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Breath acetone analysis with miniaturized sample preparation device: In-needle preconcentration and subsequent determination by gas chromatography–mass spectroscopy

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ABSTRACT

A new approach to the determination of human breath acetone with particle-packed sample preparation needle was developed. The extraction needle was packed with a copolymer of methacrylic acid and ethylene glycol dimethacrylate as the extraction medium. For the analysis of breath sample, exhaled breath was collected in a sampling bag, and 50 mL of the breath sample was extracted with the needle-type sample preparation device followed by analysis using gas chromatography–mass spectrometry (GC–MS). After the optimization of several basic extraction conditions for standard acetone samples, breath acetone concentration taken from controlled type-2 diabetic patients was determined. Furthermore, time variations of breath and urine acetone of four healthy individuals under fasting conditions were measured. Urine sample was collected in glass vial, and urine acetone concentration was also determined with the extraction needle by analyzing the corresponding headspace gas. The results demonstrated that the particle-packed extraction needle showed an excellent extraction performance for acetone in both breath and urine headspace samples, and that there is a clear correlation between the concentration of breath acetone and HbA1c level of controlled type-2 diabetic patients. The breath acetone level in controlled diabetic patients was in the range between 0.19 and 0.66 ppmv, where its concentration in medically untreated type-2 patient was 0.92 and 1.20 ppmv. The breath acetone concentration in healthy male was increased to 5.66 ppmv under the 24 h of fasting test, and a high correlation between the breath and urine acetone concentration was also observed. On the basis of the above results, the potential applications of the proposed method to the diagnosis of diabetes and/or ketoacidosis were suggested.

1. Introduction

According to the statistics by International Diabetes Federation (IDF), about 246 million people have diabetes in 2007 [1] and it can be regarded as one of the most common diseases that globally found. In case of diabetes, glucose could not be transferred into the cell and the blood glucose level will be higher due to the insulin deficiency. The energy deficiency leads to a beta-oxidation of fatty acids, producing an excess of acetyl CoA. The resulting excess of acetyl CoA synthesizes the so-called ketone bodies, i.e., acetone, acetoacetate and beta-hydroxybutyric acid. This symptom is well-known as diabetic ketoacidosis (DKA). DKA is a typical symptom found in type-1 diabetes, although it can be also found in the patients of type-2 diabetes. As the result of DKA, the resulting ketone bodies will be accumulated in the blood of the patient, and typically excreted in the urine and breath.

For the diagnosis of diabetes, glucose concentration and glycosylated hemoglobin level (HbA1c%) in blood have been used along with the concentration of the ketone bodies in urine. HbA1c% is the percentage of hemoglobin–sugar complex relative to total hemoglobin in blood, and that represents an average amount of glucose level in blood over about the last two to three months. A higher HbA1c% value means a higher glucose concentration in blood, although the value is not affected by a short-term variation that might be induced by meal and other miscellaneous factors. Therefore, HbA1c% is one of the most useful indicator of diabetes and DKA.

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Exhaled human breath contains a large number of volatile organic compounds (VOCs), especially in low concentration, that related to their blood concentrations and vapor pressure [2,3]. Analysis of the exhaled breath is a non-invasive method for clinical purposes and much attention has been paid for the determination of particular compounds that might be a biomarker of several diseases [4–6]. Acetone is normally included in human breath at ppbv level, and has been regarded as a promising biomarker of diabetes and DKA [7,8]. Generally, breath acetone concentration for diabetic patient is higher than that for healthy person, and more than 1 ppmv of breath acetone was reported [9].

Human urine analysis was also used for the diagnosis of several diseases because of its simple and inexpensive features [10,11]. Ketone bodies in urine have been widely measured for diabetic patients in clinical practice using a test strip. With this test strip, urine ketone level could be approximately monitored, where one can check the level by the color change of the strip, although the detectability is not satisfactory for the level of less than 50 mg/L (50 ppmv) for acetoacetate. Acetone is normally detected in healthy human urine at about 1 ppmv, and a higher concentration could be found from diabetic patient.

Several online detection methods have been developed for the analysis of exhaled breath sample like specific gas sensor [12,13] and mass spectrometry such as proton-transfer reaction mass spectrometry (PTR-MS) [14,15], selected ion flow tube mass spectrometry (SIFT-MS) [16] and gas chromatography–ion mobility spectrometry (GC–IMS) [17,18]. Among the developed analytical methods for VOCs, especially, gas chromatography–mass spectrometry (GC–MS) is one of the most promising techniques. Because of the high sensitivity for typical VOCs, GC–MS has been widely employed in breath analysis. However, the concentration of VOCs in the typical breath samples is quite low, thus a kind of sample preconcentration process, such as cold-trap or adsorption trap method [19,20], is still necessary before the analysis for the sensitive and accurate determination in most of the cases. These methods provide high sensitivity, however, the techniques could often require the time consuming process. Solid-phase microextraction (SPME) is one of the most advanced sampling techniques for the GC analysis [9,21,22] and has some advantages over conventional extraction methods, such as simple, solvent less and easy automation.

Needle-type sample preparation is an alternative preconcentration technique for the analysis of VOCs [23–31]. The advantages of the needle-type sampling device are: high extraction capacity, simple extraction/desorption process and the feature of repeatable use for more than 100 times without any decrease in the performance. Compared to the conventional SPME technique, the in-needle sampling device has been realized as a robust and efficient sample preparation method [29–31]. In our previous work, a copolymer of methacrylic acid and ethylene glycol dimethacrylate was synthesized and packed into a needle-shaped device as the extraction medium [25]. This particle-packed extraction needle showed a good extraction efficiency, repeatability and sample storage performance for typical VOCs in air samples. The extraction needle was also applied to the analysis of environmental tobacco smokes and smokers' breath [27], and further possibility to the breath diagnosis has been suggested.

In this study, the particle-packed extraction needle was applied to the GC–MS analysis of breath acetone taken from controlled type-2 diabetes patients. Additionally, time variations of breath and urine acetone concentration of healthy individuals were investigated under the fasting condition. Urine ketone body was also monitored with commercially available urine ketone test strip to confirm the validity of the present method.

2. Experimental

2.1. Materials

All the reagents and solvents were of analytical reagent grade and obtained from either Kishida Chemical (Osaka, Japan) or Wako Pure Chemical (Osaka, Japan). Water was purified by a Milli-Q Water Purification System (Millipore, Tokyo, Japan). For gas sampling, Tedlar Bag, was purchased from GL Sciences (Tokyo, Japan), and for urine ketone body measurements, a commercially available urine ketone test strip (Ketostix; Bayer Japan, Osaka, Japan) was also employed to additionally confirm the validity of the method. The results of test strips were visually judged and also digitally checked by photography in the computer processing.

2.2. Preparation of standards

An appropriate amount of acetone, typically 3 μL, was injected into a depressurized vacuum glass vessel of 1.0 L volume (GL Science, Tokyo, Japan). The solvent was completely vaporized in the vacuum glass vessel, and then, it was filled with N_2 gas, resulting a gaseous acetone sample of about 1000 ppmv. Next, about 10 mL of the above gas sample was collected by a glass syringe (20 mL), and injected to a Tedlar Bag of 1.0 L volume followed by the dilution process with N_2 of about 990 mL, to be the total volume of the sample 1.0 L, resulting the gas concentration of 10 ppmv. In the case of the lower concentration, a similar process was carried out once more.

All the sampling bags have two inlets, where 4 cm of TYGON tubes (5 mm i.d., 7 mm o.d.) were attached. The other ends of these tubes were capped with silicon septum, and the extraction needle was inserted into the sampling bag through the silicon septum. All the sampling bags were cleaned at least five times by pure N_2 before use along with the preliminary measurement to confirm the successful cleaning process.

2.3. Extraction/desorption procedure

The structure of the extraction needle is illustrated in Fig. 1, where a copolymer of methacrylic acid and ethylene glycol dimethacrylate was packed as the spherical particle having the diameter of 150–180 μm. The polymeric material was prepared in a similar manner as previously described [25], and packed into a section of the specially designed needle. During the sampling, the extraction needle was attached to a commercially available vacuum gas sampling device (Komyo Rikagaku Kogyo, Tokyo, Japan). The gaseous sample was introduced to the extraction needle with

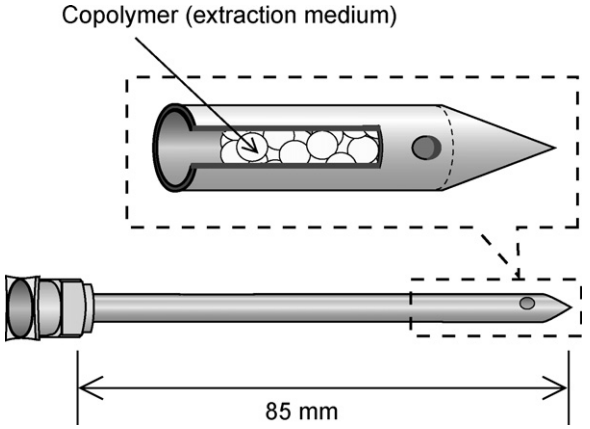

Fig. 1. Illustration of particle-packed sample preparation needle.