

# Flow-injection determination of total ammonia and total carbon dioxide in blood based on gas-diffusion separation and with a bulk acoustic wave impedance sensor

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## Abstract

A novel flow-injection (FIA) system, for the rapid and direct determination of both total ammonia ( $T_{\text{NH}_3}$ ) and total carbon dioxide ( $T_{\text{CO}_2}$ ) in clinical blood samples, has been developed. Samples were injected into a carrier stream of  $\text{H}_2\text{O}$ , then emerged with a reagent stream, where the analyte was converted into a gaseous species and diffused across a PTFE gas-permeable membrane into an acceptor stream. The trapped  $\text{NH}_3/\text{CO}_2$  in the acceptor was determined on line by a bulk acoustic wave (BAW) impedance sensor. At a through-put of 20 and 65  $\text{h}^{-1}$ , the proposed system exhibited a linear frequency response up to 200  $\mu\text{mol l}^{-1}$  ammonium and 20  $\text{mmol l}^{-1}$  bicarbonate with a detection limit of 1.0 and 10  $\mu\text{mol l}^{-1}$ , respectively. Results obtained for  $T_{\text{NH}_3}$  in serum and  $T_{\text{CO}_2}$  in plasma were in agreement with those obtained by the conventional glutamate dehydrogenase (GDH) method and gas-sensing electrode method, respectively. The effects of composition of acceptor stream, cell constant of conductivity electrode, sample volume, flow rate and potential interferences on the FIA signals were also discussed. © 1998 Elsevier Science B.V.

*Keywords:* Flow-injection analysis; Bulk acoustic wave sensor; Gas-diffusion separation; Serum ammonia; Plasma carbon dioxide

## 1. Introduction

In plasma the carbonic acid–bicarbonate equilibrium is an important buffering mechanism. Thus, acidosis and alkalosis, which cause their symptoms because of changes in the hydrogen-ion activity of the blood, should be evaluated by

measuring blood pH and blood bicarbonate values or blood pH and blood  $\text{P}_{\text{CO}_2}$ . The determination of blood ammonia is also of considerable value in clinical diagnosis [1]. For example, if any of those processes for ammonia's metabolic disposal is impaired, particularly that of urea formation by way of carbamoyl phosphate and 'the Krebs-Henseleit, ornithine-urea cycle', the level of ammonia, specially ammonium ion will rise in the

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blood, and ammonia intoxication, and chief symptoms of which are result of impaired function to the brain and include nausea and vomiting, lethargy, ataxia, convulsions, coma, and even death, may result. This situation may occur in patients with hepatomegaly and impaired liver function, since the liver is the site of the conversion of ammonium ion to urea [2]. Therefore it is particularly important to monitor the concentration of both ammonia and carbon dioxide in blood.

The most often used method for blood ammonia is the glutamate dehydrogenase (GDH) procedure [3,4], and generally, the  $\text{CO}_2$  content of plasma or serum is obtained by automated, continuous flow colorimetric methods or a manometric method [5], or by gas-sensing electrode [6]. However, they are either time consuming or expensive, suffer from inadequate sensitivity and reproducibility, or are not suitable for in situ measurements.

Flow injection analysis based on gas-diffusion separation (GD-FIA), characterized by its high selectivity, promising sensitivity, excellent precision and rapidity, has proven useful in determining volatile species and those can be selectively and quantitatively converted to a gas in complex matrices. Ammonia as well as carbon dioxide is among the species which can be advantageously determined by GD-FIA. In this technique, the sample is injected into, or merged with an alkaline/acid solution, and the  $\text{NH}_3/\text{CO}_2$  formed diffuses across a gas-permeable membrane into a recipient stream. The trapped  $\text{NH}_3/\text{CO}_2$  has been determined by spectrophotometry [7–13], by potentiometry [8,14,15] or by conductometry [8,9,13,16–21]. The conductometric method has some important advantages over the others without the sacrifice of sensitivity, selectivity and precision, the equipment as well as the reagent required is simpler and less expensive. A drawback, however, is that the high background conductivity of the recipient stream can cause an unacceptable noise level [8]. Hence, deionized distilled water, used as the acceptor, is designed to flow through a small column of mixed-bed exchanger for final purification before it enters the gas-diffusion cell [16,19], or a differential conduc-

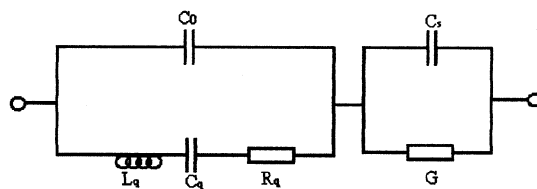


Fig. 1. Equivalent circuit of BAW-impedance sensor.  $C_0$ , static capacitance;  $L_q$ , motional inductance;  $C_q$ , motional capacitance;  $R_q$ , motional resistance of the piezoelectric quartz crystal;  $C_s$ , solution capacitance;  $G$ , solution conductance.

tivity meter is constructed for use in flow-injection systems to follow the small changes in conductance that occur in a recipient stream of high background conductivity [20], which make the FIA system more complicated and expensive. This

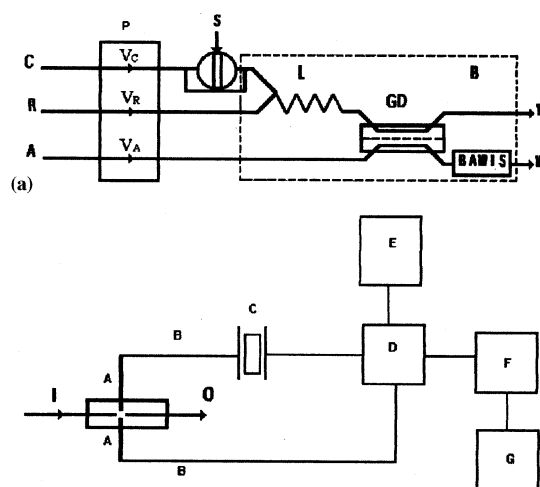


Fig. 2. (a) Schematic diagram of FIA manifold for determination of  $T_{\text{NH}_3}$  and  $T_{\text{CO}_2}$  in blood. P, peristaltic pump; S, sampling system (sample volume: 200  $\mu\text{l}$  for  $T_{\text{NH}_3}$  and 100  $\mu\text{l}$  for  $T_{\text{CO}_2}$ ); L, mixing tube (35 cm  $\times$  0.5 mm i.d.); GD, gas-diffusion cell; BAWIS, see Fig. 2(b); W, waste; B, water bath; C, carrier:  $\text{H}_2\text{O}$ ; R, reagent: 0.1 mol  $\text{l}^{-1}$  NaOH for  $T_{\text{NH}_3}$  and 0.1 mol  $\text{l}^{-1}$   $\text{H}_2\text{SO}_4$  for  $T_{\text{CO}_2}$ ; A, acceptor: 10 mmol  $\text{l}^{-1}$  boric acid + 0.5 mmol  $\text{l}^{-1}$  KCl for  $T_{\text{NH}_3}$  and 10 mmol  $\text{l}^{-1}$  Tris + 0.5 mmol  $\text{l}^{-1}$  KCl for  $T_{\text{CO}_2}$ .  $V_c$ ,  $V_r$  and  $V_a$ , are flow rates equal to 0.50, 0.50 and 0.50 ml  $\text{min}^{-1}$  for  $T_{\text{NH}_3}$ , and 0.50, 1.00 and 1.50 ml  $\text{min}^{-1}$  for  $T_{\text{CO}_2}$ , respectively. (b) Schematic diagram of BAW-impedance sensing system. I, inlet for fluid from gas-diffusion cell; O, outlet for waste solution; A, stainless-steel needles; B, leading wires; C, piezoelectric quartz crystal; D, IC-TTL oscillator; E, d.c. power supply; F, frequency counter; G, computer.

situation has led us to develop a novel detection mode as an alternative to the classical conductometry in GD-FIA.

The bulk acoustic wave-impedance sensor (BAWIS), as described previously [22,23], is constructed by connecting an AT-cut piezoelectric quartz crystal and a conductivity electrode in series, and has particular advantages over the classical conductometry. In the classical conductometric detection, a main problem is the double electric layer capacitance and Faradaic impedance, which can change the effective potential applied to the electrodes of the detector cell, and hence impede accurate measurement [24]. Although this limitation may be overcome by a multi-electrode technique or by applying electrodes with alternating potential, the apparatus tends to be more complex. In the BAWIS, however, the double electric layer is eliminated, because the resonator supplies to the electrode couple a high frequency (9 MHz) alternative electric field. Meanwhile, the small potential difference between the electrode couple is not enough to cause electrolysis. Moreover, it can detect a slight change in solution conductivity in the presence of an excess of a foreign electrolyte, and the sensitivity and accuracy are even better than those obtained in the absence of the foreign electrolyte. Compared with the normal piezoelectric sensor, in which the quartz crystal is in direct contact with the solution, the BAWIS possesses the same sensitivity to the conductivity and permittivity of the solution and a better frequency stability, lower frequency-temperature coefficient and much lower frequency dependence on the density and viscosity of the solution because only the electrode is immersed in the liquid and the crystal is out of contact with the solution. Thus, it is not surprising that BAWIS has been used so widely, e.g. for endpoint determination in titration [25], for determination of microorganism [26], and for biochemical and physiological study of blood [27,28].

To the best of our knowledge, there is no publication that combines FIA with BAW detection except a feasibility study of the combination

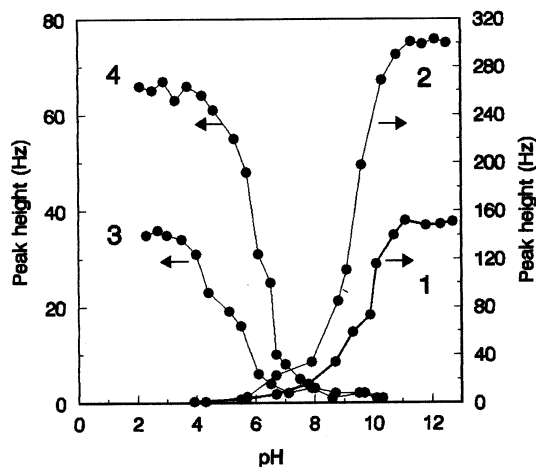


Fig. 3. Influence of pH of donor stream on the FIA signals. Samples: 1, 1.0 mmol l<sup>-1</sup> ammonium; 2, 2.0 mmol l<sup>-1</sup> ammonium; 3, 1.0 mmol l<sup>-1</sup> bicarbonate; 4, 2.0 mmol l<sup>-1</sup> bicarbonate. Sample volume: 100  $\mu$ l. Donor flow rate: 1.50 ml min<sup>-1</sup>; acceptor flow rate: 1.50 ml min<sup>-1</sup>. Other conditions as given in Fig. 2(a).

of FIA with the thickness-shear mode acoustic wave sensor by means of network analysis [29]. The present paper is a preliminary report describing the development of a novel FIA system based on the conjunction of BAWIS with GD-FIA technique, and its practical application of determination of total ammonia (T<sub>NH<sub>3</sub></sub>) and total carbon dioxide (T<sub>CO<sub>2</sub></sub>) in clinical blood samples.

## 2. Theoretical background

The proposed method described here is based on diffusion of NH<sub>3</sub>/CO<sub>2</sub> across a PTFE gas-permeable membrane from an alkaline (pH > 11)/acid (pH < 3) stream, which causes the efficient conversion of combined NH<sub>3</sub>/CO<sub>2</sub> to free NH<sub>3</sub>/CO<sub>2</sub> and creates a medium where NH<sub>3</sub>/CO<sub>2</sub> is only slightly soluble, it is trapped into a stream of diluted boric acid–KCl/Tris–KCl solution, and the trapped NH<sub>3</sub>/CO<sub>2</sub> in the acceptor is determined on line by a BAWIS.

The equivalent circuit of the BAWIS is illustrated in Fig. 1, its oscillating frequency ( $F$ ) can be expressed as [22]

$$F = F_0 \left[ 1 + \frac{\pi F_0 C_q (2\pi F_0 C_s - YG)}{G^2 + 4\pi^2 F_0^2 C_s (C_0 + C_s) - 2\pi F_0 C_0 YG} - \pi F_0 C_q R_q Y \right] \quad (1)$$

where  $F_0 = 1/(2\pi\sqrt{L_q C_q})$  is the resonant frequency of the crystal, and  $Y$  a parameter related to phase shift of the oscillator.  $G = k\chi$ ,  $k$  is the cell constant of the conductivity electrode,  $\chi$  the specific conductivity, and  $C_s = k\epsilon + C_p$ ,  $\epsilon$  is the solution permittivity,  $C_p$  the parasitic capacitance. For a diluted electrolyte solution, the change of  $F$  is chiefly dependent upon solution conductivity because the change in permittivity is negligible and other above-mentioned parameters were always kept unchanged. The sensitivity of BAWIS to conductivity, i.e. the slope of the plot of oscillating frequency  $F$  versus conductivity  $\chi$ , can be calculated by differentiating Eq. (1)  $F$  with respect to  $\chi$

$$\frac{\partial F}{\partial \chi} = \frac{\pi k F_0^2 C_q (4\pi^2 F_0^2 C_s^2 Y + 4\pi F_0 C_s G - YG^2)}{[4\pi^2 F_0^2 C_s (C_0 + C_s) - 2\pi F_0 C_0 YG + G^2]^2} \quad (2)$$

From this equation, it can be deduced that the sensitivity is determined by the solution conductivity  $G$  and the cell constant  $k$ . In the proposed GD-FIA system, conductivity of the solution flowing through the flow-cell is directly related to composition of the acceptor and concentration of total  $\text{NH}_3/\text{CO}_2$  trapped in it. Obviously, the latter is determined by the mass transfer of the  $\text{NH}_3/\text{CO}_2$  formed across the membrane and the recipient volume, i.e. that part of the acceptor stream passing the cell during diffusive sample transport across the membrane. The mass transfer ( $m$ ) is given by the equation  $m = Jt$ , where  $t$  is the residence time in the donor channel.  $J$  is the diffusive flux, it can be derived from Fick's law, based on a simple diffusion model, leading to the equation

$$J = kD(A/L) \Delta c \quad (3)$$

where  $A$  is the contact area between donor and acceptor stream,  $L$  the membrane thickness,  $D$  the diffusion coefficient of  $\text{NH}_3/\text{CO}_2$  in air,  $\Delta c$  the concentration difference and  $k$  a lump constant including gas transport in the aqueous phase, and partition of  $\text{NH}_3/\text{CO}_2$  between aqueous and

gaseous phase. At high donor flow rates, the diffusive flux is high but the transfer efficiency is low. Thus, in FIA, where sample volumes are commonly small, sufficiently low flow rates of the donor stream are appropriate and should guarantee high transfer efficiency [8]. Besides, the sample injected undergoes dispersion resulting in a peak-shaped concentration profile, the intensity and shape of which are influenced by a number of parameters of the FIA manifold.

### 3. Experimental

#### 3.1. Apparatus

Schematic diagram of the FIA manifold is illustrated in Fig. 2(a), it is composed of a LDB-M peristaltic pump (Zhejiang, China), a flow manifold of PTFE tubes (0.8 mm i.d.), a sampling system consisting of a Shimadzu SLC-6B system controller and a SIL-6B auto injector, a gas-diffusion unit, which was made of two pieces of Plexiglas with a shallow groove of 125 mm length, 3 mm width and 0.1 mm depth in each piece and a PTFE gas-permeable membrane (pore size 0.02  $\mu\text{m}$ , purchased from Jiangsu Electroanal. Instrument. Factory, China) placed between them before they were screwed together, and a BAWIS shown in Fig. 2(b). The sensor was constructed by connecting an AT-cut 9 MHz piezoelectric quartz crystal (Model JA-5, Beijing Factory No. 707) and a conductivity electrode in series to make up the feedback circuit of the IC-TTL oscillator. The oscillator was supplied with 5 V by a d.c. voltage regulator, a Iwatsu SC-72 001 universal frequency counter was used to record its oscillating frequency, and the data readings were collected and transferred to an IBM-compatible PC for further processing. MATLAB (version 4.0, The Math Works) was used to the data analysis and graphical presentation. The conductivity electrode was made from two stainless-steel needles (diameter 1 mm) inserted parallelly and oppositely in a 15  $\mu\text{l}$ -flow-through cell (5 mm  $\times$  2 mm i.d.). Design of the oscillator was given in a previous paper [30]. Temperature control ( $25 \pm 0.1^\circ\text{C}$ ) was achieved with a CS-501 super constant temperature controller (Chongqing, China).

### 3.2. Reagent

Tris(hydroxymethylamino)methane (Tris) was obtained from Shanghai Biochemical-reagent Co.

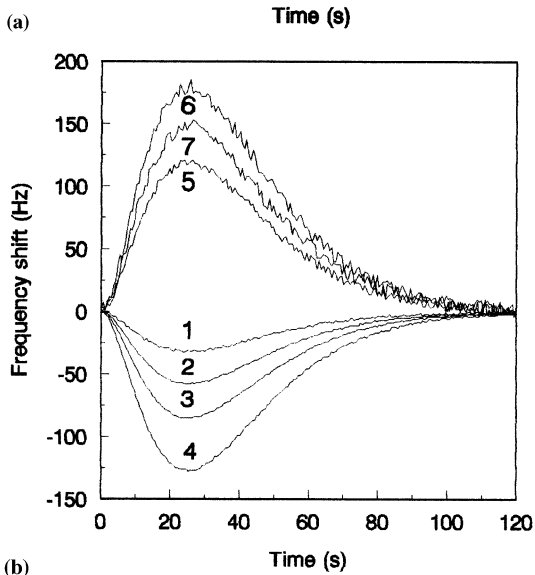
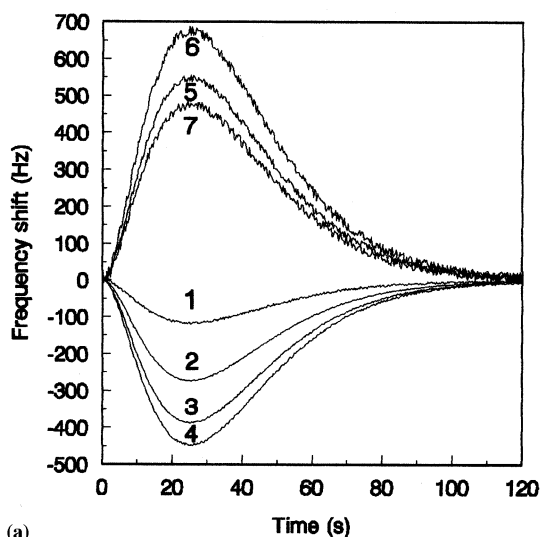


Fig. 4. FIA profiles obtained with various acceptor streams. (a) Sample:  $1.0 \text{ mmol l}^{-1}$  ammonium. Acceptor: 1,  $\text{H}_2\text{O}$ ; 2,  $1 \text{ mmol l}^{-1}$  boric acid; 3,  $5 \text{ mmol l}^{-1}$  boric acid; 4,  $10 \text{ mmol l}^{-1}$  boric acid; 5,  $0.25 \text{ mmol l}^{-1}$  HCl; 6,  $0.50 \text{ mmol l}^{-1}$  HCl; 7,  $10 \text{ mmol l}^{-1}$  HCl. (b) Sample:  $1.0 \text{ mmol l}^{-1}$  bicarbonate. Acceptor: 1,  $\text{H}_2\text{O}$ ; 2,  $1 \text{ mmol l}^{-1}$  Tris; 3,  $5 \text{ mmol l}^{-1}$  Tris; 4,  $10 \text{ mmol l}^{-1}$  Tris; 5,  $0.50 \text{ mmol l}^{-1}$  NaOH; 6,  $1.0 \text{ mmol l}^{-1}$  NaOH; 7,  $2.0 \text{ mmol l}^{-1}$  NaOH. Sample volume:  $200 \mu\text{l}$ . Donor flow rate:  $1.00 \text{ ml min}^{-1}$ , acceptor flow rate:  $1.00 \text{ ml min}^{-1}$ . Other conditions as given in Fig. 2(a).

All other chemicals were of analytical-reagent grade or better quality. Doubly distilled/deionized water was used throughout. Standard ammonium solutions were made by serial dilution of a  $0.1 \text{ mol l}^{-1}$  ammonium chloride stock solution. Standard bicarbonate solutions were made by serial dilution of a  $0.2 \text{ mol l}^{-1}$  potassium bicarbonate stock solution. The carrier stream was water, the reagent stream was  $0.1 \text{ mol l}^{-1}$   $\text{H}_2\text{SO}_4$  for  $T_{\text{NH}_3}$  determination and  $0.1 \text{ mol l}^{-1}$  NaOH for  $T_{\text{CO}_2}$  determination respectively, and for composition of the acceptor streams, see Section 4. Blood samples from healthy volunteers and identified patients were obtained directly from a clinic with informed consent.

### 3.3. Procedure

Blood samples were analyzed on the proposed GD-FIA/BAWIS system without any pretreatment except the necessary dilution for some specimens, and the value of  $T_{\text{NH}_3}$  and  $T_{\text{CO}_2}$  was calculated from the corresponding calibration graph based on their peak heights (the maximum frequency decrease), respectively. The GDH assay for  $T_{\text{NH}_3}$  was done according to the literature [3,4]. The conventional gas-sensing electrode method for  $T_{\text{CO}_2}$  was in principle identical with that of the original carbon dioxide sensor of Stow et al. [6].

## 4. Results and discussion

### 4.1. Optimization of the experimental parameters

#### 4.1.1. pH of donor stream

The pH of peripheral blood is normally within the range 7.36–7.44 [31]. In this range,  $T_{\text{NH}_3}$  exists primarily as ammonium and  $T_{\text{CO}_2}$  mainly as bicarbonate. Therefore, the conversion of both ammonium and bicarbonate into their free form,  $\text{NH}_3$  and  $\text{CO}_2$ , respectively, which can permeate through the microporous PTFE membrane, is necessary for the measurement of  $T_{\text{NH}_3}$  and  $T_{\text{CO}_2}$  with GD-FIA. Because the conversion efficiency is

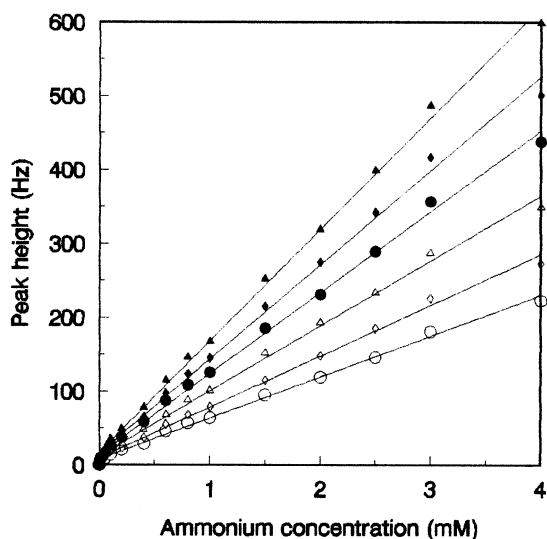


Fig. 5. Influence of KCl concentration on the sensitivity. Acceptor:  $10 \text{ mmol l}^{-1}$  boric acid + KCl ( $\text{mmol l}^{-1}$ ):  $\square$ , 0;  $\bullet$ , 0.1;  $\blacksquare$ , 0.5;  $\blacktriangle$ , 1.0;  $\triangle$ , 2.5;  $\circ$ , 5.0. Other conditions as given in Fig. 3.

dependent upon pH, the FIA signal was observed with the donor stream of various pH (adjusted with  $0.1 \text{ mol l}^{-1}$  NaOH and  $0.1 \text{ mol l}^{-1}$  HCl). As

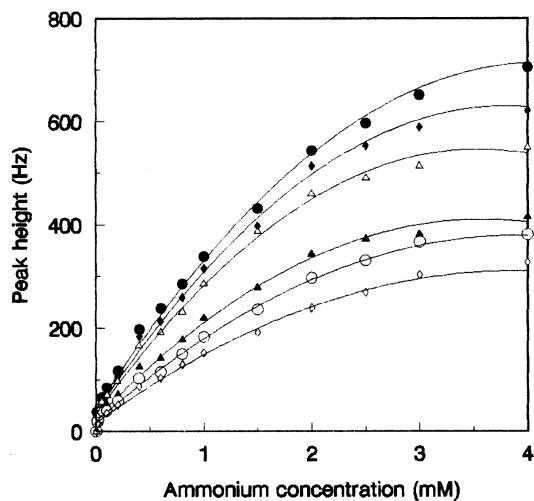


Fig. 6. Influence of cell constant of the conductivity electrode on the sensitivity. Cell constant (cm):  $\blacktriangle$ , 0.51;  $\blacksquare$ , 0.85;  $\bullet$ , 1.25;  $\triangle$ , 2.54;  $\circ$ , 3.1;  $\square$ , 4.7. Sample volume:  $100 \mu\text{l}$ . Donor flow rate:  $1.50 \text{ ml min}^{-1}$ ; acceptor flow rate:  $1.00 \text{ ml min}^{-1}$ . Other conditions as given in Fig. 2(a).

shown in Fig. 3, for ammonium standards, the signal increases with an increase in the pH of the donor stream and reaches a plateau above 11.5; for bicarbonate standards, however, the signal reaches a plateau below pH 3. Additionally, the sensitivity for the determination of ammonium is higher than that for the bicarbonate determination, which could be attributed to a higher rate of hydration or a better permeability through the microporous membrane for ammonia. In subsequent experiments,  $0.1 \text{ mol l}^{-1}$  NaOH and  $0.1 \text{ mol l}^{-1}$   $\text{H}_2\text{SO}_4$  was used as the reagent stream respectively to ensure the conversion of the analytes within the desirable pH range.

#### 4.1.2. Composition of acceptor stream

Fig. 4 shows FIA profiles of the proposed GD-FIA/BAWIS system obtained with various acceptor. For the acceptor of water and boric acid or Tris, all the injections of ammonium or bicarbonate result in a decrease in the oscillating frequencies, which is attributed to the increase in conductivity of the acceptor solution due to the dissociation of the  $\text{NH}_3/\text{CO}_2$  diffused from the donor stream. For the acceptor of HCl and

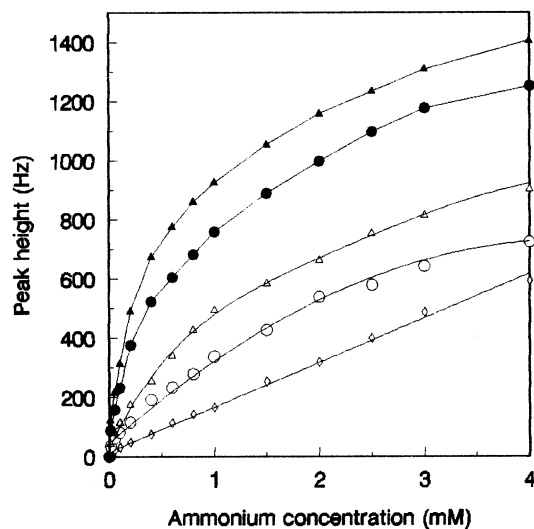


Fig. 7. Calibration graphs obtained with various FIA parameters. Sample volume ( $\mu\text{l}$ ), donor flow rate ( $\text{ml min}^{-1}$ ) and acceptor flow rate ( $\text{ml min}^{-1}$ ):  $\square$ , 100, 1.50, 1.50;  $\circ$ , 100, 1.50, 1.00;  $\triangle$ , 200, 1.50, 1.00;  $\bullet$ , 200, 1.00, 1.00;  $\blacksquare$ , 200, 1.00, 1.00;  $\blacktriangle$ , 200, 1.00, 0.50. Other conditions as given in Fig. 2(a).

Table 1  
Interference on determination of total  $\text{NH}_3$

Interferent	Interferent concentration ( $\mu\text{mol l}^{-1}$ )	$\text{NH}_3$ concentration ( $\mu\text{mol l}^{-1}$ )	
		Added	Measured
Methylamine	5	50	54.5
Dimethylamine	10	50	54.8
Diethylamine	10	50	53.5
<i>n</i> -Butylamine	25	50	54.9
Cyclohexylamine	50	50	55.2

Table 2  
Interference on determination of total  $\text{CO}_2$

Interferent	Interferent concentration ( $\text{mmol l}^{-1}$ )	$\text{CO}_2$ concentration ( $\text{mmol l}^{-1}$ )	
		Added	Measured
$\text{H}_2\text{S}$	0.20	2.00	2.14
	0.40	2.00	2.29
$\text{SO}_2$	1.00	2.00	2.17
	2.00	2.00	2.36
HF	4.00	2.00	2.18
HCOOH	4.00	2.00	2.25
$\text{CH}_3\text{COOH}$	4.00	2.00	2.31

$\text{NaOH}$ , however, an increase in the oscillating frequency was observed for injection of ammonium and bicarbonate, respectively, which is attributed to the decrease in the conductivity caused by the decrease in the concentration of  $\text{H}^+$  and  $\text{OH}^-$  in the acceptor solution, respectively. Finally, the results indicate that in terms of linearity, sensitivity and reproducibility of the response, experimentation with the acceptor of boric acid and Tris should be recommended for the determination of  $T_{\text{NH}_3}$  and  $T_{\text{CO}_2}$ , respectively. The influence of unreacted foreign electrolyte,  $\text{KCl}$ , added in the recipient solution on the sensitivity of the response was also investigated. As shown in Fig. 5, the addition of  $0.1$ – $2.5 \text{ mmol l}^{-1}$   $\text{KCl}$  into  $10 \text{ mmol l}^{-1}$  boric acid significantly improves the sensitivity, and the optimum sensitivity was obtained with an addition of  $1.0 \text{ mmol l}^{-1}$   $\text{KCl}$ . This can be regarded as a particular advantage over GD-FIA/conductometry, in which high conductivity background of

the acceptor may cause an unacceptable noise level.

#### 4.1.3. Cell constant of the electrode

Influence of cell constant of the electrode,  $k$ , was investigated under the same FIA condition while the cell constant varied from  $0.51$ – $4.7 \text{ cm}$ . Various values of  $k$  result in a marked difference in the sensitivity to the conductivity ( $\chi$ ), and alter the solution capacitance ( $C_s$ ). When  $k$  increases, the sensitivity to the conductivity increases ( $G = k\chi$ ); meanwhile, the solution capacitance also increases ( $C_s = k\epsilon + C_p$ ) which reduces the sensitivity of the frequency response of the BAWIS to solution conductance. The sensitivity of the sensor is determined by these two opposing factors. As can be seen in Fig. 6, judging by the peak heights at the same sample concentrations, the sensitivity of the proposed method increases with increasing cell constant at first, then begins to decrease. The maximum sensitivity was obtained with the cell constant  $1.25 \text{ cm}$ .

#### 4.1.4. FIA parameters

The influence of flow rate is illustrated Fig. 7. It indicates that a significant enhancement of

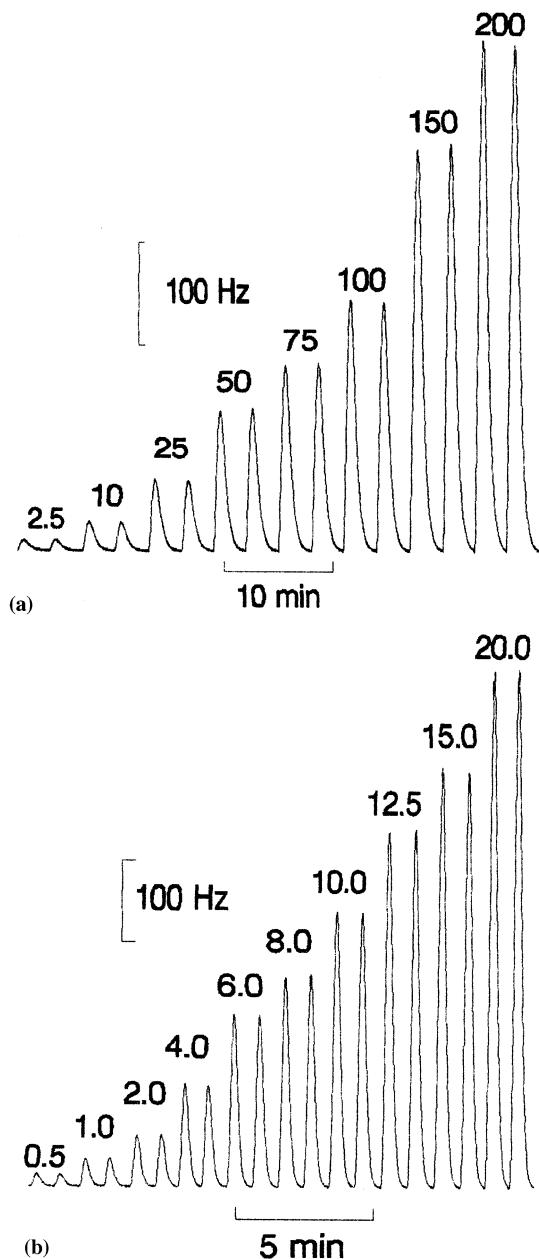


Fig. 8. Calibration run for ammonium standards (a) and bicarbonate standards (b). Numbers over the peaks refer to the concentration of ammonium and bicarbonates in  $\mu\text{mol l}^{-1}$  and  $\text{mmol l}^{-1}$ , respectively. Conditions as given in Fig. 2(a).

Table 3  
Results for  $T_{\text{NH}_3}$  in serum<sup>a</sup>

Sample	Clinical diagnosis	$T_{\text{NH}_3}$ ( $\mu\text{mol l}^{-1}$ )	
		Proposed method	GDH method
1	Healthy	$41.3 \pm 0.49$	$40.5 \pm 0.71$
2	Healthy	$29.8 \pm 0.35$	$29.3 \pm 0.55$
3	Healthy	$53.7 \pm 0.56$	$54.6 \pm 0.92$
4	Cirrhosis	$217.3 \pm 2.1$	$220.4 \pm 3.7$
5	Hepatic coma	$297.5 \pm 2.6$	$294.5 \pm 4.8$

<sup>a</sup> All measured results are average values with standard deviations from five determinations.

sensitivity may be achieved especially by decreasing the acceptor flow rate. The sampling rate, however, has to be reduced, and the linearity range become narrower. Additionally, increasing injection volume increased the intensity of the signal, but also increased the time necessary for the signal to return to baseline. With respect to the concentration of the analyte in real samples, a low flow rate and a large sample volume was chosen for  $T_{\text{NH}_3}$  determination so as to offer a high sensitivity, and for  $T_{\text{CO}_2}$  determination, a compromise between sample volume, flow rates and sampling frequency lead to the final design of the FIA manifold illustrated in Fig. 2(a), which offered the adequate sensitivity, excellent reproducibility and required throughput.

#### 4.2. Selectivity

The interference arises from the presence of those substance that can pass through the membrane and then cause change in conductivity of the acceptor solution. It has been established previously that PTFE membranes used in the FIA gas-diffusion studies are effective barriers for ionic species [7,32]. In the described method for  $T_{\text{NH}_3}$ , with the use of alkaline reagent, the interference from volatile acids is negligible; and for  $T_{\text{CO}_2}$ , the interference from volatile amines is negligible because of the use of acid reagent. A test of



Table 4  
Results for  $T_{\text{CO}_2}$  and bicarbonate content in plasma<sup>a</sup>

Sample	Clinical diagnosis	$T_{\text{CO}_2}$ (mmol l <sup>-1</sup> )		Bicarbonate content (mmol l <sup>-1</sup> )		
		pH	Proposed method	Gas-sensing electrode	Proposed method	Gas-sensing electrode
1	Healthy	7.42	25.44 ± 0.23	25.18 ± 0.51	24.28	24.03
2	Healthy	7.35	29.14 ± 0.26	28.53 ± 0.56	27.59	27.01
3	Healthy	7.45	31.71 ± 0.28	32.18 ± 0.61	30.35	30.80
4	Respiratory alkalosis due to hepatic coma	7.67	16.26 ± 0.17	16.05 ± 0.34	15.83	15.63
5	Respiratory alkalosis due to fever	7.58	18.17 ± 0.16	18.59 ± 0.38	17.59	17.99
6	Metabolic alkalosis due to vomiting	7.53	42.80 ± 0.37	43.53 ± 0.79	41.27	41.97
7	Respiratory and metabolic acidosis due to heart failure	7.06	14.37 ± 0.14	13.92 ± 0.30	12.95	12.54
8	Respiratory alkalosis and metabolic acidosis due to salicylate poisoning	7.45	12.10 ± 0.14	12.48 ± 0.28	11.58	11.95

<sup>a</sup> All measured results for  $T_{\text{CO}_2}$  are average values with standard deviations from five determinations; bicarbonate content are derived from pH and  $T_{\text{CO}_2}$ .

interference from some volatile amines on ammonia determination and interference from some volatile acids on carbon dioxide determination is given in Table 1 and Table 2, respectively. For a general consideration, the interferences would be significant if methyl-, dimethyl-, and diethylamine present at levels of 10–20% of ammonium, *n*-butylamine and cyclohexylamine at 50–100% of ammonium, H<sub>2</sub>S at 10–20% of CO<sub>2</sub>, SO<sub>2</sub> at 50–100% of CO<sub>2</sub>, and HF, HCOOH and CH<sub>3</sub>COOH at 200% of CO<sub>2</sub>, respectively. The difference may be ascribed to the different permeabilities of those substances through the microporous membrane as well as their different p*K*<sub>a</sub> values, etc. In the analysis of T<sub>NH<sub>3</sub></sub> and T<sub>CO<sub>2</sub></sub> in blood, however, it is extremely unlikely that the above-mentioned substances would be present physiologically and pathologically at such high concentrations even when some unlikely situations, e.g. methanol poisoning and paraldehyde poisoning [31], which may produce formic acid and acetic acid and therefore lead to an acidosis respectively, might be encountered. The high selectivity can be regarded as one of the important advantages of the proposed method.

#### 4.3. Analysis of samples

The described method was applied to the determination of T<sub>NH<sub>3</sub></sub> in serum and T<sub>CO<sub>2</sub></sub> in plasma respectively. A typical output, at a through-put of 20 and 65 samples per hour, for standards of ammonium and bicarbonate in the range 2.5–200 μmol l<sup>-1</sup> and 0.5–20 mmol l<sup>-1</sup>, respectively, is shown in Fig. 8, in which the frequency decrease is plotted as a positive signal to yield a conventional FIA profile. Both the calibration graphs are rectilinear over the entire working range with a detection limit of 1 and 10 μmol l<sup>-1</sup>, respectively, the regression equation for ammonium is

$$y = 3.0 + 2.49x_a \quad (r = 0.9988)$$

and for bicarbonate is

$$y = 2.1 + 32.97x_b \quad (r = 0.9981)$$

where *y* is the height of the FIA profile (the maximum frequency decrease in Hz), *x*<sub>a</sub> the concentration of ammonium in μmol l<sup>-1</sup>, and *x*<sub>b</sub> the concentration of bicarbonate in mmol l<sup>-1</sup>.

The results for  $T_{\text{NH}_3}$  and  $T_{\text{CO}_2}$  are summarized in Table 3 and Table 4, respectively, and it is shown that the results obtained by the proposed method agree with those obtained by the conventional glutamate dehydrogenase (GDH) method and the gas-sensing electrode method respectively. The average precision of the proposed method is 1.05% (RSD) for  $T_{\text{NH}_3}$  and 0.95% for  $T_{\text{CO}_2}$ , better than that of the corresponding conventional method (1.72 and 2.03%, respectively), Table 4 also contains the plasma bicarbonate values ( $[\text{HCO}_3^-]$ ), which were derived from the measured pH and  $T_{\text{CO}_2}$  according to the well recognized Henderson–Hasselbach equation

$$\text{pH} = 6.10 + \log([\text{HCO}_3^-]/(T_{\text{CO}_2} - [\text{HCO}_3^-])) \quad (4)$$

As can be seen in Table 3 and in Table 4, there is an obvious increase in blood ammonia for patients with severe liver damage, a significant increase in plasma bicarbonate for vomiting and an apparent decrease for fever, hepatic coma, heart failure or salicylate poisoning, all of these alterations has been explained pathologically [31].

## 5. Conclusion

GD-FIA provides a selective, precise and rapid determination for  $\text{NH}_3/\text{CO}_2$ . In combination with BAWIS, the method proposed here has some advantages over other detection modes, e.g. the reagents and equipment required are simpler and cheaper than that required by spectrophotometric methods, the background conductivity of the acceptor stream can improve the detection sensitivity while it may cause an unacceptable noise level in the conductometric method, and the stability of the analytical signals is better than that of the signals obtained by the conductometric method or potentiometric method. The advantages of the new method, GD-FIA/BAWIS system, should make it an attractive alternative of the methods currently in use especially to the conductometric method, and the successful development of the method for the determination of  $T_{\text{NH}_3}$  and  $T_{\text{CO}_2}$  in blood confirms the usefulness of the GD-FIA/BAWIS system for this type of clinical investigation, and indicates that this system might find

useful applications also in other assays, where  $\text{NH}_3/\text{CO}_2$  can be released by or from the species to be determined.

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