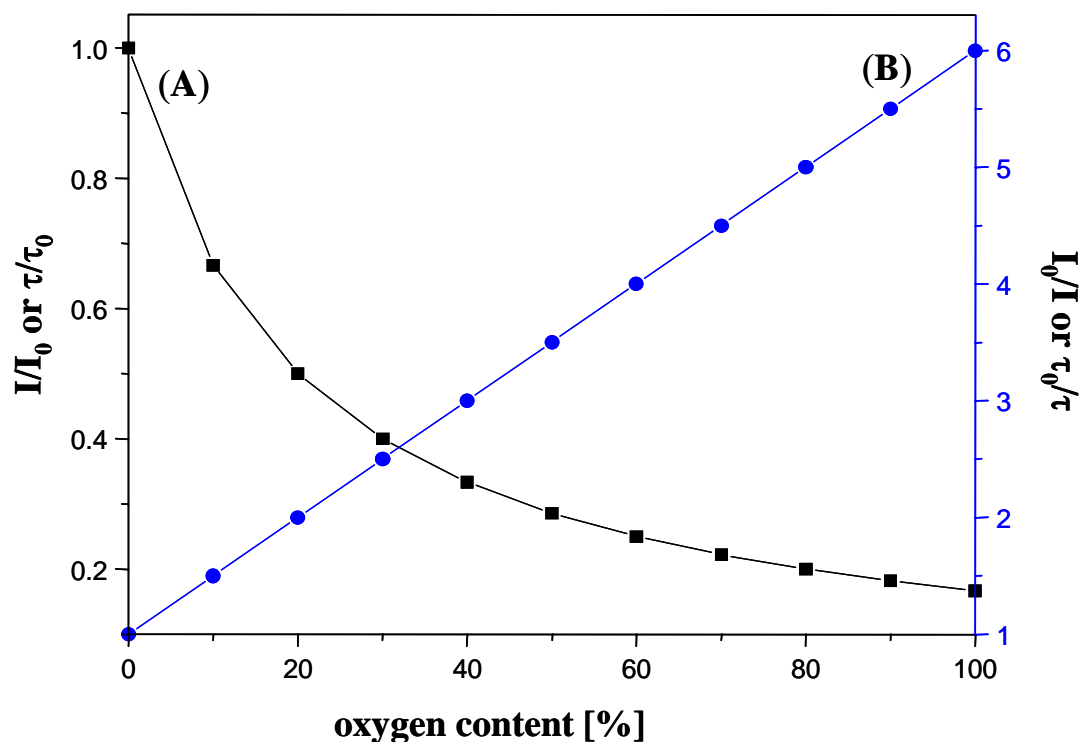I: Luminescence intensity in presence of oxygen

I_0:   Luminescence intensity in absence of oxygen

τ: Luminescence decay time in presence of oxygen

τ_0:   Luminescence decay time in absence of oxygen

K_{SV}:   Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)

$[O_2]$:   Oxygen content

Figure. 13.2 *(A) Luminescence decrease in the presence of oxygen. (B) Stern-Volmer plot.*

## 1.1.2 Major Components of Fiber-Optic Minisensors

In optical chemical sensors, the analyte interacts with an indicator and changes its optical properties. The result is either a change in the color (absorbance or spectral distribution) or the luminescence properties (intensity, lifetime, polarization). Light acts as the carrier of the information.

The major components of a typical fiber-optical sensing system are

- a light source to illuminate the sensor (laser, light emitting diode, lamps)
- an optical fiber as signal transducer (plastic or glass fiber)
- a photodetector (photodiode, photomultiplier tube, CCD-array)
- the optical sensor (indicator immobilized in a solid matrix)
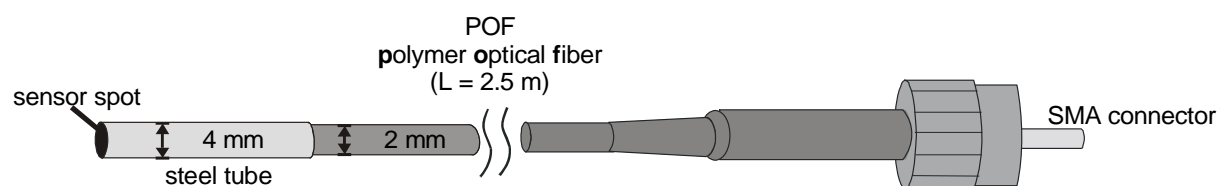
Figure 13.3 *Scheme of a minisensor.*
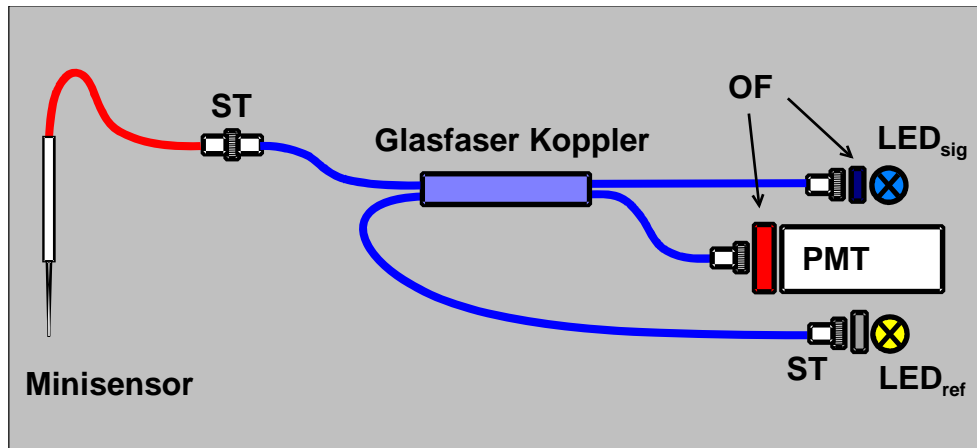
Figure 13.4 Schematic drawing of the optical setup of a measuring system with minisensors (LED: light emitting diodes, PMT: photomultiplier, OF: optical filters, ST: fiber connector).

1.1.3 Advantages of Optical Oxygen-Sensitive Minisensors

- no oxygen is consumed during the measurement;
- the signal is independent of changes in flow velocity;
- they are able to measure the oxygen content in dry gases
- they are insensible towards electrical interferences and magnetic fields;
- they are more sensitive than conventional electrodes (up to ppt-range);
- long-term stability and low drift;
- using silica fibers, it is possible to measure in samples while physically separate from the light source and detectors;
- light-conducting fibers are able to transport more information than power currents (information can be simultaneously transferred, e.g., intensity of light, spectral distribution, polarization, information such as decay time or delayed fluorescence);

1.1.4 Luminescence Decay Time

The Fibox 3 measures the luminescence decay time of the immobilized luminophore as the oxygen-dependent parameter.

$$\tau = f([O_2]) \tag{2}$$

The Fibox 3 uses the phase modulation technique to evaluate the luminescence decay time of the indicators. If the luminophore is excited with light with sinusoidally modulated intensity, its decay time causes a time delay in the emitted light signal. In technical terms, this delay is the phase angle between the exciting and emitted signal. This phase angle is shifted as a function of the oxygen concentration. The relation between decay time τ and the phase angle Φ is shown by the following equation:

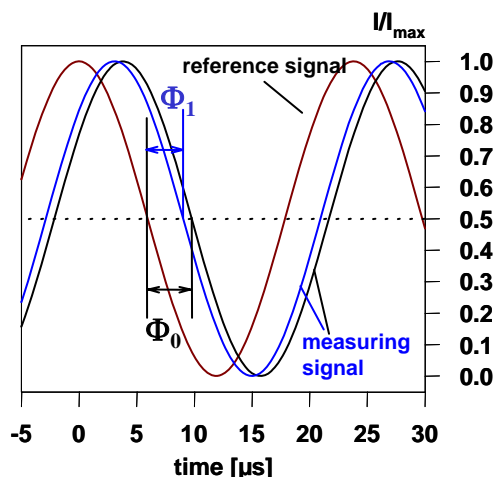$$\tau = \frac{\tan \Phi}{2\pi \cdot f_{mod}} \tag{3a}$$

$$\tan \Phi = 2\pi \cdot f_{mod} \cdot \tau \tag{3b}$$

$$\tau \equiv \tan \Phi \equiv \Phi \equiv f([O_2]) \tag{3c}$$

τ: luminescence decay time; Φ: phase angle; f_{mod}: modulation frequency
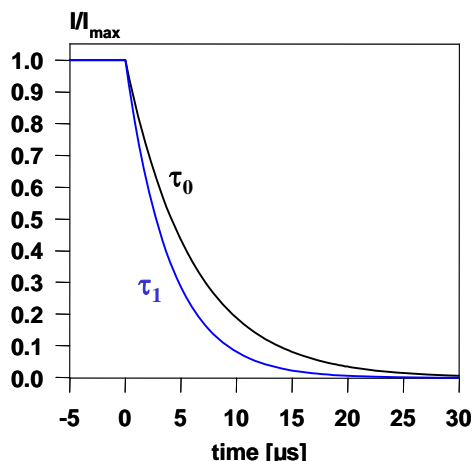
Figure 13.5 *Schematic of the single exponential decay ($t_0 > t_1$).*

Figure 13.6 *The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state*

The measurement of the luminescence decay time, an intrinsically referenced parameter, has the following advantages compared to the conventional intensity measurement:

- The decay time does not depend on fluctuations in the intensity of the light source and the sensitivity of the detector;

- The decay time is not influenced by signal loss caused by fiber bending or by intensity changes caused by changes in the geometry of the sensor;

- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ photobleaching and leaching of the indicator dye has no influence on the measuring signal;

- The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

1.1.5 Literature

If you want to find out more about this subject, we recommend the following publications.

- Wolfbeis O.S. (Ed.), *Fiber Optic Chemical Sensors and Biosensors*, Vol. **1**&**2**, CRC, Boca Raton (1991).

- Klimant I., Wolfbeis O.S., *Oxygen-Sensitive Luminescent Materials Based on Silicone-Soluble Ruthenium Diimine Complexes*, Anal. Chem., **67**, 3160-3166 (1995).

- Klimant I., Kühl M., Glud R.N., Holst G., *Optical measurement of oxygen and temperature in microscale: strategies and biological applications*, Sensors and Actuators B, **38-39**, 29-37 (1997).

- Holst G., Glud R.N., Kühl M., Klimant I., *A microoptode array for fine-scale measurement of oxygen distribution*, Sensors and Actuators B, **38-39**, 122-129 (1997).

- Klimant I., Meyer V., Kühl M., *Fiber-optic oxygen microsensors, a new tool in aquatic biology*, Limnol. Oceanogr., **40**, 1159-1165 (1995).

- Klimant I., Ruckruh F., Liebsch G., Stangelmayer A., Wolfbeis O.S., *Fast Response Oxygen Microsensors Based on Novel Soluble Ormosil Glasses*, Mikrochim. Acta, **131**, 35-46 (1999).

1.2 Oxygen Conversion Formulas

Please note:

These conversion formulas are only valid in aqueous solutions and humidified air. These formulas have to be modified if measurements have to be performed in organic solvents or solutions with high salinity.

% saturation

% air-saturation

Default setting of the instrument.

% oxygen-saturation

$$\% \, O_2 = \% \ \text{air - saturation} \cdot \frac{20.95}{100} \tag{4}$$

0.2095: volume content of oxygen in air

ppm in the gaseous phase:

$$\text{ppm}[O_2] = \% \ \text{air} - \text{saturation} \cdot \frac{20.95}{100} \cdot 10000 = \% \, O_2 \cdot 10000 \tag{5}$$

$$1\text{ppm} = \frac{1}{1000000} = \frac{1\text{mg}}{1\text{kg}} = \frac{1\mu\text{L}}{1\text{L}} = \frac{1}{10000}\% $$

Partial pressure of oxygen
in hPa

$$p_{O_2}[\text{hPa}] = \left(p_{\text{atm}}[\text{hPa}] - p_W(T)[\text{hPa}]\right) \cdot \frac{\% \ \text{air - saturation}}{100} \cdot 0.2095 \tag{6}$$

in mbar

$$p_{O_2}[\text{mbar}] = \left(p_{\text{atm}}[\text{mbar}] - p_W(T)[\text{mbar}]\right) \cdot \frac{\% \ \text{air - saturation}}{100} \cdot 0.2095 \tag{7}$$

in Torr

$$p_{O_2}[\text{Torr}] = \left[\left(p_{\text{atm}}[\text{mbar}] - p_W(T)[\text{mbar}]\right) \cdot \frac{\% \ \text{air - saturation}}{100} \cdot 0.2095\right] \cdot 0.75 \tag{8}$$

Please note:
1 mbar = 1 hPa = 0.750 Torr
Oxygen Concentration

in mg/L

$$c_{O_2}[\text{mg / L}] = \frac{p_{\text{atm}} - p_W(T)}{p_N} \cdot \frac{\% \ \text{air - saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \tag{9}$$

in ppm = mg/L (in case the density is equal to 1 kg/L)

$$c_{O_2}[\text{ppm}] = c_{O_2}[\text{mg / L}] = \frac{p_{\text{atm}} - p_W(T)}{p_N} \cdot \frac{\% \ \text{air - saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \tag{10}$$

in μmol/L

$$c_{O_2}[\mu mol/L] = c_{O_2}[mg/L] \cdot \frac{1000}{M(O_2)} = c_{O_2}[mg/L] \cdot 31.25$$

$$= \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\% \text{ air - saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000000 \cdot \frac{1}{V_M}$$

(**11**)

p_{atm}: actual atmospheric pressure

p_N: standard pressure (1013 mbar)

0.2095: volume content of oxygen in air

$p_W(T)$: vapor pressure of water at temperature T given in Kelvin

$\alpha(T)$: Bunsen absorption coefficient at temperature T; given in $cm^3(O_2)/cm^3$

$M(O_2)$: molecular mass of oxygen (32 g/mol)

V_M: molar volume (22.414 L/mol)

1.3   Temperature-Dependent Constants Affecting the Oxygen Content

1.3.1   Water Vapor Pressure

As shown in equation 4 - 11, the water vapor pressure p_w influences the oxygen partial pressure of air-saturated water and water vapor-saturated air.

Oxygen partial pressure in dry air:

$$p(O_2) = p_{atm} \cdot 0.2095$$

(**12**)

$p(O_2)$: oxygen partial pressure in dry air at a barometric pressure p_{atm}
0.2095: volume content of oxygen in air.

Oxygen partial pressure in air-saturated water and water vapor-saturated air:

$$p(O_2)' = (p_{atm} - p_W(T)) \cdot 0.2095$$

(**13**)

Temperature variations strongly affect water vapor pressure, and thus influence the oxygen partial pressure as shown in equation 13.

Table 13.1 Variation of water vapor pressure $p_W(T)$ with temperature.

θ [°C]	0	5	10	15	20	25	30	35	40	50
T [K]	273	278	283	288	293	298	303	308	131	323
$p_W(T)$ [mbar]	6.1	8.7	12.3	17.1	23.3	31.7	42.4	56.3	73.7	123.3

A convenient fitting function is given by the Campbell equation 14:

$$p_W(T) = \exp\left(A - \frac{B}{T} - C \cdot \ln T \right)$$

(**14**)

where T is the temperature in Kelvin and A, B and C constants given in Figure 13.7
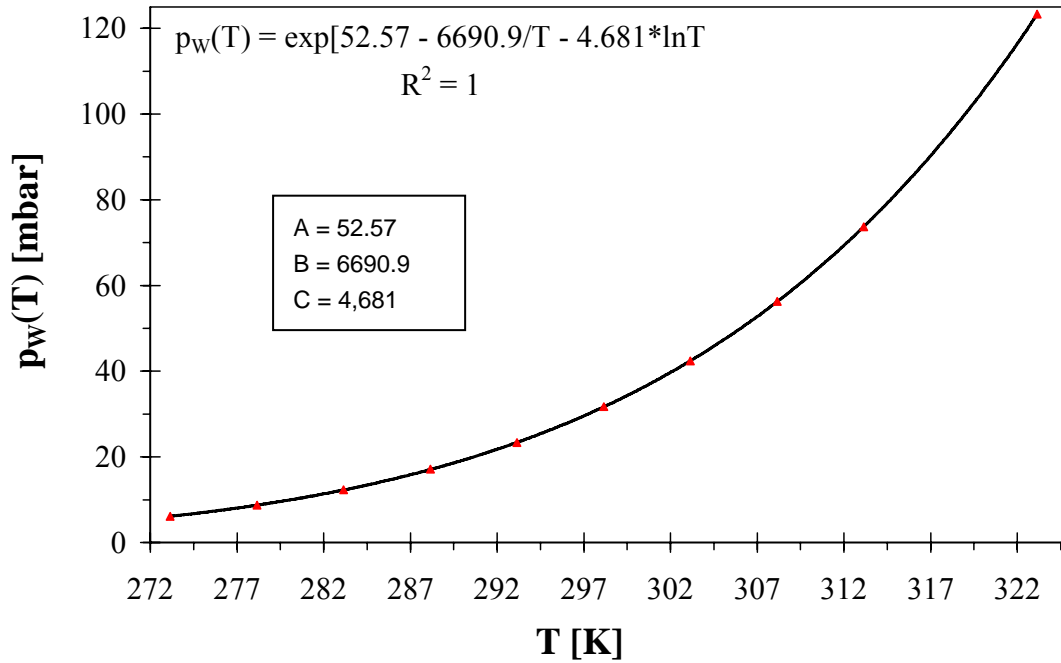
Figure. 13.7 *Variation of water vapor pressure with temperature. R^2 is the square of the correlation coefficient.*

1.3.2 Bunsen Absorption Coefficient

The solubility of oxygen in water is temperature-dependent and can be described using the Bunsen absorption coefficient $\alpha(\theta)$ and the oxygen partial pressure $p(O_2)$ according to equation 15. With increasing temperature, the solubility of oxygen in water decreases.

$$c_S(p,\theta) = \frac{p(O_2) - p_W(T)}{p_N}\alpha(\theta)$$
(**15**)

$c_S(p,\theta)$: temperature-dependent solubility of oxygen in water, given in (cm^3 (O_2) / cm^3)

$p(O_2)$: oxygen partial pressure

p_N: standard pressure (1013 mbar)

$\alpha(\theta)$: Bunsen absorption coefficient, given in (cm^3 (O_2) / cm^3)

Table 13.2 Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature.

θ [°C]	0	5	10	15	20	25	30	35	40	50
$\alpha(\theta)\cdot 10^3$	49.01	42.94	38.11	34.17	31.01	28.43	26.30	24.63	23.16	20.85

The data in Table 13.2 can be described by two forms of equations.

The first form of equation to describe the temperature-dependent variation of the Bunsen absorption coefficient $\alpha(\theta)$ is obtained by fitting a general power series to the values in Table 13.2. A fourth degree polynomial fit can be chosen, yielding equation 16.

$$10^3\alpha = a + b\cdot\theta + c\cdot\theta^2 + d\cdot\theta^3 + e\cdot\theta^4$$
(**16**)

where θ is the temperature in °C and a - e the coefficients calculated by standard curve fitting procedures as given in Figure 13.8.

$$\alpha(\theta)*10^3 = 48.998 - 1.335*\theta + 2.755*10^{-2}*\theta^2 - 3.220*10^{-4}*\theta^3 + 1.598*10^{-6}*\theta^4$$

a = 48.998
b = -1.335
c = 2.755 * 10^{-2}
d = -3.220 * 10^{-4}
e = 1.598 * 10^{-6}
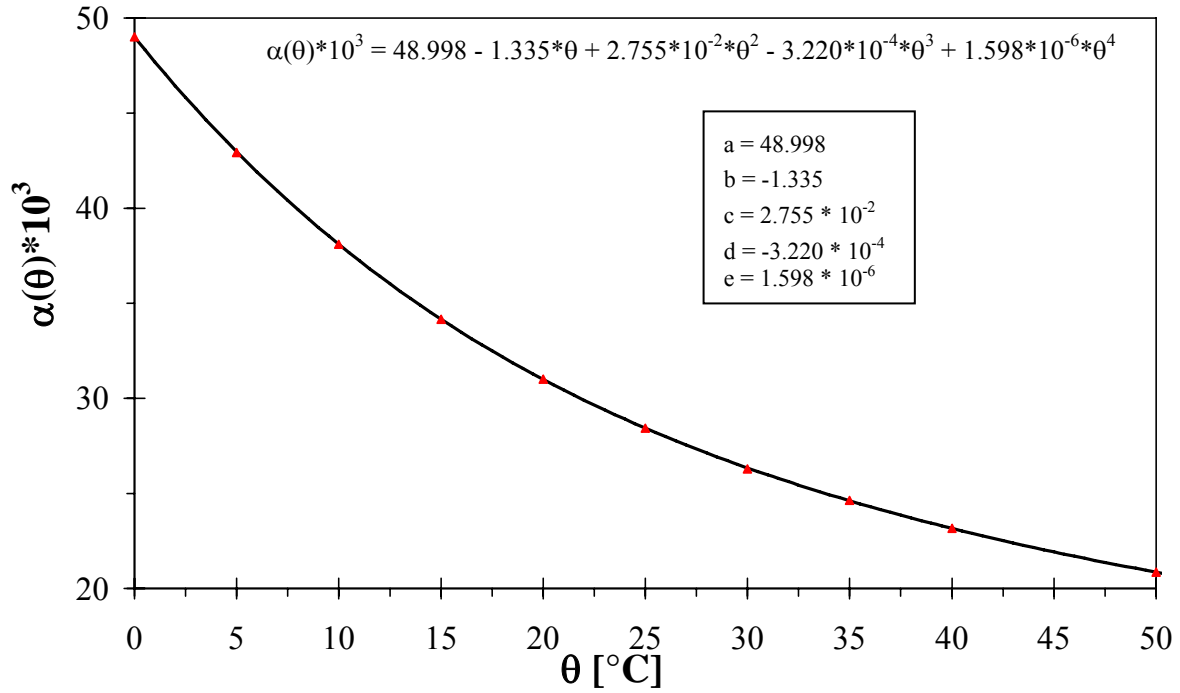
Figure 13.8 *Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature. R^2 is the square of the correlation coefficient.*

The other form of equation to describe the variation of α with temperature can be derived from a thermodynamical correlation and gives an equation of the form

$$\ln 10^3 \alpha = \frac{A}{T} + B \cdot \ln T + C \tag{17}$$

where A, B and C are constants and T is the temperature in K. For oxygen dissolved in water we find by fitting the equation to the values of α in Table 13.2 that A = 8.553 * 10^3, B = 2.378 * 10, and C = -1.608 * 10^2.

Values of α calculated from eqns. 16 and 17 for the same temperature agree within ± 0.5 %.

The Bunsen absorption coefficient, however, is not a very practical measure. Values of $\alpha(\theta)$ have therefore to be converted to mg/L, and the method for doing this is best illustrated by an example.

Example: Calculation of the oxygen content ($c_S(p_{atm},\theta)$ in air-saturated water at a temperature θ of 20°C.

Equation 17 allows the solubility of oxygen in air-saturated fresh water to be calculated for any temperature and pressure provided that the values of the Bunsen absorption coefficient $\alpha(T)$ and the vapor pressure $p_W(T)$ at the particular temperature are known. Equation 16 or 17 can be used to obtain α, and p_W can be calculated from equation 14. The oxygen content c_S of air-saturated water can be calculated according to

$$c_S(p_{atm},\theta) = \frac{p_{atm} - p_W(\theta)}{p_N} \cdot 0.2095 \cdot \alpha(\theta) \cdot \frac{M_{O_2}}{V_M}. \tag{18}$$

In equation 18, p_{atm} is the actual atmospheric pressure corrected for the contribution of the water vapor pressure p_w and related to standard pressure p_N. The corrected pressure is multiplied by 0.2095, the volume content of oxygen in air, by $\alpha(\theta)$ and by the molecular mass of oxygen (M_{O2}) divided by the molar volume V_M.

At a given atmospheric pressure of 1013 mbar (p_{atm} = p_N) and a temperature of 20 °C the oxygen content can be calculated according to equation 19 and results in

$$c_S(1013\text{mbar},20°C) = \frac{1013 - 23.3}{1013} \cdot 0.2095 \cdot 0.031 \cdot \frac{32 \cdot \text{g/mol}}{22.414\text{mol/L}} = 0.009\text{g/L} = 9.06\text{mg/L} \qquad \textbf{(19)}$$

Table 13.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C. The calculated value for c_s at a temperature of 20.0 °C agrees with the tabulated value of 9.08 mg/L.

Figure 13.9 shows the temperature-dependent oxygen solubility in air-saturated fresh water.

$$c_S(O_2) \text{ [mg/L]} = p_N - p_W(T) / p_N * 0.2095 * \alpha(\theta)*10^3 * M_{O2}/V_M$$
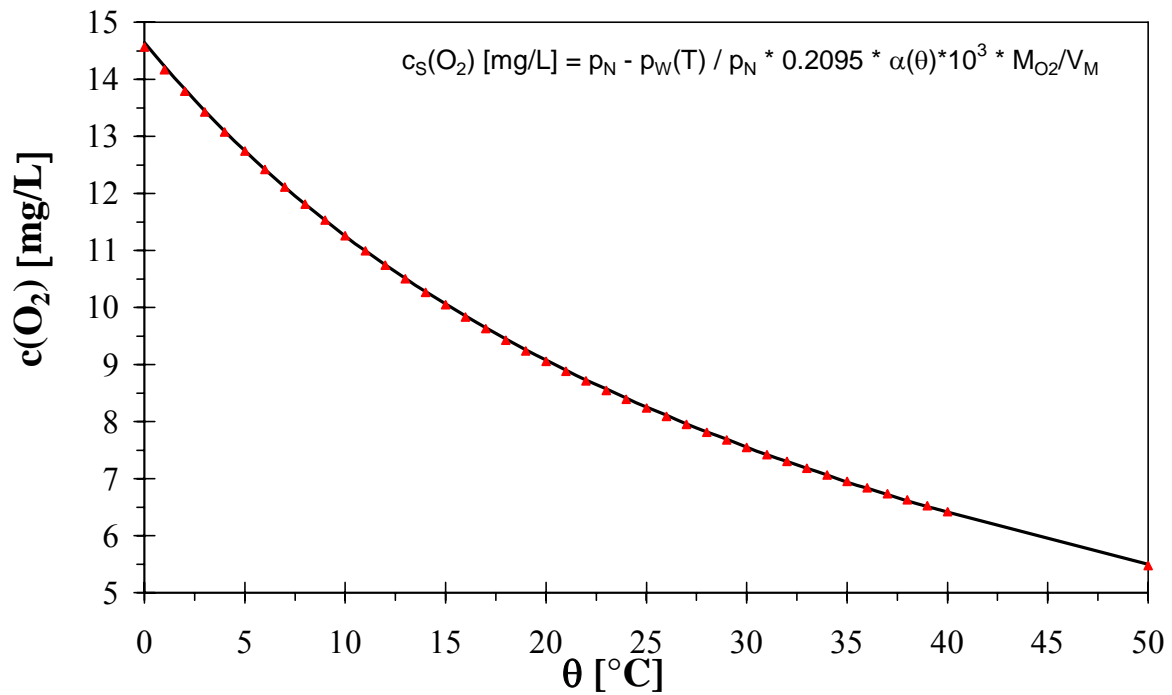
Figure 13.9 *Dependence of the oxygen solubility in air-saturated fresh water on temperature.*

Table 13.3 Oxygen solubility in air-saturated fresh water [mg/L].

T [°C]	$c_S(T)$	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
0	14.	64	60	55	51	47	43	39	35	31	27	23
1		23	19	15	10	06	03	99	95	91	87	83
2	13.	83	79	75	71	68	64	60	56	52	49	45
3		45	41	38	34	30	27	23	20	16	12	09
4		09	05	02	98	95	92	88	85	81	78	75
5	12.	75	71	68	65	61	58	55	52	48	45	42
6		42	39	36	32	29	26	23	20	17	14	11
7		11	08	05	02	99	96	93	90	87	84	81
8	11.	81	78	75	72	69	67	64	61	58	55	53
9		53	50	47	44	42	39	36	33	31	28	25
10		25	23	20	18	15	12	10	07	05	02	99
11	10.	99	97	94	92	89	87	84	82	79	77	75
12		75	72	70	67	65	63	60	58	55	53	51
13		51	48	46	44	41	39	37	35	32	30	28
14		28	26	23	21	19	17	15	12	10	08	06
15		06	04	02	99	97	95	93	91	89	87	85
16	9.	85	83	81	70	76	74	72	70	68	66	64
17		64	62	60	58	56	54	53	51	49	47	45
18		45	43	41	39	37	35	33	31	30	28	26
19		26	24	22	20	19	17	15	13	11	09	08
20		08	06	04	02	01	99	97	95	94	92	90
21	8.	90	88	87	85	83	82	80	78	76	75	73
22		73	71	70	68	66	65	63	62	60	58	57
23		57	55	53	52	50	49	47	46	44	42	41
24		41	39	38	36	35	33	32	30	28	27	25
25		25	24	22	21	19	18	16	15	14	12	11
26		11	09	08	06	05	03	02	00	99	98	96
27	7.	96	95	93	92	90	89	88	86	85	83	82
28		82	81	79	78	77	75	74	73	71	70	69
29		69	67	66	65	63	62	61	59	58	57	55
30		55	54	53	51	50	49	48	46	45	44	42
31		42	41	40	39	37	36	35	34	32	31	30
32		30	29	28	26	25	24	23	21	20	19	18
33		18	17	15	14	13	12	11	09	08	07	06
34		06	05	04	02	01	00	99	98	97	96	94
35	6.	94	93	92	91	90	89	88	87	85	84	83
36		83	82	81	80	79	78	77	75	74	73	72
37		72	71	70	69	68	67	66	65	64	63	61
38		61	60	59	58	57	56	55	54	53	52	51
39		51	50	49	48	47	46	45	44	43	42	41
40		41	40	39	38	37	36	35	34	33	32	31

Example:: $c_S($**20.0°C**$)$ = **9.08 mg/L**

1.3.3 Dependence on the Salt Concentration

Table 13.4 gives values of the concentration of dissolved oxygen at several temperatures in solutions with various chloride concentrations. Increasing the salt concentration leads to a decrease in oxygen solubility. This behavior is characteristic for the solubility of many nonelectrolytes - it is the phenomenon known as the ***salting-out effect***.

Instead of chlorinity [Cl⁻] - the amount of chloride in parts per thousand - which was used as a measure of the amount of salt in water, the term salinity is often used. If salinity is preferred as a measure of salt concentration, then the conversion from g/L can be readily made using equation 20.

$$S = 1.805[Cl^-] + 0.03 \tag{20}$$

where S is the salinity in [‰] or [g/1000g].

Table 13.4 Solubility of oxygen in water as a function of temperature and salt concentration (Total pressure = 760 torr)

T [°C]	*Oxygen solubility [mg/L]*					
[Cl⁻] (g/1000g)	**0**	**4**	**8**	**12**	**16**	**20**
0	14.5	13.9	13.3	12.6	12.0	11.3
10	11.3	10.8	10.4	9.9	9.5	9.0
20	9.1	8.8	8.5	8.1	7.8	7.4
30	7.5	7.3	7.0	6.7	6.4	6.1

The effect of increasing the salt concentration on the vapor pressure is negligible small as shown in Table 13.5.

Table 13.5. Variation of solution vapor pressure (p$_W$) with salt concentration

T [°C]	*Vapor pressure of solution (torr)*			
[Cl⁻] (g/1000g)	**0**	**9**	**18**	**26**
0	4.6	4.5	4.4	4.4
10	9.2	9.1	8.9	8.8
20	17.5	17.3	17.0	16.7
30	31.8	31.4	30.9	30.4

The dependence of oxygen solubility on salt concentration can also be obtained from equation 14 except that now values calculated from either equation 21 or 22 have to be used for calculation of the Bunsen absorption coefficient. Equation 21 differs from equation 16 by an additional forth degree polynomial term for chlorinity.

$$10^3 \cdot \alpha = a + b \cdot \theta + c \cdot \theta^2 + d \cdot \theta^3 + e \cdot \theta^4 - [Cl^-] \cdot (p + q \cdot \theta + r \cdot \theta^2 + s \cdot \theta^3 + t \cdot \theta^4) \tag{21}$$

where θ is the temperature in °C, a - e are the coefficients used in equation 16 and p - t are new constants given in Table 13.6. The values of these new constants are obtained by fitting the polynomial to experimental data in the ranges $0 \leq \theta \leq 30$°C and $0 \leq [Cl^-] \leq 20$‰. To obtain an oxygen solubility from the Bunsen absorption coefficient, the same procedure as described previously is used (s. equation 18, page 74).

An alternative equation to compensate the Bunsen absorption coefficient by the salt concentration displays equation 22.

$$10^3 \cdot \alpha = \exp\left[\left(A + \frac{B}{T} + C \cdot \ln T + D \cdot T\right) - [Cl^-] \cdot \left(P + \frac{Q}{T} + R \cdot \ln T + S \cdot T\right)\right] \tag{22}$$

where T is the temperature in Kelvin, and A - D and P - S are the coefficients given in Table 13.6. They are based on measurements for $273.1 \leq T \leq 308.18$ K and $0 \leq [Cl^-] \leq 30$‰ and is therefore more extensive than equation 21. Both equations give values of $10^3 \cdot \alpha$ which agree to better than ± 1 %.

Table 13.6 Values of the coefficients in equations **21** and **22**.

Eqn. 21	a	$4.900 * 10$	p	$5.516 * 10^{-1}$
	b	-1.335	q	$-1.759 * 10^{-2}$
	c	$2.759 * 10^{-2}$	r	$2.253 * 10^{-4}$
	d	$-3.235 * 10^{-4}$	s	$-2.654 * 10^{-7}$
	e	$1.614 * 10^{-6}$	t	$5.362 * 10^{-8}$
Eqn. 22	A	-7.424	P	$-1.288 * 10^{-1}$
	B	$4.417 * 10^{3}$	Q	$5.344 * 10$
	C	-2.927	R	$-4.442 * 10^{-2}$
	D	$4.238 * 10^{-2}$	S	$7.145 * 10^{-4}$

Seawater has a typical salinity of 35 ‰ (35 g / 1000 g) or a chloride content of about 19 ‰, and therefore falls within the scope of both equations.