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Microanalysis of Carbon Monoxide in Blood by Head-Space Capillary Gas Chromatography

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ABSTRACT: A gas chromatographic procedure for the determination of carboxyhaemoglobin in blood was improved by using a capillary system in combination with a micro thermal conductivity detector. This system is very sensitive with high resolutions. The analytical time is reduced to approximately two minutes. Using sulphuric acid as the liberating agent of carbon monoxide, a gradual and reproducible release of carbon monoxide was seen. This shortens the time in emergency cases for sample preparation.

KEYWORDS: forensic science, carbon monoxide, blood, capillary gas chromatography (GC-TCD)

Several methods for the determination of carboxyhaemoglobin (COHb) in blood have been published and reviewed by Blackmore [*1*]. Gas chromatography is the most accurate because it is only minimally affected by decomposition of the blood. However, for the procedures described up till now, packed columns have been used to separate carbon monoxide (CO) from other permanent gases, including nitrogen, oxygen and carbon dioxide [*2–4*]. The use of capillary systems greatly improve the separation efficiency and shorten the analysis time. Therefore, this paper describes the use of a capillary Molecular Sieve column in combination with a Micro Thermal Conductivity detector (TCD). This detector has a much higher sensitivity than the commonly used packed TCDs. The combination gives comparable results to those obtained by Goldbaum et al. [*4*] even when using a split injection system. These authors, using a packed Molecular Sieve column and an extraction chamber attached to the GC apparatus were able to quantitate COHb levels down to 0,5% in small blood samples (0,02 to 0,5 mL). This elegant method however uses an on-line system and therefore has the disadvantage of gradual accumulation of water and carbon dioxide causing a decreased resolution of CO from other gases. Such problems are not expected with the use of a split injector and a capillary system.

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Materials and Methods

Equipment

A Perkin Elmer 900 gas chromatograph, equipped with a 25 m capillary PLOT fused silica column coated with Molsieve 5A (0.32 mm internal diameter) (Chrompack, The Netherlands) was used. The column was held isothermally at 80°C. Detection was realized by a Micro-TCD detector from Chrompack. The detector temperature was adjusted to 130°C and the bridge temperature to 210°C. Helium was used as carrier gas (3 mL/min). A Varian DS-650 data system (Varian Associates, CA) handled the chromatograms. The split injector was held at 100°C and the split ratio adjusted to 1/10. To calibrate the gas chromatograph, a certified mixture of 1520 ppm CO in air, purchased from UCAR (Belgium), was used.

Reagents

Two liberating agents were tested. In one series of experiments, 2N sulphuric acid was used, containing 1,5% saponin to ensure complete lysis and microscopic fragmentation of red cell membrane. In another series of experiments, a solution containing potassium hexacyanoferrate(III) (3%), sodium carbonate (4%), sodium hydrogen carbonate (4%) and saponin (1.5%) was used. The pH of this solution was approximately 9.

COHb standards were prepared with fresh human blood initially containing 0.4% COHb. Endogenous CO was removed by bubbling pure oxygen through the blood for two hours. A portion of the cleaned blood was saturated with CO, obtained from l'Air Liquide (Belgium), by placing it in a sealed flask. CO gas was bubbled through the blood for half an hour followed by a nitrogen stream during 5 minutes. Appropriate dilutions

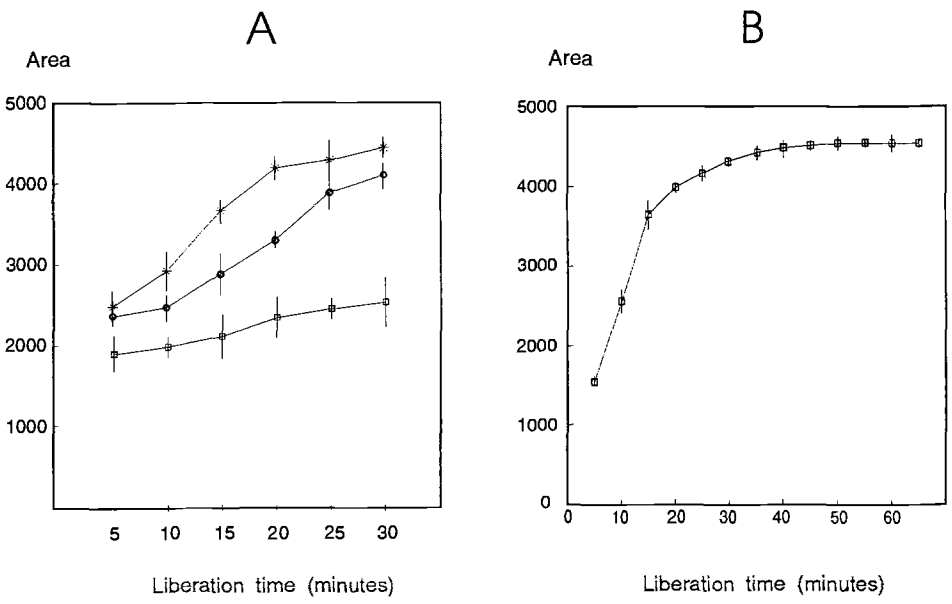

FIG. 1—*Liberation of CO from whole blood using the potassium hexacyanoferrate(III) mixture at 40°C* (A; □), *at 50°C* (A; ○) *and at 60°C* (A; ∗) *and using the sulphuric acid mixture* (B). *The blood samples have a COHb level of approximately 10%.*

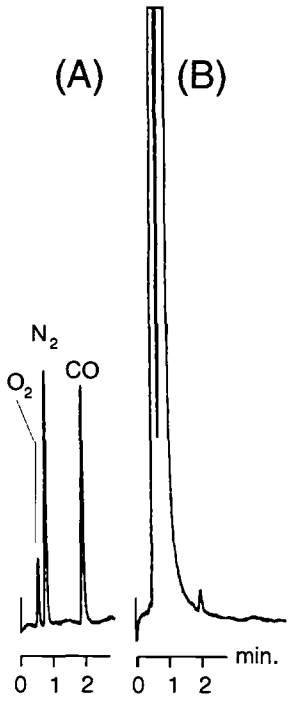

FIG. 2—*Gas chromatogram of a mixture of carbon monoxide in air* (A)*; gas chromatogram of 50 μL blood sample containing 0.4% COHb* (B).

were then made to yield COHb concentrations of 2, 4, 6, 8, 10, 20, 40, 60 and 80%. These standards are stable for approximately four weeks, when kept at 4°C.

Analytical Procedure

To measure the carboxyhaemoglobin concentrations, CO was liberated from blood by placing an appropriate amount (50 μL to 1 mL) in a crimp top vial, which was then sealed with a fluorosilicon septum. Using two hypodermic needles inserted in this septum, the vial was flushed for half a minute with purified helium. One needle was removed and an appropriate amount (twice the blood volume) of the liberation agent added through the other. Then the latter was removed and the vial stirred magnetically. In the case of sulphuric acid, this was done at room temperature for forty minutes. In the case of the potassium hexacyanoferrate(III) solution, the liberation was realized by stirring the mixture for varying liberation times on a heated plate, kept at different temperatures (40, 50 or 60°C). In all experiments, 200 μL of the head space vapor was then injected on the column.

The per cent saturation of the blood was calculated by the ratio of the peak areas obtained from untreated and saturated blood:

$$A_c/A_s \times 100 = \% \text{ saturation}$$

A_c = area of CO peak from untreated blood

A_s = area of CO peak from CO-saturated blood

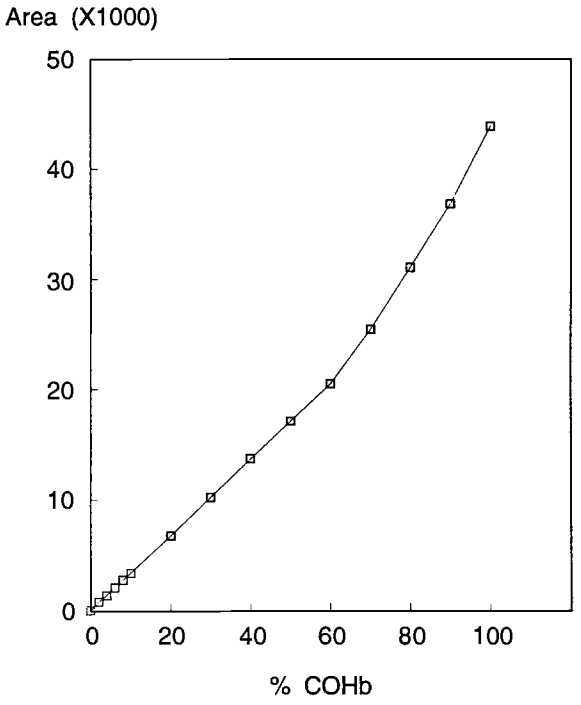

FIG. 3—*Peak area versus % COHb for different COHb levels.*

Saturation of the blood was realized by placing an aliquot of the same blood sample in a sealed flask. Using two hypodermic needles, one placed in the blood and the other placed above, CO gas was gently bubbled through the blood for half an hour followed by a stream of nitrogen during 5 minutes. After saturation the needles were removed and the blood was handled in the same way as described for the blood samples.

Results and Discussion

Figure 1*A* and 1*B* show the liberation of CO from whole blood using the potassium hexacyanoferrate(III) reagent and the sulphuric acid mixture respectively. The former procedure only allows complete liberation of CO at temperatures above 60°C. Although this is achieved within 25 minutes, we prefer the sulphuric acid method for its better reproducibility and more gradual release of the gas at room temperature.

The gas chromatographic procedure was checked for its efficiency. Figure 2*A* shows

TABLE 1—*Fourteen day and within-day reproducibility for different COHb levels.*

% COHb	Within-day reproducibility	Fourteen day reproducibility
2.14	0.03	0.04
5.79	0.03	0.06
10.36	0.04	0.05
21.19	0.09	0.11

TABLE 2—*Accuracy of COHb analyses at varying liberation times using the sulphuric acid mixture as liberating agent.*

Liberation times	Measured COHb levels at different liberation times							
	10′		20′		30′		40′	
% COHb	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	2.12	0.09	2.11	0.10	2.15	0.07	2.14	0.03
6	5.81	0.11	5.82	0.09	5.71	0.06	5.79	0.03
10	10.39	0.13	10.36	0.11	10.32	0.13	10.36	0.04
20	21.17	0.15	21.23	0.17	21.26	0.10	21.19	0.09

a chromatogram of a mixture of carbon monoxide in air. High resolutions and excellent peak shapes are obtained within a short analysis time of about two minutes, for example a reduction of the analytical time by a factor 2 to 4 in comparison with the methods described by Guillot et al. [*3*] and Goldbaum et al. [*4*].

The linearity of the detector response was checked by the analysis of blood dilutions of varying COHb concentrations. Figure 3 shows a linear relationship between peak area and the COHb concentration from 0 to 65%. For higher values a deflection of the curve is seen. The reproducibility of the method was measured by analyzing four samples containing different COHb concentrations during fourteen days. Within-day reproducibility was measured by analyzing the same samples, 10 times during the same day. The results are shown in Table 1.

The detection limit was calculated to be less than 0.02% COHb. Since this level is far below the normal range for non-smokers (0.4 to 0.7%) [*5*], smaller sample volumes than one mL can be used. Figure 2*B* shows a chromatogram obtained from a 50 μL blood sample containing a physiological COHb level (0.4%). The results are comparable with those obtained by Goldbaum et al. [*4*] but the method does not necessitate the use of an on-line extraction chamber thus excluding the disadvantages of the latter. No decrease of resolution was observed even with the daily use of the capillary system for months. Due to the split injection system accumulation of water can be prevented. The lower amount of substances injected on the capillary column is compensated by the much higher sensitivity of the micro thermal conductivity detector.

In addition to a rapid analysis, it is also possible to reduce the liberation time of 40 minutes. On condition that a strictly timed procedure for both unsaturated and saturated blood samples is followed, incomplete liberation can provide satisfactory results as well. Table 2 shows the results obtained for four standard samples on which different liberation times (10 min–40 min) were applied prior to their analysis. Each analysis was performed ten times. COHb levels remain very comparable with only some greater variations for shorter preparation times. This can be useful in an emergency case.

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