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Determination of Carbon Monoxide in Blood by Gas Chromatography Using a Thermal Conductivity Detector

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ABSTRACT: A method is described for the gas chromatographic quantitation of carbon monoxide by means of thermal conductivity detection. Carbon monoxide is released from blood samples as small as 0.02 mL using a unique extraction chamber. The method was compared to a standard gas chromatographic and spectrophotometric method of carbon monoxide quantitation. It was comparable to the former with all samples evaluated and apparently more reliable than the latter with decomposed samples.

KEYWORDS: toxicology, carbon monoxide, blood, chromatographic analysis

Quantitation of carboxyhemoglobin (COHb) is a frequently required and important analysis in the forensic toxicology laboratory. There are several classical methods available for this purpose, including gasometric [*1*], colorimetric [*2*], infrared [*3*], and spectrophotometric [*4,5*].

A gas chromatograph (GC) method using a thermal conductivity detector (TCD) for the quantitation of carbon monoxide (CO) in blood was developed by Dominguez et al [*6*] and later modified by Goldbaum et al [*7*]. Subsequent modifications included alterations in the procedures employed for the release [*8*], detection [*9*], and quantitation [*10*] of CO.

Although GC methods which use a TCD demonstrate a good quantitative response to CO, they generally have relatively high detection limits. Because of this, large blood samples or involved extraction procedures and sample preparation are required. We report the development of a procedure that utilizes an extraction chamber in which detectable quantities of CO are released from blood samples as small as 0.02 mL.

Materials and Methods

Equipment

A Packard Model 417 Becker gas chromatograph equipped with a TCD and a 1.8-m by 3.2-mm (6-ft by 1/8-in.) inside diameter (ID), U-shaped, stainless steel column containing

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250- to 180-μm (60 to 80) mesh, molecular sieve 5A was maintained isothermally at 50 to 70°C. The temperature of the injection port was approximately 28°C and the detector current was 200 or 250 mA. A single channel Autolab Minegrator Integrator (Spectra-Physics) was used for integrating areas under the peaks.

Liberation of CO was achieved in a simple extraction chamber designed for this purpose. A photograph of this chamber is presented in Fig. 1 and a diagrammatic representation in Fig. 2. It was constructed by attaching a four-way stopcock to a glass chamber with a capacity of approximately 5 mL. A septum holder (Kontes, K-672800) approximately 25.4 mm (1 in.) in length, fitted with a rubber septum, was attached to the chamber and used as an injection port. The stopcock was connected to both the carrier gas and the GC column. Use of the stopcock permitted helium to bypass the chamber and enter the column or to pass through the chamber and onto the column. Mixing of the contents of the chamber was achieved by placing two small magnetic stir bars in the chamber and positioning the chamber over a magnetic stirrer or by placing the chamber on a vortex mixer. Blood samples and the liberating agent were delivered into the chamber by means of a micro-syringe and a tuberculin syringe, respectively.

Reagents

The liberating agent was a mixture of lactic acid (85%), hydrochloric acid (37%), and water in a ratio of 2:2:1 (v/v). One millilitre of *n*-octanol was added to one-hundred millilitres of liberating agent.

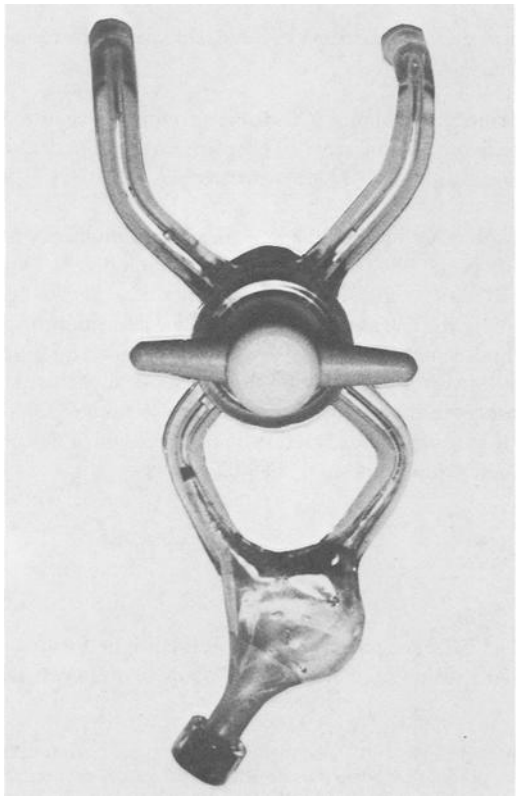

FIG. 1—*The CO extraction chamber.*

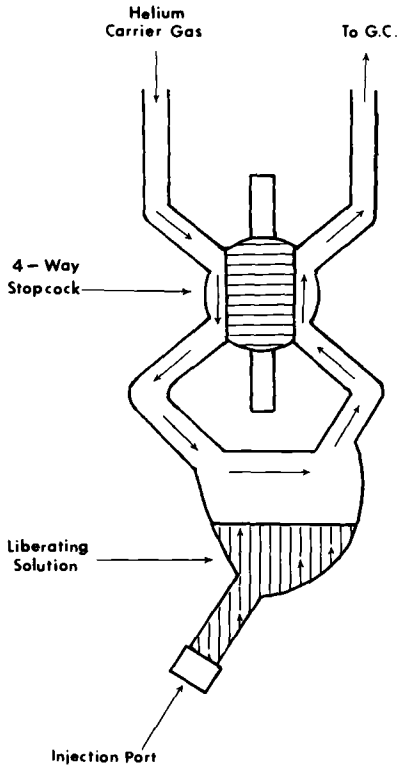

FIG. 2—*Schematic of the CO extraction chamber.*

The COHb standard solution was prepared and standardized by a modification of the method of Collison et al [*9*]. One volume of a fresh, whole blood sample was diluted with five volumes of 0.9% (w/v) sodium chloride (NaCl) and then centrifuged. The aqueous layer was discarded and the red blood cells (RBC) were suspended in 0.9% NaCl and then centrifuged. The aqueous layer was discarded and the RBC collected. To 1 volume of packed RBC were added 0.1 volume of a 12% solution of octylphenoxydecaethanol and 3 volumes of pH 8.5 borate buffer (6.18 g of $Na_2B_4O_71OH_2O$ and 55 mL of 0.2*N* sodium hydroxide (NaOH) diluted to 500 mL with water [H_2O]. This solution was mixed for 5 min, to allow for complete hemolysis, and then centrifuged. The resulting clear hemoglobin (Hb) solution was removed and transferred to a 50-mL syringe equipped with a three-way Teflon® stopcock. Approximately 35 mL of CO was added to the syringe, which was rotated for 15 min to saturate the Hb. This was repeated once again and followed by the addition of 35 mL of helium to the syringe. The syringe was rotated for 1 min to remove dissolved CO from this solution and again the excess gas was expelled.

This 100% saturated COHb solution was stored, air-free, in a syringe at 4°C. The CO concentration of this fully saturated standard reference solution was calculated by determining the Hb concentration by a cyanmethemoglobin method [*11*] in which the blood sample was incubated with the Drabkin's reagent for 90 min as suggested elsewhere [*12*] and by assuming the CO binding capacity of 1 g of Hb to be 1.39 mL [*13*].

Analytical Procedure

With the stopcock positioned so that the carrier gas passed through the chamber into the GC column, 1 mL of the liberating agent was injected through the rubber septum into the

reaction chamber. The chamber was suspended over a magnetic or vortex stirrer for 2 min to release dissolved gases from the solution. The stopcock was then adjusted to isolate the helium filled chamber, that is, to prevent gases from passing from the chamber onto the GC column. A blood sample (0.02 to 0.5 mL) was injected through the rubber septum into the extraction chamber containing the liberating agent. The contents of the reaction chamber were shaken for 5 min so that the CO was released from the hemoglobin into the helium above the solution. The stopcock was then adjusted so that the carrier gas passed through the chamber and carried the liberated CO onto the GC column. At the end of the analysis, the chamber was closed off and the solution in the chamber was withdrawn with a syringe. The chamber was then used for the next sample.

The CO binding capacity of the blood sample was determined as previously described for the preparation of the COHb standard solution. The analytically determined volume of bound CO [*1*] in the sample was divided by the theoretical combining capacity of that volume of blood. This resulted in the percent of total Hb present as COHb, that is, degree of saturation. For example, if a 50-μL blood sample with a total Hb concentration of 8 g/100 mL was determined to contain 2.69 μL of bound CO, the COHb saturation was calculated to be 48.4%.

Standard Curve

The undiluted COHb standard solution was used to construct a standard curve. Four different volumes, 20, 30, 50, and 70 μL, of this stock solution containing calculated CO volumes of 1.9, 2.7, 4.6, and 6.3 μL, respectively, were used. Five aliquots of each volume were analyzed using the analytical procedure.

Comparison of Methods

The concentration of COHb in antemortem blood samples was determined by three methods: (a) the GC/TCD method described above in the analytical procedure section, using blood samples from 0.05 to 0.5 mL; (b) the gas chromatographic/flame-ionization detector (GC/FID) method of Collison et al [*9*], by which CO is catalytically converted to methane, which is then determined; and (c) the spectrophotometric method using the CO-Oximeter-182 as directed by the manufacturer [*14*].

In addition, the GC/TCD and CO-oximeter methods were used to determine the CO content of fresh and stored postmortem blood samples.

Statistical Analysis

Linear regression analysis, by the method of least squares standard error of the estimates as well as the 95% confidence intervals for slopes and y intercepts, were determined as described by Freund [*15*].

Results and Discussion

The GC/TCD procedure described readily separated CO from other common gases such as O_2, N_2, and CH_4 (Fig. 3). The gradual accumulation of water and carbon dioxide on the GC column caused a decreased resolution of CO from these other gases. When this occurred, the oven temperature and carrier gas flow rate were adjusted to reestablish the necessary resolution. If the resolution could not be improved by these adjustments, the column was regenerated by overnight heating at 250°C.

The use of the unique extraction chamber described allowed for a quantitative transfer of CO released by the liberating agent onto the GC column. Thorough mixing of the blood with

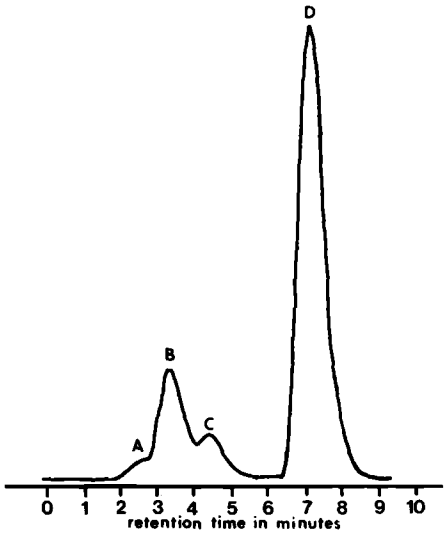

FIG. 3—*Typical chromatogram:* (a) *oxygen,* (b) *nitrogen,* (c) *methane,* (d) *carbon monoxide.*

the acid liberating agent was necessary to completely liberate the Hb bound CO. This was easily achieved by placing the chamber on a vortex mixer for 5 min. This method of liberating CO and transferring it onto the column minimized the errors and losses observed in other methods caused by sample manipulation.

The results obtained from the analysis of the standard COHb solution by means of the GC/TCD method are presented in Table 1. The precision of the method is indicated by the coefficient of variation (CV) which was less than 2% in all cases. The linearity of the GC/TCD response to CO volume is demonstrated in Fig. 4. A straight line with a correlation coefficient r of 0.998 was obtained when GC area units were plotted as a function of CO content. The limits of prediction at the 0.95 level are indicated as parallel lines in Fig. 4.

The results obtained from the analysis of 20 antemortem blood samples by the 3 different methods used are presented in Table 2. Graphic comparisons of the data obtained by the GC/TCD method with those obtained by the GC/FID and CO-oximeter are presented, respectively, in Figs. 5 and 6. The slopes and y intercepts obtained for these two comparisons do not differ significantly, at a 95% confidence interval, from the expected slope of 1.0 and the expected y intercept of 0. Therefore, the data demonstrate that the GC/TCD method developed is comparable to these existing methods evaluated.

Although this correlation did not reveal a significant difference between the GC/TCD and CO-oximeter methods, inspection of Figs. 5 and 6 reveals a greater scatter of data points when the results obtained by GC/TCD method were compared to the CO-oximeter results

TABLE 1—*Analysis of the standard solution of COHb.*

Volume of Blood, μL	CO Content, μL	Area Units (± standard deviation)	CV, %
20	1.9	806 ± 9	1.1
30	2.7	1082 ± 19	1.7
50	4.5	1929 ± 37	1.9
70	6.3	2656 ± 20	0.75

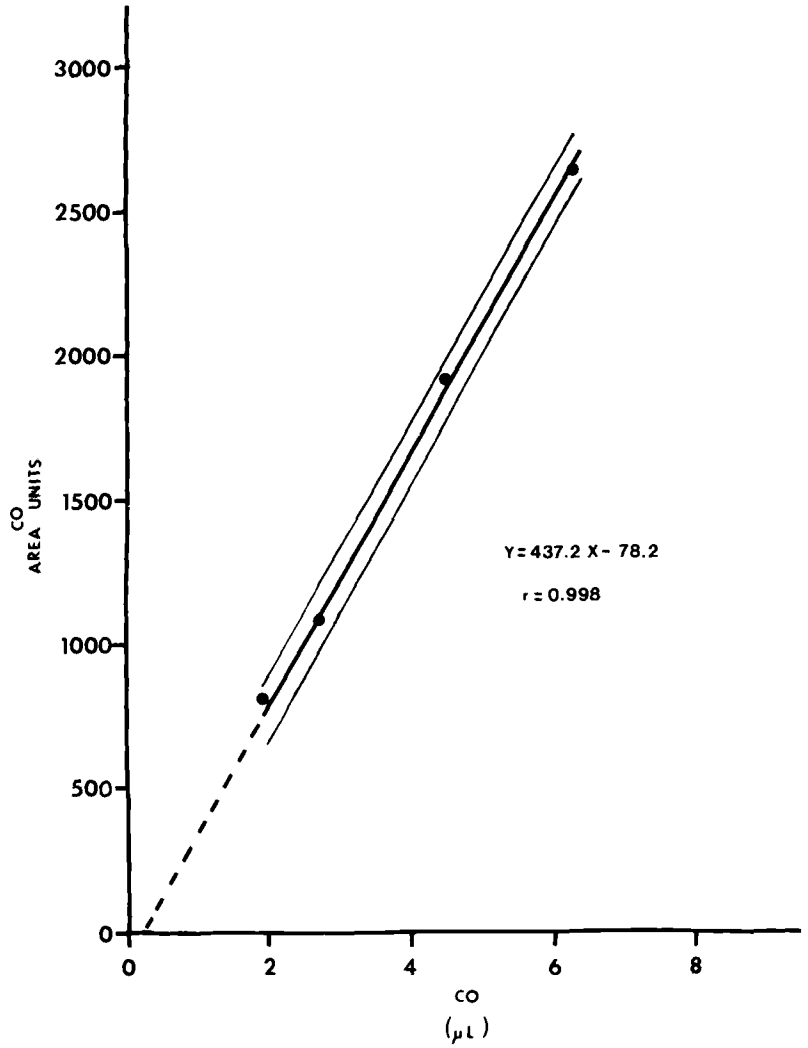

FIG. 4—*Standard curve for the GC/TCD method.*

than when they were compared to the GC/FID results. This is reflected in the standard errors of the estimates (Se) for the two comparisons. The Se for GC/TCD versus GC/FID is 0.178 and for GC/TCD versus Co-oximeter is 0.561.

The results obtained from the analysis of postmortem blood samples by the GC/TCD and Co-oximeter methods are presented in Table 3. Graphic comparisons of these methods are presented in Fig. 7. For those samples without any signs of decomposition, the results obtained by the two methods were well correlated; the observed slope and y intercept did not differ significantly at the 0.95 confidence level. However, a comparison of the results obtained from the analysis of blood samples with signs of decomposition demonstrated a difference between the two methods. At the 95% confidence level, the range for the observed slope was 1.71 to 2.57 and for the observed y intercept was 4.06 to 13.7. Since the expected slope of 1.0 and the expected y intercept of 0 do not fall within these ranges, we conclude that the results obtained by these two methods differ significantly. The difference (higher COHb val-

TABLE 2—*Comparison of COHb results obtained by three procedures.*

Sample	COHb (% Saturation) GC/FID	GC/TCD	CO-Oximeter
1	0.50	0.50	0.30
2	0.65	0.60	0.30
3	0.70	0.90	0.40
4	0.85	0.75	1.0
5	0.90	1.2	0.80
6	1.0	0.90	1.6
7	1.1	1.2	0.60
8	1.2	1.2	0.40
9	1.5	1.3	1.2
10	1.7	1.5	2.4
11	2.2	1.9	0.6
12	2.3	2.2	2.8
13	2.7	2.5	2.3
14	2.8	3.0	2.4
15	3.0	2.7	2.6
16	3.2	3.2	2.5
17	4.0	4.0	3.7
18	5.4	5.6	4.6
19	6.0	5.7	5.8
20	8.3	8.4	7.6
Mean	2.50	2.46	2.20
Standard deviation	2.07	2.07	1.98

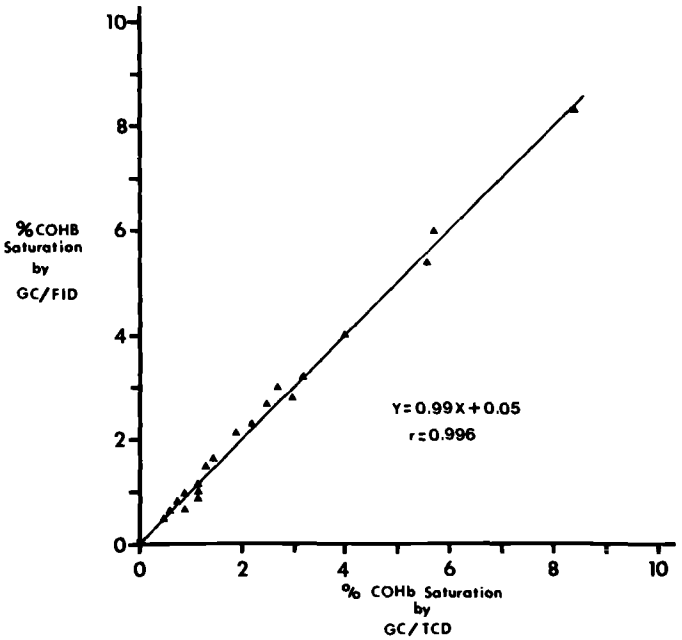

FIG. 5—*Comparison of GC/TCD results with those obtained by GC/FID.*

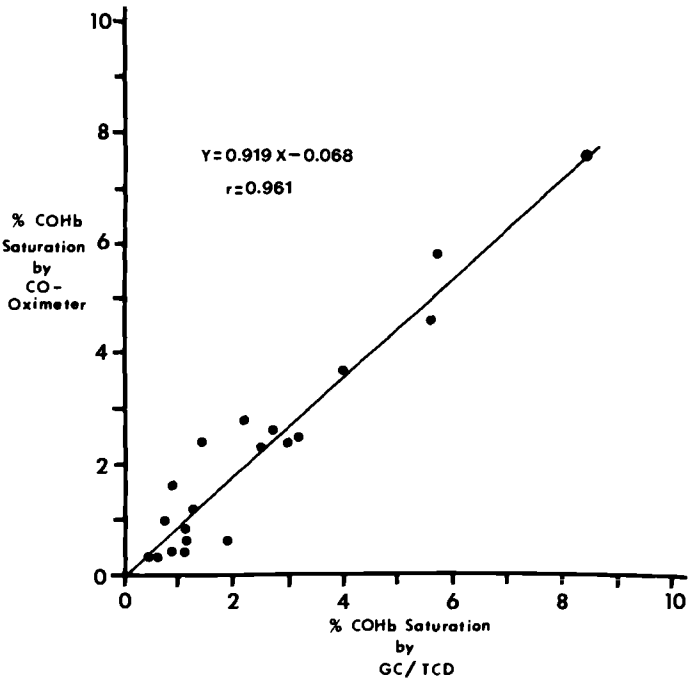

FIG. 6—*Comparison of GC/TCD results with those obtained with the CO-oximeter.*

TABLE 3—*Comparison of GC/TCD and CO-oximeter results obtained from the analysis of postmortem blood samples.*

Sample	COHb (% Saturation) GC/TCD	CO-Oximeter
	NO DECOMPOSITION	
1	0.8	1.7
2	0.8	4.0
3	1.0	0.7
4	12.5	17.0
5	14.0	15.0
6	14.5	18.5
7	45.0	48.0
8	58.0	62.0
Mean	18.3	20.9
Standard deviation	21.6	22.5
	DECOMPOSITION	
1	2.0	10.5
2	3.0	17.0
3	3.5	15.0
4	3.5	15.0
5	6.0	26.0
6	26.0	64.0
Mean	7.33	24.6
Standard deviation	2.70	20.0

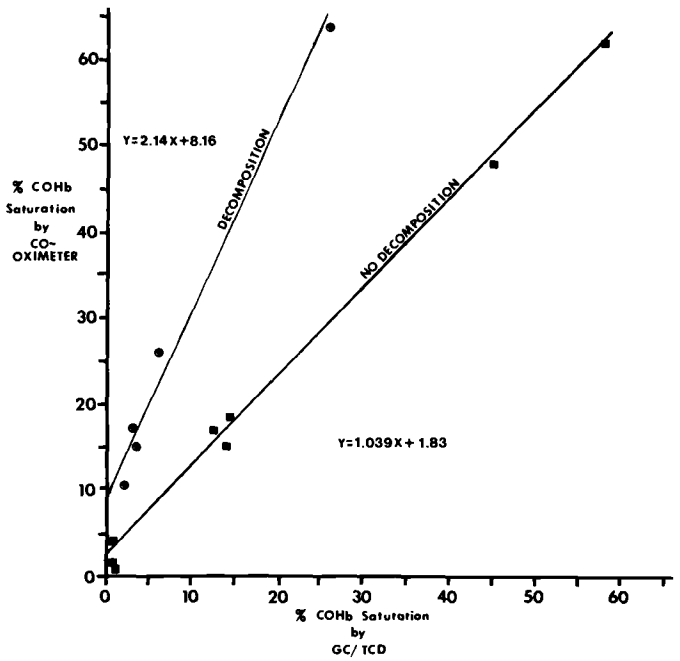

FIG. 7—*Comparison of GC/TCD and CO-oximeter results obtained from the analysis of postmortem blood samples with or without decomposition.*

ues obtained by the CO-oximeter than by the GC/TCD method) may be due to the presence of interfering pigments or high concentrations of methemoglobin. The problems associated with the use of spectrophotometric methods for the quantitation of COHb in aged blood samples have been described previously [*16*].

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