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DETERMINATION OF CARBON MONOXIDE IN BLOOD BY MEANS OF MICRODIFFUSION

OZNACZANIE TLENKU WĘGLA WE KRWI METODĄ MIKRODYFUZJI

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There was developed a little-work-consuming and non-expensive microdiffusion method of carboxyhaemoglobin determination in blood, in low range concentration. It can be used in the assessment of passive smokers' exposure to carbon monoxide. The amount of blood neccessary for the determination was 0.15 ml. An average precision of the developed method of the carbon monoxide determination (converting into HbCO) for the concentration ranged from 1 to 5%, from 3 to 12% and from 5 to 20% was respectively 5.96, 4.09 and 2.14.

INTRODUCTION

Carbon monoxide is present in blood mainly as a complex with haemoglobin, i.e. carboxyhaemoglobin (HbCO), some of it being a free form. An integral indicator of the exposure of people (including smokers) to exogenous CO may be its general contents in blood, both in the free form and in the complex with heamoglobin, as well as the contents of the HbCO itself in blood.

The aim of this paper is to develop an easy, little-work-consuming and cheap microdiffusional method of HbCO determination and to limit the amount of blood taken in the least invasive way, e.g. from a finger tip or an ear-lobe.

It was assumed that it would be possible to use the developed method for the assessment of passive smokers' exposure to CO present in the air polluted with smoke, i.e. the blood determination in low range concentrations, on the level which is only a little higher than the physiological concentration of HbCO (about 1%).

In this paper a microdiffusional method was used. CO determination in blood, by means of this method, required an adequate modification of the Conway's vessels, because of CO freeing at the time of the determination (after adding a freeing reagent to the sample).

It was impossible to use original vessels with closely adjusted glass plate. Developing of this method required using a particular freeing reagent and a reagent absorbing CO which is released in blood samples.

The developed method is an alternative to other methods of determining low concentration of CO in blood, such as chromatographic (with indispensable catalytic

methanation) or spectroscopic method [1, 3–5], which require highly technical equipment and are used for mass determinations.

METHOD

This work involved using modified *Conway's* vessels (Figure 1). As an analytical reaction based on chemisorption of the CO in 1% solution of sodium-silver salt p-sulfamylbenzoic acid was applied. As a result a quantitatively coloured silver sol. determined by means of spectrophotometric method, was created [3].

The course of the analytical reaction (taking the quantity into consideration) was examined with the use of standard CO solutions in the air. Afterwards calibration of the developed method of CO determination in blood was carried out on appropriately prepared samples.

Blood samples (group A) received from the District Blood Donor Station in Katowice (expired samples) were haemolysed by adding distilled water (in the volume ratio of 1:4). In order to prepare calibration solutions of HbCO in blood, part of the heamolysate was oxygenated (oxygenated blood) and the other part was saturated with pure CO (carboxygenated blood) in Zajcew's washer and mixed in appropriate proportions.

0.75 ml of haemolysate was poured into the internal cylinders of the Conway's vessel and 0.3 ml of 1% solution of sodium-silver salt p-sulfamylbenzoic acid in its external part. Next they were tightly closed with silicon rubbers. Afterwards 0.4 ml of 10% solution of $K_4[Fe(CN)_6]$ was added to the haemolysate with syringes, in order to free the CO without opening the vessels.

The samples were stored for 24 hours in room temperature and in darkness. After the time the absorbance was measured at 426 nm.

RESULTS

The results of this project include the above-described method and the findings of the carried out calibration of the developed method.

The construction of the reactional vessels (modified *Conway's* vessels)

For the construction of the reactional vessels used during this work, instead of original *Conway's* vessels, there were used vessels consisting of glass containers made of dark glass (14 by 33 mm and volume 5 ml), in which there were placed cylinders made of glass functioning as internal vessels (10 by 18 mm). The internal vessel did not create one part with the external one, but it leaned against a glass distance ball, which on the one hand limited the possibility of its movements, and on the other hand provided an unlimited contact of the freed CO and the analytical reagent. The vessels were closed with silicon rubber and tightened with hole cap.

The calibration of the method

The results of the calibration of the CO determination (both in the air and in blood haemolysates) were presented in Table I as simple regression coefficients *a* and *b* describing particular calibration curves also estimation parameters used calibration

procedure, as correlation coefficient r, standard error of estimation *Sy*, error of method *Sxo* and precision of method *vxo*.

The calibration of CO in the air

The calibration of the CO determination in the air was carried out within the concentration range of CO from 0.008 to 0.040% v/v, at 25 C (curves 1 and 2). The amount of CO in the samples of its solutions in the air (from 4.59×10^{-4} to 2.29×10^{-3} mg) was compatible with the amount of CO which would be freed from 3 drops (0.15 ml) of blood if the concentration of HbCO was 1.5 to 7.5%.

The calibration of CO in blood haemolysates

The calibration was carried out in solutions of haemolizates for three concentration ranges of CO, present in blood converting into HbCO, from 1 to 5% (v/v) (curves 3–6), from 3 to 12% (curves 7–10) and from 5 to 20% (curves 11 and 12) in optimal conditions fixed on the basis of the calibration of CO determination in the air.

DISCUSSION AND CONCLUSIONS

It seems that the developed method, according to our adopted assumptions, distinguishes from other alternative methods of CO determination, due to shorter time of preparation of the samples for the analysis (avoiding long storage of the samples at night in order to finish an analytical reaction), a small amount of blood needed to perform the determination and low costs.

This method may be applied in research of individual passive smokers' exposure to CO present in smoke, without a need to take venous blood from the examined. Although the composition of capillary blood is not fully compatible with the composition of venous blood, it seems to have little significance where exposure to CO is marginal.

No similar methods of CO determination in blood have been found in any scientific journals, except for the microdiffusional method of CO determination by *Bela* and *Long Boso* [2], which in fact varies considerably from the first one. Besides in this method much more blood is used and the determinations with other reagents and different methodology are possible only with higher concentrations of CO in blood.

The modification of *Conway's* vessels proposed here, gives an opportunity to add a freeing reagent to the samples of blood haemolysate, placed in the internal part of the vessel, after it is tightly closed which helps to avoid CO losses leading to wrong results. The modified *Conway's* vessels can be applied in determination of other substances by means of microdiffusion method where the addition of relasing have to be freed from the material which was determined with the help of an additional reagent.

In the results of the determinations presented in the paper (converting into HbCO – Table I), both combined CO and free-form CO (which appears as a product of alternative HbCO dissociation) were taken into account.

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OZNACZANIE TLENKU WĘGLA WE KRWI METODĄ MIKRODYFUZJI

Streszczenie

Opracowano mało pracochłonną i niekosztowną mikrodyfuzyjną metodę oznaczania HbCO we krwi w niskich zakresach stężeń przeznaczoną do oceny narażenia biernych palaczy na tlenek węgla. Niezbędna do oznaczeń ilość krwi wynosiła 0.15 ml. Średnia precyzja opracowanej metody oznaczania tlenku węgla w przeliczeniu na HbCO w zakresie stężeń od 1 do 5%, od 3 do 12% i od 5 do 20% wynosiła odpowiednio 5,96; 4,09 i 2,14.

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