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Development of a Compact and Simple Gas Chromatography for Oral Malodor Measurement

Akatsuki Murata,* Anton Rahardjo,† Yuki Fujiyama,‡ Takayuki Yamaga,† Mariko Hanada,§ Ken Yaegaki,‖ and Hideo Miyazaki†

**Background:** Volatile sulfur compounds (VSCs) in oral air are the only type of gases correlated with the strength of oral malodor. We developed a compact and simple gas chromatograph (GC) equipped with a newly invented indium oxide semiconductor gas sensor (SCS) for measuring the concentrations of VSCs in mouth air. We have assessed the correlation between measurements with a GC-SCS and those with a regular GC.

**Methods:** Oral air samples from randomly selected volunteers were analyzed with both a GC-SCS and a GC with a flame photometric detector (FPD), which is specific to VSCs, and GC-SCS measurements were compared to those obtained by GC-FPD. Subsequently, oral air samples before and after mouthrinsing with 5% ethanol mouthwash were analyzed to determine the effect of ethanol on VSC measurements by GC-SCS.

**Results:** There were strong correlations between VSC concentrations determined using these two gas chromatography methods (hydrogen sulfide, $R = 0.821$, $P < 0.0001$; methyl mercaptan, $R = 0.870$, $P < 0.0001$; and dimethyl sulfide, $R = 0.770$, $P < 0.0001$). Although GC-SCS can differentiate ethanol and VSCs in oral air samples after mouthrinsing, GC-SCS measurements demonstrated higher values than those obtained by GC-FPD; however, this discrepancy improved over time due to the reduced effect of ethanol.

**Conclusion:** The results suggest that GC-SCS may be useful for the diagnosis of halitosis. *J Periodontol* 2006;77:1142-1147.

**KEY WORDS**
Chromatography, gas; halitosis; indium oxide; semiconductors; sulfur compounds.

Halitosis is caused by odorous compounds in breath or mouth air. Although more than 200 volatile compounds are found in human breath, only volatile sulfur compounds (VSCs) have been found to have a good correlation between concentration and organoleptic values.\(^1\)\(^2\)\(^3\) No other compounds have been reported to be correlated with organoleptic values. Furthermore, VSCs involve very strong and unpleasant malodor.\(^4\) Therefore, quantitative analysis of VSC by a gas chromatography (GC) equipped with a flame photometric detector (FPD) is considered one of the most reliable measurements for diagnosing halitosis. GC-FPD measurement is very dependable because of its specificity to VSCs; the GC method is originally highly objective and reproducible. However, GC-FPD is a sophisticated piece of equipment that requires an experienced operator. Furthermore, the device is costly and very large. Therefore, it is impractical to use it for routine examinations in dental practices.

Instead of the GC-FPD procedure, organoleptic measurements are performed for the assessment of oral malodor. This method is carried out simply by sniffing the breath and scoring the level of oral malodor by preference.\(^5\) Organoleptic measurement does not require special equipment and detects all compounds involving odor. Such measurement by a
trained examiner is considered an effective method to determine oral malodor, but its objectivity is lower compared to machine analyses. Portable VSC detectors, such as a sulfide monitor, are widely used for the quantitative measurement of oral malodor. These have sufficient sensitivity to detect H₂S but also detect other volatiles existing in human oral air, even though they are not malodorous. The electric nose technique has recently been introduced, but the equipment is extremely costly. This technique cannot determine volatile chemicals precisely, and it is difficult to distinguish mouth-air compounds from others present using this equipment. The mouth-air sample will be contaminated with a certain amount of respiratory air by this sampling procedure.

We have developed a compact and simple GC equipped with a newly invented indium oxide (In₈O₃) semiconductor gas sensor (SCS), which is highly sensitive to all kinds of VSCs. GC-SCS measures each VSC separately, whereas other devices cannot detect each separately. We have previously demonstrated that the GC-SCS procedure was highly reproducible in the measurements of standard VSCs. However, the accuracy of GC-SCS measurement for oral air has not yet been evaluated. In this study, we examined the precision of GC-SCS measurements of oral air samples by comparing them to those obtained by GC-FPD procedure.

**MATERIALS AND METHODS**

**Sampling of Oral Air**

Oral air samples were obtained from 77 randomly selected volunteers (51 males and 26 females) who agreed to participate in the study. Their ages ranged from 17 to 52 years (mean age: 34.6 ± 8.7 years). This study was carried out in 2002 and was approved by the Ethics Committee of Niigata University Graduate School of Medical and Dental Sciences. Informed consent was obtained from each subject.

The volunteers were asked to refrain from oral activity, including eating, drinking, toothbrushing, and mouthrinsing, before testing. Oral air samples from 59 subjects were analyzed.

For sampling, a three-way stopcock was incorporated between a 20-cm length of polytetrafluoroethylene (PTFE) sampling tube (3.3-mm outside diameter) and the GC-FPD, and a 1-ml disposable syringe was connected to the other arm of a three-way stopcock (Fig. 1).

Before each analysis, subjects were instructed to keep their mouths closed and to breathe through the nose for 30 seconds. The sampling tube was inserted into the center of the oral cavity through the lips and teeth, and the lips remained closed around it. After making sure saliva did not enter the tube, 15 ml oral air was aspirated with a gas-tight syringe connected to the outlet of the autoinjector of the GC-FPD, as reported previously. A 10-ml sample of the air was automatically transferred into the GC-FPD column and chromatographed. Immediately after aspirating 15 ml oral air into the autoinjector, 1 ml oral air was aspirated twice by the syringe. Because of the dead-space effect in the syringe, the first aspirated sample was abandoned, and 0.5 ml oral air from the second sample was injected into the GC-SCS.

A single trained examiner performed all measurements for each gas chromatograph to avoid interoperator variation.

**Clinical Application of GC-SCS**

When the effect of mouthwash on VSC production is evaluated by VSC monitors in vivo, ethanol...
Figure 2.
Typical chromatogram of GC-SCS. Hydrogen sulfide (A), methyl mercaptan (B), and dimethyl sulfide (C) were detected separately; the concentrations were 360, 205, and 9 ppb, respectively.

contained in the mouthwash always interferes with VSC determination. Ethanol gives an extremely high reading in VSC measurements by sulfide monitors. To determine the influence of ethanol on VSC measurement by GC-SCS in vivo, oral air samples from 11 subjects were taken before (baseline) and immediately after mouthrinsing with 5 ml 5% ethanol. Further oral air samples were taken at 60 and 120 minutes after mouthrinsing, and the change in magnitude of the measurements was determined.

VSC monitors detect many other organic volatile compounds and alcohols. Thus, with these devices, it is difficult to judge the clinical effect of mouthwashes, toothpastes, or other oral-hygiene products on VSC production in vivo. For the evaluation of the usefulness of GC-SCS in clinical research for oral hygiene products, the effect of zinc chloride-containing mouthwash, which is one of the most effective mouthwashes in reducing VSC production in the mouth, was examined with the GC-SCS, and the results were compared to those obtained by GC-FPD.

Oral air samples were taken from seven subjects before and immediately after mouthrinsing with 0.1% zinc chloride-containing mouthwash® and were analyzed with both GC-SCS and GC-FPD as described above.

Gas Chromatography Equipped With an In$_2$O$_3$ Semiconductor Sensor
GC-SCS (280 × 400 × 130 mm, 5.5 kg) equipped with a data-handling software system** was used for VSC measurement in oral air. The GC-SCS was connected to a personal computer to show the chromatogram. Each VSC was separated on a PTFE column (internal diameter, 5 mm; length, 300 mm). The temperature of the column was maintained at 40°C. The carrier gas was atmosphere filtered using 5 g silica gel†† and 6 g activated charcoal§§ at a flow rate of 9.8 ml/minute with a built-in motorized pump. The

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# Bee Brand, Osaka, Japan.
** ABILIT, Osaka, Japan.
†† Packed with 5,5'-oxydipropionitrile (ODPN) 25% Uniport HP on an 80-100 mesh, GL Sciences, Tokyo, Japan.
§§ Fuji Silysia, Kasugai, Japan.
$\#$ Kuraray, Osaka, Japan.
immediately after mouthrinising (Fig. 3B). Although GC-FPD indicated 0 ppb, GC-SCS detected comparatively high VSC concentrations in some cases. Additionally, GC-SCS measurements were higher than those obtained with GC-FPD at 60 minutes (Fig. 3C), although Pearson correlation coefficients yielded high values \( r = 0.822 \) [\( P < 0.0001 \)] for \( H_2S \) and \( r = 0.921 \) [\( P < 0.0001 \)] for \( CH_3SH \). The discrepancy improved at 120 minutes after mouthrinising, perhaps because the influence of the mouthwash had decreased substantially (Fig. 3D). These results suggest that ethanol may affect accurate measurement with the GC-SCS. However, the effect of ethanol is very much lower than in a sulfide monitor*** (data not shown).

It is widely reported that zinc chloride-containing mouthwash has a very strong immediate effect on reducing VSC concentration in oral air.\(^{14}\) We evaluated the effect of zinc chloride-containing mouthwash on VSC production in the mouth. Our results showed that VSC concentrations measured by both GC-SCS and GC-FPD were dramatically decreased in all cases (Fig. 4), although some measurements by GC-SCS were higher than those by GC-FPD. Ethanol in the mouthwash might have interfered with GC-SCS measurements.

CONCLUSIONS
It is suggested that the GC-SCS can be used for the diagnosis of halitosis and in clinical studies. However, one should note that mouthrinising with ethanol-containing mouthwash may affect accurate measurement with the GC-SCS.

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REFERENCES

Correspondence: Dr. Hideo Miyazaki, Division of Preventive Dentistry, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, 951-8514 Japan. Fax: 81-25-227-0807; e-mail: hideomy@dent.niigata-u.ac.jp.

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