TECHNICAL NOTE

Yoshinao Katsumata,[1] M.D.; Minoru Aoki,[1] M.S.; Keizo Sato,[1] M.D.; Osamu Suzuki,[2] M.D.; Masakazu Oya,[1] M.D.; and Shoichi Yada,[1] M.D.

A Simple Spectrophotometry for Determination of Carboxyhemoglobin in Blood

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ABSTRACT: A simple spectrophotometric method for determination of carboxyhemoglobin (HbCO) in blood is described. Blood is dissolved in sodium carbonate solution at a dilution near 250-fold. The diluent contains sodium hydrosulfite providing the two-component system HbCO-reduced hemoglobin (HHb). The percentage of HbCO is calculated from absorbance values at 532 and 558 nm measured after the addition of sodium hydroxide, which makes the solution completely clear. Results obtained by the present method are in satisfactory agreement with those by the oxygen electrode method.

KEYWORDS: pathology and biology, spectroscopic analysis, carbon monoxide, carboxyhemoglobin

The measurement of carbon monoxide in blood is essential for diagnosis of carbon monoxide poisoning. The most accurate methods for measuring carbon monoxide in blood by gas chromatography [*1-3*] are too demanding for routine or emergency measurement in clinical laboratories. The oxygen electrode method determining oxygen equivalent to carbon monoxide in blood reported by the authors [*4*] is much simpler and reliable [*5*], but each sample requires separate measurement of hemoglobin capacity if blood samples contain methemoglobin (Met-Hb). Although the spectrophotometric method described by Klendshoj et al [*6*] based upon the two-component system carboxyhemoglobin (HbCO)-reduced hemoglobin (HHb) is simple and rapid and can be applied to blood samples containing Met-Hb, it is inaccurate at low values of HbCO. Recently, Rodkey et al proposed an accurate spectrophotometric method [*7*] based upon the same two-component system. It works well with fresh blood samples, but the turbidity of the hemolysates may cause errors in some blood samples, especially in denatured ones.

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[1]Senior lecturer, forensic chemist, graduate student, assistant professor, and professor, respectively, Department of Legal Medicine, Nagoya University School of Medicine, Nagoya 466, Japan.
[2]Assistant professor, Department of Legal Medicine, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan.

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We reported a simple spectrophotometric method for determination of HbCO in blood based upon the two-component system HbCO and HHb in sodium hydroxide solution [8]. The method is fairly accurate and the turbidity of the hemolysate is negligible in strong alkaline solution. However, the method requires a recording spectrophotometer and is inaccurate at low values of HbCO. In the present study, we propose a new accurate method for determination of HbCO based upon the same two-component system.

Materials and Methods

Materials

Heparinized venous blood samples were kindly supplied by the clinical laboratory of Nagoya University Hospital after necessary determinations were made. Oxyhemoglobin (HbO_2) and HbCO standards were prepared as described earlier [4].

Methods

HHb and HbO_2 were determined by the oxygen electrode method [4], Met-Hb by the method of Sato et al [9], and total hemoglobin (total Hb) by the cyanmethemoglobin method [10].

HbCO in blood was determined by the present method, by the oxygen electrode method [4], and by the double wavelength method described by Ramieri et al [11]. Determinations were made in duplicate. Spectrophotometric measurements were made with the Hitachi 557 spectrophotometer (Hitachi, Ltd., Tokyo, Japan).

Recommended Procedure

1. Add about 2 mg of solid sodium hydrosulfite to a cuvette containing 2.5 mL of 0.1% sodium carbonate.
2. Add 10 μL of blood and 0.2 mL of 5*N* sodium hydroxide into the cuvette, with mixing after each addition.
3. After letting the solution stand for 5 min, read the absorbancies at 532 and 558 nm (A_{532} and A_{558}) against water as a blank.
4. Calculate the percentage of HbCO by the following equation:

$$\text{HbCO\%} = (2.44 - A_{558}/A_{532}) \times 67 \quad (1)$$

Results

The absorption curves of HbCO and HHb after the addition of sodium hydroxide are shown in Fig. 1. The wavelength showing the maximum difference between the two spectra was 558 nm and the isobestic points where the two spectra crossed each other were at 510, 532, 544, and 563 nm. Since the accuracy of the determination by this method is strongly dependent on the reproducibility of the wavelength setting, the wavelengths 544 and 563 nm in which the absorption curves have steep slopes were inadequate for the reference wavelength. The isobestic point 532 nm was chosen for the reference wavelength in the present study since it was nearer to 558 than 510 nm.

The ratios A_{558}/A_{532} of ten blood samples containing pure HHb or HbCO prepared as described above were shown in Table 1. From these ratios, Eq 1 was introduced, provided that the ratio changes linearly with HbCO concentration in blood.

The stability of the absorbance values at 558 and 532 nm was tested because blood samples were exposed to extremely alkaline solution in this method. As shown in Fig. 2, the ab-

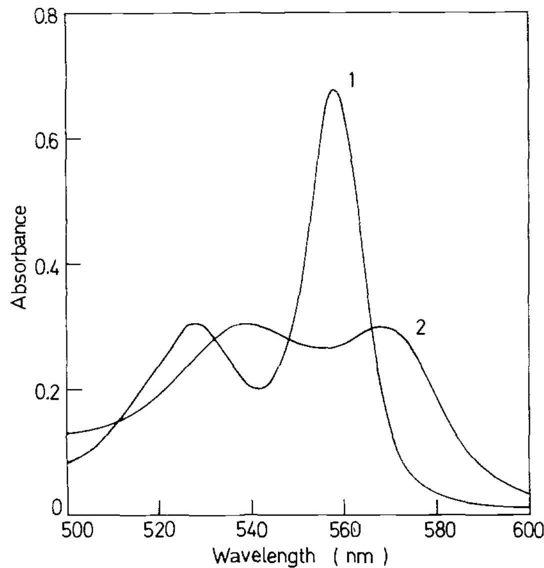

FIG. 1—*Absorption spectra of HHb* (1) *and HbCO* (2) *in the presence of sodium hydroxide.* (1) *Forty microlitres of HbO_2 blood were added to 10 mL of 0.1% sodium hydrosulfite and sodium hydroxide as described in the procedure.* (2) *The same hemolysate was bubbled with carbon monoxide for a few seconds and was treated as above.*

TABLE 1—*The ratio A_{558}/A_{532} of ten blood samples containing 0 or 100% of HbCO.*

HbCO, %	n	A_{558}/A_{532}
0	10	2.438 ± 0.006
100[a]	10	0.948 ± 0.002

[a]The hemolysates of the blood samples containing around 97% HbCO were further bubbled with carbon monoxide for a few seconds.

sorbance values at both 558 and 532 nm reached maximum within 5 min and did not change for at least 20 min with both HHb and HbCO.

The ratio A_{558}/A_{532} did not change significantly when A_{532}, the isobestic point, was changed from 0.2 to 0.8 with HHb and HbCO.

The determination of HbCO was repeated six times with three blood samples prepared from pure HbO_2 and HbCO by the present method. Results were 1.4 ± 0.1, 46.0 ± 0.3, and 97.3 ± 0.3% as the mean plus or minus the standard deviation.

The present method was compared with the oxygen electrode method using standard bloods containing 0 to 97% HbCO. These bloods were prepared by mixing pure HbO_2 and HbCO and were analyzed by both methods. Results are shown in Fig. 3. There was an extremely good correlation between the two methods.

Since absorbance values of 558 and 532 nm could vary with a slight change in wavelength setting, changes in absorbance values with changes in wavelength setting were examined using three bloods containing 0, 53, and 95% HbCO (Table 2). Absorbance values near 558 nm did not change significantly between 557.6 and 558.4 nm with three bloods. The values near 532 nm changed around 0.004 with changes in wavelength of 0.2 nm when blood con-

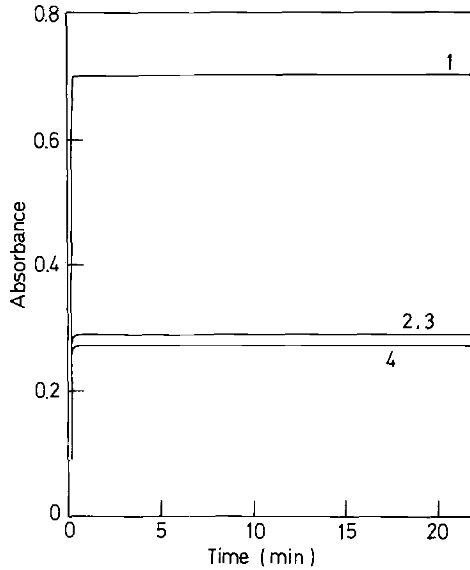

FIG. 2—*Stability of the absorbances at 558 and 532 nm with HHb and HbCO.* (1) A_{558} *of HHb.* (2) *and* (3) A_{532} *of HHb and HbCO.* (4) A_{558} *of HbCO.*

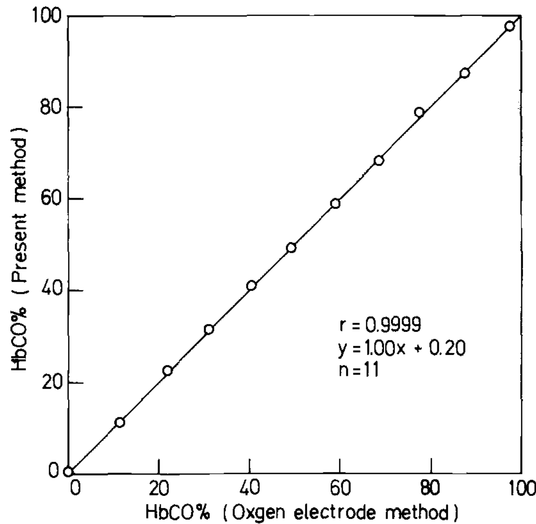

FIG. 3—*Comparison of results by the present method and the oxygen electrode method for measurement of HbCO in eleven blood samples containing 0 to 97% HbCO.*

tained 0% HbCO, though changes in the values were much smaller when blood contained 53 or 95% HbCO. Therefore, care must be taken so that the wavelength near 532 nm is adjusted finely to give the value of 2.44 for A_{558}/A_{532} with pure HbO_2 when accurate determination is required for blood containing 0 to 10% HbCO. The spectrophotometer used in the present study was equipped with a microcomputer, and the error of wavelength setting was ± 0.2 nm. Usually, the wavelength to give 2.44 for A_{558}/A_{532} varied day to day from 531.8 to 532.2 nm but was stable at least 12 h once the equipment was turned on.

TABLE 2—*Effect of changes in wavelength setting on absorbance values near 558 and 532 nm with blood samples containing 0, 53, and 95% HbCO. Blood samples were treated as described in the Recommended Procedure section.*

	Absorbance		
Wavelength	0% HbCO	53% HbCO	95% HbCO
557.6	1.022	0.705	0.442
557.8	1.023	0.706	0.443
558.0	1.023	0.707	0.443
558.2	1.022	0.707	0.444
558.4	1.020	0.707	0.444
531.6	0.426	0.430	0.430
531.8	0.422	0.429	0.432
532.0	0.419	0.428	0.433
532.2	0.415	0.427	0.435
532.4	0.411	0.427	0.436

The present method was compared with the double wavelength method, which is accurate at low concentrations using standard bloods containing 0 to 10% HbCO (Fig. 4). There was a good correlation between the two methods.

The present method and the oxygen electrode method were compared using blood from six fire victims (Table 3). These bloods contained 4.4 to 17.8% of Met-Hb. The sum of HHb, HbO_2, and HbCO determined by the oxygen electrode method and Met-Hb determined photometrically by the method of Sato et al [9] was nearly the same as total Hb determined by the cyanmethemoglobin method [10]. Therefore, these determinations seemed to be made accurately in each sample. The HbCO% values determined by the present method were similar to those determined by the oxygen electrode method although there were differences by 4 to 8% between them in some bloods.

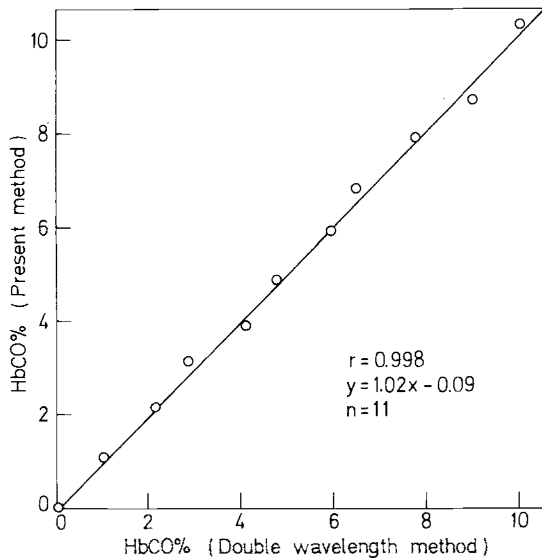

FIG. 4—*Comparison of results by the present method and the double wavelength method for measurement of HbCO in eleven blood samples containing 0 to 10% HbCO.*

TABLE 3—*Comparison of the results of the present method to those of the oxygen electrode method for determination of HbCO using samples from six fire victims.*

		Total Hb, g/dL		HbCO, %	
Case	Met-Hb, %	Method I[a]	Method II[b]	Present Method	Oxygen Electrode Method
1	14.7	19.0	18.8	43.8	39.6
2	6.8	21.2	21.2	76.4	68.3
3	11.6	6.4	6.5	29.8	31.5
4	17.8	21.6	21.4	65.1	61.3
5	4.4	7.6	7.3	60.4	59.5
6	7.6	14.7	14.1	8.2	8.4

[a]Cyanmethemoglobin method.
[b]Sum of the four hemoglobin derivatives determined by the oxygen electrode (HHb, HbO_2, and HbCO) and the method of Sato et al [9] (Met-Hb).

Discussion

For determination of HbCO in blood, various spectrophotometric methods are widely used since they are simple, rapid, and useful for routine or emergency measurement in clinical laboratories. In practice, however, subjects of the determination are often old denatured blood samples. The hemolysates of such samples are usually turbid and contain varying amounts of Met-Hb. Although Met-Hb can be effectively converted to HHb in the two-component system HbCO-HHb [6, 7], turbidity of the solution may cause determination errors.

In the two-component system HbCO-HHb in strong alkaline solution reported by the authors [8], the turbidity of the solution is usually negligible. The present method is based upon the same system with the following modifications; the concentration of sodium hydroxide in the solution was raised fourfold, all of the procedure was done in a cuvette to eliminate the pouring steps that cause the loss of HbCO [11], and the calculations were made using absorbance values at 532 and 557 nm instead of those at two absorbance maxima between 500 to 600 nm. In addition, this method only requires an ordinary spectrophotometer without a recording system and is very accurate (see Figs. 3 and 4).

When blood contains 0 to 10% HbCO, the wavelength setting should be adjusted finely to get accurate values although the values are stable at slight changes in wavelength setting when blood contains a significant amount of HbCO (see Table 2).

The oxygen electrode method determines carbon monoxide in blood and can be applied to denatured blood containing Met-Hb [5]. The results in Table 3 indicate that the present method can also be applied to denatured blood although there may be some interference associated with blood specimens containing Met-Hb.

This method is simple, accurate, and applicable to denatured blood and useful in forensic science.

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Address request for reprints or additional information to
Yoshinao Katsumata, M.D.
Department of Legal Medicine
Nagoya University School of Medicine
Nagoya 466, Japan