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Direct analysis of human breath ammonia using corona discharge ion mobility spectrometry

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ABSTRACT

In this study, ammonia in human breath was directly determined using corona discharge ionization ion mobility spectrometry (CD-IMS) technique with several important advantages including high sensitivity, low cost, high speed, and ease of maintenance. The temperature effect on the ammonia signal was evaluated too. The results indicated that the best temperature for the investigation of breath ammonia was 150 °C. The analytical results showed that the linear dynamic range was between 12 and 810 ppb and the detection limit was 6.6 ppb. The relative standard deviation (RSD) was obtained to be 5, 3, and 3 for 290, 348, and 522 ppb, respectively. The amounts of ammonia in breath of eight healthy volunteers were measured. The values were between 236 and 1218 ppb. Also, the inequality in breath ammonia levels was scrutinized over a 6 h working day for three healthy volunteers. The results showed a drop in breath ammonia from the morning amount to the mid-day measurement and then, a progressive increase while the day continued. In addition, the amounts of ammonia were determined to be 1494–1553 ppb in exhaled breath of two renal failure patients. The results obtained in this work revealed that the method was conveniently established without any considerable sample pretreatment for direct analysis of ammonia in human breath.

1. Introduction

There are at least 1000 trace volatile compounds (VCs) in human breath and their determination can be helpful in clinical diagnosis and therapeutic monitoring [1]. Volatile compounds found in human breath are linked to various physiological conditions as they represent the products of metabolism in human body [2]. In other words, the human breath can be considered as an information source of the health status of the body because some of the volatile compounds detected in human breath can be directly correlated to specific diseases. In fact, the composition of substances in the exhaled air is representative of blood borne concentrations detected through gas exchange at the blood/breath interface in the lungs [3]. Therefore, breath analysis has the potential of substituting the blood and urine analyses for many compounds. Modern breath analysis began in the 1970s when researchers identified more than 200 components in human breath using gas chromatography (GC) [4,5]. Ammonia is one of the most important compounds existing in human breath, urine, and blood. The main sources of ammonia in human body are the deamination of amino acids, transamination of most amino acids with α-ketoglutaric acide to form glutamic acide, and operation of glutaminase enzyme in the kidney. In addition, this compound can be produced during purine and pyrimidine catabolism, the action of intestinal bacteria on the non-absorbed dietary amino acids, and the action of monoamine oxidase enzyme. The ammonia is absorbed into the portal circulation, taken up by the liver and converted into urea, via the urea cycle [6,7]. In fact, ammonia is one of the harmful substances normally made harmless by the liver and kidneys and the dysfunction of these organs causes that blood ammonia cannot be filtered out of the body, properly. It can be source of damage to the nervous system and causes the hepatic encephalopathy. When ammonia amount is increased in blood, it is diffused into the lungs and can be exhaled in breath. In addition, a method for recognizing the diseases that originated from liver and kidneys dysfunction (such as uremia, acidosis and edema) is the quantification of breath ammonia levels [8].

Based on the above discussion, an accurate, simple, sensitive, and portable method is essentially needed for the analysis of ammonia trace in the human breath. So far, several analytical methods including photoacoustic spectroscopy [8–10], selected ion flow tube mass spectrometry (SIFT-MS) [11–14], gas chromatography with different detectors such as flame ionization detector or mass spectrometry (MS) [4,5] have been used for the determination of trace amounts of breath ammonia. However, these methods are time consuming and laborious in addition to their high prices. Consequently, it is not possible to apply these methods as the field analyzers for routine breath analysis.

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In 1970s, ion mobility spectrometry (IMS) under the name of "plasma chromatography" was introduced by Karasek for the trace detection of organic compounds [15]. To date, IMS has been used as an accepted analytical method for the identification and quantification of trace compounds such as explosives, chemical weapons and illicit drugs [15]. Really, very important advantages including portability, low cost, high speed, ease of maintenance, and high sensitivity encourage scientists to use this technique. In contrast to MS, IMS can be used for the separation of ions based on the ion shape in addition to the ion mass and charge, which are used in MS. Therefore, IMS can help users distinguish between isomeric species. It can be considered as a particularly important feature in proteomics research. Additionally, another advantage of IMS relative to MS is its application capability under atmospheric pressure; therefore no vacuum equipments are needed.

Ionization source is one important part of an ion mobility spectrometer which produces ions at the ambient pressure. A variety of ionization sources including ^{63}Ni, corona discharge, photo ionization, and electrospray ionization have been used in IMS instruments. Baumbach and co-workers [16–19] published some papers on using IMS equipped with a ^{63}Ni ionization source for the determination of ammonia in human breath. Recently, the breath analysis by multi-capillary columns coupled to IMS for the identification of volatile organic compounds retained in uremia was also investigated by Pagonas et al. [20]. However, they have explored only qualitative analysis; so an exhaustive study on the quantification of breath ammonia is needed to be investigated. On the other hand, in the gas analysis, corona discharge as a nonradioactive ionization source is a good choice for producing the ions from analytes in IMS. Previous research [21] indicates that corona discharge has a higher ion current about one order of magnitude relative to ^{63}Ni, resulting in a lower detection limit and a wider dynamic range. In addition, ^{63}Ni-IMS requires a regular leak test due to its radioactivity property which limits the capability of the instrument for field analysis of breath ammonia in hospitals or other clinical centers.

Considering the discussions above, the present work intended to develop the corona discharge ionization ion mobility spectrometry (CD-IMS) for the analysis of ammonia in the human breath. Ion mobility spectra of various standard gaseous solutions were obtained and the analytical parameters such as dynamic range, detection limit, and reproducibility were obtained using CD-IMS for ammonia analysis. In addition, some real human breath samples were analyzed for different healthy and patient volunteers. The obtained results were satisfactory, revealing the capability of CD-IMS as a suitable field analyzer.

2. Experimental

2.1. Instrumentation

The ion mobility spectrometry used in this study was equipped to corona discharge (CD) as the ionization source. The instrumentation of this apparatus can be found in the work reported previously [22]. In brief, the CD-IMS contains various parts such as IMS cell, heating oven, power supplies, pulse generator, and a computer for data processing. The IMS cell includes reaction and drift regions separated by an ion gate. A Faraday cup type collector was used for ion current collection. After passing the nitrogen gas through a 13× molecular sieve (Fluka), it was introduced into the IMS cell as both the carrier and the drift gases. The spectrometer could operate in the positive mode with various drift fields. The optimum experimental conditions for obtaining the ion mobility spectra of the samples are given in Table 1.

Table 1
Typical operating conditions of CD-IMS during the experimental runs.

Operating parameters	Setting
Needle voltage	+9.50 kV
Target electrode voltage	+7.00 kV
Drift field	437 V cm^{-1}
Drift gas flow (N_2)	700 mL min^{-1}
Drift temperature	150 °C
Injection port temperature	150 °C
Carrier gas flow (N_2)	300 mL min^{-1}
Drift tube length	11 cm
Shutter grid pulse	0.2 ms
Scan time	20 ms

2.2. Sample preparation

Ammonia solution (25%) was purchased from Merck (d = 1.93 g mL^{-1}). For the preparation of stock standard sample, 0.5 μL of the solution was transferred into a cleaned 2-L vessel. The vessel was heated using a heater-stirrer (100 °C) to evaporate the sample and to prevent the ammonia adsorption on the vessel wall. To make a known concentration of the sample vapor, the appropriate volume of the stock standard sample was transferred to another vessel. By using a 5-mL syringe, the prepared standard samples were introduced into the injection port of the instrument and then transferred into the ionization region by the carrier gas.

3. Results and discussion

3.1. Ion mobility spectrum

The background ion mobility spectrum obtained at the optimum operation conditions (Table 1) is shown in Fig. 1. According to this figure, it is observed that the background spectrum contained two ion peaks named RIP_1 and RIP_2. These ion peaks, known as reactant ions, can react with analyte molecules and convert them to ions clusters. For the calculation of reduced mobility values of these cluster ions, nicotinamide with the reduced mobility of 1.85 cm^2 V^{-1} s^{-1} [23] was used as an internal calibrant and the K_0 for the ion peaks was determined relative to this value according to Eq. (1):

$$\frac{K_0(\text{unknown})}{K_0(\text{standard})} = \frac{t_d(\text{standard})}{t_d(\text{unknown})} \quad (1)$$

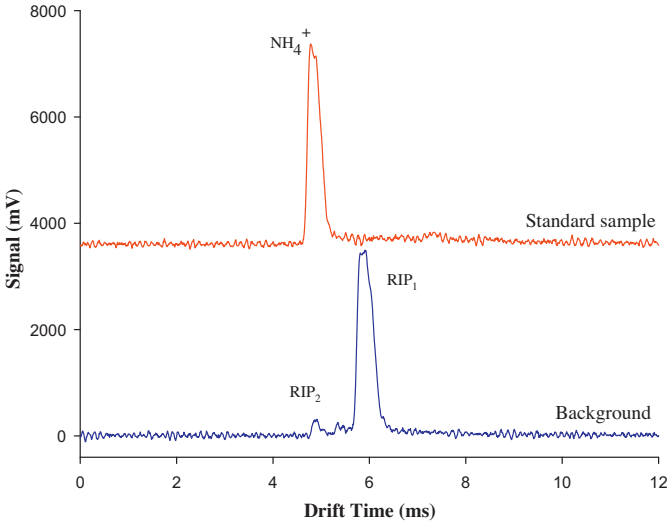

Fig. 1. The ion mobility spectrum of standard ammonia solution compared with that of the background obtained by CD-IMS in positive mode.