Zander, Mertzlufft (eds.): The Oxygen Status of Arterial Blood,
pp. 152–156 (Karger, Basel 1991)

Oxymetric Determination of the Concentrations of Hb and its Derivatives COHb and MetHb

R. Zander

Institute of Physiology and Pathophysiology, Mainz University, FRG

Introduction

Modern multi-wavelength oxymeters (so-called CO- or Hem-oxymeters) offer the possibility of measuring not only the O_2 saturation of a blood sample but also the concentrations of hemoglobin (cHb, g/dl), carboxyhemoglobin (cCOHb, %), and methemoglobin (cMetHb, %). Whereas the Hb concentration can be determined using several other methods and types of equipment, the oxymeter is today the method of choice for measuring the derivatives, COHb, and MetHb. For this reason the accuracy of determination of cHb, cCOHb, and cMetHb was examined using the CO-oxymeter 2500 (Ciba Corning) as being representative of other, similar equipment.

Methods

Heparinized venous blood was investigated in smokers and non-smokers. The CO-oxymeter was calibrated daily according to the manufacturer's instructions and used to determine cHb (g/dl), cCOHb (%) and cMetHb (%). For determining the Hb concentration, the blood was either diluted or concentrated (centrifugation) to produce variations in cHb. The possible influence of the MCV of the erythrocytes on the results was investigated in blood samples that had been diluted with hypotonic (0.45 g/dl) or hypertonic (3.6 g/dl) NaCl solutions. The reference method used for determining cHb was the alkaline hematin D-575 method.

Blood samples were quantitatively mixed within airtight syringes by a magnetic stirrer to produce defined concentrations of COHb and MetHb. The starting material was blood that had been equilibrated with 20% CO in N_2 for 30 min, giving a proportion of COHb (taking into account the physically dissolved CO) of (theoretically) 101.6%. Blood samples with a high proportion of MetHb were produced by adding a 1 M solution of $NaNO_2$.

To examine the possible influence of pH on the cCOHb and cMetHb data, blood samples were equilibrated at 37 °C at different CO_2 partial pressures; different BE values were produced by adding HCl or NaOH.

Results

The determination of the Hb concentration was in very good agreement with the alkaline hematin D-575 method. Table 1 shows a summary of the means from 10 individual determinations. This agreement was valid over a wide concentration range as well as for blood samples whose erythrocyte volumes (MCV) had been artificially varied. The largest difference between the two methods was only 1.4%.

The results for the determination of COHb and MetHb in mixtures of defined compositions are summarized in table 2 as the means of 10 individual measurements. In no case the difference between actual and expected values for COHb and MetHb was more than 1%.

The pH of the blood sample apparently has a slight effect on the measurement of cCOHb as shown in blood samples from 5 smokers in figure 1. In all cases the measured cCOHb increased slightly with increasing pH. A blood sample whose COHb concentration should be 6% at a pH of 7.4 gave an erroneous value of 6.6% when measured at a pH of 7.6.

Table 1. Determination of Hb concentration (g/dl) with a CO-oxymeter: comparison with the alkaline hematin D-575 method. Means ± standard deviations from 10 individual measurements.

Material		cHb (g/dl)	
		CO-oxymeter	alk. hematin
Dil. blood:	hypotonic	7.48 ± 0.04	7.51 ± 0.04
	hypertonic	7.60 ± 0	7.61 ± 0.07
Normal blood		15.11 ± 0.19	15.30 ± 0.05
Conc. blood:	hypotonic	20.82 ± 0.08	20.87 ± 0.10
	hypertonic	21.72 ± 0.25	21.41 ± 0.15

Table 2. Determination of cCOHb (%) and cMetHb (%) with a CO-oxymeter. Quantitative mixture of MetHb-blood (addition of 1 M $NaNO_2$), O_2Hb-blood (pO_2 = 100 mmHg) and COHb-blood (20% CO in N_2) 1 + 2 + 1, in an airtight syringe by a magnetic stirrer. Means ± standard deviations from 10 individual measurements.

	cO_2Hb (%)	cCOHb (%)	cMetHb (%)
Starting values			
MetHb-blood	9.23	1.13	83.57
O_2Hb-blood	96.2	2.53	0.23
COHb-blood	0	'101.6'	0
Expected value	50.41	26.95	21.01
Measured value	49.25	26.48	21.48
	± 0.22	± 0.23	± 0.25

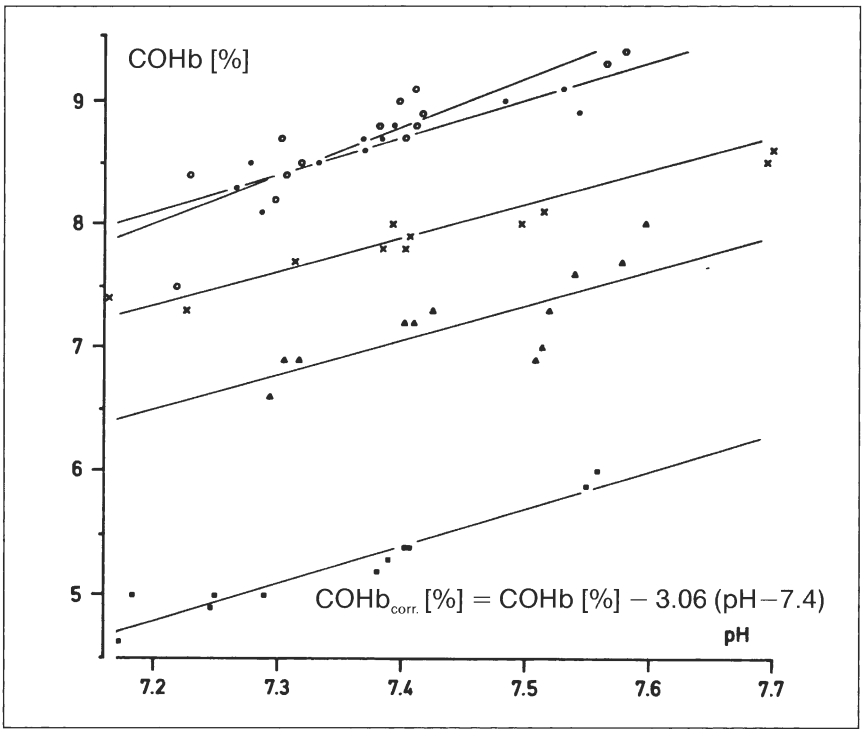

Fig. 1. Concentration of COHb (%) measured in an oxymeter as a function of pH in blood taken from 5 smokers. The measured value is clearly a function of pH, i.e. the COHb becomes overestimated with increasing pH and vice versa.

A possible correction factor for this error can be derived from the relationship shown in figure 1.

A very slight dependence of the determination of cMetHb on pH is shown in figure 2. With increasing blood pH values, the MetHb concentration is underestimated. A blood sample giving a MetHb concentration of 0.7% at a pH of 7.6 should have shown a value of 1.0% at a pH of 7.4. The appropriate correction factor is shown in figure 2.

Discussion

If a modern multi-wavelength oxymeter is available, this can be used to determine the Hb concentration. The results presented here show that this can be done with sufficient accuracy. It may therefore be possible in the future to determine changes in Hb concentration quickly and on the spot, for example during surgery, particularly since only microlitre quan-

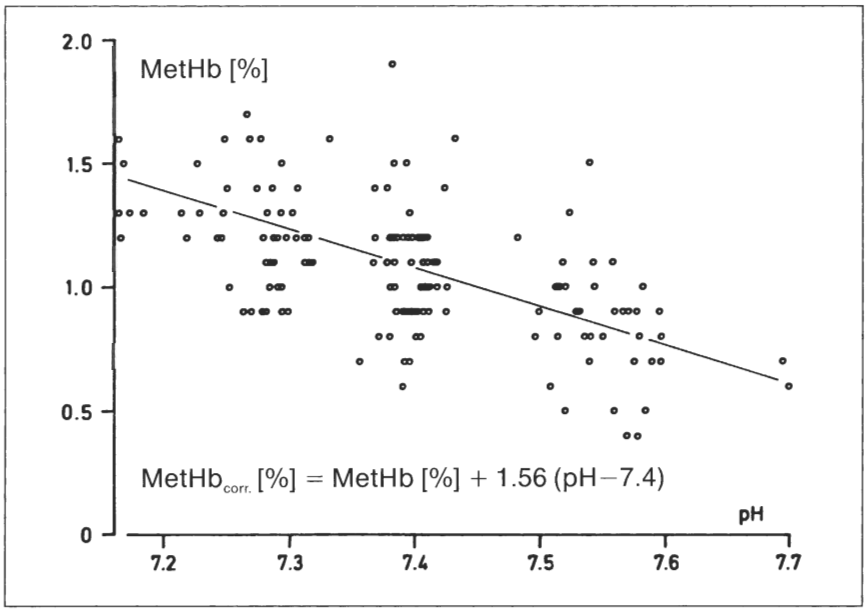

Fig. 2. Concentration of MetHb (%) measured in an oxymeter as a function of pH in blood samples. The measured value is clearly a function of pH, i.e. the MetHb becomes underestimated with increasing pH and vice versa.

tities of blood are required. On the other hand, oxymetry is the method of choice for determining COHb and MetHb in terms of accuracy, ease of use and sample volume. This is evident from the data presented here. The slight pH-dependency found is of importance theoretically but not clinically.

Summary

The determination of cHb (g/dl), cCOHb (%) and cMetHb (%) using modern multi-wavelength oxymeters can be recommended on grounds of ease of handling and accuracy. For the determination of the derivatives COHb and MetHb such oxymeters can be considered to be the method of choice.

Prof. Dr. R. Zander, Institut für Physiologie und Pathophysiologie der Universität Mainz, Saarstraße 21, D-6500 Mainz (FRG)