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

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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.

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Halitosis is caused by odorous compounds in breath or mouth air. Although more than 200 volatile compounds are found in human breath,[1] only volatile sulfur compounds (VSCs) have been found to have a good correlation between concentration and organoleptic values.[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

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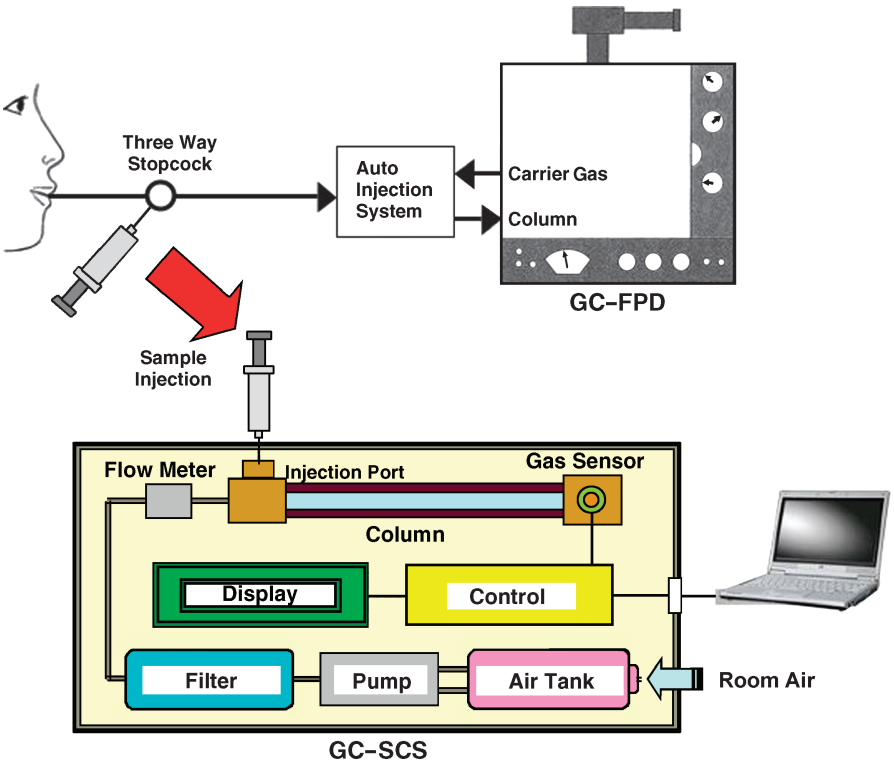

Figure 1.
GC-SCS and sampling system. The compositions of GC-SCS are shown. For both measurements by GC-SCS and GC-FPD, a 20-cm length of sampling tube and a 1-ml disposable syringe were connected to the autoinjection system through a three-way stopcock.

trained examiner is considered an effective method to determine oral malodor,[6] but its objectivity is lower compared to machine analyses.[7,8] Portable VSC detectors, such as a sulfide monitor,¶ are widely used for the quantitative measurement of oral malodor. These have sufficient sensitivity to detect H_2S but also detect other volatiles existing in human oral air, even though they are not malodorous.[9,10] 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.[11]

We have developed a compact and simple GC equipped with a newly invented indium oxide (In_2O_3) semiconductor gas sensor (SCS), which is highly sensitive to all kinds of VSCs.[12] 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.[12] 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.[13] A 10-ml sample of the air was automatically transferred into the GC-FPD column and chromatographed.[13] 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

¶ Interscan, Chatsworth, CA.

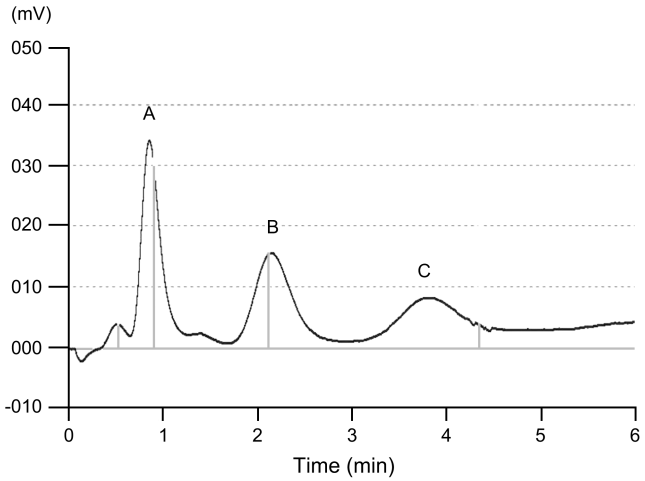

Figure 2.
Typical chromatogram of GC-SCS. Hydrogen sulfide (A), methyl mercaptan (B), and dimethyl sulfide (C) were detected separately; the concentrations were 360, 205, and 9 ppb, respectively.

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,[14] 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_2O_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.[12] 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

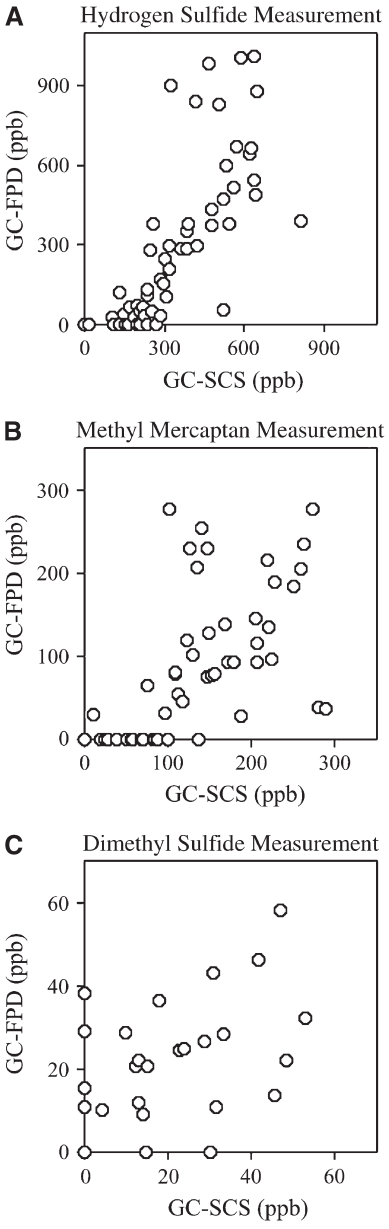

Figure 3.
The relationship for each VSC concentration between measurements with the GC-SCS and those with the GC-FPD. Oral air samples from 59 subjects were analyzed: ***A)*** *hydrogen sulfide; r = 0.821 (P <0.0001);* ***B)*** *methyl mercaptan; r = 0.870 (P <0.0001); and* ***C)*** *dimethyl sulfide; r = 0.770 (P <0.0001).*

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

Bee Brand, Osaka, Japan.
** ABILIT, Osaka, Japan.
†† Packed with β,β′-oxydipropionitrile (ODPN) 25% Uniport HP on an 80-100 mesh, GL Sciences, Tokyo, Japan.
‡‡ Fuji Silysia, Kasugai, Japan.
§§ Kuraray, Osaka, Japan.

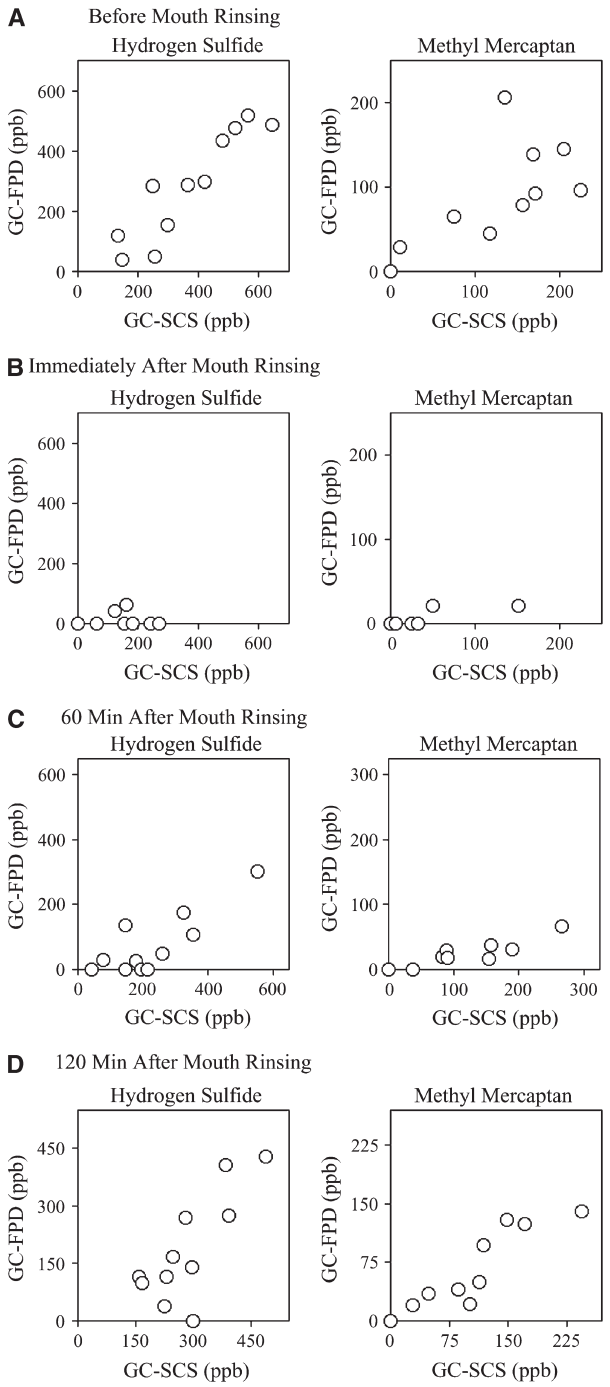

Figure 4.
The relationship for each VSC concentration between measurements with the GC-SCS and those with the GC-FPD. Oral air samples from 11 subjects were taken right before, immediately after, and 60 and 120 minutes after mouthrinsing with mouthwash containing 5% ethanol: ***A)*** *before mouthrinsing: hydrogen sulfide; r = 0.920 (P <0.0001), methyl mercaptan; r = 0.727 (P <0.01);* ***B)*** *immediately after mouthrinsing: hydrogen sulfide; r = –0.151, methyl mercaptan; r = 0.781 (P <0.01);* ***C)*** *60 minutes after mouthrinsing: hydrogen sulfide; r = 0.822, (P <0.01), methyl mercaptan; r = 0.921 (P <0.0001); and* ***D)*** *120 minutes after mouthrinsing: hydrogen sulfide; r = 0.780 (P <0.01), methyl mercaptan; r = 0.914 (P <0.0001).*

GC-SCS system was calibrated with standard VSCs, prepared by a permeater‖‖ and analytical-grade permeation tubes.

Gas Chromatography Equipped With a Flame Photometric Detector

GC-FPD¶¶ was used for VSC measurement in oral air. Each VSC was separated on a PTFE column (internal diameter, 3.2 mm; length, 3.1 m),## using nitrogen as the carrier gas. The autoinjector was placed between the column and the injection port.[13] Throughout the study, the VSC concentrations were expressed in parts per billion (ppb).

Statistical Analysis

To determine the association between VSC values measured with these two gas chromatography methods, Pearson correlation coefficients were used when highly positive correlations were expected by bivariable distribution.

RESULTS

As shown in Figure 2, each VSC peak was determined separately by GC-SCS and GC-FPD. Fifty-nine samples were analyzed with GC-SCS and GC-FPD, and their relationship is shown in Figure 3. Pearson correlation coefficients for H_2S, CH_3SH, and $(CH_3)S_2$ between both methods were 0.821 (P <0.0001), 0.870 (P <0.0001), and 0.770 (P <0.0001), respectively.

Oral air samples from 11 subjects were taken before, immediately after, and 60 and 120 minutes after mouthrinsing with 5% ethanol. The relationships are shown in Figure 4. As $(CH_3)_2S$ was measured in only six subjects and in very low concentrations at baseline analysis and was not detected within 120 minutes after mouthrinsing except in three cases, $(CH_3)_2S$ data were not considered for statistical analysis. Pearson correlation coefficients at the baseline analysis (Fig. 4A) were r = 0.920 for H_2S (P<0.0001) and r = 0.727 for CH_3SH (P<0.01). VSC measurements obtained with both gas chromatography methods were substantially decreased immediately after mouthrinsing (Fig. 4B). Pearson correlation coefficients were r = –0.151 for H_2S and r = 0.781 for CH_3SH (P<0.01). Although Pearson correlation coefficients yielded high values (r = 0.822, P <0.001 for H_2S and r = 0.921, P <0.0001 for CH_3SH) at 60 minutes, GC-SCS measurements are obviously higher than those obtained with GC-FPD (Fig. 4C); however, GC-SCS measurements came close to GC-FPD measurements at 120 minutes (Fig. 4D). Pearson correlation coefficients were r = 0.780 for H_2S (P<0.01) and r = 0.855 for CH_3SH (P<0.0001).

‖‖ GASTEC, Ayase, Japan.
¶¶ Shimadzu, Kyoto, Japan.
Packed with 1,2,3-Tris(2-cyanoethoxy)propane (TCEP) 25% Shimalite on 80-100 mesh with acid-washed, dimethyl dichlorosilane (AW-DMCS) treatment, Shimadzu.

Oral air samples were taken from seven subjects before and immediately after mouthrinsing with 0.1% zinc chloride-containing mouthwash, and the results are shown in Figure 5. VSC measurements obtained with both gas chromatography methods were substantially decreased compared to the measurements before mouthrinsing (Fig. 5).

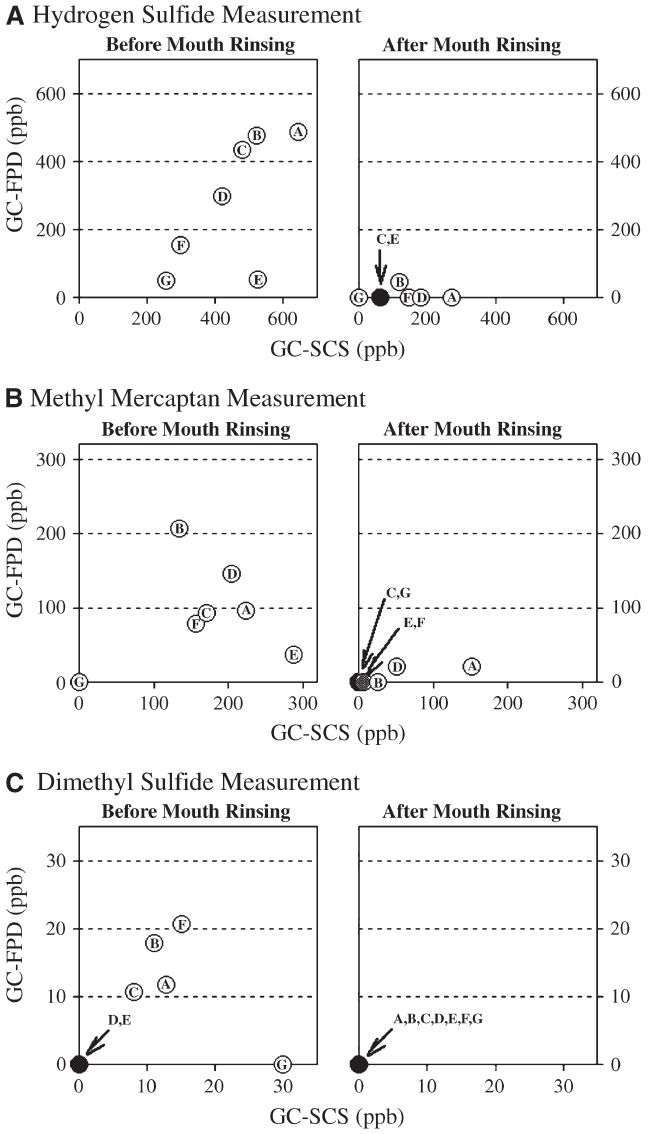

Figure 5.
The relationship for each VSC concentration between measurements with the GC-SCS and those with the GC-FPD. Oral air samples were taken from seven subjects right before and immediately after mouthrinsing with mouthwash containing 0.1% zinc chloride: ***A)*** *hydrogen sulfide;* ***B)*** *methyl mercaptan; and* ***C)*** *dimethyl sulfide. Each letter indicates an individual subject. Some measurements, such as subjects C and E after mouthrinsing with hydrogen sulfide, subjects C, G, E, and F after mouthrinsing with methyl mercaptan, subjects D and E before mouthrinsing with dimethyl sulfide, and all of the subjects after mouthrinsing with dimethyl sulfide demonstrated the same results; these data, therefore, are identified as a closed or shaded circle.*

DISCUSSION

A strong correlation was demonstrated between the organoleptic score and VSC concentration in oral air. Organoleptic assessment by experienced examiners is considered the gold standard for measuring oral malodor.[5] However, among machine determinations of VSC concentrations, the GC-FPD system, which is specific to VSCs, is most reliable. The GC-FPD system can measure individual VSC concentration and provides a readout of each VSC concentration in oral air. The ratio of CH_3SH to H_2S of intraoral origin is characteristic.[15] When H_2S is detected as the predominant VSC in oral air, physiological halitosis is suggested. When both H_2S and CH_3SH are detected as the predominant VSCs, periodontal diseases are suggested. $(CH_3)_2S$ is increased in liver conditions but is usually a minor component of VSC.[16] H_2S and CH_3SH are highly toxic. Some studies have reported that CH_3SH causes severe damage to periodontal tissues and cells.[17-20] CH_3SH may not only be responsible for oral malodor but may also contribute to the pathogenesis of periodontal disease. Therefore, it is very important to measure individual VSC concentrations for diagnosis and treatment rather than to measure total VSC concentration, as is usually the case with VSC monitors. On the other hand, GC-FPD is extremely costly. We have developed a compact and simple GC-SCS for measuring individual VSC in oral air. The cost of GC-SCS is 15% to 25% of a conventional GC-FPD. In addition, GC-SCS does not require hydrogen and carrier gas, which are essential for GC-FPD.

In this study, we compared GC-SCS measurements with those obtained using GC-FPD, and the GC-SCS results corresponded with the results from the GC-FPD (Fig. 2). Pearson correlation coefficients between concentrations measured with both methods yielded high values, such as r = 0.821 for H_2S (P <0.0001), r = 0.870 for CH_3SH (P <0.0001), and r = 0.770 for $(CH_3)_2S$ (P<0.0001), respectively. These values indicate sufficient performance as a measuring device of VSCs.

VSC monitors with a semiconductor gas sensor detect not only VSCs but also other volatile compounds.[10] Ethanol is contained in many mouthwashes, and VSC monitors therefore often indicate a very high value after mouthrinsing. Ethanol interferes with the accurate measurement of semiconductor gas sensors. We analyzed oral air samples before and after mouthrinsing with 5% ethanol to determine the effect of ethanol on VSC measurements by GC-SCS. Oral air samples were taken serially before and after mouthrinsing. VSC measurements were transiently decreased

immediately after mouthrinsing (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 mouthrinsing, 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 mouthrinsing with ethanol-containing mouthwash may affect accurate measurement with the GC-SCS.

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