Analysis of Ammonia in Blood by Gas Permeation/Gas Chromatography

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It was the purpose of this paper to report a new analytical method for direct determination of blood ammonia (NH₃) by gas permeation/gas chromatography (GC). The apparatus was mainly composed of GC and a thermoregulator, in which a permeation plate assembly used for a measurement of urine NH₃ was installed. A blood sample of 1.0 ml was injected into the stream of pH 7.4, 0.01 M Tris/HCl buffer solution and carried in two the permeation plate assembly with the buffer solution. Ammonia in the sample was permeated from the buffer solution through the membrane into GC carrier gas (He). The permeated NH₃ was determined by GC. The precision for the measurement was within 6% in terms of coefficient of variation. A minimal detection limit of NH₃ was 0.25 μg in the injected sample. An analytical time required for NH₃ measurement was less than 13 min. Correlation between the proposed method and the indophenol method was 0.99 (n=17) in terms of correlation coefficient.

Introduction

In general, NH₃ in blood has been chemically analyzed by several methods such as the ion selective electrode method and micro-diffusion method. However, these analytical methods had to be conducted in the high alkaline solution (pH 11–13), but NH₃ measurement in blood would be obviously preferable to use the neutral zoned solution (pH 6–8) wherein the blood tissue was not destroyed.

In this view, direct determination of blood NH₃ was investigated by gas permeation/gas chromatography.

Materials and Methods

APPARATUS

Flow diagram of apparatus and cross-sectional view of permeation plate assembly (PPA) and gas permeation membrane (GPM) that were used for a determination of urine NH₃ were shown in Fig. 1 and Fig. 2, respectively.

The apparatuses were mainly composed of GC (I), thermoregulator in which the PPA was installed (II), sample injection port (III), pump system (IV) and preparation system for carrier solution (V).

The PPA was installed in the thermoregulator. The GPM used in the assembly was Fluoropore (Sumitomo Electric Ind., Tokyo) of 0.45 μm pore size. The membrane was sandwiched in between two stainless steel plates. Passage for gas or liquid was an U-shaped ditch carved in a circle on surface of the each steel plate. Two thin plates of silicone rubber for gas tight sealing were inserted separately between the steel

Key words: Gas permeation/gas chromatography, Direct determination, Blood ammonia, Gas analyzer
Fig. 1 Flow diagram of apparatus
A, Flow meter; B, TCD detector; C, Separation column; D, Precolumn; E, Pressure control valve; F₀, Permeation plate assembly (I); F₁, Permeation plate assembly (II); G, Mixing coil; H, Recorder; I₀, I₁, Sample injection ports; J, Pressure gauge; K, Gas cylinder (He); L, Reservoir for the carrier solution; M, Gas cylinder (N₂); N, High pressure pump.

Fig. 2 Permeation plate assembly
Left, Permeation plate; Right, Permeation membrane and silicone plate. A, B, Outlet and/or inlet ports of carrier gas and carrier solution; a, Screw hole; b, Joint; c, Groove; d, Stainless steel plate.
plate and the membrane.

The details regarding the apparatus were described as followed:

(I) GC: Shimazu 3BTGC (Kyoto); Column (C)/Length 1.0 m, I. D. 3 mm, Temperature 80°C, Packed material 80/100 mesh chromosorb 103 (GC Ind. Co., LTD, Tokyo); Precolumn (D)/Length 100 mm, I. D. 5 mm, Packed material 60/80 mesh silica gel (GC Ind. Co., LTD), Temperature 80°C; Carrier gas (K)/He.

(II) Thermoregulator in which a PPA was installed: Thermoregulator/Air bath (GC Ind. Co., LTD), Size 270 mm × 190 mm × 100 mm; PPA (F0, F1)/100 mm φ, 7 mm thick, round shaped stainless steel.

(III) Sample injection port: Hitachi-163 glass insert sample injector (I0, I1)/Volume 1.0 ml.

(IV) Pump system: Pump (N)/Maximum flow rate 2.0 ml/min, Maximum pressure 150 kg/cm².

(V) Preparation system for the carrier solution: Reservoir (L)/Volume 1 l, Double spaced reservoir which N₂ bubbler was placed in the inside chamber and hot water could be poured into the outside one; N₂ cylinder (M) for N₂ bubbling.

OPERATION and PROCEDURE

At first, N₂ from a cylinder (M) was carried into the pH 7.4, 0.01 M Tris/HCl buffer solution, and O₂ and CO₂ in the solution were degased by bubbling N₂. The carrier solution was previously warmed till the PPA temperature with hot water. The carrier solution was carried into the PPA with a pump (N) and wasted to the outside of the thermoregulator via mixing coil (G) and the PPA.

Ammonia in blood was determined as followed; Under the optimum condition of GC, blood sample was injected into the pH 7.4, 0.01 M Tris/HCl buffered carrier solution through a sample injection port. The sample was carried into the PPA via mixing coil. A part of NH₃ in the sample was permeated into GC carrier gas from the carrier solution in the PPA according to NH₃ permeation mechanism shown in Fig. 3. The permeated

NH₃ was carried into GC with carrier gas and was determined by GC. Next, amount of NH₃ in blood was calculated by using a relationship between amount of injected NH₃ and peak area of permeated NH₃.

CHEMICAL and SAMPLES

All chemicals used were GR grade of WAKO CHEMICAL Co. (Osaka).

1/15 M phosphate buffer solution; 1/15 M KH₂PO₄ solution and 1/15 M Na₂HPO₄ solution were mixed at arbitrary rate. This solution was used as the carrier solution at pH ranged from 5.3 to 7.0.

0.01 M Tris/HCl (tris (hydroxymethyl) aminomethane-hydrogen chloride); 0.01 M Tris solution and 0.01 M HCl solution were mixed at arbitrary rate. This solution was also used as the carrier solution at pH ranged from 7.0 to 9.0.

Reagents for NH₃ measurement by an indophenol (IP) method; All reagents used were prepared according to a preparation method described by H. Okuda et al.₁⁰.

Ammonia standard; Ammonia gas with a purity of 99.98 % was used as NH₃ standard. The gas was purchased from SETA-GAYA SANSO Co., LTD (Tokyo). Ammonia gas was diluted with N₂ and used for experiments. Also, concentration of NH₃ standard was calculated by using the equation of state of gas.

Blood samples; Whole blood samples of normal, uremia and acute atrophy of the liver were used. Their samples were treated as followed; 1.0 ml of blood was hemolyzed with 1.0 ml of water and the solution was
centrifuged at 1000×g for 10 min. The supernatant was used as the blood sample A for the experiments. The blood sample B without the pretreatment was also used for NH₃ determination in blood.

Results and Discussion

THE OPTIMUM CONDITION AND CHROMATOGRAM OF NH₃ STANDARD SAMPLE

Gas permeation was mainly affected on a PPA temperature, flow rate of GC carrier gas (GCFR; or GC inlet pressure)/flow rate of the carrier solution (CSFR; or pump outlet pressure) ratio and a difference of the gas concentration between the both phases. The experimental condition by which more amount of NH₃ should be rapidly permeated into GC carrier gas from the carrier solution at middle temperatures (30–80°C), was investigated about these factors with 1.5 μg of NH₃ in the NH₃ standard.

As a result of the experiment, the optimum condition on 30 ml/min GCFR (1.0 kg/cm² GC inlet pressure), 0.30 ml/min CSFR (2.5 kg/cm² pump outlet pressure) and 50°C PPA temperature was obtained to it.

Under the optimum condition, the sample injected was stayed for about 3 min in the PPA and the times required for the detections of permeated N₂ and NH₃ from the sample of NH₃ standard were 9 min and 13 min after an injection of the sample, respectively. Also, NH₃ in the NH₃ standard sample was completely separated from N₂. Therefore, NH₃ was determined by the proposed method under the optimum condition.

EFFECT OF pH OF THE CARRIER SOLUTION ON GAS PERMEATION

Under the optimum condition, pH effect of the carrier solution on NH₃ permeation was investigated with 1.5 μg of NH₃ in the NH₃ standard sample. The more the pH of the carrier solution was increased, the more the amount of permeated NH₃ was increased. This effect could be explained by the face that NH₃/NH₄⁺ concentration ratio on chemical equilibrium (NH₃+H⁺⇌NH₄⁺) was more increased, and then the pH of the solution became more alkaline. However, an effective pH of the carrier solution to direct determination of blood NH₃ was selected because a pH of blood was generally about 7.4.

CALIBRATION CURVE

Under the optimum condition, 0.2–100 μg of NH₃ in the NH₃ standard samples were injected from a sample injection port, and a relationship between amount of NH₃ injected and peak area of permeated NH₃ was investigated. The peak area was calculated by a measurement of peak width at half height.

The same equations about two PPAs; (Amount of injected NH₃, μg)=0.0118×(Peak area of permeated NH₃, mm²)+0.001; were obtained.

The calibration curves were linear up to 100 μg of injected NH₃ and the minimal detection limits were also to 0.25 μg in the injected NH₃. These curves were estimated to be practical for the blood NH₃ measurement because about 1.2 μg of NH₃ was resolved in 1.0 ml of blood.

CHROMATOGRAM OF BLOOD SAMPLE AND DETERMINATION OF BLOOD NH₃

Generally, O₂, CO₂ and small amounts of CO and N₂ except for NH₃ were resolved in blood. However, as shown in typical chromatogram of blood sample of Fig. 4,

![Fig. 4 Typical chromatogram of blood sample](image-url)
only two peaks were recorded. One was a mixed peak of O₂, CO₂ and N₂, and other was that of NH₃. This fact was based on a reason that their retention times of O₂, N₂ and CO₂ were equal to one another under the optimum condition by the proposed method, and their compounds could not be separated from each other. Also, very small amount of CO could not be detected under the condition. Therefore, blood NH₃ was estimated to be determined without the interference of their gas compounds.

Ammonia in 1.0 ml of blood samples A and B were determined by the present method. The results were shown in Table 1. Determined values of NH₃ in the samples A and B were essentially identical. Therefore, NH₃ in the blood sample was estimated through the only one step in which the sample was directly injected into the pH 7.4, 0.01 M Tris/HCl buffered carrier solution without the pretreatment of that.

A precision by the present method was within 6% in terms of coefficient of variation.

Also, the values depending on the proposed method were in agreement with the values depending upon the IP method, and the correlation coefficient was 0.99 (n=17). Furthermore, the time required for the blood NH₃ measurement was 13 min and the time was about 40 min shorter than that of the IP method.

EVALUATION OF THE PROPOSED METHOD FROM CONTINUOUS SAMPLE INJECTION

When blood sample of 1.0 ml without the sample pretreatment were continuously injected into the pH 7.4, Tris/HCl carrier solution at intervals of 7 min, the continuous chromatographic patterns of permeated NH₃ and (N₂+CO₂+O₂) from the blood samples were shown in Fig. 5.

When 8 samples of blood per 1 hr were injected into the apparatus and 60 blood samples were continuously analyzed with the apparatus, during that time, an ability of the apparatus for being capable of the NH₃ measurement was kept constant. After 60 blood samples had been continuously analyzed, two peaks of NH₃ and (N₂+CO₂+O₂) were overlapped each other, and blood NH₃ could not be determined with it as shown in Fig. 5-A. This trouble is explained that the separability of the chromosorb column is decreased by an adsorption of such gaseous compounds as moisture. However, as shown in Fig. 5-B, the separability of the column was recovered by
heating the column till 200°C and eluting their gaseous compounds adsorbed in the column.

**Conclusion**

The analyses of blood ammonia through the use of gas permeation/gas chromatography were studied.

For ammonia measurement in blood by the proposed method, an analytical time required was 13 min and the precision was within 6% in terms of coefficient of variation. The results of the proposed method were in good agreement with those of the indophenol method and the correlation coefficient was 0.99 (n=17).

In all biochemical analysis it is necessary to adopt the method by which tissue is not destroyed. In such cases it would be obviously preferable to use the method proposed here.

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ガスパリメーション・ガスクロマトグラフィーによる血中アンモニアの分析

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体液のpHは通常中性付近（pH 6〜8）にある。そのpH領域で体液成分を測定可能とすることは、試料の前処理操作を不要とし、臨床検査等のルーチンワークにおける測定の簡易化あるいは体液中の酵素活性の測定等の際に、便利かつ有効である。

そこで、当該pH領域での体液成分中のアンモニアの直接定量法、すなわち膜分離とGCによる2段分離分析法を考案、検討した（腎、肝機能検査としての血中アンモニアの測定、血清クライシンアミノペプチダーゼ活性の測定等に利用）。

本法による①測定要時間は18分以内、②測定精度は変動係数として6％以内、③標準的実験方法とされているインドフェノール法との相関も良好であった（r=0.99, n=17）。④検体処理能力は、時間当り8検体処理の割合で、総数60検体の連続測定が可能であった。