Z. Klin. Chem. Klin. Biochem.
12. Jg. 1974, S. 535–538

Quantitative Determination of Carbon Monoxide in Blood by Gaschromatography

By *H.-J. Klimisch, K. Meißner* and *H. Wernicke*

Forschungsinstitut der Cigarettenindustrie e.V. (Direktor: Prof. Dr. W. Dontenwill) 2 Hamburg

(Eingegangen am 16. Mai/12. August 1974)

The gaschromatographic determination of CO in blood has been simplified by using carbon molecular sieve columns for separation of gases. Regeneration of the columns is necessary only after 1000 and more analyses. Moreover, a more simple procedure for evaluating the CO concentration was developed. This procedure is suitable for routine determination in the sensitivity range of 0.2–100 % COHb in blood or 0.25 µl/l to ml/l in air.

Durch Verwendung einer Kohlenstoffmolekularsiebsäule zur Trennung der Gase wurde die gaschromatographische Bestimmung des Kohlenmonoxids im Blut stark vereinfacht, so daß sich das Verfahren auch für Routinebestimmungen eignet. Mehr als 1000 Analysen lassen sich mit einer Säule quantitativ auswerten. Außerdem wurde eine einfachere Auswertung der CO-Konzentration entwickelt. Das Verfahren ist in breiten Empfindlichkeitsbereichen von 0,2–100 % COHb im Blut bzw. 0,25 µl/l bis ml/l in Luft anwendbar.

A number of procedures, such as gasometry, calorimetry, and spectrophotometry have been published for the quantitative determination of CO in blood (1). These methods show some disadvantages, e.g. low sensitivity and time consuming operation. Recently, gaschromatographic methods have been developed, which permit more sensitive, more selective and more rapid measurements. Interest in the determination of low CO-concentrations in gases or blood is growing, because of the increasing problem of air pollution and the observation that CO is produced endogenously as metabolic product.

The use of FID instead of TCD following the hydrogenation of the CO to CH_4 (2–5) marked a considerable improvement of gaschromatographic procedures. The gaschromatographic methods described so far involve a considerable amount of work in order to obtain reproducible results in the sensitive measuring range. Either the CO set free from the blood has to be concentrated on columns by cooling with liquid nitrogen (4), or the whole blood gas has to be injected into the gaschromatographic system during a longer interval of CO-release. Hereby, different peak areas have to be evaluated by integrators (3). If silicate molecular sieve columns (e.g. 5A or 13X) are used, it is necessary to first dry the gas sample and remove CO_2 (3). Nevertheless, the columns have to be regenerated very often. When measuring in very sensitive ranges, the regeneration temperature should not exceed 250°C (3, 5). A regeneration time of 24–48 hours is required (3).

We have simplified the gaschromatographic method considerably by using carbon molecular sieve columns (CMS) (6). Alterations due to contamination of numerous compounds can be avoided and regeneration becomes necessary only after 1000 analyses and more.

Moreover, a more simple evaluation of the CO-concentration in blood gas has been elaborated. This procedure is suitable for routine determination of CO in concentrations of 0.25 µl/l to % ranges or of 0.2 to 100 % COHb.

Material and Methods

Reagents

Solution I: 2 g $K_3[Fe(CN)_6]$ + 1 g saponin were dissolved in bidistilled water to 100 ml.

Solution II: 0.35 g lactic acid were diluted with bidistilled water to 100 ml. Anticoagulation liquid: Blood sample tubes of 4 ml were coated with about 6 mg EDTA (Greiner, Nürtingen, Germany).

Defoaming agent: Silicone anti-foam (Carl Roth, Karlsruhe, Germany).

Release of CO from blood

1 ml of solution I and 1 ml of solution II were transferred into a reaction vessel of 5 ml (fig. 1) and 5 drops of antifoam were added. The closed vessel was sparged with nitrogen (40 ml/min) for 2 min to remove CO, and 100 µl of the blood sample were then injected through the septum. After a reaction time of 1 min the CO released was rinsed with nitrogen (40 ml/min) into a polyethylene bag (Alkotest bag of Messrs. Dräger AG, Lübeck, Germany) for exactly 3 min; final volume of the bag = 120 ml.

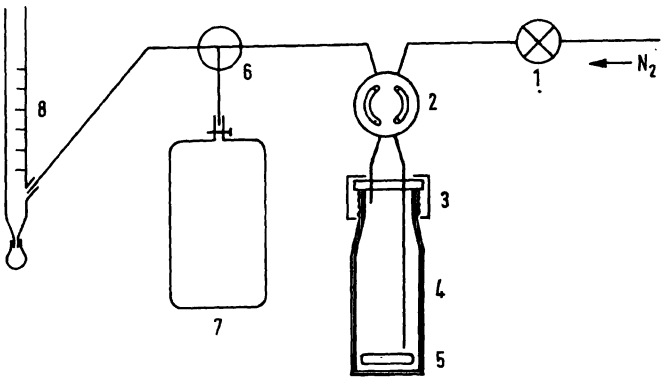

Fig. 1. Equipment for the release of CO from blood
1 flow controller Millaflow
2 four-way ball valves Whitey
3 cap with septum
4 reaction vessel 5 ml
5 magnetic stirrer
6 three-way cock
7 PVC bag
8 bubble meter

By a flow control system and a flow meter the nitrogen stream was exactly adjusted at a flow rate of 40 ml/min and passed via valves into the reaction vessel with the connected sample bag.

Gaschromatographic determination of CO

Principle of the method: The gas mixture was injected into the gaschromatograph via a gas sampling loop (fig. 2). Separation occurred on a 1 m column with carbon molecular sieve. Subsequently, the separated CO was hydrogenated to CH_4 via a column filled with a Nickel catalyst. The preparation of these columns has been previously described in detail (4, 5). The gaschromatograph used was a Varian 1200 with FID.

The separating column, SS 1 m × 1/8 " o.d., packed with carbon molecular sieve Sorptophase CMS, 100–120 mesh (W. Seifert K.G., Düsseldorf-Oberkassel, Germany) was activated at 200°C. 0.5 ml of the gas mixture were injected via a loop. The gas mixture was separated at 30°C; the column was fastened in a thermostatic heating bath filled with polypropylene glycol. Subsequently, the separated CO was hydrogenated at 300°C on a column in the gaschromatograph, SS 0.5 m × 1/8 "o.d., packed with 10 % nickel on Chromosorb P, 60–80 mesh.

The flow rate of the carrier gas was maintained at H_2–23 ml/min, that of the air at 350 ml/min. The retention time of CO hydrogenated to CH_4 was 110 s (fig. 4). After 18 min of retention time CH_4 hydrogenated from CO_2 was eluted from the column. However, 3–4 injections could have been done before CO determination; there is no interference with the measuring accuracy, and no alteration of the retention time.

Calibration and calculation of % COHb

A linear correlation between peak height of the hydrogenated CO peak and the CO concentration was observed with CO calibration gases (Linde A.G., Lohhof, Germany) of 1, 10 and 50 µl/l and 10, 50 and 200 ml/l CO. For the determination of 0–100 % COHb the range of 0–50 µl CO was calibrated with the calibration gases 1, 10 and 50 µl/l CO at a sensitivity of $10^{-12}/16$ of the amplifier. The calibration curve of figure 3 was calculated from mean values of 5 measured concentrations. This range corresponds to a COHb value of 0–28.5 % in case of 160 g/l Hb in blood. Values of 28.5–100 % were obtained at a sensitivity of $10^{-12}/64$, values of 0–5 % COHb at a sensitivity of $10^{-12}/4$. As different amplifier ranges showed proportional values, all CO values could be taken from the same calibration curve (fig. 3).

The CO content of the blood gas determined gaschromatographically was converted to µg CO/0.1 ml blood. The % COHb value could be calculated by measuring the hemoglobin content of the same blood sample (Cyanhemoglobin method "Merckotest", Merck A.G., Darmstadt, Germany). According to this method the COHb value was determined in smokers and nonsmokers as well as in untreated and smoke-exposed rats and hamsters (7).

Results and Discussion

Our observations with respect to accuracy and reproducibility of the method are in conformity with results of *Porter & Volman* (2). A quantitative reduction of CO to CH_4 occurred at as low as 270°C. We have chosen a temperature of 300°C, because a higher temperature increases stability and decreases the noise level. For investigations of the total CO release from the blood, a series of 1–100 % COHb of the total CO release from the blood, a series of 1–100 % COHb was analyzed by diluting blood with 100 % COHb, prepared by a method of *Stove* (8). An average value of 99.7 % could be detected in the calibration curve (fig. 3). A prolongation of the rinsing time for 4 or 5 min did not cause an increase of the CO value. These results agree with findings of *Collison* (3) that 99.5–99.7 % of CO are released

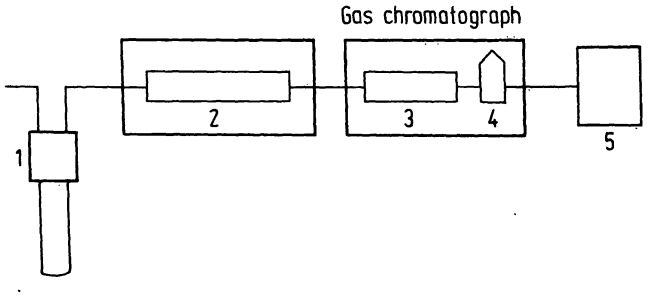

Fig. 2. Flow diagram for the gaschromatographic system.
1 gas sampling loop
2 separating column in a heated bath filled with polypropylene glycole
3 hydrogenation column } GC-apparatus
4 flame ionisation detector } GC-apparatus
5 recorder

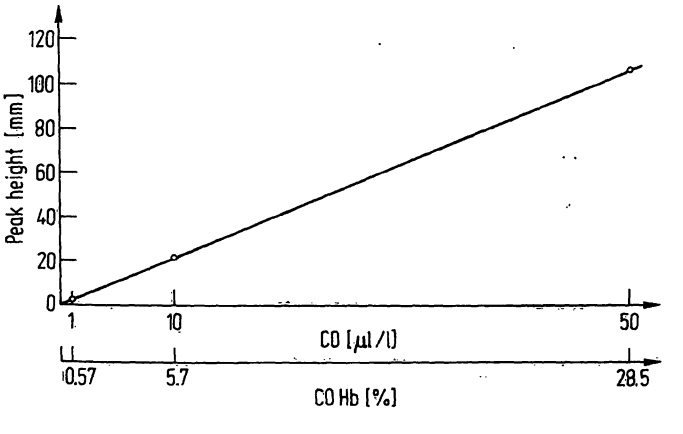

Fig. 3. Calibration curve for the determination of CO in blood. % COHb calculated on 160g/lHb.

during the first 3 min. A blank test from all reagents did not produce a measurable CO value.

To check the accuracy of the determination of the CO rinsing gas volume, 200 µl of CO were injected by means of a gastight syringe into each of 6 bags which had to contain 120 ml N_2 according to the described rinsing procedure. The CO content was determined gaschromatographically after mixing. There was a deviation of ± 1 % of the volume to be required. We have chosen the method of estimating the CO concentration in rinsing gas N_2 because only the final volume of CO and N_2 has to be known. Procedures with adsorption columns for CO at temperatures of liquid nitrogen (4) or total or partial injection of the CO/N_2 gas mixture for the time of CO relase from COHb blood seemed to be inaccurate and associated with an expenditure of work.

This chromatographic method also detected CO_2. It was not investigated whether this method is also suitable for a simultaneous determination of bicarbonate. When no elution of CO_2 is desired, a column (1/4" o.d. by 100 mm long) packed with molecular sieve 13X can be switched between gas sample bag and sampling loop. CO_2 is adsorbed on the column.

A considerable advantage of this method lies in the large sensitivity range of 0.2–100 % COHb and in the great measuring stability of the columns. The use of our Varian 1200 has proved that linear proportional signals of the amplifier were obtained in the sensitivity ranges of $10^{-12}/4$ and $10^{-12}/64$. Therefore, only one calibration curve between 1 and 50 µl/l CO was necessary. Other instruments would have to be examined with regard to this finding (9).

Without obtaining alterations of the retention time 1000 and more analyses can be evaluated with the described column. Hence a reactivation and calibration of the column is not necessary very often. In the measuring range approaching 1 µl/l at a sensitivity of $10^{-12}/4$, positive and negative deflections of the recorder baseline were found before the methane peak (fig. 4). Deflections may be referred mainly to alterations of thermal conductivity of the FID flame. These alterations are e.g. caused by nitrogen present in the injected sample (10). However, these peaks have no influence on the measuring accuracy of the methane peak. In the measuring range toward 50 µl/l at a sensitivity of $10^{-12}/16$ only slight alterations could be detected (fig. 5). The accuracy of this method is primarily established by the reproducibility of measuring the volume of the blood sample and the blood gas. Repeated measurements of a calibration gas for the gaschromatographic system showed a coefficient of variation of CV = 0.25 % whereas repeated measurements of the same blood sample for the whole analytical system showed a coefficient of variation of CV = 2 %.

The detection limit for the determination of COHb is 0.2 %. This could be diminished, e.g. by increasing the

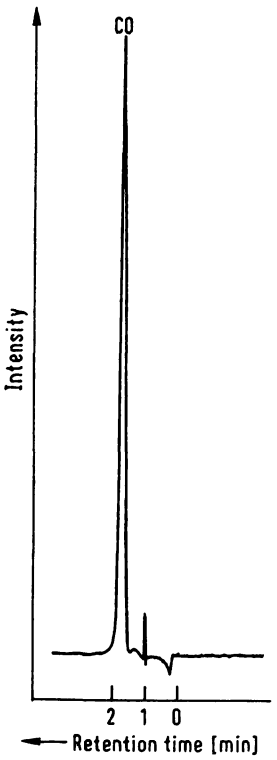

Fig. 4. Gaschromatogram of a 100 µl blood sample of 1% COHb = 1.75 µl CO in blood gas. Sensitivity of GC amplifier $10^{-12}/4$; CO_2 was adsorbed on a molecular sieve column 5A.

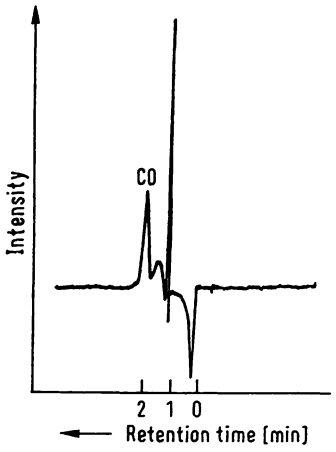

Fig. 5. Gaschromatogram of a 100 µl blood sample of 41% COHb (70,7 µl/l CO in blood gas). Sensitivity of GC amplifier $10^{-12}/16$; CO_2 was adsorbed on a molecular sieve column 5A.

injection volume from 0.5 to 5 ml. However, these ranges are without practical interest. The detection limit for CO is 0.1 µl/l in 4 ml of the gas sample volume according to *Grieder* and *Buser* (4) and 1 µl/l in 5 ml according to *Porter* et al. (2).

With this method we succeeded in carrying out COHb measurements in a wide sensitivity range. In the blood of untreated hamsters and rats 0.2–0.4 % COHb could be detected. The COHb value of smoke-exposed rats or hamsters ranged between 41–46 %. Investigations of the COHb saturation in swine and other mammals are reported elsewhere (11).

References

1. "Air Quality Criteria for Carbon Monoxide": U.S. Dept. of Health, Education and Welfare, Washington, March 1970.
2. Porter, K. & Volman, D. (1962), Anal. Chem. *34*, 748–49.
3. Collison, H. A., Rodkey, F. L. & O'Neal, J. D. (1968), Clin. Chem. *14*, 162–171.
4. Grieder, K. & Buser, H. (1971), Beitr. Tabakforsch. *6*, 36.
5. Klimisch, H.-J. & Meißner, K. (1972), Beitr. Tabakforsch. *6*, 216.
6. Klimisch, H.-J., Meißner, K. & Wernicke, H., Lebensmittel-Unters. & Forsch., in press.
7. Dontenwill, W., Chevalier, H.-J., Harke, H.-P., Lafrenz, U., Reckzeh, G. & Schneider, B. (1973), J. Natl. Cancer Inst. *51*, 1781–1832.
8. Stove, H. W. & Pelletier, M. F., Automat. Anal. Chem. Technicon Symp., 3rd, 1967, Publ. 1968, *1*, 431.
9. Blackmore, D. J. (1970), Analyst *95*, 439.
10. Boček, P. & Janák, J. (1971), Chromatogr. Rev. *15*, 111.
11. Klimisch, H.-J., Chevalier, H.-J., Harke, H.-P. & Dontenwill, W., Toxicology in press.

Dr. H.-J. Klimisch
2000 Hamburg 54
Gazellenkamp 38