A “Dual-acceptor Channel” Membraneless Gas-diffusion Unit for Simultaneous Determination of Ethanol and Acetaldehyde in Liquors Using Reverse Flow Injection

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A new design of membraneless gas-diffusion unit with dual acceptor channels for separation, collection and simultaneous determination of two volatile analytes in liquid sample is presented. The unit is comprised of three parallel channels in a closed module. A sample is aspirated into the central channel and two kinds of reagents are introduced into the other two channels. Two analytes are isolated from the sample matrix by diffusion into head-space and absorbed into the specific reagents. Non-absorbed vapor is released by opening the programmable controlled lid. The unit was applied to liquors for measurement of ethanol and acetaldehyde using reverse flow injection. Dichromate and nitroprusside were exploited as reagents for colorimetric detection of ethanol and acetaldehyde, respectively. Good linearity ranges ($r^2 >0.99$) with high precision (RSD <2%) and high accuracy (recovery: 90 - 105%) were achieved. The results were compared to the results by GC-FID and no significant difference was observed by paired t-test (95% confidence).

Keywords Membraneless gas-diffusion unit, dual-acceptor channel, reverse flow injection, simultaneous determination, ethanol, acetaldehyde, liquor

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Introduction

Various designs of membraneless units have recently been reported for gas-liquid separation, collection and determination of volatile compounds.1-4 The idea of these units is the same as the conventional gas-diffusion (GD) and pervaporation (PV) units but employs no membrane. Nomenclature of the membraneless units are different based on their reservoir configurations. The first design with rectangular shape was called “membraneless gas-diffusion” (MGD) unit. It was presented as having two parallel channels (donor and acceptor) separated by a thin wall.1 The channels are connected by the head-space above where diffusion of gaseous analyte occurs. Application to a liquid sample, such as for the determination of ethanol in liquors, was carried out. The MGD unit was found to provide greater mass transfer than the typical GD. The problem due to malfunction of the membrane caused by exposure to samples or reagents no longer existed. Another MGD unit was proposed for the determination of ethanol in gasoline.2 By this design, residual ethanol vapor in the head-space that caused signal tailing was eliminated by manual opening of the unit’s lid. A similar design as the original MGD unit1 was also independently reported by Mornane et al.3 However, the unit was denoted as a “thin layer distillation” (TLD) unit since the authors believed that it was more descriptive terminology.3 It was useful for analysis of ammonia and high molecular weight amines. In 2011, Almeida et al.4 explored the potential use of a re-designed MGD unit with a “bathtub” for avoiding flooding between the channels. This bathtub-MGD was integrated with a multi-syringe flow analysis for determination of ammonia in environmental water samples.

The extensive change to the non-membrane configuration was made for direct analysis of a solid sample. The unit was named the “membraneless vaporization” (MBL-VP) unit.6,7 The shape
of the vaporization section was modified from channel to cylindrical chamber. A solid sample was inserted into the chamber. The analyte was converted into gaseous form inside the unit for separation and collection of the gas into a reagent stream for further on-line detection. The applications were demonstrated for analysis of calcium in supplement products and cement. Later in 2013, donor and acceptor of the MBL-VP unit were redesigned as “cone-shape chamber” for analysis of liquid samples. The unit was coupled to both flow injection (FI) and sequential injection (SI) in order that precise volumes of solutions could be loaded into the chambers without overflow. It is noticed that configurations of the membraneless units mentioned above were modified accordingly to their application purposes. Nonetheless, all designs are effective for the separation and determination of only a single volatile analyte.

Herein, a newly designed membraneless unit is presented for gas–liquid separation, collection and simultaneous determination of two volatile analytes in a liquid sample. The rectangular unit is comprised of three parallel grooves. A sample is aspirated into the central channel while two kinds of acceptors are introduced into the other two channels. The unit is called “dual-acceptor channel” MGD unit. The unit body is covered with the lid, which is designed for automatically opening to release non-absorbed vapor. We selected ethanol and acetaldehyde as the model volatile analytes to demonstrate the utility of the unit. Both analytes are key compounds for the quality control of liquor manufacturing. For simultaneous analysis of these analytes, gas chromatography (GC) was effective because it provided high accuracy. However, GC normally requires a long analysis time, which is tedious for routine work. The FI is more attractive due to its capability for “on-line” measurement. The enzymatic FI was proposed for simultaneous determination of both analytes. However, enzyme activity was gradually reduced after several injections. The present MGD unit is therefore coupled to the FI with simple colorimetric detection. Reagents were injected through six-port valves into the acceptor channels based on reverse FI for minimizing reagent consumption. Acidified dichromate was used as the chromogenic reagent for the ethanol detection. Dissolved nitroprusside in morpholine was chosen as a specific reagent for the acetaldehyde detection. Application of the developed method to liquors and validation were studied.

Experimental

Reagents and chemicals

All chemicals used were of analytical-reagent grade. Deionized-distilled water was employed throughout. Mixed working standard solutions of ethanol (1 to 40% (v/v)) and acetaldehyde (0.5 to 10 mmol L⁻¹) were freshly prepared by dilution from stock standards of 99.8% ethanol (Riedel-de Haën, Germany) and 99.5% acetaldehyde (Riedel-de Haën). Standard acetaldehyde was standardized accordingly to iodometry prior to further dilution. An acceptor solution for ethanol was prepared by dissolving of 5.9 g of potassium dichromate (AnalaR, Australia) in 100 mL of 0.4 mmol L⁻¹ sulfuric acid (Riedel-de Haën). An acceptor solution for acetaldehyde was prepared by dissolving of 2.5 g of sodium nitroprusside dihydrate (AnalaR) in 100 mL of 10% (v/v) morpholine (Fluka, Germany).

Design of the dual-acceptor channel MGD unit

Photograph and schematic diagrams of the dual-acceptor channel MGD unit are presented in Fig. 1. The unit is made of acrylic in rectangular shape (25 mm width × 70 mm length × 20 mm height) and is composed of three parallel grooves (Fig. 1(A)) with equivalent dimension (2 mm width × 50 mm length × 5 mm height). The central channel accommodates a sample. The other channels are exploited for loading of two acceptors (Fig. 1(B)). Each channel is separated by the 1 mm-thick barrier and is designed in a “bathtub” pattern, similar to Almeida et al. for ceasing liquid overflow and cross contamination. The solutions are delivered in and out of the channels via connecting ports (Fig. 1(D)). The unit is covered with an acrylic lid (15 mm width × 70 mm length × 2 mm thickness) without any bolt (Fig. 1(C)). To prevent gas leakage during the determination, a silicone gasket (0.5 mm thickness)
is mounted along two lateral sides of the unit (Fig. 1(D)). The lid automatically slides to open or close position using our "in-house" software (Visual Basic 6.0™) with a control module.

### Table 1 Steps of manipulation of the final flow system with the dual-acceptor channel MGD unit for simultaneous determination of ethanol and acetaldehyde

| Step | P₁ | P₂ | Valve position | Duration time/s | Function |
|------|----|----|----------------|-----------------|----------|
| 1    | On | On | SV S Load IV₁ Load Close | 0 – 60 | Fill sample and acceptors into MGD unit |
| 2    | On | Off| SV S Inject IV₁ Load Close | 61 – 120 | Propel AS₁ to detector/trap AS₂ inside acceptor channel |
| 3    | On | Off| SV H₂O Inject IV₁ Inject Close | 121 – 150 | Propel AS₂ to detector |
| 4    | On | Off| SV H₂O Inject IV₁ Inject Open | 151 – 160 | Releasing of residual vapor |

### Set-up and steps of manipulation of the flow manifolds

The manifolds of the preliminary system and the final system with the dual-acceptor channel MGD unit for simultaneous analysis of ethanol and acetaldehyde are shown in Figs. 2(A) and 2(B), respectively. Both systems were assembled using 1.0 mm i.d. PTFE tubing. Tygon™ pump tubes (0.95 mm i.d., Ismatech, Switzerland) were utilized with peristaltic pumps. A 12-channel peristaltic pump, P₁ (IPC-12, Ismatech, Switzerland) was used for propelling the sample (S) and acceptor streams (in Fig. 2(A)) or carrier streams C₁, C₂ and C₃ (in Fig. 2(B)) in and out of the unit. For the final system, a switching valve, SV (V101L, Upchurch Scientific, USA) was used for selection of either sample or water to the central channel. Acidified dichromate (AS₁) and dissolved nitroprusside in morpholine (AS₂) were loaded into six-port valves, IV₁ and IV₂ (Upchurch Scientific, USA) using a 4-channel peristaltic pump, P₂ (REGLO, Ismatech, Switzerland) integrated into the acceptor channels via PTFE tubing, depicted as dash line (--.--).
Phills, US) was used for detection.

Steps of manipulation of the final system (Fig. 2(B)) as summarized in Table 1 was started by introduction of AS1 and AS2 to fill the acceptor channels. At the same time, the sample was aspirated into the central channel by P1 with time-based injection (60 s, 1.2 mL/min). After that, IV1 was turned to “inject” position for delivering AS1 toward the detector for the ethanol analysis. IV2 was still situated at “load” position since beginning sample aspiration in order to “trap” the AS2 zone inside the channel. After trapping (60 s), IV2 was switched to “inject” for propelling AS2 to the detector for the acetaldehyde determination. The lid of the unit was automatically opened for 10 s in the last step for removal of residual vapor. All liquid delivery devices were automatically controlled by our developed software (Visual Basic 6.0®).

Results and Discussion

Investigation of the appropriate flow manifold

The simple manifold (Fig. 2(A)) was employed for preliminary study on the feasibility of the new design MGD unit. Mixed standards (ethanol + acetaldehyde) and their specific acceptors were directly and continuously aspirated into the channels. All solutions were propelled and sucked out of the unit using the same flow rate (1.2 mL/min) and the same pump (P1) to maintain the liquid level. The outlet tube from each acceptor channel was connected to the individual spectrophotometer for parallel determination of the analytes. Linear calibrations were obtained (1 to 40% (v/v) ethanol and 0.5 to 10 mmol L⁻¹ acetaldehyde) with good linearity ranges \( r^2 > 0.99 \). However, the system was not cost-effective since two detectors were exploited. In addition, this system consumed large amounts of acceptor solutions (2.9 mL/analysis for each acceptor). To reduce reagent consumption, the preliminary system was adapted to the final system (Fig. 2(B)). Two six-port valves were therefore merged by Y-connection and became a single line for delivering two reacted zones to the same detector. This is more cost-effective than the preliminary one. By this manifold, the valves were necessarily injected at different times to prevent overlapping of two chasing zones. IV1 was first turned to “inject” to deliver AS1 to the detector for ethanol determination. At the same time, IV2 was located at “load”. This caused trapping of AS2 inside the unit with continuous flow of sample. As shown in Fig. 2(C), sensitivity for the acetaldehyde determination is enhanced around 2-fold, compared to the preliminary system. This is important for real application since the acetaldehyde content in liquor is very low. With this flow strategy, the observed first signal corresponded to the peak for the ethanol determination followed by the second peak for the acetaldehyde determination. In terms of reagent consumption, only an aliquot of 1.2 mL/analysis for each acceptor was injected. This volume is reduced by almost 2-fold from the volume consumed by the preliminary system. Therefore, this reverse FI was regarded as the appropriate manifold and was exploited for further studies.

Automated lid opening for residual vapor releasing

In the previous work, residual vapor in the head-space of the unit was found to cause baseline shift and signal tailing because of memory effect, especially at high concentrations of analytes. Manual opening of the unit’s lid was carried out for residual gas removal. In this work, the lid is designed for automated opening using a control module comprised of a robot arm, step motor and control board. The arm touches the belt that is attached to the top of the lid (Fig. 3(A)). When the arm was rotated clockwise, the lid was dragged to open (Fig. 3(A)). An interval time of 10 s was suitable for lid opening as residual vapor was completely removed (no baseline shift). After that, the lid was pulled back to close the unit for the next analysis, as the arm was rotated anti-clockwise. We tested feasibility of this control module by consecutive aspirations of 20% (v/v) standard ethanol \( n = 10 \). As expected, the well-defined profiles without baseline shift were obtained as the lid was opened after every analysis (Fig. 3(B)) while the baseline was shifted as the lid was permanently closed (Fig. 3(C)). Precision was also improved (RSDlid open = 1.24% and RSDlid close = 4.97%).
Optimization study

Chemical and physical parameters were optimized. Results are presented in Fig. 4 (A to H) and are discussed as the following details. For the ethanol measurement, the absorbance increased as the concentration of dichromate was increased up to 0.2 mmol L\(^{-1}\) and then reached its plateau. This concentration was therefore selected for minimizing reagent consumption. The concentration of 4.0 mmol L\(^{-1}\) H\(_2\)SO\(_4\) was chosen as the highest signal was obtained at this level. For the acetaldehyde analysis, increasing nitroprusside and morpholine concentrations led to increased absorbance readings. The concentration of 2.5% (w/v) nitroprusside and 10% (v/v) morpholine were selected as a compromise between sensitivity and chemical usage. For the effect of morpholine concentration, the signal was increased up to 10% (v/v) then became significantly decreased. This is because morpholine (secondary amine) can induce the solution to become more alkaline at higher concentrations and the reaction between nitroprusside and morpholine.

Fig. 4  Summary of results of optimization study. Results (A) to (D) are the concentration effects of dichromate, sulfuric acid, nitroprusside and morpholine, respectively. (E) Effect of aspirated sample volume. (F) Effect of acceptor volume. (G) Effect of flow rate of carrier streams. (H) Effect of trapping time. Standard ethanol (10% (v/v)) and acetaldehyde (8 mmol L\(^{-1}\)) were studied (n = 3).
at higher volumes (>1.2 mL), the signals were slightly decreased. 1.2 mL was thus chosen. For the effect of acceptor volumes, dispersion was noticed at 0.6 and 0.8 mL min⁻¹. This brought differences (ethanol: 1.0% from the label value is illegal accordingly to Table 2.  By the statistical paired t-test,¹⁶ there was no evidence of significant differences (ethanol: \( t_{\text{critical}} = 2.20, \quad t_{\text{calculated}} = 0.71 \), acetaldehyde: \( t_{\text{calculated}} = 2.20, \quad t_{\text{critical}} = 0.78 \)). This indicates that the proposed system was successfully validated. Analysis speed of this method (23 samples h⁻¹) is also faster than GC-FID (8 samples h⁻¹). The acetaldehyde concentrations were found to be lower than the regulation limit and the ethanol percentages were also deviated within ±1 degree from the label contents. Analytical recoveries were found ranging from 90 to 105% (Table 2). This suggests that the MGD unit is useful for separation of two volatile analytes from a sample matrix.

**Features of the method and the new MGD unit**

The effect of aspirated sample volume was investigated by varying aspiration time when the flow rate of P1 was kept constant at 1.2 mL min⁻¹. For all investigated volumes, neither drying nor flooding of the liquid in the central channel of the MGD unit was found. The signal increased dramatically when the volume was increased up to 1.2 mL. Higher volumes caused slight enhancement of the signals. This is due to limited reagent effective and robust for releasing residual vapor.

**Application to liquors: validation and recovery study**

The developed system (Fig. 2B) was applied to liquors of various kinds to investigate broad application. All samples were directly aspirated into the MGD unit without any filtration or dilution. In Thailand, the acetaldehyde level must be below 160 ppm (ca. 4 mmol L⁻¹) and deviation of the ethanol content greater than ±1.0% from the label value is illegal accordingly to the Department of Excise.¹⁵ Results are compared to the contents obtained by GC-FID as summarized in Table 2. By the statistical paired t-test,¹⁶ there was no evidence of significant differences (ethanol: \( t_{\text{critical}} = 2.20, \quad t_{\text{calculated}} = 0.71 \), acetaldehyde: \( t_{\text{calculated}} = 2.20, \quad t_{\text{critical}} = 0.78 \)). This indicates that the proposed system was successfully validated. Analysis speed of this method (23 samples h⁻¹) is also faster than GC-FID (8 samples h⁻¹). The acetaldehyde concentrations were found to be lower than the regulation limit and the ethanol percentages were also deviated within ±1 degree from the label contents. Analytical recoveries were found ranging from 90 to 105% (Table 2). This suggests that the MGD unit is useful for separation of two volatile analytes from a sample matrix.

**Conclusions**

A new design MGD unit with “dual-acceptor channel” was reported for the first time for separation, collection and simultaneous determination of two volatile analytes in a liquid sample. The unit lid has allowed for greater ease of opening. This is very effective and robust for releasing residual vapor.

Features of the new design MGD unit are compared to the other membraneless units as shown in Table S1 (Supporting Information). The most remarkable characteristic of our unit is its design as having dual acceptor channels inside the same unit, which allows for separation, collection and simultaneous analysis of two volatile analytes in a liquid sample. The sliding lid has allowed for greater ease of opening. This is very effective and robust for releasing residual vapor.

**Table 2  Recovery and comparison of the ethanol and acetaldehyde contents in various types of liquors, determined by this work and GC-FID**

| Code/type          | Ethanol content, % (v/v) | Recovery, % | Acetaldehyde content/mmol L⁻¹ | Recovery, % |
|--------------------|--------------------------|-------------|--------------------------------|-------------|
|                    | Label                   | This work⁶ | GC-FID⁴                       | This work⁶ | GC-FID⁴ |
| S1/Beer            | 5                       | 4.9 (±0.01) | 5.3 (±0.04)                   | 91.2        | 1.3 (±0.02) | 1.5 (±0.03) | 90.0 |
| S2/Beer            | 5                       | 5.3 (±0.02) | 5.9 (±0.05)                   | 90.9        | 1.9 (±0.01) | 1.6 (±0.02) | 92.4 |
| S3/Lychee wine⁵    | —                       | 9.5 (±0.09) | 10.4 (±0.08)                  | 90.1        | 3.9 (±0.01) | 4.2 (±0.03) | 90.5 |
| S4/Lychee wine⁵    | —                       | 10.1 (±0.12)| 9.8 (±0.07)                   | 99.2        | 2.7 (±0.06) | 2.4 (±0.07) | 98.4 |
| S5/Red wine        | 14                      | 13.0 (±0.05)| 13.3 (±0.09)                  | 93.4        | 3.2 (±0.06) | 2.9 (±0.02) | 95.2 |
| S6/Red wine        | 14                      | 14.3 (±0.01)| 14.1 (±0.06)                  | 105         | 2.2 (±0.04) | 2.7 (±0.01) | 97.2 |
| S7/Red wine        | 14                      | 13.6 (±0.04)| 13.2 (±0.11)                  | 99.5        | 2.4 (±0.06) | 2.7 (±0.06) | 96.8 |
| S8/Brandy          | 25                      | 24.1 (±0.14)| 25.1 (±0.09)                  | 101         | 2.1 (±0.05) | 2.4 (±0.06) | 102 |
| S9/Brandy          | 25                      | 25.9 (±0.22)| 24.2 (±0.43)                  | 93.2        | 1.9 (±0.06) | 1.8 (±0.08) | 98.1 |
| S10/Brandy         | 25                      | 24.2 (±0.23)| 24.7 (±0.16)                  | 95.3        | 2.4 (±0.07) | 2.6 (±0.07) | 90.9 |
| S11/Whisky         | 35                      | 34.5 (±0.49)| 35.1 (±0.33)                  | 97.8        | 2.6 (±0.06) | 2.2 (±0.08) | 102 |
| S12/Whisky         | 40                      | 39.1 (±0.45)| 40.9 (±0.23)                  | 103         | 2.3 (±0.09) | 2.8 (±0.04) | 105 |

a. Reported as mean ± SD (n = 3). b. Analytical procedure of the validating GC-FID were adapted from J. F. Brien et al.¹¹ c. Values obtained by this work. d. S3 and S4 are “lab-made” wines from Department of Biology, KMITL (the label ethanol contents are not available).

Features of the method and the new MGD unit

Under the optimal parameters, linear calibrations of ethanol (1 to 40% (v/v)) and of acetaldehyde (0.5 to 10 mmol L⁻¹) were obtained with good linearity ranges (\( r^2 >0.99 \)) with high precision (\( \% \text{RSD}_\text{calculated} = 1.97 \% (\text{v/v}) \) and \( \% \text{RSD}_\text{acetaldehyde} = 1.85 \% (\text{mmol L}^{-1}) \), \( n = 10 \) replicates). Limits of detection (3σ) were 0.62% (v/v) and 0.41 mmol L⁻¹ for ethanol and acetaldehyde, respectively. This allowed application of our method to real liquor samples. This work utilizes a six-port valve coupled to the acceptor channel for injection of acceptors instead of direct aspiration to the unit. Reagent consumption and waste production is therefore minimized. Throughput of 23 samples h⁻¹ (with simultaneous measurement of two analytes) was obtained.

**Conclusions**

A new design MGD unit with “dual-acceptor channel” was reported for the first time for separation, collection and simultaneous determination of two volatile analytes in a liquid sample. The unit lid was enabled for automated opening using an “in-house” control module. This is very convenient for releasing residual vapor. The unit was successfully applied to releasing residual vapor. The reduced chemical consumption and waste production was achieved. The method also provided high precision and accuracy.

*morpholine is inhibited by acetaldehyde itself in a strong alkaline solution.*¹⁴
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Supporting Information

Supporting information includes absorption spectra of blue-colored products (Fig. S1) and comparison of the characteristics of the membraneless units (Table S1). This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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