The Present of the Detection of Carbon Monoxide Poisoning

Jing Ding [a], Hao Xu [b], Chaokang Gu [c], Hao Guo [a, *]

a Chongqing Institute of Forensic Science, Chongqing, China.

b Parallel first author: Department of Radiotherapy, Fudan University Shanghai Cancer Center, Shanghai, China.

c Reel Solar Power Inc, San Jose, California, USA.

* CORRESPONDING AUTHOR E-mail: hao.guo1982@hotmail.com, Phone: 86-23-63764388.

ABSTRACT Carbon monoxide (CO) is a colorless, odorless and non-irritant toxic gas that is easily absorbed through the lungs. The essence of carbon monoxide's toxicity is its competitive action with oxygen for binding to hemoglobin. The affinity of hemoglobin for carbon-monoxide is 200-250 times as great as its affinity for oxygen, and the bond of carbon-monoxide is almost 25,000 times stronger than the oxygen bond. Tissue hypoxia is the result of the impaired release of oxygen from the carboxyhaemoglobin (COHb), as well as a decrease in the body's oxygen-carrying capacity. Carbon monoxide shows an affinity to other proteins within the prosthetic group of hem proteins (myoglobine and some mitochondrial enzymes).

CO toxicity is one of the common types of poisoning, and CO poisoning remains a common cause of both suicidal and accidental deaths in reported many countries. As a consequence, determination of the percent carboxyhemoglobin (COHb%) level in postmortem blood is a common analysis performed in toxicology laboratories. Therefore, the detection of carboxyhemoglobin (COHb) in blood is important in clinical and legal medical fields. In this paper, the methods such as Gas chromatography (GC), Headspace-gas chromatography-mass spectrometry (HS-GC-MS), CO-oximeter, UV/Vis spectrophotometry, Fourier transform infrared micro-spectroscopy (FTIR) and other kinds of analytical methods used for determination of COHb in blood during recent thirty years were reviewed. The advantages and application conditions of these methods were analyzed to provide consultation for further research.

KEY WORDS toxicology analysis, carbon monoxide poisoning, carboxyhemoglobin (COHb), determination method, forensic science

1. INTRODUCTION

Carbon monoxide (CO) is a colorless, colorless, odorless and non-irritant toxic gas. It is highly toxic to the blood and nervous system. Binding of CO to hemoglobin reduces the oxygen-carrying capacity of hemoglobin, leading to the tissue necrosis due to hypoxia. Severe cases are fatal. CO poisoning is one of the common causes of poisoning. It is used as a regular indicator of death in coal mine accidents. The concentration of carboxyhemoglobin (COHb) in the blood can be used to determine the degree of CO poisoning. Therefore, the detection of COHb in blood is of great significance in clinical medicine, forensic toxicological analysis and so on.

In recent years, the detection of COHb has made great progress, mainly in the improvement of methods and instruments [1]. At present, in the field of clinical monitoring and toxicological analysis, the common methods of analyzing COHb in blood of CO poisoning are gas chromatography, headspace gas chromatography, oximetry, spectrophotometry and infrared spectroscopy. In this study, the research and application of the CO poisoning detection methods in the past 30 years were reviewed.

2. GAS CHROMATOGRAPHY

Guillot used a headspace gas chromatographic method to quantify the CO level in blood as early as 1981 by calculating the CO level in the blood using the standard CO peak area ratio in the blood. Goldbaum et al. [2] used a gas chromatograph (GC/TCD) with a thermal conductivity detector in 1986 to detect CO levels in blood. Since headspace gas chromatography is more sensitive to the detection of spoiled blood, it is possible to separate CO from a mixed gas containing O_2, CO_2, N_2, etc., using a chromatographic column. This method was much more sensitive than conventional thermal conductive devices from Goldbaum's study. In 1992,

Yuzhong et al. [3] used a headspace sampler and a gas chromatograph (HS / GC/TCD) with a TCD detector to determine the CO content in the bioassay of CO poisoning. This method not only detected the organ COHb saturation (COHb%). but also showed a difference within 10% for measurement between the actual value of COHb% in the blood and that of the blood at room temperature under vacuum storage after 14 months. In 1994, Van Dam J et al. [4] used the HS / GC/TCD method to measure the COHb% of blood, increasing the sensitivity of the assay and reducing the analysis time to about 2 minutes. In 2002, Anna et al. [5] used a gas chromatograph (GC/FID) with a flame ionization detector to quantify the CO content in blood. This method was highly sensitive (0.01 nmol/ml) and accurate (1.5% precision), which was suitable for low concentration of CO analysis.

The normal GC method sometimes has a problem in the detection work. When the cadaveric blood was heated or the freezing temperature surpassed -30℃, the methemoglobin (MetHb) in the blood could be increased or the red blood cell could be oxidized to MetHb because of not saving the blood in time [6-8]. Since MetHb did not bind CO, a high concentration of MetHb weakened the CO binding capacity [9], resulting in decrease of denominator in the formula (COHb% = peak area of blood test / peak area of saturated blood × 100%) and increase of COHb%. In order to solve the above problems, Lewis et al. [10] studied a new GC/TCD method for the determination of COHb% in blood. The new GC/TCD method was based on the original GC/TCD method by adding reducing agent, sodium hydrosulfite reductant, which effectively reduced the MetHb content. Experiments showed that this method could accurately detect COHb% when the blood was heated or frozen.

3. HEADSPACE GC-MS

Mass spectrometry is a new type of separation and detection technology developed in recent years. At present, the mass spectrometry technique applied in the analysis of CO poisoning is mainly headspace-gas chromatography-mass spectrometry (HS/GC/MS). It has been reported in the literature [11-12] that the storage conditions and time had little effect on this method. COHb% detected was consistent with that when CO poisoning happened. This method has high sensitivity and efficiency. It is available for detection of the corruption of blood and a variety of biological organ tissue samples. This method has been successfully applied in forensic CO poisoning cases.

However, if the HS/GC/MS method is to be used as a standard method in practical work, it is necessary to further investigate the influencing factors of HS/GC/MS quantitative analysis. Therefore, Varlet et al. [13] quantified the CO content in human blood by modified HS/GC/MS method in 2012 and validated the new method with accuracy curve. Hao et al. [14] investigated in detail the factors affecting the CO concentration in blood, including headspace volume, heating temperature, heating time, acid addition method, and methods of preparing saturated blood samples, by HS/GC/MS method. At the same time, the HS/GC/MS method was compared with the conventional spectrophotometric method for the same sample. They concluded that the HS/GC/MS method had more advantages in the determination of the corrupt sample. This method is suitable for testing some materials from complicated cases in China and provides the court with a reliable data basis.

4. SPECTROPHOTOMETRY

Katsumata et al. quantitatively determined CO content in blood by spectrophotometry as early as 1982. According to the literature [15], ultraviolet (UV) spectrophotometry commonly used in the detection of CO poisoning includes single wavelength method (improved Akigu - ancient village law), such as absorption point method, simultaneous equations method, dual wavelength method and derivative spectrum method [16-20]. These methods mainly used seven kinds of calculation formulae (Table 1). The experiment proved that the above methods could calculate the COHb% in fresh blood with an accurate range more than 30%.

At present, the UV spectrophotometry is the most widely used in the detection of CO poisoning, because of its simple, rapid and reproducible characteristics. Many forensic drug inspection departments are using UV spectrophotometry for detection of COHb% in CO poisoning cases in Taiwan [21], Hong Kong [22], Sichuan [23], Guangdong [24], Shanxi [25], Jinan [26], Shanghai [27], Beijing [28], Heilongjiang [29], Chongqing [30], Jiangxi[31] and Hubei [32] in China. In other countries such as India [33], South Korea [34], Japan [35], Greece [36], Germany [37], England and Wales[38], Turkey [39], USA [40], UV spectrophotometry is also applied in similar cases.

5. BLOOD OXYGEN METER METHOD

Blood oxygen meter is a special automatic spectrophotometer. It can measure different absorption peaks of hemoglobin derivatives. Total hemoglobin (THb), oxyhemoglobin (HbO_2), MetHb, and COHb were measured by oximetry at the same time by Ohshima et al. [52-53]. The COHb%, COHb% = COHb / THb, THb = HHb + HbO_2 + COHb + MetHb [54], was calculated by using an oximeter to select the absorbance peak with a spectrophotometer for six different wavelengths and the concentration of each component in THb was determined from six lines. Because of its small sampling (<l00 μl), fast response (<l min) and no requirement of any pre-treatment and so on [55], blood oxygen meter was applied

widely in clinical testing and forensic toxicological cases [56-61] .

Table 1 UV spectrophotometry calculation formulae

Number	Author	Formula
1	Liu Yao[41]，Qiao Jing[42]	$COHb\% = [1.5 \times (A-B)/(C-D)] \times 100\%$
2	Lu Huiming[43]	$COHb\% = [(A_x - A_0)/(A_{100} - A_0)] \times 100\%$
3	He Langchong[44]	$COHb\% = [(A_{420}/A_{430})_X - (A_{420}/A_{430})_0]/ [(A_{420}/A_{430})_{100} - (A_{420}/A_{430})_0] \times 100\%$
4	Chen Juyi[45]	$COHb\% = [(1.317 \times A_{420} - 0.988 \times A_{432})/(0.844 \times A_{420} + 0.982 \times A_{430})] \times 100\%$
5	Zhang Jieke[46]，Jiang Yan[47]	$COHb\% = (\Delta A_X/\Delta A_{100}) \times 100\%$
6	Yun Keming[48,51]	$COHb\% = (A_{538}/A_{555})/0.458\text{-}1.692$
7	Yang Ya[49]	$COHb\% = 0.71 \times (A_{419}/A_{425.5})_x - 1.2$
8	Zhang Jingjing[50]	$COHb\% = [0.94(A_{420}/A_{430})_x - 0.69)] \times 100\%$

6. INFRARED SPECTROSCOPY

1994 Moria et al [62] used Fourier transform infrared spectroscopy to determine COHb absorption peak. From the infrared spectroscopy, we can observe that a weak absorption peak at 1969 cm^{-1} due to the stretching vibration of Fe <-CO. The stretching vibration of N-H and >C=O and the bending of -NH resulted in a strong absorption peak appeared at 1544 cm^{-1}. The COHb% can be obtained from the absorption peak area ratio between 1969 cm^{-1} and 1544 cm^{-1} for the detection of CO poisoning blood. In 1996, Mendelson et al. [63] measured the absorption peaks of two hemoglobin derivatives (COHb and MetHb) using pulsed multi-wavelength visible-near-infrared spectroscopy. In 1999, Venkatesh et al. [64] measured the absorption peak of hemoglobin derivatives using Fourier transform Raman spectroscopy. Zhang Pingli et al. [65] used the Raman spectroscopy to measure different saturations of COHb. The experimental results showed that this method was rapid, simple, direct, and non-destructive for samples. It was expected to be a new method for detecting COHb%.

7. OTHER METHODS

Other CO poisoning analysis and detection methods are capillary electrophoresis and oxygen electrode methods. Durkin et al. [66] detected COHb concentration in cadavers by capillary electrophoresis method in 1998. The experiment successfully segregated reduced heme and CO-bound heme in standard hemoglobin by capillary electrophoresis. Thus capillary electrophoresis method is used for the detection of CO poisoning blood. Y. KATSUMATA et al. [67] detected COHb concentration in blood samples by oxygen electrode method. The method is to use the electrode analyzer with a recorder (oxygen electrode equipped with a glass bottle for adding red blood cells and potassium ferricyanide used as a release agent). When there was COHb in the blood, CO was released from COHb. After adding the red blood cell, CO replaced O_2 from oxygenated hemoglobin (HbO2). Potassium ferricyanide would not release O_2 from the HbO_2 in the cells. O_2 concentration was proportional to CO concentration in blood. Thereby HbCO concentration was obtained by detecting O_2 concentration in the sample blood. The oxygen electrode method is suitable for detecting the HbCO concentration in the deteriorated blood after the fire disaster. At present, the above two methods used in CO poisoning detection were reported little.

8. OUTLOOK

From the current application of domestic and international methods for detection of CO poisoning, we believe that the UV-visible spectrophotometry is still the most commonly used method for present and future. Headspace GC-MS method was applicable to most of the CO poisoning detection of the corrupt samples and complicated cases in China. Those methods such as using oximeter[68], Fourier transform infrared spectroscopy[69] and Raman spectroscopy will be further investigated, which would be widely used in clinical and forensic CO poisoning detection.

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