ELSEVIER

Analytica Chimica Acta 475 (2003) 27–35

ANALYTICA CHIMICA ACTA

www.elsevier.com/locate/aca

Portable oral malodor analyzer using highly sensitive In_2O_3 gas sensor combined with a simple gas chromatography system

Mariko Hanada [a,*], Hiroshi Koda [a], Kazuo Onaga [a], Katsuyuki Tanaka [a], Takahiro Okabayashi [b], Takeshi Itoh [b], Hideo Miyazaki [c]

[a] *FiS Inc., 3-36-3 Kitazono, Itami, Hyogo 664-0891, Japan*

[b] *Takasago Electric Industry Co. Ltd., 9-14, 2-Chome, Minami-senba, Chuo-ku, Osaka 542-0081, Japan*

[c] *Division of Preventive Dentistry, Department of Oral Health Science, Graduate School of Medical and Dental Sciences, Niigata University, 2-5274 Gakkocho-Dori, Niigata 951-8514, Japan*

Received 2 May 2002; received in revised form 16 September 2002; accepted 26 September 2002

Abstract

A portable oral malodor analyzer was developed for quantitative detection of volatile sulfur compounds (VSCs) in mouth air using a combination of a semiconductor gas sensor and a compact gas chromatography system. We found that In_2O_3 doped with Au is the optimum sensing material of the gas sensor for obtaining a high sensitivity to measure all the VSCs in mouth air with low concentration ranges. A specific column design, simple sampling system and signal processing method were applied for achieving a portable analyzer without using a standard pressurized carrier gas. This analyzer can easily measure the concentration of essential components of oral malodor individually and quantitatively over a wide range from 50 to 1000 ppb (v/v).

Keywords: Halitosis; Volatile sulfur compound; Hydrogen sulfide; Methyl mercaptan; Gas sensor; Indium oxide

1. Introduction

Volatile sulfur compounds (VSCs), such as H_2S, CH_3SH and $(CH_3)_2S$, in breath are well known as the main causes of oral malodor [1], and the concentration ratio of each VSC differs depending on the type of disease, which produces oral malodor. For example, the concentration ratio of CH_3SH and H_2S is higher in patients with periodontitis than those without [2]. Therefore, the quantitative analysis of components of oral malodor is extremely beneficial to halitosis treatment, not only for measuring the intensity of oral malodor but also inferring the causes of oral malodor, and verifying the effect of a treatment. Conventionally, diagnosis of oral malodor is performed by a dentist through organoleptic measurement. However, intra- and inter-examiner reliability or reproducibility to assess patients' mouth air is not always high, and it is difficult to evaluate the concentration of VSC quantitatively. In order to solve these problems, various attempts have been made to measure oral malodor with apparatuses. Conventionally, a gas chromatography (GC) equipped with FPD detector is used for quantitative measurement of VSC [1]. But since this apparatus is large, expensive, uses high-pressurized gas and requires special knowledge

* Corresponding author. Tel.: +81-72-7801800; fax: +81-81-727850073.
E-mail address: mariko@fisinc.co.jp (M. Hanada).

0003-2670/02/$ – see front matter
PII: S0003-2670(02)01038-3

for operation, it is not suitable for general practitioners in dental clinics. Instead of GC, several simple detectors have been developed using electrochemical gas sensors (Halimeter^{TM}, Interscan, USA) [3], a zinc oxide semiconductor gas sensor [4], or a tin oxide semiconductor gas sensor [5]. However, their performances were not sufficient because they could not measure the concentration of VSC components separately, and the selectivity for VSC was not enough due to the influence of humidity or interfering gases.

In order to develop an effective oral malodor analyzer as a medical instrument, we have developed a simple GC system using a highly sensitive metal oxide semiconductor gas sensor as the detector. Semiconductor gas sensor shows high sensitivity to low concentration of gases compared with other types of gas sensors. This feature is a big advantage when it is used as the detector for low concentration of VSC in mouth air. In addition, because semiconductor gas sensors have widely been used to detect combustible gases, reliability was well documented and the technology of industrial production to make it inexpensive was already established. For these reasons, we have chosen the semiconductor gas sensor as the detector of this system. With the combination of a simple GC system and a semiconductor gas sensor, a new portable oral malodor analyzer was realized.

2. Experimental

2.1. Structure of GC system

The configuration of the developed analyzer is shown in Fig. 1. This apparatus consists of a carrier gas pump, a carrier gas purifying filter, a sample injection part, a column with a heater, a detector, a controller, and a display. The data for analysis is obtained by the computer connected to the exterior port. The dimensions of the analyzer are 400 mm × 280 mm × 130 mm, and the weight is 5.5 kg.

2.1.1. Column

The column for the GC analysis was made of a Teflon^{TM} tube (5 mm i.d., 300 mm length) packed with 25% ODPN supported on Celite (GL Science). A sensor was directly connected to the outlet of the column, and the whole part was placed in a copper tube. The copper tube is surrounded with a silicone rubber heater and the column temperature is regulated by a thermistor and temperature control circuit.

2.1.2. Carrier gas

Ambient air was used for the carrier gas. The air was supplied from ambient air by a pump, and purified by silica gel and charcoal filters. The filters were made of Teflon^{TM} tube (6 mm i.d., 100 mm length,) filled with silica gel (10–20 mesh, 5 g) and active charcoal (GC20/42, KURALAY CHEMICAL, 6 g).

2.1.3. Detector

For VSCs detection, we have studied the metal oxide semiconductor gas sensor as a detector. The metal oxides, which were the gas sensing materials, were obtained by hydrolyzing chloride salts and by calcinating at 500 °C in air for 1 h. For the materials with noble metal catalysts, aqua regia (a mixture of hydrochloric acid and nitric acid, HCl:HNO_3 (v/v) = 3 : 1) solution of noble metal was added to the metal oxides in concentration of 0.15 wt.%, and then re-calcined at the same condition. The sensing material was processed into a paste with terpineol. The structures of the sensing element and the gas sensor are shown in Fig. 2. A Pt wire electrode was placed at the center

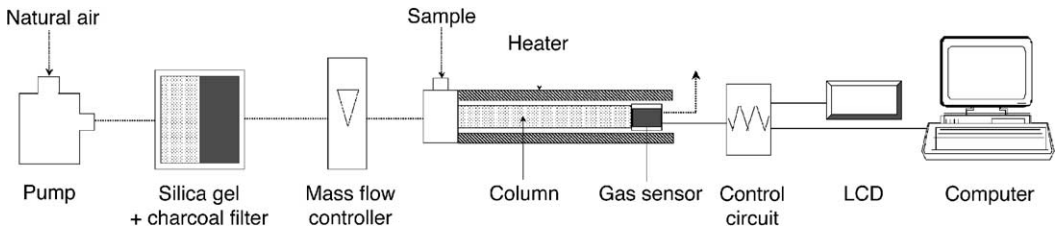

Fig. 1. Schematic diagram of the analyzer.

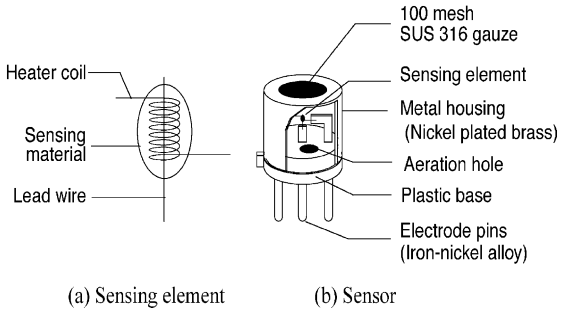

Fig. 2. Structure of: (a) the sensing element, and (b) the sensor.

of a coiled Pt wire (0.02 mm diameter) as the heater and the electrode. The electrodes and a heater were coated with the paste of the sensing material so as to give a 0.3 mm × 0.5 mm diameter bead shape element, followed by heating at 700 °C for 30 min. The sensing element was placed in a housing through which gas can flow from the upper mesh and the lower aeration hole. The temperature of the sensing element was controlled at 400 °C by applying a sufficient voltage to the heater.

2.2. Measurement conditions

The standard VSC gases containing H_2S, CH_3SH and $(CH_3)_2S$ in air were prepared by using a Permeater™ (GASTECH, PD-1B). The concentration of the gas was in the range of 50 to 1000 ppb (v/v) in air. The mixture of H_2S, CH_3SH and $(CH_3)_2S$ was adjusted as follows: (1) the predetermined concentration of each gas was prepared by the Permeater™. (2) The above three gases were injected into a Teflon™ gas bag and mixed. (3) The final gas concentration was calculated with the predetermined concentration in (1) and the volume of mixed gas in (2).

For measuring the oral malodor, the air inside the oral cavity was sampled directly with a syringe after closing the mouth for 30 s. The amount of sample injected to the analyzer was 0.5 cm^3.

The column temperature was 35 °C and the carrier gas flow rate was 10 cm^3/min. Fig. 3 shows the operation circuit of the detector. A load resistance (RL) was connected serially to the sensor, and the change of voltage across the RL (V_{RL}) was measured as a function of time. By recording the V_{RL} in a computer, chromatograms were obtained. The circuit voltage

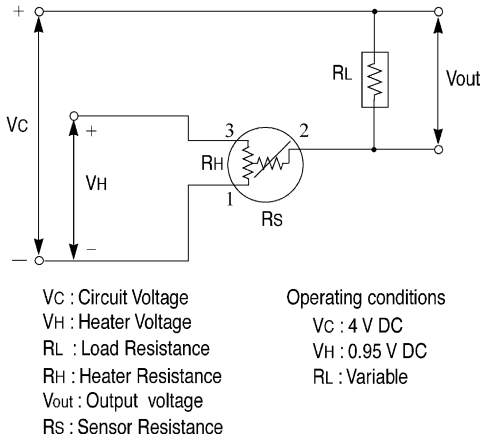

Fig. 3. Operation circuit and operating conditions of the detector.

(V_c) was 4 V. The duration of one measurement was 8 min.

3. Results and discussion

3.1. System design

The structure of the analyzer was designed to be compact and portable. By using a short and straight column with a heater, the thermostatic vessel conventionally required for a gas chromatography is eliminated, realizing a miniaturization of the apparatus. In addition, we did not use a high-pressurized gas but natural ambient air as a carrier gas, therefore, a portable apparatus was obtained. When using air as the carrier gas, impurities and water in the ambient air may influence the stability of the sensor output and the sensitivity with respect to VSC. To eliminate these influences, the air was purified with silica gel and charcoal filters. Influences of impurities, such as ethanol, hydrogen or carbon monoxide which may exist in dental clinics, were almost eliminated by the charcoal filter. Water in the ambient air could not be eliminated completely, but abrupt humidity change was reduced by the silica gel filter, and the sensor output was kept stable throughout measurement. As mentioned later, the developed sensor showed rather small dependency on humidity, consequently the measurement results were not seriously influenced with humidity. Thus, the influences of impurities and water in the ambient air were dimin-

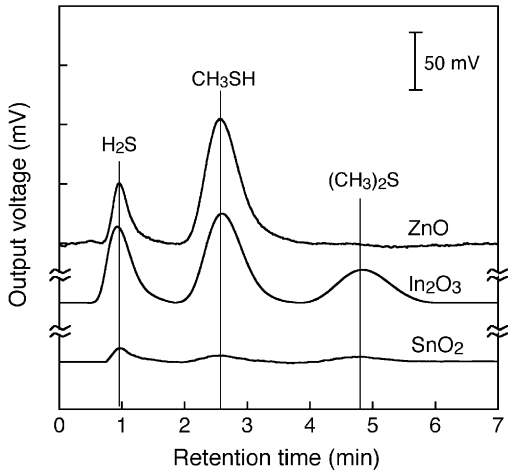

Fig. 4. Chromatograms using different kinds of metal oxide sensors without addition of noble metal catalysts for a gas mixture consisting of 1 ppm of each of the following gases: H_2S, CH_3SH, and $(CH_3)_2S$.

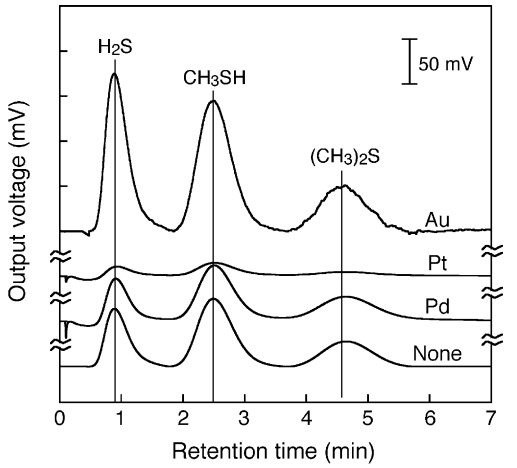

Fig. 5. Chromatograms using indium oxide sensors with and without noble metal catalysts, for a gas mixture consisting of 1 ppm of each of the following gases: H_2S, CH_3SH, and $(CH_3)_2S$.

ished to a negligible level by silica gel and charcoal filters.

3.2. Investigation of gas sensor

The performance of the sensor should: (1) be sensitive to all of the H_2S, CH_3SH and $(CH_3)_2S$ gases; (2) be as highly sensitive as possible for each gas of 100 ppb; and (3) not be saturated until 1000 ppb. To obtain the optimum sensing material, the sensitivity was examined for three types of metal oxide gas sensors made of SnO_2, In_2O_3 and ZnO. The chromatograms are shown in Fig. 4 for a gas mixture consisting of 1 ppm each of H_2S, CH_3SH and $(CH_3)_2S$. SnO_2 is commonly used as a gas sensing material for commercial sensors, but the sensitivity to all VSCs was too low to use as a detector of the oral malodor analyzer. ZnO was highly sensitive to H_2S and CH_3SH but almost insensitive to $(CH_3)_2S$, therefore, ZnO is also not suitable for a detector in this study. In contrast to these, In_2O_3 showed enough high sensitivity to all the three types of gases, and we have selected $In_2O_3$for the base material.

We have studied to increase the sensitivity by doping some noble metal catalysts to In_2O_3. The results are as shown in Fig. 5. The sensitivity to H_2S and CH_3SH increases dramatically with the addition of Au in contrast with Pd and Pt. The thiol group strongly attaches to the gold surface, therefore, the amount of absorbed thiols increases and metal–molecule complex is formed accompanied by the transfer of electrons [6,7]. The increase in sensitivity by Au is supposed to be due to the absorptive ability of Au for thiols. From the above results, we concluded that an Au added indium oxide sensor is optimal as a detector of an oral malodor analyzer.

The sensor as the detector of GC system is required for a high sensitivity and a fast response speed for good separation of each gas. To find the optimum operating temperature of the Au added indium oxide sensor, the sensitivity and the response speed to 300 ppb of H_2S were measured at 320, 370, 370 and 420 °C. The results are as shown in Fig. 6. The sensitivity was defined by the ratio of sensor resistance in the air and in the gas. With an increase of the operating temperature, the sensitivity to H_2S decreased and the response speed increased. The response speed at 400 and 420 °C was much faster than that at 320 and 370 °C, and the sensitivity was high enough to detect VSCs even in a low concentration. There was no remarkable difference between 400 and 420 °C. Taking into account sensitivity, response speed and the thermal durability, it was decided that 400 °C was the optimal operating temperature.

The influence of humidity to the Au added indium oxide sensor was studied. Fig. 7 shows the relationship between the absolute humidity and the sensor

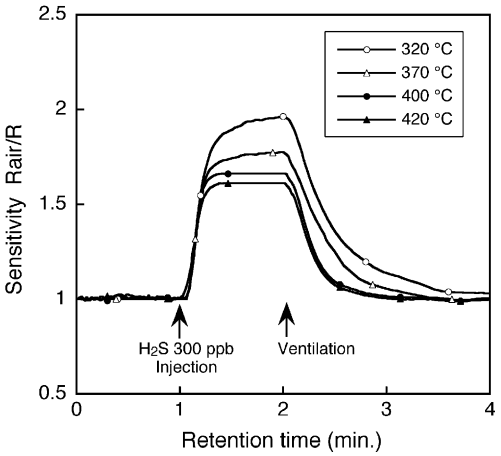

Fig. 6. Response of the Au added indium oxide sensor for the change in H_2S concentration.

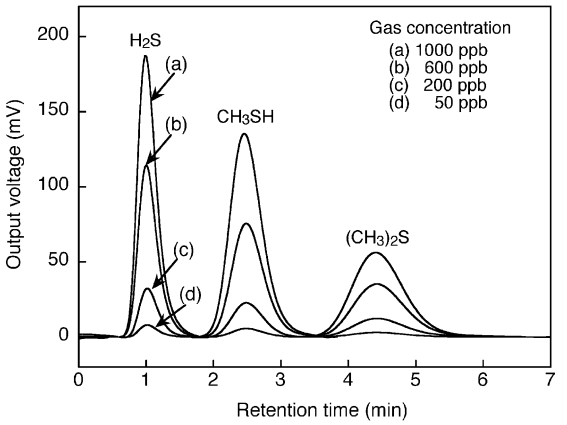

Fig. 8. Chromatograms for VSCs in concentrations of 50–1000 ppb of each of the three gases.

resistance in the air and in gas. The sensor resistance was rather influenced by the humidity, but the sensitivity was not. In the developed analyzer, VSC concentration is calculated through peak area. Though the sensor sensitivity has a large effect on the peak area, the sensor resistance has almost no influence. Accordingly, the influence of the ambient humidity to the analyzer accuracy was quite small.

3.3. Quantification of VSC

The chromatograms at different concentrations of VSCs are as shown in Fig. 8. Peak area of each gas is proportional to gas concentration in the range of 0–1000 ppb, and the relationship between the gas concentration (x ppb) and peak area (y mV s) are expressed with the following equations:

H_2S : $y = 3.26x$

CH_3SH : $y = 4.44x$

$(CH_3)_2S$: $y = 3.20x$

The concentrations of VSCs in mouth air were determined with the computer, and the above equations were used for calibration of the analyzer. The peak areas at 50 ppb are above 100 mV s, which provide a sufficient electrical resolution for detection. It is clear that this analyzer can detect the concentration of each VSCs over a wide range from 50 to 1000 ppb quantitatively.

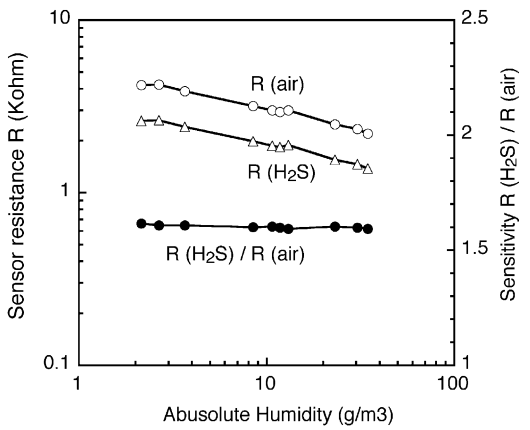

Fig. 7. Absolute humidity dependency of the Au added indium oxide sensor.

3.4. Influence of interference gases

It is known that various kinds of gases are present in human breath and such gases may effect the accuracy in the quantitative measurement of VSCs. We measured chromatograms of 10 ppm of H_2, CO, ammonia, isoprene, acetaldehyde, acetone and ethanol as possible gases in human breath. The results are as shown in Fig. 9(a) and (b). The peak of isoprene overlaps with the peak of H_2S and the peak of acetaldehyde overlaps with the peak of CH_3SH. However, they were

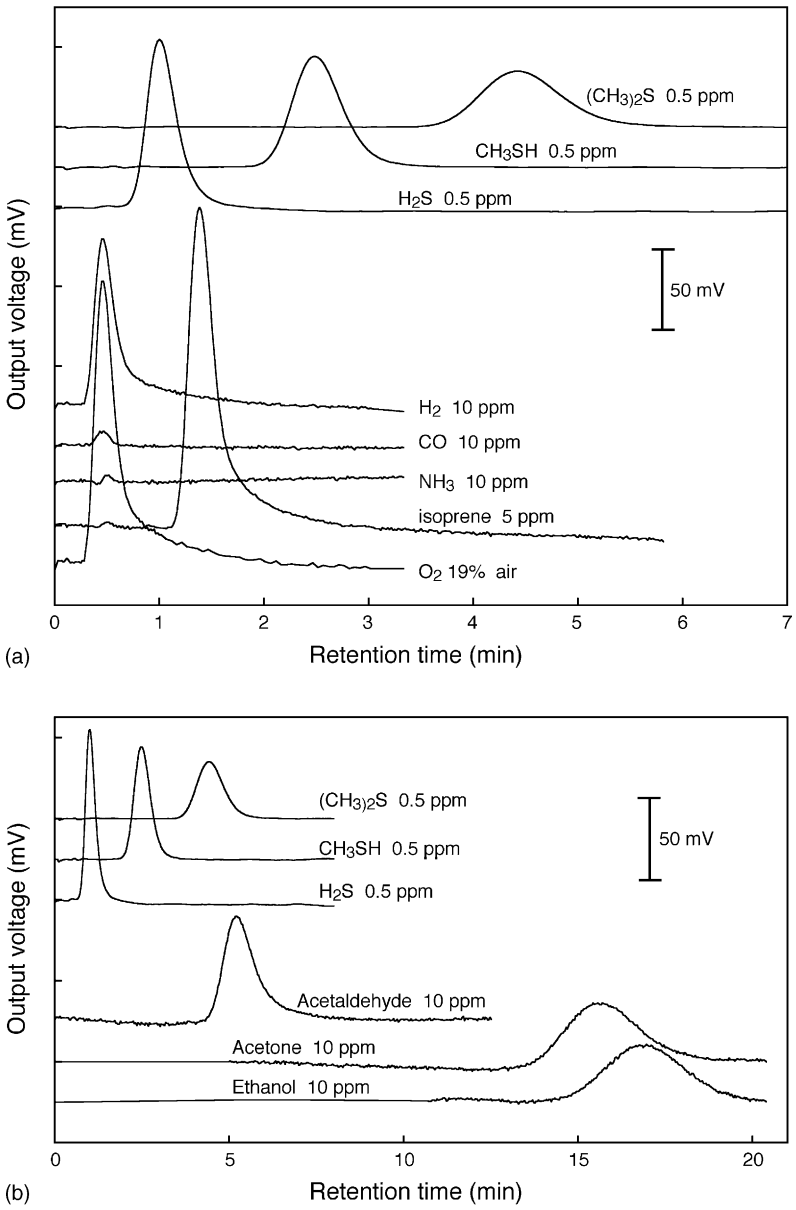

Fig. 9. Chromatograms of VSCs and various interference gases in human breath: (a) H_2, CO, NH_3, isoprene and O_2 in 19% air; (b) acetaldehyde, acetone and ethanol.

not influential in VSC analysis because the concentrations of these gases in human breath are actually much lower than 10 ppm, and the sensitivities to these gases are much lower than those to VSCs.

Besides the above-mentioned gases, oxygen concentration in the breath must also be considered. O_2 concentration in human expired air is approximately 16%. When human breath was examined, a peak caused by the reduction of O_2 concentration in the breath compared with the natural air always appeared. We call this peak "the background peak". As O_2 concentration in a mouth cavity is somewhat higher than

16, 19 of O_2 in N_2 was examined. The result is as shown in Fig. 9(a). Since the background peak overlaps with the peak of H_2S considerably, it is necessary to separate these two peaks for accurate quantification of H_2S. We have studied a method to separate these two peaks explained in the following paragraph.

We examined the influence of brushing teeth with toothpaste, rinsing with mouthwash solution, chewing gum, smoking a cigarette and drinking beverages. A person who did not have oral malodor took these substances and two measurements were performed at 1 and 10 min after taking these substances. No interfering gas was detected after drinking beverages, such as coffee, green tea, black tea and black tea with lemon. Interfering gases were detected 1 min after brushing teeth with toothpaste, rinsing with mouthwash solution, chewing gum, or smoking a cigarette. But in all the cases, they decreased to a negligible level within 10 min. From this result, it is found that the VSC analysis can be performed without influence from these substances after a lapse of 10 min.

3.5. Data analysis

To determine the concentration of H_2S, it is indispensable to eliminate the influence of "the background peak" mentioned in the foregoing paragraph. For this purpose, we studied a method of separating a chromatogram of mixed VSC gases into each individual peak by software treatment. The separation of the peaks was performed on the basis of the fact that the shapes of the peaks are similar regardless of the concentration as shown in Fig. 8. At first, the height of "the background peak" that is not influenced by

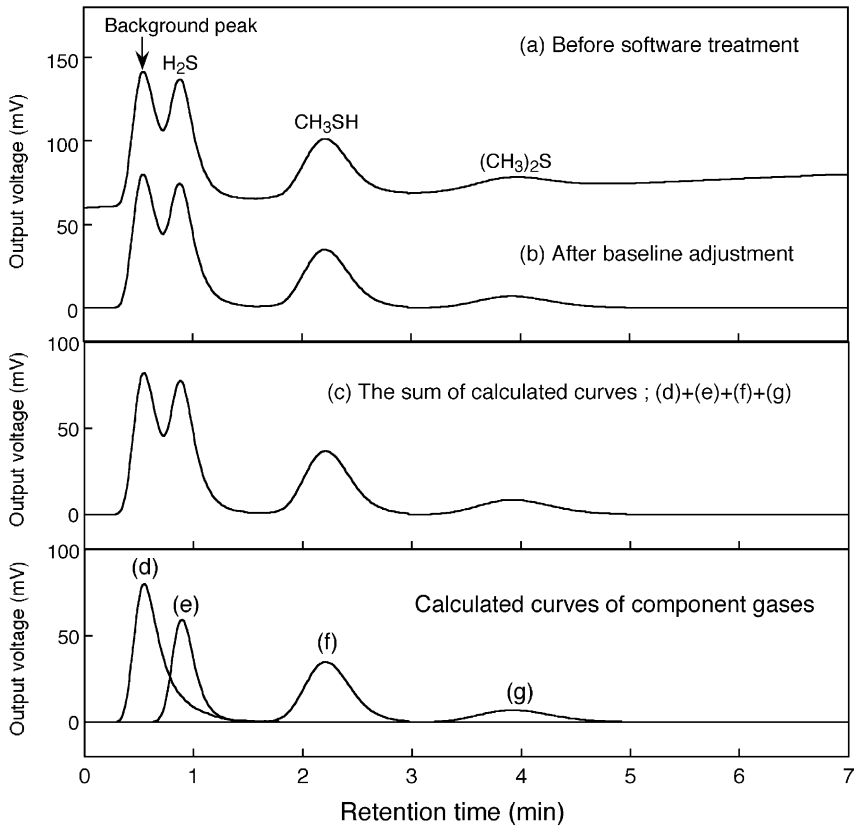

Fig. 10. An example of data analysis of a breath measurement: (a) a chromatogram of a breath sample before software treatment; (b) a chromatogram after baseline correlation; (c) a reproduced chromatogram; the sum of calculated curves (d + e + f + g); (d–g) calculated curves obtained by peak separation of (d) the background peak, (e) H_2S, (f) CH_3SH and (g) $(CH_3)_2S$.

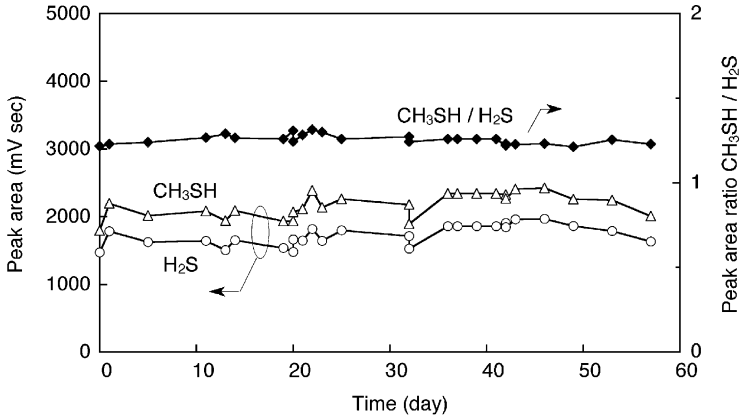

Fig. 11. Long-term stability of the peak areas and the area ratio of 500 ppb of H_2S and CH_3SH without calibration.

the presence of H_2S was measured. Next, the area of "the background peak" was calculated using the peak height and a predetermined shape of the peak. The peak area of H_2S was calculated from the area of "the background peak" and the sum of two areas. Basically, the peak separation of VSC components and other interfering gases was also performed in the same way, but they were rarely influential in the VSC analysis. The gas concentrations were calculated from the area of each individual peak using the equations shown in Section 3.3.

The following is an example of a breath malodor measurement and its data analysis. Fig. 10(a) is a chromatogram of a breath measurement. It has four peaks, "the background peak", H_2S, CH_3SH and $(CH_3)_2S$ peaks, respectively. Fig. 10(b) is the chromatogram after baseline correlation. The baseline of chromatogram (a) increases slightly with time. A stable baseline is desirable, but sometimes, the baseline changes gradually after power on, or caused by the effect of ambient temperature changes. To enable measurements even in such cases, a baseline correlation was performed.

The curves (d–g) in Fig. 10 are individual chromatograms of "the background peak", H_2S, CH_3SH and $(CH_3)_2S$ separated by software treatment. In this example, by calculating the peak areas of these chromatograms, the concentration of H_2S, CH_3SH, $(CH_3)_2S$ were determined as 320, 270, and 120 ppb, respectively using the equations shown in Section 3.3. The chromatogram (c) is a reproduced chromatogram obtained by adding the chromatograms (d–g). The calculated chromatogram (c) corresponds well to the real chromatogram (b). It proves that the peak separation method is effective.

3.6. Long-term stability

The long-term stability of the peak area of 500 ppb of H_2S and CH_3SH and the peak area ratio of CH_3SH and H_2S of the developed analyzer was tested over 60 days. Fig. 11 shows variation in peak areas and the peak area ratio of H_2S and CH_3SH without calibration. The peak areas were stable, but some slight fluctuations were observed. However, the errors of determined concentration for both the gases were within ±15% without calibration; it is thought that the accuracy of the analyzer is within the allowable limit for practical use. In addition, the peak area ratio of CH_3SH and H_2S, which is an important factor for diagnosis of halitosis, was almost stable. The stability of this analyzer was considered to be sufficient for an oral malodor analyzer.

4. Conclusion

We have developed a new oral malodor analyzer using a GC system with a highly sensitive semiconductor gas sensor as the detector. By using specific column design and not using a high-pressurized carrier gas, this analyzer is portable, economical and easy to use. It can evaluate the concentrations of H_2S, CH_3SH and $(CH_3)_2S$ in human mouth air individually and quantitatively over a wide range from 50 to 1000 ppb. Thus,

the newly developed analyzer will be effective to diagnose halitosis and to evaluate its treatment outcomes in dental clinics.

References

[1] J. Tonzetich, Arch. Oral Biol. 16 (1971) 587–597.
[2] K. Yaegaki, K. Sanada, J. Periodont. Res. 27 (1992) 233–238.
[3] US Patent 4,017,373.
[4] M. Shimura, Y. Yasuno, M. Iwakura, Y. Shimada, S. Sakai, K. Suzuki, S. Sakamoto, J. Periodontol. 67 (1996) 396–402.
[5] E. Maita, in: D. van Steenberghe, M. Roscmberg (Eds.), Bad breath: A Multidisciplinary Approach, Lieven University Press, Belgium, 1996, Chapter 19.
[6] E. Emberley, G. Kirczenow, Phys. Rev. B58 (1998) 10911.
[7] S.N. Yaliraki, A.E. Roitberg, C. Gonzalez, V. Mujica, M.A. Ratner, J. Chem. Phys. 111 (1999) 6997.