รายงานวิจัยฉบับสมบูรณ์

โครงการ การพัฒนาเครื่องตรวจวัดค่าการนำไฟฟ้าแบบไม่สัมผัสสารละลายและวัดค่าทางแสงร่วมกับการใช้อุปกรณ์ไอระเหยแบบไม่ใช้เมมเบรนในระบบการไหลสำหรับหาปริมาณของซัลไฟต์และเอทานอลในไวน์

โดย ดร.ฐิติรัตน์ แม้นทิม

เดือน ปี ที่เสร็จโครงการ: มิถุนายน 2561

สัญญาเลขที่ TRG5880170

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและ
มหาวิทยาลัยศรีนครินทรวิโรฒ

บทคัดย่อ

รหัสโครงการ : TRG5880170

ชื่อโครงการ : การพัฒนาเครื่องตรวจวัดค่าการนำไฟฟ้าแบบไม่สัมผัสสารละลายและวัดค่าทางแสงร่วมกับการใช้อุปกรณ์ไอระเหยแบบไม่ใช้เมมเบรนในระบบการไหล สำหรับหาปริมาณของซัลไฟต์และ เอทานอลในไวน์

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บทคัดย่อ:

งานวิจัยนี้ได้เสนอการใช้อุปกรณ์ไอระเหยแบบไม่ใช้เมมเบรน (MBL-VP unit) ในระบบการไหลสำหรับหาปริมาณของเอทานอลและซัลไฟต์ทั้งหมดในไวน์ขาว ระบบการไหลประกอบไปด้วยอุปกรณ์ MBL-VP ซึ่งมีถ้วยรูปกรวยสำหรับสารละลาย 3 ถ้วย และเครื่องตรวจวัดที่สร้างขึ้นเอง 2 ชนิดคือ เครื่องตรวจวัดทางแสงชนิดพีอีดีดี (Paired Emitter-Detector Diodes: PEDD) และเครื่องตรวจวัดค่าการนำไฟฟ้าแบบไม่สัมผัสสารละลาย (Capacitively Coupled Contactless Conductivity Detector: C4D) ในระบบการไหลนั้นสารละลายตัวอย่างจะถูกผสมกับสารละลายกรดก่อน แล้วจึงนำสารละลายตัวอย่างที่ผสมกับกรดแล้ว ปริมาตร 200 ไมโครลิตร ส่งไปยังถ้วยใส่สารละลายตัวให้ไอระเหย (donor reservoir) ของอุปกรณ์ MBL-VP ซึ่งมีถ้วยสำหรับใส่สารละลายตัวรับไอระเหย (acceptor reservoir) 2 ถ้วย โดยแต่ละถ้วยจะบรรจุสารละลายตัวรับไอระเหยหรือตัวรับแก๊ส ปริมาตร 200 ไมโครลิตร ได้แก่ สารละลายเปอร์แมงกาเนตในกรด และน้ำปราศจากไอออน การระเหยกลายเป็นไอของเอทานอลและแก๊สซัลเฟอร์ไดออกไซด์ (SO_2) ที่เปลี่ยนมาจากซัลไฟต์ จาก donor reservoir ใช้เวลา 15 วินาที หลังจากนั้นสารละลายตัวรับไอระเหยทั้งเอทานอล และ SO_2 จะถูกนำส่งไปตรวจวัดแยกกันที่เครื่องตรวจวัด PEDD และ C4D ตามลำดับ โดยใช้กระแสของตัวพาที่เป็นอากาศ (air carrier stream) การฟอกจางสีของสารละลายตัวรับเปอร์แมงกาเนตในกรด เกิดจากปฏิกิริยารีดักชั่นด้วยเอทานอลที่ถูกดูดซึมอยู่ในสารละลายเปอร์แมงกาเนต ส่งผลให้สัญญาณของ PEDD สูงขึ้น ขณะที่การละลายของแก๊ส SO_2 ในสารละลายตัวรับที่เป็นน้ำปราศจากไอออนจะส่งผลให้สัญญาณของการนำไฟฟ้าที่ตรวจวัดด้วยเครื่อง C4D สูงขึ้น การตรวจวัดเอทานอลได้ความเป็นเส้นตรงของกราฟมาตรฐาน ($\Delta V = ((4.0\pm0.03)\times10^{-2})x-((1.85\pm0.35)\times10^{-2})$: $r^2 = 0.999$) ในช่วงความเข้มข้น 5.0 ถึง 15.0 %(V/V) และการตรวจวัดซัลไฟต์ ได้ความเป็นเส้นตรงของกราฟมาตรฐาน ($\Delta V = ((0.64\pm0.015)\times10^{-2})x-((3.25\pm1.64)\times10^{-2})$: $r^2 = 0.999$) ในช่วงความเข้มข้น 10 ถึง 200 มิลลิกรัมต่อลิตร กระบวนการวิเคราะห์เอทานอลและซัลไฟต์นี้สามารถตรวจวัดได้รวดเร็ว โดยใช้เวลาวิเคราะห์ทั้งหมด 2.5 นาที ได้ร้อยละการคืนกลับ 81-104 % และ 88-110 % สำหรับการวิเคราะห์เอทานอลและซัลไฟต์ตามลำดับ วิธีที่พัฒนาขึ้นนี้ประสบความสำเร็จในการสอบเทียบกับวิธีมาตรฐาน ได้แก่ วิธีแก๊สโครมาโทกราฟีสำหรับการวิเคราะห์เอทานอล และวิธีการไทเทรตแบบไอโอโดเมตรีสำหรับการวิเคราะห์ซัลไฟต์

คำหลัก : การระเหยแบบไม่ใช้เมมเบรน; เอทานอล; ซัลไฟต์; เครื่องตรวจวัดทางแสงชนิดพีอีดีดี; เครื่องตรวจวัดค่าการนำไฟฟ้าแบบไม่สัมผัสสารละลาย

Abstract

Project Code : TRG5880170

Project Title : Development of dual C4D-photometric detector coupled with membraneless vaporization unit in flow system for simultaneous determination of sulfite and ethanol in wine

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Project Period : 1st July 2015 – 30th June 2017

This work presents the use of a single membraneless vaporization unit (MBL-VP unit) in a flow system for concurrent determination of ethanol and total sulfite in white wine. The flow system comprises a MBL-VP unit with three cone-shaped reservoirs and two in-house detectors, a paired emitter-detector diodes (PEDD) and a capacitively coupled contactless conductivity detector (C4D). Wine sample is first acidified in the flow system. Then a 200-μLsample is delivered to the donor reservoir of the MBL-VP unit in which the two acceptor reservoirs contain 200-μL of the gas acceptor reagents, viz. acidic permanganate solution and deionized water. Vaporization of ethanol and SO_2(g) (converted from sulfite) from the donor into the acceptor reservoirs is carried out for 15 s. The acceptor solutions are then sequentially transferred by an air carrier stream for separate detection at the PEDD and C4D detectors. Decolorization of the permanganate solution from the reduction reaction with absorbed ethanol gives an increase in the PEDD signal, whereas dissolution of SO_2(g) in the water acceptor leads to an increase in conductivity detected by the C4D. Linear calibrations were obtained in the range of 5.0-15.0 % (v/v) for ethanol ($\Delta V = ((4.0\pm0.03)\times10^{-2})x-((1.85\pm0.35)\times10^{-2})$: $r^2 = 0.999$) and 10-200 mg L^{-1} for sulfite ($\Delta V = ((0.64\pm0.015)\times10^{-2})x-((3.25\pm1.64)\times10^{-2})$: $r^2 = 0.999$). The analysis is rapid with total analysis time of 2.5 min. Percentage recoveries were 81-104% and 88-110% for ethanol and sulfite, respectively. The method was successfully validated with gas chromatography and iodometric titration for the determination of ethanol and sulfite, respectively.

Keywords : Membraneless vaporization; Ethanol; Sulfite; Paired emitter-detector diodes detector; Contactless conductivity detector

Objectives

1. To develop the a single MBL-VP unit with 3 reservoirs coupled with the flow system for concurrent determination of ethanol and total sulfite.
2. To develop a detection system consists of a PEDD detector for monitoring ethanol concentration and a C4D detector for monitoring total sulfite.
3. To use less toxic reagent ($KMnO_4$ reagent) and deionized water as gas acceptor liquids for analysis of ethanol and total sulfite, respectively.
4. To apply the developed 3 reservoirs MBL-VP unit couple with the flow system using PEDD and C4D detectors for concurrent determination of ethanol and total sulfite in white wine

1. Chemicals and reagents

Preparation of standard and reagent solution

All chemicals in this work were analytical reagent grade and solutions were prepared indeionized Milli-Q® water (resistivity 18.2 MΩ·cm). Stock standard sulfite solution was prepared by dissolving Na_2SO_3 (Merck Millipore, Germany) in 100.0 mL of 0.1% (w/v) Na_2EDTA (Fisher Scientific, UK) to give ca. 10,000 mg L^{-1} sulfite solution. The accurate concentration of this stock solution was determined by titration with standardized iodine solution. For calibration sulfite standard solutions were freshly prepared from the 10,000 mgL^{-1} sulfite stock solution by aliquoting appropriate volume to give a series of sulfite standards containing 0.1% (w/v) Na_2EDTA, 0.4 mol L^{-1} NaOH (Merck Millipore, Germany) and 10 % (v/v) ethanol. The concentration range of the sulfite standards was 10-200 mg L^{-1}. Working standards for ethanol (5-15 %(v/v)) were prepared by diluting absolute ethanol (99.5% (v/v) Merck Millipore, Germany) with a solvent comprising 0.1% (w/v) Na2EDTA, 0.4 mol L^{-1} NaOH and 100 mg L^{-1} sulfite.

The optimized acceptor solution for ethanol (0.8 mmol L^{-1} permanganate) was prepared by dissolving 0.032 g of potassium permanganate (Ajax Chemicals, Australia) in 25 mL of 4.0 mol L^{-1} H_2SO_4 (Lab-Scan, Thailand) and diluted ten-fold with 4.0 mol L^{-1} H_2SO_4. The acceptor solution for sulfite was deionized (DI) water.

Preparation of wine samples

The developed method was performed using seven white wine samples, which were purchased from a supermarket in Bangkok, Thailand. Sample pre-treatment was required by dilution by factor of two in 0.4 mol L-1 NaOH for degrading bound form of sulfite and in a

0.1% (w/v) EDTA for stabilizing sulfite solution. Samples were left to stand for 15 minutes before injecting into the flow system.

Preparation for recovery study

The recovery of ethanol was studied by spiking absolute ethanol to obtain final concentration of 2.5 % (v/v) into samples. For recovery study of sulfite, 10,000 mg SO_2 L^{-1} sulfite stock solution was spiked into diluted sample to obtain the final concentration at 25 mg SO_2 L^{-1}. All solutions were prepared in 0.4 mol L^{-1} NaOH for degrading bound form of sulfite and 0.1% (w/v) EDTA for stabilizing sulfite solution. Samples were left to stand for 15 minutes before injecting into the flow system.

2. Materials and methods

The 3-cone reservoir membraneless vaporization unit

The three-reservoir membraneless vaporization unit or “MBL-VP” unit is a cylindrical acrylic unit (60 mm-outer diameter and 45 mm-inner diameter) with adjustable screw lid (45 mm-diameter). The base of this unit consists of three small cone-shaped reservoirs for holding microliter volumes of donor and acceptors with the same size (12 mm-diameter and 8 mm-height with 300 μL-volume). The lid is screwed down towards the base when used. The bottom level of the lid controls the volume of the headspace above the three cone-shape reservoirs. An air vent hole was drilled for release the pressure inside unit. At the bottom of a reservoir, there are drilled channels for connecting the cone reservoirs to the flow system with ports located at the bottom of each reservoir. The ports are the inlet and outlet. The schematic diagram of MBL-VP unit is shown in Figure 1. Diffusion of volatile compounds takes place across the headspace above the reservoirs. The headspace volumes are adjustable in a range of 4.8 - 39.8 mL by screwing the lid to change the height of headspace. A tiny hole was drilled above the acceptor reservoirs for fitting a small tube (1.0 mm-i.d. with 20 cm-length) to regular air vent flow.

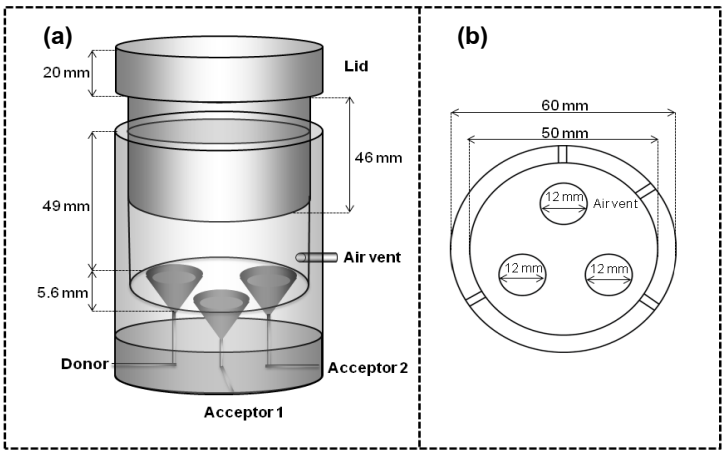

Figure 1 Schematic diagram of the three-reservoir MBL-VP unit. Side view of three-dimensional MBL-VP unit (a) and top view of the MBL-VP unit (b).

Fabrication of PEDD flow-cell detector

A simple flow-cell optical detector was constructed based on 'paired emitter-detector diode' or PEDD. In this research, PEDD photometric detector was employed for monitoring the decolorization of permanganate after reacted with ethanol. PEDD consists of two LEDs aligned opposite to one another across a flow channel as shown in Figure 2a and 2b. The two LEDs are placed on a flow cell holder (Figure 2c) to direct the light into the flow cell. In this work, two indium gallium nitride light emiiting diode (InGaN LED) with wavelength of 525 ± 35 nm (green light emission LED, Kingbright, Taiwan) was used. A flow-through cell with 10-mm pathlength (178.010-QS, Hellma Analytics, German) was placed between the two LEDs. The first LED acts as light source connected to 100 Ω resister as a current-limiting load to the LED. The potential of 8 V was applied to LED emitter by DC power supply (BEST, model PS-1502DD, China). The second LED acts as the light detector. The digital multimeter with 10^{12} Ω impedance (Fluke, model 8845A, USA) is used for measuring the current from the LED detector. The more light illuminating the LED detector, the high the output voltage. LabVIEW program was used to record the signal data on a personal computer.

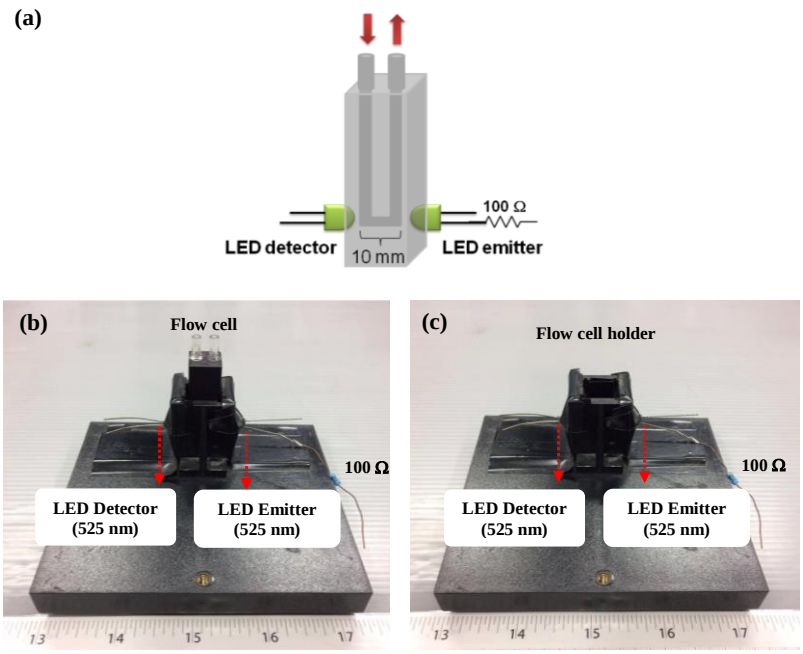

Figure 2 Schematic drawing of PEDD flow-cell detector (a), photograph of PEDD (b), and photograph of the PEDD flow cell holder (c).

In-house C4D

C4D is frequently used as a detector in separation technique especially capillary electrophoresis. In this work, we adopted and built an in-house C4D for quantitative analysis. The in-house C4D employed in this work is shown in Figure 3a and 3b. Two silver electrodes (high purity silver paint, SPI Supplies, USA) was installed on the outer surface of insulating tube to construct C4D cell with electrodes in cylindrical alignment. The length of each electrode is 1 cm. The two metal electrodes are separated with a gap of 1 mm. In this work, PEEK (polyether ether ketone tube, Upchurch Scientific) tube with internal diameter (i.d.) of 1 mm was employed as an insulator. Thus, the test solution is separated from the electrodes by a thin layer of insulating wall tube. C4D cell was placed inside a metallic box. One of silver electrode was used as the input electrode connected to the function generator. The other second electrode is as the output electrode connected to pre-amplifier applied for AC current measurement.

Figure 3b illustrates a photograph of instrumental setup of the C4D cell used in this work for detection of sulfite. A function generator was employed for applying sinusoid voltage of 20 V with a frequency of 20 kHz to the C4D cell. The response signal obtained from the C4D cell was processed by an amplifier and a rectifier. Then, the resulting signal was monitored by a digital multimeter.

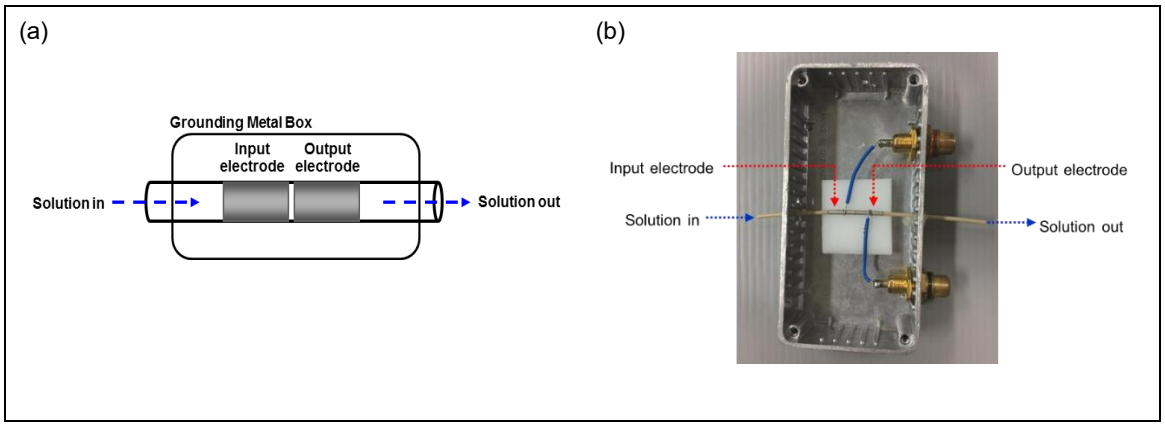

Figure 3 The schematic illustrations of drawing C4D (a) and photograph of C4D (b).

Flow system and operation

The design of the flow system employed in this work is shown in Figure 4. The system is divided into two sections comprising the flow injection analysis (FIA) section and the sequential injection analysis (SIA) section, respectively. The MGC-MPV LMPro (version 5.2) software was used for controlling all the electrical components. The FIA unit is used for sample on-line acidification and transportation of the acidified sample into the MBL-VP unit.

The SIA section is used for handling of the liquid acceptor zones into and out of the MBL-VP unit. PTFE tubing (1.0 mm i.d., VICI, Switzerland) is employed for all the flow lines.

As shown in Figure 4, the FIA section comprises two peristaltic pumps, PP1 and PP2 (Ismatec, Switzerland), a 6-port injection valve IV (model 5020, Rheodyne, USA) with a 200 μL sample loop and a 4-port switching valve SV (model V-101D, Upchurch Scientific, USA). Tygon^{TM} pump tubes were used for PP1 and PP2. The SIA section comprises two sets of SIA with syringe pumps SP1 and SP2 (Hamilton-MVP, Japan), each fitted with a 5.00-mL syringe barrel, selection valves SLV1 and SLV2, PTFE holding coil (1.5 mm i.d., 188 cm in length) and the PEDD and C4D detectors. For the PEDD, two green LEDs (525 ± 35 nm, Kingbright, Taiwan) were used with a 10 mm flow-through cuvette cell (Figure 2). LabVIEW 8.0^{TM} software was used for recording the PEDD signal. This cell was employed for measuring ethanol in the acceptor solution. The C4D flow cell is a PEEK tubing (1 mm i.d., 1.6 mm o.d., 15 cm length) with two silver painted electrodes (Figure 3). An AC potential (20 Vpp, 20 kHz) is fed into one electrode from a function generator (GW Instek, SFG-2104, Taiwan). The AC current flowing between the two electrodes is monitored at the second electrode. The current is amplified and rectified by a custom built electronics unit (Bangkok High Lab Co., Ltd., Thailand). The final DC voltage output, proportional to the AC current, is recorded by a signal recorder (e-corder 210, eDAQ, Australia). This cell was used for the determination of sulfite dissolved in the water acceptor.

The flow system in Figure 4 is operated according to the optimized sequence in Table 1. A precise volume of the liquid acceptors (200 μL of acidic permanganate and deionized water) is introduced simultaneously into the acceptor reservoirs A1 and A2 using the two SIA modules. At the same time as these acceptor aliquots are introduced into the cones A1 and A2, the streams of sample and 1.5 M H_2SO_4 solution are merged and mixed employing pump PP1 of the FIA unit. This acidified stream of sample flows into the 200-μL IV loop. This 200-μL plug of acidified sample is then injected into an air carrier stream and drawn into the donor cone D of the MBLVP unit using the peristaltic pump PP1. After vaporizing and diffusion from the donor for 15 s, the two acceptor solutions are simultaneously withdrawn from cones A1 and A2 to the PEDD and C4D detectors, respectively, again using the two SIA modules. At the same time, the pump PP2 and the switching valve SV of the FIA section are operated to withdraw the spent 200-μL sample from the donor cone D to waste W2. PP2 is left on for 90 s for evacuating the residual gas from the headspace of the MBL-VP unit to waste W2. This operating cycle takes 2.5 min. Before starting the next cycle, a cleaning cycle is operated using the same procedure as shown in Table 1 but with the sample bottle in the FIA section replaced by a bottle of deionized water. For cleaning the flow lines in the SIA sections, 200 μL volumes of permanganate solution and deionized water are delivered into cones A1 and

A2. After the cleaning process, analysis of the next sample is carried out according to the procedure in Table 1.

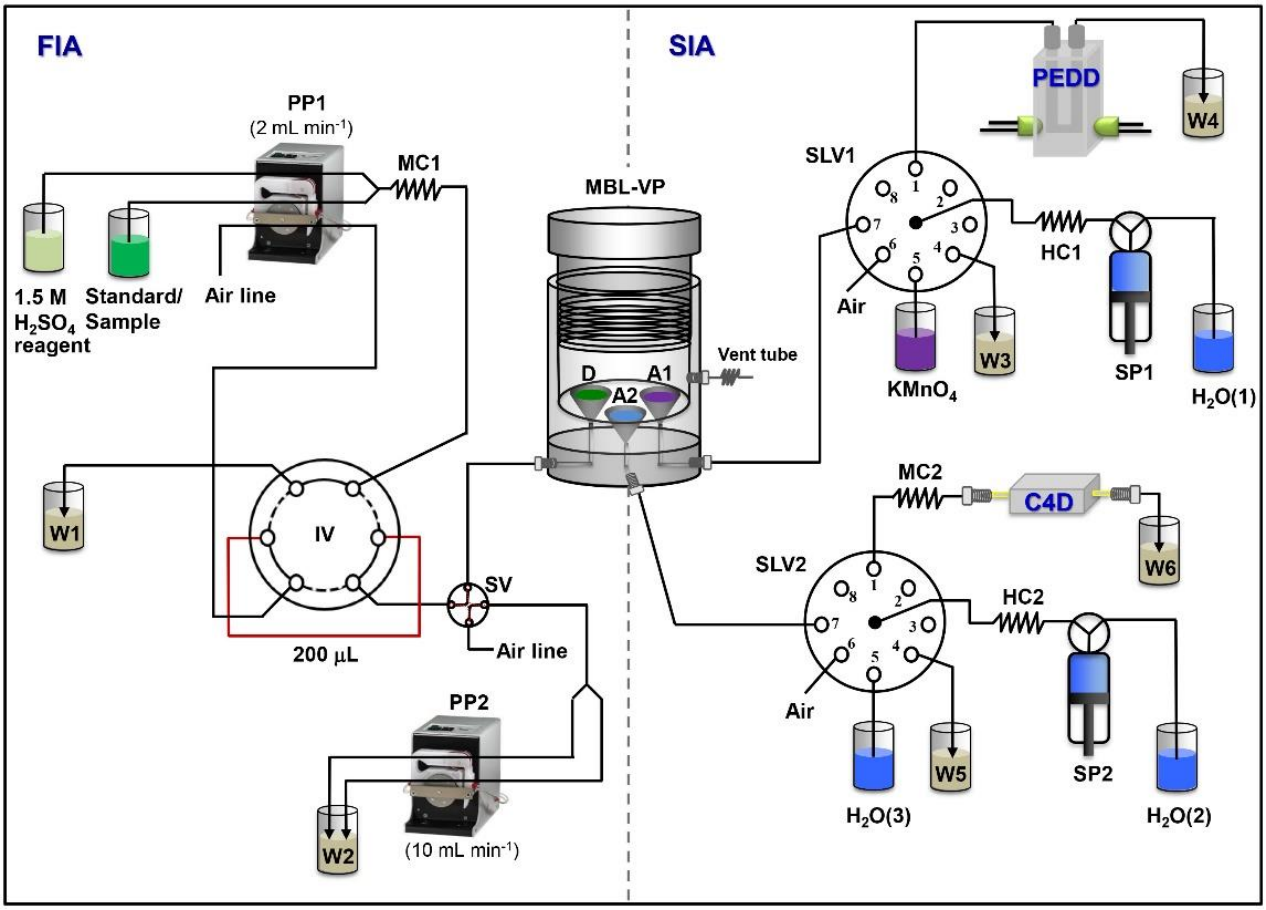

Figure 4 Schematic illustration of the flow manifold for simultaneous analysis of ethanol and sulfite. The system consists of a FIA section and a SIA section connected to the membraneless vaporization (MBL-VP) unit. SP1, SP2: syringe pump; PP1, PP2: peristaltic pump; IV: injection valve; SV: switching valve; D: donor reservoir; A1, A2: acceptor reservoir; SP: syringe pump; W1-W6: waste; MC1, MC2: mixing coil; HC: holding coil; PEDD: Paired Emitter-Detector Diodes Detector; C4D: Capacitively Coupled Contactless Conductivity Detector.

Table 1 Operation procedure of the MBL-VP unit in conjunction with the two flow systems.

Step	FIA section					SIA section				
	Action	PP1	PP2	IV position	SV position	Action at SP1	SP1[a] (Flow rate)[b]	Action at SP2	SP2[a] (Flow rate)[b]	Duration (s)
1	Draw out vapor from MBL-VP unit	OFF	ON	Load	To W2	• Aspirate 1,500 μL of H_2O(1) into syringe	↓ (12)	• Aspirate 1,500 μL of H_2O(2) into syringe	↓ (12)	7.5
2	Draw out vapor from MBL-VP unit	OFF	ON	Load	To W2	• Aspirate 165μL air into HC1 • Changing SLV1 position	↓ (3)	• Aspirate 165 μL air into HC2 • Changing SLV2 position	↓ (3)	3 3
3	Mixing of sample and H_2SO_4 into 200 uL injection loop by PP1	ON	OFF	Load	To W2	• Aspirate 200 μL $KMnO_4$ into HC1 • Changing SLV1 position	↓ (3)	• Aspirate 200 μL H_2O(3) into HC2 • Changing SLV2 position	↓ (3)	4 3
4	Mixing of sample and H_2SO_4 into 200 uL injection loop by PP1	ON	OFF	Load	To W2	• Dispense 365μL of air and $KMnO_4$ zone to A1 reservoir	↑ (3)	• Dispense 365 μL of air and H_2O(3) zone to A2 reservoir	↑ (3)	7 3
5	Inject donor zone to rest in reservoir D	ON	OFF	Inject	To D reservoir	• $KMnO_4$ zone rest in • A1 reservoir	hold	• H_2O(3) zone rest in • A1 reservoir	hold	15
6	Sample held in reservoir D	OFF	OFF	Load	To D reservoir	• Gas diffusion process for A1:	hold	• Gas diffusion process for A2:	hold	15
7	Sample held in reservoir D	OFF	OFF	Load	To D reservoir	• Draw the following zone from A1 into HC1: (130 μL HP air+200 μL $KMnO_4$+165μL air in line) • Changing SLV1 position	↓ (3)	• Draw the following zone from A2 into HC2: (130 μL HP air+200 μL $KMnO_4$+165 μL air in line) • Changing SLV2 position	↓ (3)	10 3
8	Draw out donor solution and vapor from MBL-VP unit	OFF	ON	Load	To W2	• Send the following zone to PEDD: (130 μL HP air+200 μL $KMnO_4$+165μL air in line) and clean tube line to W4	↑ (1.8)	• Send the following zone to C4D: (130 μL HP air+200 μL $KMnO_4$+165 μL air in line) and clean tube line to w6	↑ (1.8)	16.5
9	Draw out vapor from MBL-VP unit	OFF	ON	Load	To W2	-	hold	-	hold	60

Total analysis time 2.50 min

[a] (↓) syringe piston down; (↑) syringe piston up. [b] flow rate with a unit of mL min^{-1}

PP1, PP2: peristaltic pump1 (2 mL min^{-1}), peristaltic pump2 (10 mL min^{-1}). IV: injection valve. SV: switching valve. SLV: selection valve D: donor reservoir. A: acceptor reservoir. SP: syringe pump. W1, W4, W6: waste. HC: holding coil. HP: headspace. PEDD: Paired Emitter-Detector Diodes Detector. C4D: Capacitively Coupled Contacless Conductivity Detector.

3. Results and discussion

Signal profile and zone sequence

The flow system in Figure 4 was set-up and used for preliminary tests. Initial optimization was carried out to obtain conditions giving reasonable signal profiles of ethanol and sulfite.

The first test was carried out using standard ethanol solution (see Section 2.1) or a negative control solution (100 mg L^{-1} sulfite, 0.1% (w/v) Na_2EDTA and 0.4 mol L^{-1} NaOH) as the donor. The procedure in Table 1 was employed. Although sulfite signal at the C4D was not recorded in this experiment, cone A2 was loaded with the sulfite acceptor (200 µL of deionized water) for ensuring that the system was operated at constant pressure inside the MBL-VP unit. The negative control solution was first introduced into the donor cone D of the MBL-VP unit, in which a 200 µL zone of the permanganate acceptor (0.8 mmol L^{-1} $KMnO_4$ in 4.0 mol L^{-1} of H_2SO_4) was held in cone A1. After 15 s, the acceptor zone was pushed through to the PEDD for signal recording. The second cycle was then operated following the procedure in Table 1 using 10 % (v/v) ethanol standard solution. We obtained signal profiles for the negative control and standard ethanol as shown in Fig. 3a. The figure also shows the sequence of the air, acceptor and water plugs from acceptor cone A1 as they reside in the SIA holding coil HC1 (see Fig.2). It should be noted that the signal (V, in volt) from the PEDD decreases linearly with increasing absorbance of a sample. Thus for the ethanol determination, decrease in the permanganate concentration in the acceptor A1 leads to higher PEDD signal. As expected, the height of the blank ($HA1_B$) is smaller than the height for 10% (v/v) ethanol ($HA1_S$) since the reduction reaction of $MnO_{4\,(aq)}^-$ by absorbed ethanol vapor leads to decrease of the purple color in the acceptor zone and subsequent increase in the PEDD signal. We also tested the flow system with a series of ethanol standard solutions (2.5 - 12% (v/v) ethanol). The difference in the signal heights between the standard and the negative control was used for the constructing of the calibration plot. A linear calibration was satisfactorily obtained from this preliminary work [$((HA1_S - HA1_B),\Delta V) = ((3.32\pm0.07)\times10^{-2})$ %(v/v) ethanol + $((0.21\pm0.5)\times10^{-2})$: $r^2 = 0.999$].

A second experiment was carried out using the same flow system in Figure 4 for a series of sulfite standard solutions (25-200 mg L^{-1}). The operating procedure in Table 1 was used. In an operating cycle, 200-µL aliquot of deionized water was delivered into the acceptor cone A2 for 15 s to trap the SO_2 gas. Although the ethanol signal was not recorded in this experiment, cone A1 was loaded with 200-µL aliquot of the permanganate acceptor. Figure 5b shows the sequence of the air, acceptor and water plugs from acceptor cone A2 as they

reside in the SIA holding coil HC2 (see Figure 4) and examples of signal profile of the negative control sample (10% (v/v) ethanol, 0.1%(w/v) Na_2EDTA and 0.4 mol L^{-1} NaOH) and a 100 mg L^{-1} sulfite standard solution. The signal ($HA2_B$) for the negative control sample was not different to the signal of the deionized water. Compared to the blank signal, $HA2_B$, the signal for the 100 mg L^{-1} standard $HA2_S$ was 7.5 times greater (note the comment concerning the scale for $HA2_B$ in Figure 5b) indicating there was a significant amount of SO_2 gas converted from sulfite trapped by the water acceptor. It was found that the calibration of sulfite was also linear over the concentration range of the standards employed: $((HA2_S - HA2_B), \Delta V) = ((0.65\pm0.02)\times10^{-2})$ mg L^{-1} sulfite – $((3.81\pm1.50)\times 10^{-2})$: $r^2 = 0.999$.

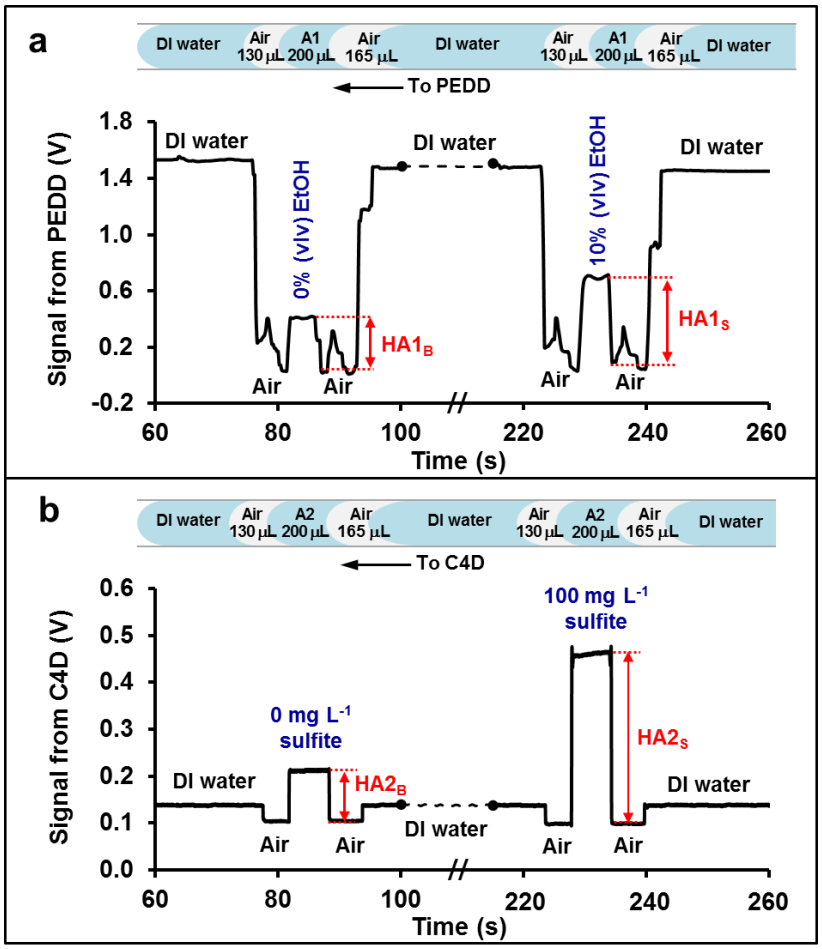

Figure 5 Examples of signal profiles obtained (a) from PEDD for permanganate measurement from acceptor A1 (0 and 10% (v/v) ethanol) and (b) from C4D for water conductivity from acceptor A2 (0 and 100 mg L-1sulfite).

Note: C4D signals for 0 mg L-1 sulfite are displayed at 1.5 times greater than the actual voltage.

Investigation of effect of ethanol and sulfite contents on acceptor solutions

Our method employs a separate series of standard solutions for ethanol and sulfite, respectively. However the presence of other volatile compounds in a sample can affect vaporization efficiencies of each volatile species. Most wines contain ethanol from 7 to 21 % (v/v) and the amount of ethanol will affect the sensitivity of the sulfite analysis. We investigated, using the flow system in Figure 4, standard sulfite solutions containing various amount of ethanol from 0 – 20 % (v/v), respectively. It was found (data not shown) that the sensitivity of analysis increased as the ethanol content was raised from 0 to 5%, but remained constant for ethanol concentration $\geq$ 5 % (v/v). We therefore prepared the sulfite standards in 10 % (v/v) ethanol.

We also investigated the effect of sulfite on the vaporization of ethanol. We did not observe (data not shown) any significant change in the PEDD signal for a 10% (v/v) ethanol standard with addition of sulfite from 25 to 200 mg L^{-1}, respectively.

Optimization

- Reagent concentration

For the system in Figure 4 measurement is performed after a fixed time, not at the equilibrium state. Thus concentrations of the reactants, $KMnO_4$ and H_2SO_4, employed for the ethanol analysis are crucial. Analysis using a series of ethanol standard solutions (5-15 % (v/v)) were carried out for various concentrations of $KMnO_4$ and H_2SO_4 of the acceptor solution.

The results in Figure 6a show that the sensitivity of the ethanol analysis increased when the concentration of $KMnO_4$ was raised from 0.2 to 0.6 mmol L^{-1}, reaching a constant value at 0.8 mmol L^{-1} permanganate. Thus for the routine procedure the concentration of $KMnO_4$ in the acceptor solution was set at 0.8 mmol L^{-1}. The oxidation of ethanol by permanganate also requires acidic condition. The results in Fig. 4b show that that the sensitivity of analysis increased with increasing acid concentration, especially when the concentration of H_2SO_4 was increased from 3 to 4 mol L^{-1}. For optimal sensitivity, permanganate was prepared in 4 mol L^{-1} H_2SO_4.

The concentration of H_2SO_4 in the reagent stream in the FIA section (Figure 4) was also optimized. The role of H_2SO_4 is important for conversion of the so-called 'sulfite preservatives' to SO_2 gas. In the optimization study, a sulfite standard solution at 100 mg L^{-1} was merged to mix with a stream of solution of H_2SO_4 at various concentrations using the flow injection (FIA) system in Figure 4. The results in Figure 6c clearly show that without the acid, *i.e.* merging the standard sulfite with a stream of deionized water, the C4D signal was

low (~0.32 volts). The signal increased approximately 1.75 times when using 1 mol L^{-1} H_2SO_4. However when employing 2 mol L^{-1} H_2SO_4 the signal observed was not significantly different, indicating sufficient excess of acid was already achieved with 1.5 mol L^{-1}. We therefore chose 1.5 mol L^{-1} H_2SO_4 in the reagent stream of the FIA section in Figure 4 to ensure sufficient excess acid.

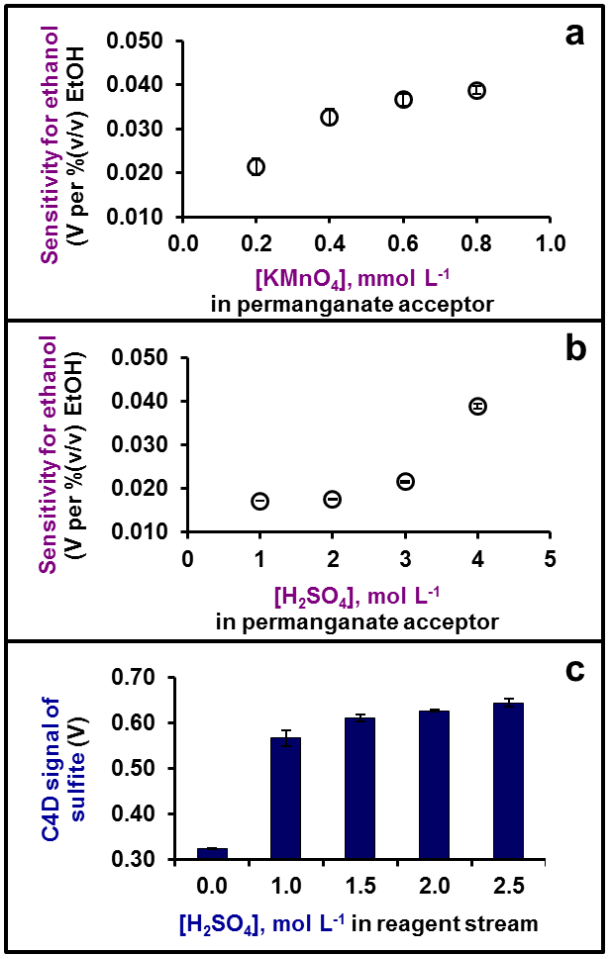

Figure 6 Results of optimization of reagent concentration. (a) [$KMnO_4$] in permanganate acceptor, (b) [H_2SO_4] in permanganate acceptor and (c) [H_2SO_4] in FIA reagent.

- Volume of donor, acceptor and headspace

The volumes of donor and acceptor solutions were optimized. As shown in Figure 1, each cone has a diameter of 12 mm and a height of 8 mm. The calculated volume of the cone is just over 300 µL. The donor volume was thus varied from 100 to 350 µL. Although 300 µL is the capacity of the cone it was found that a cone could accommodate a slightly larger volume of a liquid. When loading 350 µL of liquid we observed a slight concave shape of the liquid surface above the cone which remained stable.

The effect of the volume of donor was studied using samples containing both ethanol and sulfite. We operated the flow system in Figure 4 using the optimized acceptor solutions

as described previously with the acceptor volumes fixed at 200 µL. A standard solution containing 10% (v/v) ethanol and 100 mg L^{-1} sulfite in 0.1% (w/v) Na_2EDTA and 0.4 mol L^{-1} NaOH was loaded into cone D. The procedure in Table 1 was modified in order to introduce various volumes of this standard into cone D. The results are shown as bar graphs in Figure 7a and Figure 7b for ethanol and sulfite, respectively. The solid purple bars in Figure 7a are the PEDD signals obtained for the donor standard solution at various volumes. The larger the bar graphs the smaller is the absorbance of permanganate solution, *i.e.* larger amount of ethanol vapor was adsorbed by the acceptor solution, leading to greater decolorization of the permanganate. The signals (purple striped bar) for samples without ethanol (*i.e.* negative ethanol control) are not different indicating that none of the constituents in the negative control contributed to the decolorization of the permanganate. The difference in signal heights (ΔV) between the ethanol standard and negative control are also shown in Figure 7a. As expected, when we increased the donor volume from 100 to 350 µL, ΔV for ethanol significantly increased from 0.17 to 0.52 V.

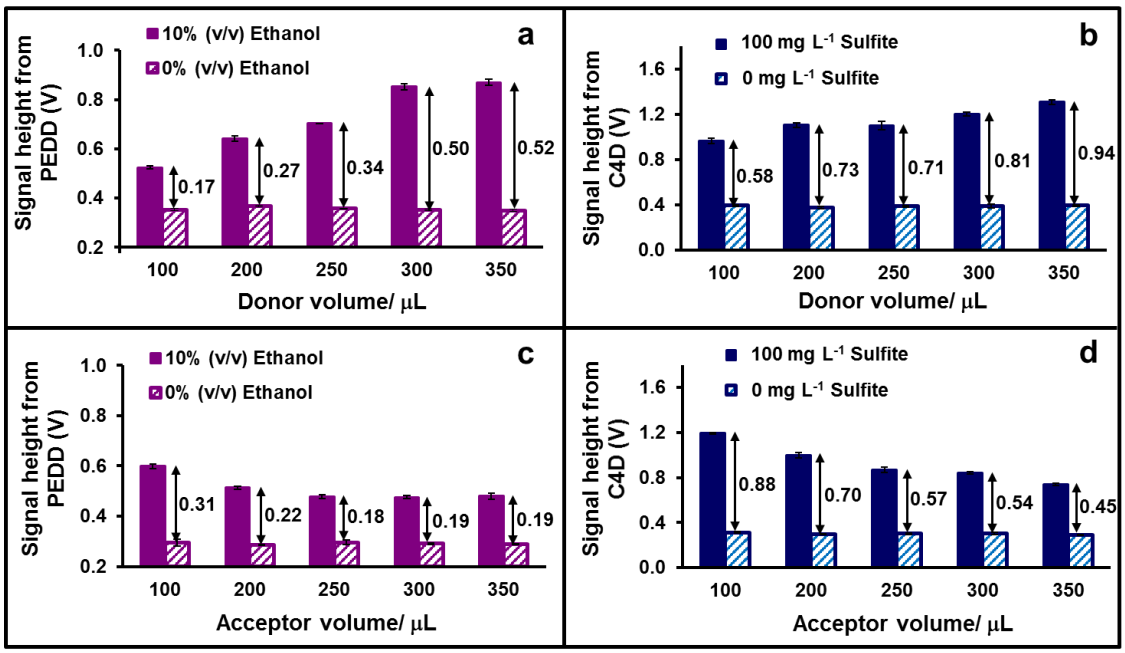

Figure 7 Bar graphs of the effect of volume of donor on (a) PEDD signal and (b) C4D signal and the effect of acceptor volume on (c) PEDD signal and (d) C4D signal. The PEDD data are for standard ethanol (10% (v/v)) and its negative control (0% (v/v)). The C4D data are for the standard sulfite (100 mg L^{-1}) and its negative control (0 mg L^{-1}).

Figure 7b shows the results for the sulfite measurements where the bar graphs are the signals from the C4D detector. The larger the signal means the greater the amount of SO_2 adsorbed in the deionized water acceptor. The signals for the negative control sulfite samples are almost constant indicating that other volatile components in the sample (*e.g.* ethanol) have little effect on the conductivity of the water. Increasing the donor volume leads to increase in the sulfite detection. In this work, the donor volume of 200 µL was selected as it provided sufficient sensitivity for wine analysis.

The operating procedure in Table 1 was also modified to investigate the effect of the acceptor volume. The volumes of acceptor solutions in cone A1 (acidic permanganate) and in cone A2 (deionized water) were varied from 100 to 350 µL. The donor volume was fixed at 200 µL. Results in Figure 7c and Figure 7d show that the difference in signal (ΔV) between the standard solution and the negative control decreased as the acceptor volumes were increased, both for ethanol and sulfite. This decrease is due to dilution effect. Using a fixed donor volume and fixed diffusion time, there would be the same quantity of the volatile gases adsorbed by the acceptor solutions. This amount of gas would become more diluted as it dissolves into increasing acceptor volume from 100 to 350 µL, respectively. Although a volume of 100 µL gave the largest signal we did not choose this volume since it was sometimes observed that breakage of the acceptor zone occurred during the SIA procedure. Therefore 200 µL was selected as the optimum acceptor volume for both the permanganate solution and deionized water.

The headspace volume is also another factor that may affect the sensitivity of the analysis. Measurements were carried out using a standard mixture containing 10% (v/v) ethanol and 100 mg L^{-1}sulfite in 0.1% (w/v) Na_2EDTA and 0.4 M NaOH. Operation of the flow system in Figure 4 followed the procedure in Table 1. The donor volume and acceptor volumes were fixed at 200 µL. Signals for ethanol and sulfite of the standard and negative controls were measured for headspace volumes from 4.8 to 17.5 mL, respectively. Results (data not shown) show that there was no significant change in the observed signal with headspace volume. Large headspace volume would however require longer suction time to remove the residue sample gases. But higher imprecision in the measurements (1.0 - 3.24% RSD) were observed for both ethanol and sulfite for volumes less than 11 mL. This may be due to a buildup of pressure within the headspace. We therefore chose 11 mL as the optimum headspace volume, as at this volume the %RSD was less than 1 for both ethanol (0.92 %RSD) and sulfite (0.95 %RSD), respectively.

- Diffusion time

Diffusion time is defined as the time selected for vaporization of ethanol and sulfite from the donor reservoir and diffusion across the headspace to the acceptor reservoirs A1 and A2, respectively. Measurement of the diffusion time commences at the completion of the the loading of the donor into reservoir D (step 6 in Table 1). As expected, the sensitivity of the ethanol analysis improved when increasing the diffusion time from 10 to 30 s (see Table 2). The sensitivities for sulfites were comparable for diffusion times of 10 and 15 s but improved as the diffusion time was increased from 15 to 60 s. However the linear working range decreased with increased diffusion time. A diffusion time of 15 s was selected since it provided sensitivity of analysis, linear working range and analysis times suitable for wine samples.

Table 2 Effect of diffusion time on the sensitivity of ethanol and sulfite analysis[a]

Diffusion times/s	Ethanol			Sulfite			Analysis time/ min
	Working range/ %(v/v)	calibration curve	r^2	Working range/ mg L^{-1}	Calibration curve	r^2	
10	2.5-17	$y = (2.9\pm0.43)\times10^{-2}x + (3.73\pm0.49)\times10^{-2}$	0.999	25-200	$y = 0.7\pm0.015)\times10^{-2}x - (6.23\pm1.87)\times10^{-2}$	0.999	2.42
15	1.0- 15	$y = (3.9\pm0.67)\times10^{-2}x - (0.49\pm0.57)\times10^{-2}$	0.999	10-200	$y = (0.64\pm0.015)\times10^{-2}x - (3.25\pm1.64)\times10^{-2}$	0.999	2.50
30	0.5-12	$y = (4.5\pm1.1)\times10^{-2}x - (0.40\pm0.73)\times10^{-2}$	0.998	10-150	$y = (1.1\pm0.01)\times10^{-2}x - (1.83\pm0.86)\times10^{-2}$	0.999	2.75
60		-		5-75	$y = (1.8\pm0.024)\times10^{-2}x - (2.6\pm1.03)\times10^{-2}$	0.999	3.25

[a] Standard ethanol and sulfite were prepared separately. All ethanol standards contain sulfite at 100 mg L-1. All sulfite standards contained 10% (v/v) ethanol.

- Analytical feature

Using the optimum condition, calibration curve is linear in the range of 5.0-15.0 % (v/v) for ethanol (*e.g.,* $\Delta V = ((4.0\pm0.03)\times10^{-2})$ % (v/v) ethanol – $((1.85\pm0.35)\times10^{-2})$: r^2 = 0.999)) and 10-200 mg L^{-1} sulfite (*e.g.*, $\Delta V = ((0.64\pm0.015)\times10^{-2})$ mg L^{-1} sulfite – $((3.25\pm1.64)\times10^{-2})$: r^2 = 0.999), respectively. These working ranges are suitable for the levels of ethanol

and sulfite found in most white wines. The system provides a lower limit of quantitation (LLOQ = 3SD of intercept/slope) of 0.26 % (v/v) ethanol and 7.68 mg L^{-1} sulfite, respectively. The system also provides satisfactorily good precisions with %RSD less than 1 for both ethanol (%RSD = 0.24 - 0.92, n = 6) and sulfite (%RSD = 0.11 - 0. 95, n=6), for analysis of a standard mixture containing 10% (v/v) ethanol and 100 mg L^{-1} sulfite. The method provides rapid analysis with sample throughput of 24 samples h^{-1} (analysis time = 2.5 min).

- Wine analysis and validation

This proposed method was applied to the determination of ethanol and sulfite contents in seven samples of white wine as shown in Table 3. Ethanol contents obtained from our method were compared with a gas chromatographic method with flame ionization detector (GC-FID). Sulfite contents were conpared with an iodometric titration method. According to paired *t*-test, ethanol contents obtained from our method are not significantly different to the contents as obtained from the GC-FID method (t_{stat} = 1.24, $t_{critical}$ = 2.45 at P = 0.05). Similarly for the sulfite analysis, there is no significant difference between the results obtained from our method and the iodometric method (t_{stat} = 0.40, $t_{critical}$ = 2.45 at P = 0.05).

We also analyzed four samples of red wines. However the results were significantly different from values obtained using the comparison method. For the ethanol analysis, the values obtained from our method were slightly greater than that using GC-FID method, ranging from 2.9 to 10.1%. As for the sulfite data, results from our method did not agree well with the results from the iodometric method. There was also no clear trends in the differences between the two methods. These results suggest that volatile matrices of red wine are more complicated than in white wines. Further investigation is needed for analysis of red wines using this developed method.

Table 3 Determination of ethanol and total sulfite in white wines by the developed method and by comparison methods.

Diffusion times/s	Ethanol			Sulfite			Analysis time/ min
	Working range/ %(v/v)	calibration curve	r^2	Working range/ mg L^{-1}	Calibration curve	r^2	
10	2.5-17	$y = (2.9\pm0.43)\times10^{-2}x + (3.73\pm0.49)\times10^{-2}$	0.999	25-200	$y = (0.7\pm0.015)\times10^{-2}x - (6.23\pm1.87)\times10^{-2}$	0.999	2.42
15	2.0- 15	$y = (3.9\pm0.67)\times10^{-2}x - (0.49\pm0.57)\times10^{-2}$	0.999	10-200	$y = (0.64\pm0.015)\times10^{-2}x - (3.25\pm1.64)\times10^{-2}$	0.999	2.50
30	0.5-12	$y = (4.5\pm1.1)\times10^{-2}x - (0.40\pm0.73)\times10^{-2}$	0.998	10-150	$y = (1.1\pm0.01)\times10^{-2}x - (1.83\pm0.86)\times10^{-2}$	0.999	2.75
60		-		5-75	$y = (1.8\pm0.024)\times10^{-2}x - (2.6\pm1.03)\times10^{-2}$	0.999	3.25

4. Conclusion

We present development of a flow system suitable for quality control in production of white wine. The system offers concurrent determination of two important components required to be reported on the labels of wine, *viz.* ethanol and total sulfite. A membraneless vaporization unit with three cones was employed for simultaneous vaporization of ethanol and sulfur dioxide (produced from acidification of sulfite). Vaporization and diffusion inside the membraneless unit allows detection of the two volatile compounds adsorbed in selective acceptor liquids without interference from the wine matrices. The method is green chemistry employing environmentally friendly gas acceptors, *i.e.,* permanganate solution and deionized water for trapping vapors of ethanol and sulfur dioxide, respectively. This is also the first use of a PEDD for monitoring the decolorization of permanganate by ethanol. Sulfite is determined based on the change in conductivity of the water acceptor by adsorbed SO_2 using a C4D detector.

Output from this project research

Manuscript for submission to international journal

Title: Concurrent determination of ethanol and total sulfite in white wine using on-line membraneless gas-liquid separation flow system

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Volume 9 | Number 43 | 21 November 2017 | Pages 6085–6214

Analytical Methods

rsc.li/methods

ISSN 1759-9679

ROYAL SOCIETY OF CHEMISTRY

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Cite this: *Anal. Methods*, 2017, **9**, 6107

Green analytical flow method for the determination of total sulfite in wine using membraneless gas–liquid separation with contactless conductivity detection†

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A green analytical flow method was developed for the determination of total sulfite in white wine. The method employs the membraneless vaporization (MBL-VP) technique for gas–sample separation allowing direct analysis of wine. Sulfite in an aliquot of sample was converted to SO_2 gas *via* acidification. Dissolution of the gas into the water acceptor led to a change in the conductivity of the acceptor which was monitored using a 'capacitively coupled contactless conductivity detector' (C4D) flow cell. Only a minute amount of common acid (100 μL of 1.5 mol L^{-1} H_2SO_4) is used. The MBL-VP unit was incorporated into the flow system to separate the SO_2 gas from the wine sample using the headspace above the donor and acceptor compartments as a virtual membrane. The method provides a linear working range (10–200 mg L^{-1} sulfite) which is suitable for most wines with calibration equation $y = (0.056 \pm 0.002)x + (1.10 \pm 0.22)$ and $r^2 = 0.998$. Sample throughput is 26 samples h^{-1}. The lower limit of quantitation (LLOQ = 3SD of blank per slope) is 0.3 mg L^{-1} sulfite for 20 s diffusion time with good precision (%RSD = 0.8 for 100 mg L^{-1} sulfite, $n = 10$). We also present a simple modification of the MBL-VP unit by the addition of a third cone-shaped reservoir to provide two acceptor zones leading to improvement in sensitivity of more than three-fold without use of heating to enhance the rate of diffusion of SO_2.

Received 1st August 2017
Accepted 22nd September 2017

DOI: 10.1039/c7ay01879g

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1. Introduction

Sulfite is a common preservative used in wine production to stabilize the product by preventing oxidation and bacterial growth.[1,2] Apart from its use in wine production, sulfite is also used in other food industries to preserve food appearance, color and aroma. Although sulfite is considered not toxic to human health when employed as recommended, it has been found that there are some adverse effects for some individuals ingesting sulfite at certain levels. These effects include sudden allergic reactions with symptoms such as dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhea. Sulfite consumption by asthmatic patients can trigger bronchoconstriction.[3–5] Thus, the level of sulfite in food and beverages must be strictly controlled to the levels determined by the legislation of each country[6] or as set by the World Health Organization (WHO).[7] According to the WHO, the acceptable daily intake of sulfite (expressed as SO_2 equivalent) is 0.7 mg kg^{-1} body weight.

There have been various methods available for the determination of sulfite in food and beverages. The most common is the AOAC method which employs an optimized Monier-Williams method.[8] The method is laborious requiring an initial distillation process. After mixing the sample with hydrochloric acid, the sample mixture is refluxed to convert sulfite into SO_2 gas. Nitrogen gas is used to purge the generated SO_2 into a hydrogen peroxide solution for converting the SO_2 gas to sulfuric acid which is later titrated with a standardized sodium hydroxide solution. Apart from the AOAC titrimetric method with the distillation process, there are other methods that have been developed based on different principles including spectrometric methods,[9,10] chemiluminescent methods[11,12] and electrochemical methods.[13,14]

Automated methods for direct analysis of sulfite based on flow injection analysis (FIA) and FIA-related methods, with the use of an on-line gas-diffusion (GD) unit to separate SO_2 gas

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7ay01879g

from the sample matrix, have been developed by several groups.[6,11,12,15–18] The SO_2 gas generated from the sample diffuses from the donor stream through the porous hydrophobic membrane of the GD unit to dissolve into an acceptor stream flowing on the other side of the membrane. Detection of the dissolved SO_2 can be carried out in various ways including absorption spectrometry,[6,15,16] chemiluminescent spectrometry,[11,12] potentiometry,[17] and amperometry.[18] Although gas diffusion through a porous membrane has made quantitative analysis of sulfite simpler and more friendly to operate than the conventional AOAC method, it is known that use of the membrane-based gas–liquid separation has drawbacks such as contamination and clogging of the membrane surface, leading to the limited lifespan and subsequent replacement of the membrane. To overcome this problem we employed the so-called 'membraneless vaporization' (MBL-VP) technique to provide a more effective method for direct determination of total sulfite in wine.

The membraneless vaporization technique was first presented in 2006 (ref. 19) as an on-line technique for gas–sample separation in flow analysis. A MBL-VP unit comprises two separate reservoirs for the donor and acceptor liquids. The two regions are connected *via* an air headspace, which acts as a pseudo-membrane, allowing only volatile analytes to diffuse from the liquid donor phase to the acceptor phase. Donor and acceptor reservoirs have been designed and constructed in various configurations to fit the types of samples, such as liquid[19–24] or solid samples.[25,26] Most MBL-VP devices have been employed in continuous flow methods, such as FIA[19–21,25,26] or multisyringe flow injection analysis (MS-FIA).[24] For discontinuous flow or zone-fluidics (ZF) mode of operation,[27] Ratanawimarnwong *et al.*[22] in 2013 presented a new design of a MBL-VP unit for sequential injection analysis (SIA). The device consisted of two cone-shape reservoirs for holding donor and acceptor aliquots under a common headspace. Aeration at the donor reservoir was employed to accelerate the gas diffusion process.[22] Unlike the first design in 2006 of the membraneless extraction module,[28] MBL-VP units were designed for complete automation and are therefore suitable as on-line gas-separation devices for flow analysis systems. Donor and acceptor liquids are transported to and out from MBL-VP units *via* flow control.

In this work, we utilized the MBL-VP technique using the cone-shaped design for quantitative analysis of total sulfite in wine. The wine sample was acidified in-line. A 200 μL aliquot of the acidified sample was delivered into a cone reservoir (donor cone). Diffusion of SO_2 gas from the donor cone into the MBL-VP headspace and subsequent dissolution of the gas into the pure water acceptor resting in two other reservoirs (acceptor cones) led to a change in the pH of the water. This was monitored as a change in the conductivity of the acceptor liquids using a 'capacitively coupled contactless conductivity' detector (C4D detector).[29–32] To the best of our knowledge, this is the first time that sulfite has been determined using the concept of online MBL-VP for gas–liquid separation. C4D is also new for detection of sulfite in wine. Our method is considered as 'Green Analytical Chemistry' (GAC)[33] since the liquid acceptor is chemical reagent-free. Pure water was shown to be a suitable liquid acceptor. The only required chemical is for converting sulfite to SO_2 gas. Dilute sulfuric acid is used in only a small volume. This method generates no waste from employment of membranes. We also investigated an added feature of the cone-shaped reservoirs of the MBL-VP unit. A second cone-shaped acceptor reservoir was added to the original two-cone device,[22] thereby increasing the surface area of the acceptor zone with subsequent improvement in sensitivity and precision.

2. Experimental

2.1 Chemicals and reagents

All chemicals and reagents were analytical reagent grade and solutions were prepared in deionized Milli-Q® water (resistivity 18.2 MΩ cm^{-1}). Stock standard sulfite solution was prepared by dissolving Na_2SO_3 (Merck Millipore, Germany) in 100.0 mL of 0.1% (w/v) Na_2EDTA (Fisher Scientific, UK) to give *ca.* 10 000 mg L^{-1} sulfite stock solution. The accurate concentration of this stock solution was determined by titration with standardized iodine solution.

The working sulfite standards were freshly prepared from the 10 000 mg L^{-1} sulfite stock solution by aliquoting appropriate volumes to give a series of sulfite standards (10 to 200 mg L^{-1}). To each aliquot, 1.00 mL of 5% (w/v) Na_2EDTA (Fisher Scientific, UK), 5.00 mL of 4 mol L^{-1} NaOH (Merck Millipore, Germany), and 7.50 mL of 99.5% (v/v) ethanol (Merck Millipore, Germany) were added. Deionized water was added to each standard mixture to make a final volume of 50.0 mL.

2.2 Preparation of wine samples

Six wine samples were purchased from local supermarkets in Bangkok. A 100.0 mL sample of each wine sample was diluted with 150.0 mL of water. An aliquot of 38.75 mL of the diluted sample was accurately transferred into a glass bottle. To this sample 1.00 mL of 5% (w/v) Na_2EDTA (Fisher Scientific, UK), 5.00 mL of 4 mol L^{-1} NaOH (Merck Millipore, Germany), 5.00 mL of 99.5% (v/v) ethanol (Merck Millipore, Germany) and 0.25 mL of water were added. The sample mixture was mixed thoroughly before analysis for sulfite content using the developed method and a comparison method employing a membrane gas-diffusion system.[16]

For the recovery study, a sample was prepared according to the above procedure with addition of 0.25 mL of 5000 mg L^{-1} sulfite standard instead of 0.25 mL of water.

2.3 Membraneless vaporization unit with 3 cone-shaped reservoirs

The MBL-VP unit with the 3 cone-shaped reservoirs is shown in Fig. 1a. The unit is made of transparent acrylic polymer (Perspex®) and comprises a base section and an adjustable screw-top upper section. The current unit has 3 reservoirs instead of 2 reservoirs as in the original design (Fig. 1b).[22] Reservoir D is used for holding a plug of the donor (standard or sample). Reservoirs A1 and A2 are used for holding two separate plugs of the acceptor (deionized water). The volume of each cone reservoir is 260 μL. Therefore the maximum volume

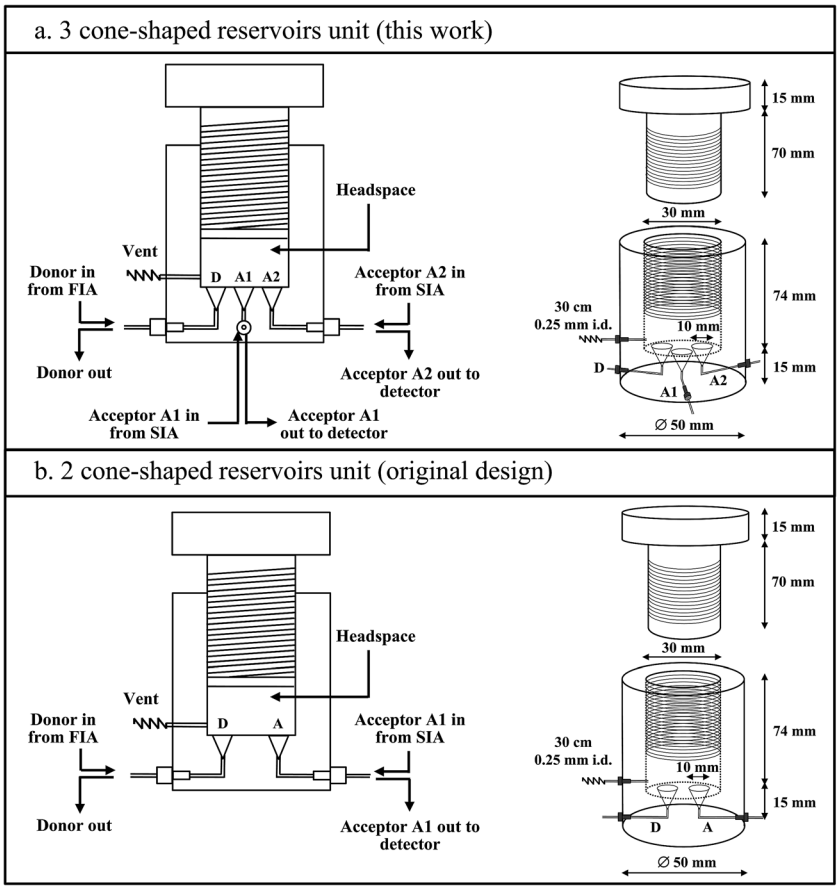


Fig. 1 Schematic diagrams (2-dimension and 3-dimension) of the new membraneless vaporization unit (a) with 3 cone-shaped reservoirs for improving the sensitivity as compared with the original unit (b) with 2 cone-shaped reservoirs. D: donor reservoir. A1, A2: acceptor reservoirs.

delivered into the cone reservoir is 200 μL to avoid overflow and subsequent cross-contamination. The volume of the headspace above the reservoirs can be varied from 2.82 to 10.60 mL by adjusting the number of turns of the screw-top lid. Similar to the previous design, a vent tubing (0.25 mm i.d., 30 cm long) was also fitted to the new unit (Fig. 1a and b) to prevent excessive build-up of pressure within the headspace.

2.4 The flow system and its operation

The schematic diagram of the entire MBL-VP flow system is shown in Fig. 2. The system is divided into two sections: the 'FIA section' (left frame, Fig. 2) and the 'SIA section' (right frame, Fig. 2). The MBL-VP unit connects the two sections. PTFE tubing (1.0 mm i.d., VICI, Switzerland) is employed in all the flow lines. The FIA section is used for the preparation of the donor solution and its transportation into and out of reservoir D of the MBL-VP unit. The SIA section is used to control the flow of the plugs of the two acceptor zones into and out of the reservoirs A1 and A2.

In the FIA section, peristaltic pump PP1 (Ismatec/ISM827, Switzerland) is used to acidify the standard/sample stream by merging with a flow of sulfuric acid (1.5 mol L^{-1}) to convert sulfite ion into SO_2 gas. In one analysis cycle, the system is operated using the steps shown in Table 1S.† In step 8 (Table 1S†), the acidified sample flowing through the 200 μL sample loop installed at the 6-port injection valve IV (Upchurch Scientific, USA) is delivered, *via* the selection valve SV (Upchurch Scientific, USA), into the donor reservoir D by the air carrier line of the peristaltic pump PP1.

In the SIA section, a commercial SIA system (FIAlab 3500, USA) with one syringe pump SP and a selection valve SLV1 was employed for the control of zone fluidics. The SIA section is used to meter and transport two water zones (100 μL each) into the acceptor reservoirs A1 and A2 in step 4 and step 8 (Table 1S†) for trapping the SO_2 gas diffusing from the donor reservoir.

The detection system of the acceptor plugs is a C4D unit.[29–31] The C4D flow cell is a PEEK tubing (1 mm i.d., 1.6 mm o.d.) with a total length of about 15 cm. Silver conductive ink (SPI Supplies, USA) was painted on the exterior of the tube to form two separate conducting bands. The two cylindrical silver bands (electrodes) are 0.2 ± 0.05 mm apart.[26] An AC potential (20 V_{pp}, 20 kHz) is fed into one electrode from a function generator (GW Instek, SFG-2104, Taiwan). The AC current flowing between the two electrodes is monitored at the second electrode. The current is amplified and rectified by a custom built electronics unit (Bangkok High Lab Co., Ltd, Thailand). The final output DC voltage, proportional to the AC current, is recorded by a signal recorder (e-corder 210, eDAQ, Australia).

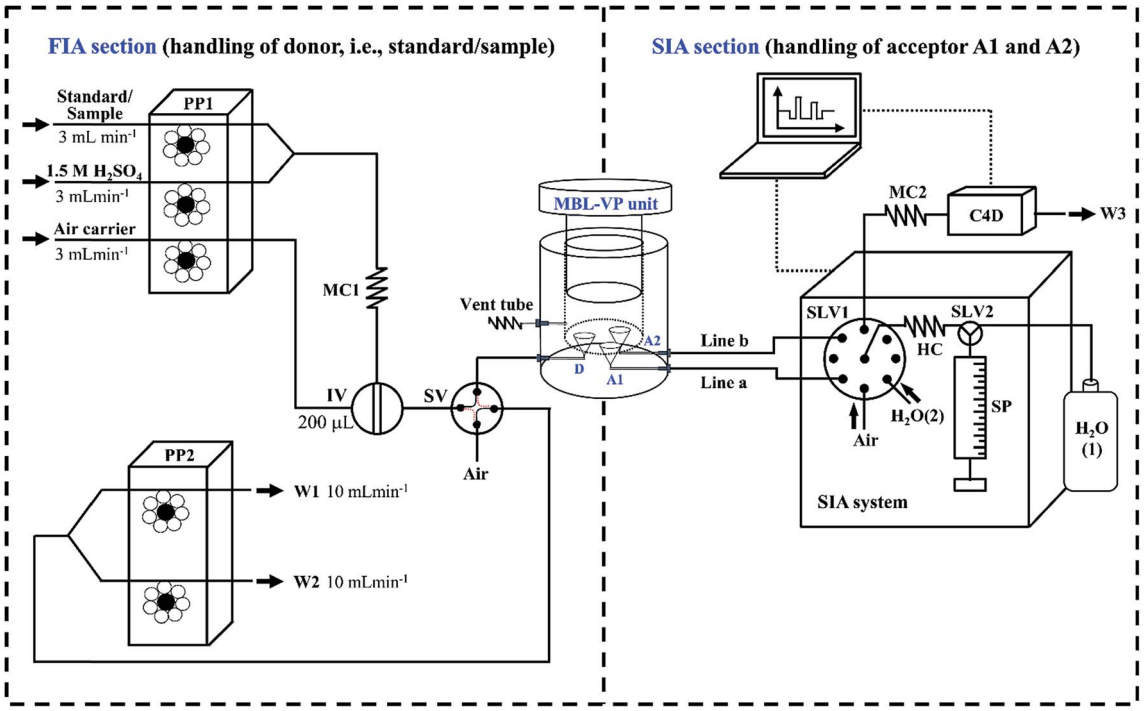


Fig. 2 The flow system with the new MBL-VP unit (3 cone-shaped reservoirs) for determination of sulfite in wine. PP1, PP2: peristaltic pumps, IV: injection valve, SV: switching valve, D: donor reservoir, A1, A2: acceptor reservoirs, SP: syringe pump, SLV1, SLV2: selection valves, W1, W2, W3: wastes, HC: holding coil (200 cm, 1 mm i.d.), MC1, MC2: mixing coils (100 cm, 1 mm i.d., 50 cm 1 mm i.d.), C4D: capacitively coupled contactless conductivity detector.

3. Results and discussion

3.1 Design of liquid handling and zone stack, signal profile and calibration

The design of the flow system is shown in Fig. 2. The flow system consists of both FIA and SIA sections. The FIA section is used for the handling of sample, generation of SO_2 gas and the delivery of the donor plug into the MBL-VP unit. The SIA section is employed to control the flow of the acceptor plugs (Fig. 2) into and out of the MBL-VP unit with liquid segments sequentially moved *via* use of the selection valve SLV1 and syringe pump SP. After holding the acceptor plug (water) in cone A1 for 10 s, this acceptor zone is first withdrawn from the MBL-VP unit to rest in the holding coil HC (step 10 in Table 1S†), while the acceptor plug (water) in cone A2 is still in the reservoir of the MBL-VP unit for further trapping of the SO_2 gas. The A2 aliquot is held inside the MBL-VP unit for a total of 20 s before withdrawing to rest inside the holding coil together with the zone from reservoir A1 (step 11 in Table 1S†). Fig. 3a shows the stack of zones of acceptors A1 and A2 with the three air plugs as they reside in the SIA holding coil HC. On pushing the zone stack from the holding coil into the PEEK tubing of the C4D detector, a sequence of two signals (with sulfite as the donor solution) from the acceptor plug A2 and the acceptor plug A1 is observed, as seen in Fig. 3b. Similar profiles for varying concentrations of sulfite standards are shown in Fig. 1S (in the ESI†). The sum of the peak heights ($H_{A1} + H_{A2}$ in Fig. 3b) was used for constructing the calibration graph. It was found that the calibration was

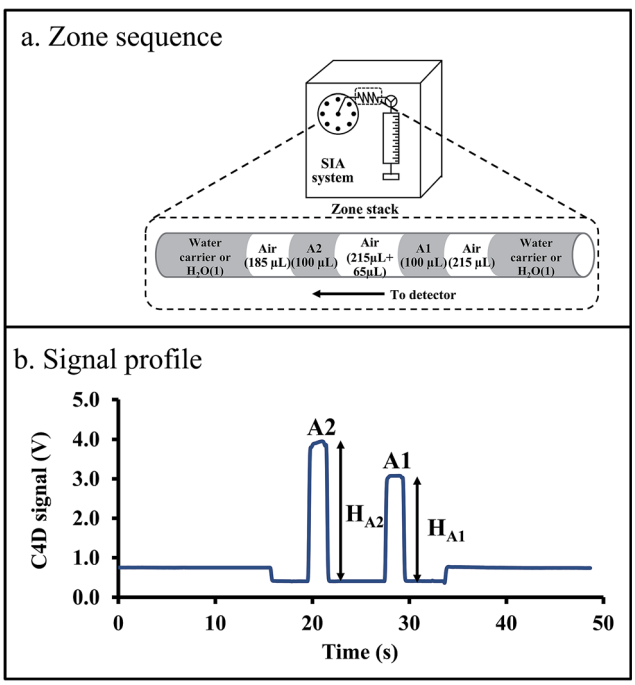


Fig. 3 (a) Zone sequence in holding coil HC of the SIA section and (b) example of a signal profile obtained from one cycle corresponding to the zone sequence, with signals of A1 and A2 and signal heights $H_{A1} + H_{A2}$, respectively.

satisfactorily linear over the range 10 to 200 mg L^{-1} of sulfite [*e.g.*, $y = (0.056 \pm 0.002)x + (1.10 \pm 0.22)$: $r^2 = 0.998$].

3.2 Effect of the surface area and volume of the acceptor on sensitivity

Increasing the surface area of the acceptor for the same volume should enhance the sensitivity of analysis. In this work this is accomplished by employing two acceptor cones as depicted in Fig. 1a. The flow system in Fig. 2 was used to compare the performance between the new unit (Fig. 1a) and the previous design (Fig. 1b). The volume of the donor was 200 μL, the same as for the single-acceptor cone of the previous design[22] (Fig. 4(i)). The acceptor volume was 200 μL in the single-acceptor unit (Fig. 4(i)). However for the two-acceptor cones of the current design, the acceptor volume was divided into two 100 μL portions (Fig. 4(ii)). The operation steps for analysis are as shown in Table 1S,† but with modified operation of the SIA section suitable for the single-acceptor cone (data not shown). The total diffusion times employed for the units in Fig. 4(i) and (ii) were the same at 20 s, respectively.

When using the acceptor volume of 200 μL, the available surface area of this acceptor was 0.656 cm^2 (Fig. 4(i)). As shown in Fig. 4(ii), the surface area for the same volume of acceptor was increased to 0.828 cm^2, when this volume is divided into two portions in cone A1 and A2 for the current MBL-VP unit (Fig. 1a). By increasing this surface area, it was found that the sensitivity (slope in Fig. 4a) was significantly improved by 3.8 times when using the two-acceptor cone design (Fig. 4(ii)), as compared to the single-acceptor cone design (Fig. 4(i)). With this increase in the sensitivity, the precision was also improved. For example, we observed that the precision (%RSD) for 50 mg L^{-1} sulfite was improved from 4.78% to 0.57%. We therefore selected to use the modified unit with two-acceptor cones (as shown in Fig. 1a) for further development. Even though the single-acceptor cone design can be modified to have a shallower reservoir with a larger surface area of acceptor, its construction is not easy. Use of such a trough design will lead to difficulties in loading and draining of the acceptor liquid at microliter volumes.

Increasing the surface area of the acceptor liquid in the cone by increasing the volume of the liquid may appear to increase the sensitivity of analysis. However this enhancement can be offset by the dilution factor. As shown in Table 1, we observed that when the surface area of the acceptor was increased from 0.828 cm^2 to 1.312 cm^2 (condition 1 *vs.* condition 3) by increasing the volume of the acceptor liquid in each acceptor cone from 100 μL to 200 μL (see Table 1), the sensitivity decreased by a factor of 1.9. Thus, 'condition 1' in Table 1 (inset, Fig. 4(ii)) giving the best sensitivity was therefore employed in this work.

3.3 Effect of the surface area of the donor on sensitivity

It is expected that the surface area of the donor will also have an effect on the sensitivity of the analysis. Two donor volumes, 100

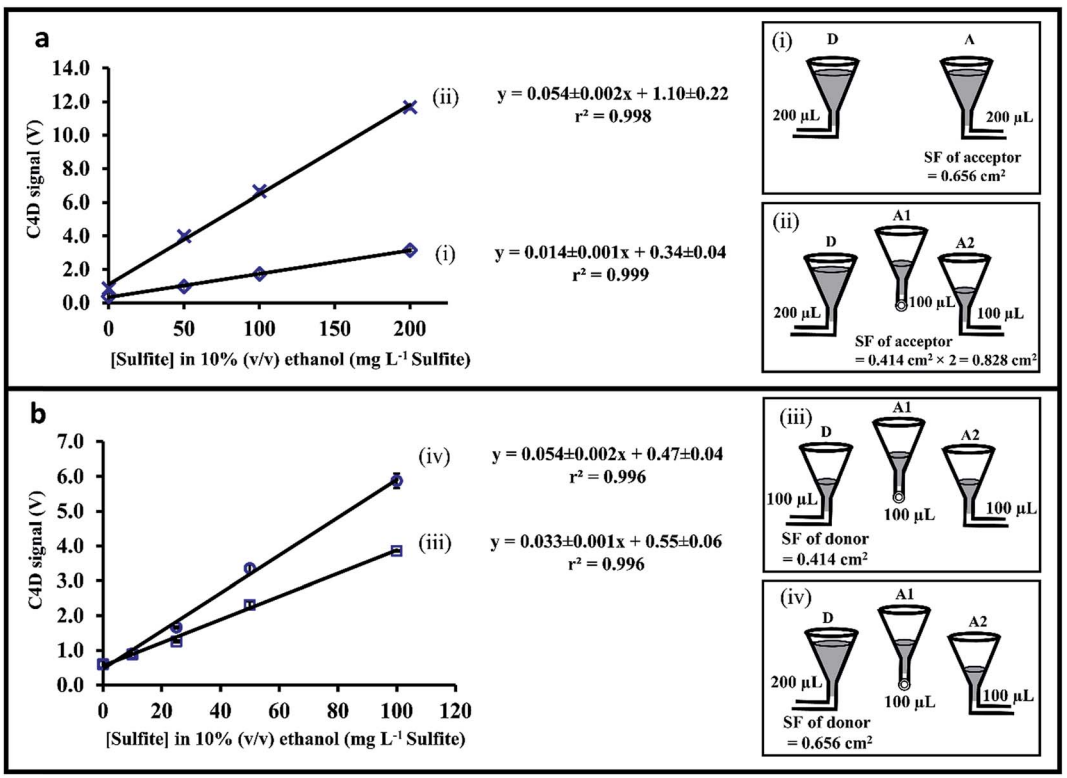
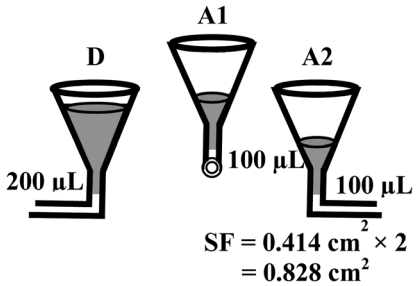
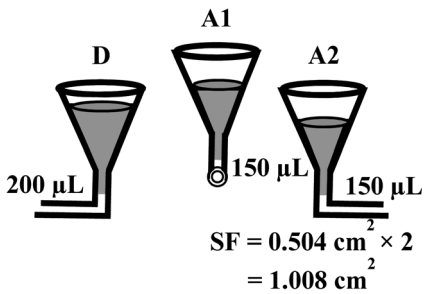
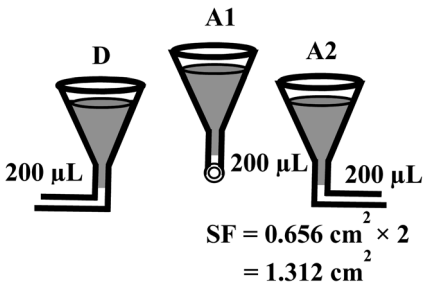


Fig. 4 (a) Effect of the surface area of the liquid acceptor on sensitivity and (b) effect of the surface area and volume of the liquid donor on sensitivity. SF: surface area. Note: the calibrations (ii) and (iv) were obtained using the same conditions but on two different days.

Table 1 Decrease of the sensitivity enhancement with increased surface area by dilution factor

Condition	Variation in the surface area and volume of the acceptor	Calibration equation	Coefficient of determination, r^2
1		$y = (0.056 \pm 0.002)x + (1.10 \pm 0.22)$	0.998
2		$y = (0.041 \pm 0.001)x + (0.89 \pm 0.14)$	0.999
3		$y = (0.028 \pm 0.001)x + (0.57 \pm 0.13)$	0.997

µL and 200 µL, were selected with surface areas of 0.414 cm^2 and 0.656 cm^2, respectively (Fig. 4b). The volume of the acceptor phase was fixed at 100 µL for each acceptor cone (see Fig. 4(iii) and (iv)). The data in Fig. 4b show that the larger the donor surface area, together with the concomitant increase in volume, the higher is the sensitivity. For all work, the volume of the donor liquid in reservoir D (260 µL capacity) is set at 200 µL.

3.4 Optimization

3.4.1 Diffusion time and aeration. For our membraneless systems, diffusion time is one of the parameters that affect the sensitivity.[19,22] The results in Table 2 show that as the total diffusion time is increased from 20 to 80 s, sensitivity improved from (0.056 ± 0.001) to (0.078 ± 0.002) V L mg^{-1} respectively. However, increase in the diffusion time would lead to decrease in the sample throughput. As shown in Table 2, the sample throughput was reduced from 26 to 18 samples h^{-1}. As a compromise between sample throughput and sensitivity, condition 1 with the shortest total diffusion time of 20 s was selected.

In our previous work employing the MBL-VP unit, with 2 cone-shaped reservoirs (Fig. 1b) for the analysis of ethanol,

Table 2 Effect of diffusion time on the sensitivity and sample throughput[a]

Condition	Diffusion time (s) A1	A2	Total	Linear equation	r^2	LOD (3SD of blank per slope)	Throughput (h^{-1})
1	10	20	20	$y = (0.056 \pm 0.001)x + (0.90 \pm 0.10)$	0.998	0.30	26
2	30	40	40	$y = (0.064 \pm 0.002)x + (0.96 \pm 0.13)$	0.999	0.28	22
3	70	80	80	$y = (0.078 \pm 0.002)x + (1.01 \pm 0.12)$	0.999	0.24	18

[a] The experiments were carried out using a series of sulfite standards (10–100 mg L^{-1}) prepared in 10% (v/v) ethanol.

aeration of the donor plug improved the sensitivity up to 13 ± 4%.[22] In this study, experiments were carried out to determine the effect and necessity of aeration for the new unit with 3 cone reservoirs. This MBL-VP unit has two acceptor reservoirs. When the two acceptor reservoirs contain the same liquid volume, there is twice the surface area to trap the gas. Thus it was anticipated that aeration may not be necessary. This hypothesis was tested for repetitive injections of a sulfite standard at 100 mg L^{-1} using the flow system in Fig. 2 equipped with either the current MBL-VP design or the original design (Fig. 1). For the aeration, a flow rate of 1 mL min^{-1} air was used for 20 s.

The data in Fig. 5 show that aeration of the donor plug improved the sensitivity for both designs of the MBL-VP unit. The percentage improvement is almost the same: 35% increase for the 2 cones unit and 37% increase for the modified 3 cones unit. However it is observed that aeration leads to poorer precision of measurements. For the 3-cones unit with aeration, %RSD increased from 0.83% to 2.2%. Taking both factors into consideration, it was decided not to employ aeration, since the increase in efficiency of the new unit was already sufficient.

3.4.2 Acid concentration. Sulfur dioxide gas is produced from sulfite by acidifying the wine sample. This process is carried out by the FIA section of the flow system (left frame of Fig. 2). Sulfuric acid was selected as it has a lower vapor pressure than hydrochloric acid[34] in order to reduce the baseline signal due to diffusion of the acid from the donor cone to the acceptor cones. Sufficient acid is required to convert all the sulfite to sulfur dioxide (maximum concentration of sulfite in this work is 200 mg L^{-1}) and also to neutralize the sodium hydroxide added to the sample (0.4 mol L^{-1}). Fig. 6a shows the plot of the C4D signal for a 200 mg L^{-1} standard sulfite sample for various concentrations of sulfuric acid in the acid flow line (Fig. 2). The presence of a sufficient amount of acid is indicated by constant signal peak heights. This is observed for acid concentration >0.10 mol L^{-1}. In order to ensure that there is sufficient acid, sulfuric acid at 1.5 mol L^{-1} was selected as the suitable concentration for sulfur dioxide production.

3.4.3 Effect of ethanol content. Ethanol is the major volatile component in wine and can vary from 8 to 15% (v/v). Ethanol can be taken up by the water acceptor phase, which may affect the sensitivity of the sulfur dioxide analysis. Calibration curves were constructed for standard sulfite solutions containing ethanol from 0 to 30% (v/v). The results are shown in Fig. 6b. It is observed that the sensitivity of analysis increases with ethanol concentration, but remains constant for ethanol concentration >10% (v/v). It is also interesting to note that the coefficient of determination (r^2) of the calibration is closer to 1 with increase in ethanol content. Without aeration the presence of co-existing volatile compounds such as ethanol could help to improve volatilization (*via* a non-ideal effect) of the generated SO_2 from the donor to the acceptor. This leads to improvement in terms of precision (data not shown) and sensitivity. With improvement in the precision, the coefficient of determination r^2 also improved as we increased the concentration of ethanol in the donor. In this work the standard sulfite solutions were prepared with 15% (v/v) ethanol content.

3.4.4 Headspace volume. The volume of the headspace can be adjusted by varying the height of the screw-top lid of the MBL-VP unit (Fig. 1a). The volume of headspace will affect the concentration of SO_2 gas during the diffusion process. To determine the optimal volume of the headspace, analysis of a standard solution of sulfite (100 mg L^{-1}) was carried out for headspace volumes of 2.82 to 10.60 mL, respectively. Diffusion times for the acceptor plugs A1 and A2 were fixed at 10 s and 20 s, respectively (see Table 1S†). The flow rate for venting of the headspace was 20 mL min^{-1} for 90 s. The results are shown in Fig. 6c.

Fig. 6c shows that the C4D signal decreases with increasing volume of the headspace due to the dilution effect arising from the increased volume. The imprecision of measurements ($n = 3$) also varied with the volume of the headspace. The buildup of the pressure within the headspace due to the vaporization of sulfur dioxide and ethanol is highest for 2.82 mL volume, leading to possible venting fluctuation through the vent line (%

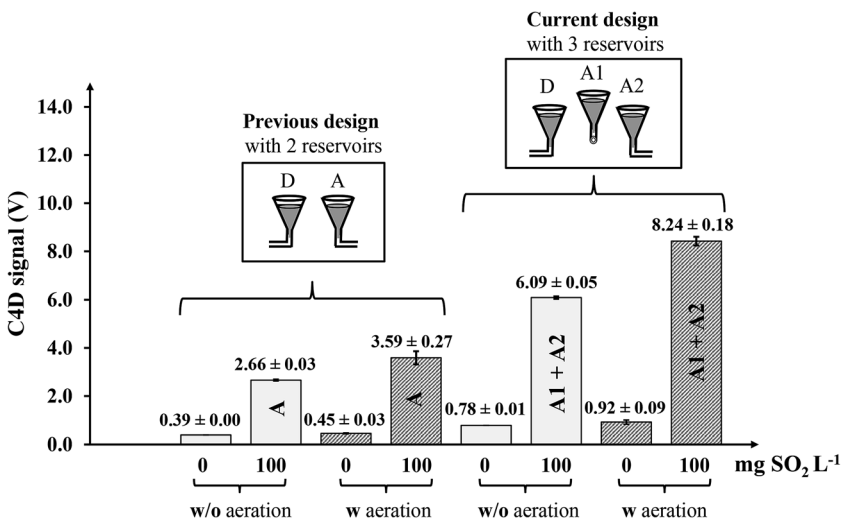


Fig. 5 Effect of aeration on signal size and precision investigated using previous[22] and current designs of the MBL-VP unit.

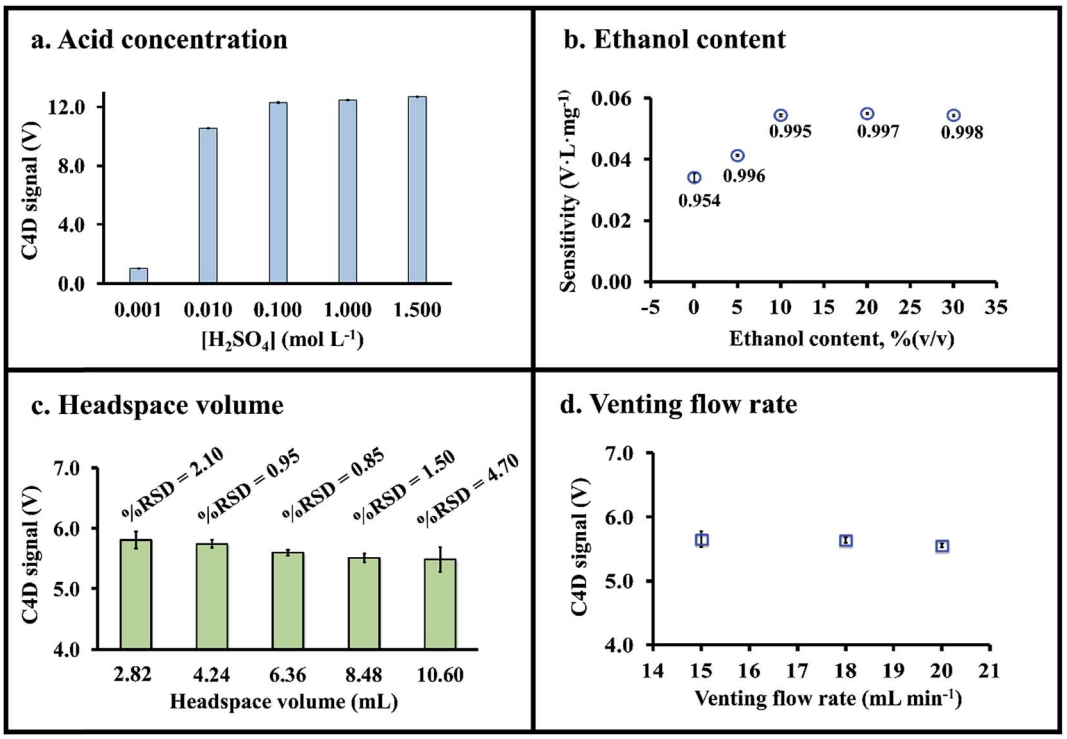


Fig. 6 Optimization using a sulfite standard at 200 mg L^{-1} for (a) and at 100 mg L^{-1} for (c) and (d). Note: (i) the sulfite standard employed in (a, c, and d) was prepared in 10% (v/v) ethanol. (ii) Values under the data points in (b) are the coefficients of determination (r^2) of the calibration for sulfite standards from 10 to 200 mg L^{-1}.

RSD = 2.10). The precision improved for headspace volumes of 4.24, 6.36 and 8.48 mL, respectively (%RSD 0.95, 0.85 and 1.5). However when the headspace volume was set to the maximum volume of 10.60 mL, %RSD increased to 4.7%. Although the headspace was flushed with up to 30 mL of air (20 mL min^{-1} for 1.5 min), it was found that there was still some SO_2 residue. In this work, a headspace volume of 6.36 mL was selected as a compromise between signal size and precision.

3.4.5 Flow rate for venting of the headspace. In the flow system shown in Fig. 2, peristaltic pump PP2 is used to remove any residual volatile gas from the headspace. Air and residual volatile gases are flushed out to wastes W1 and W2 (Fig. 2, FIA section). The venting flow rate is the sum of the flow of the two pumping tubes. In this work three venting flow rates, 15, 18 and 20 mL min^{-1}, were tested using the final selected headspace volume of 6.36 mL (see Section 3.4.4). The venting time was fixed at 1.5 min for all flow rates. The results in Fig. 6d for analysis of a standard solution of sulfite (100 mg L^{-1}) clearly show that the C4D signals are not significantly different for the three flow rates, indicating that there is no carryover of SO_2 gas. It may also be noticed in Fig. 6d that the precision improved when increasing the venting flow rate to 20 mL min^{-1}. This flow rate was therefore selected as the optimal condition.

3.5 Analytical features

Using the optimized parameters (Table 2S†), the MBL-VP flow system in Fig. 2 provides a linear working range of 10–200 mg L^{-1} sulfite. A typical calibration line is $y = (0.056 \pm 0.002)x + (1.10 \pm 0.22)$, with $r^2 = 0.998$, where y is the C4D signal in volts. This working range is suitable for sulfite analysis of most wines and also for regulatory purposes (*e.g.* labeling is required for sulfite content >10 mg L^{-1} (ref. 35)). Our working range is comparable with another membrane-based GD-FIA method[16] and with a GD-SIA method,[6] both employing formaldehyde and pararosaniline as reagents for spectrometric detection. Our throughput is 26 samples h^{-1} which is also similar to the throughput of the GD-FIA method employed as our comparison method.[16] Our throughput is 1.6 times faster than that of the GD-SIA method.[6] The lower limit of quantitation (LLOQ = (3SD of blank)/slope) is 0.3 mg L^{-1} sulfite. Our method is very precise with %RSD as low as 0.8 (for 100 mg L^{-1}, $n = 10$). The C4D detection is reagent-free with use of only pure water as the acceptor. Generation of sulfur dioxide gas in the donor zone uses only 100 μL of 1.5 mol L^{-1} sulfuric acid.

3.6 Application to wine samples

The MBL-VP flow system (Fig. 2) was applied to analyze six wine samples and the results compared with a GD-FIA method (Table 3). Applying the statistical paired t-test for the white wine, the calculated t-value (0.086) was lower than the critical t-value (2.57) for $P = 0.05$, indicating that there was absence of systematic differences between the results of the two methods. Measured recoveries for the white wines were between 84 and 97% ($n = 18$). All 6 white wines did not contain sulfite exceeding the permitted levels of 300 mg L^{-1} sulfite as set by the Thai Industrial Institute.[36] However all samples were found to

Table 3 Sulfite content in white wine and red wine determined by MBL-VP and GD-FIA methods

	Sulfite content (mg L^{-1})	
Sample	MBL-VP (this work)	GD-FIA[16]
White wine #1	118.0 ± 0.2	116.0 ± 1.2
White wine #2	110.0 ± 1.1	111.0 ± 1.2
White wine #3	72.2 ± 1.3	74.1 ± 1.2
White wine #4	69.6 ± 1.8	67.9 ± 0.6
White wine #5	121.0 ± 2.0	123.0 ± 1.6
White wine #6	85.5 ± 0.4	83.4 ± 1.2
Red wine #1	116.0 ± 1.9	86.7 ± 0.7
Red wine #2	116.0 ± 0.9	89.3 ± 0.5
Red wine #3	51.1 ± 0.1	24.5 ± 0.5
Red wine #4	34.8 ± 1.6	19.3 ± 0.2

contain sulfite higher than 10 mg L^{-1}, and were properly labeled as containing sulfite in accordance with the regulation.[36]

We also analyzed the sulfite content of four red wines. However the results were statistically different from the results using the comparison method which employed a membrane. The data from our membraneless vaporization method were always significantly higher than those using the membrane-based gas diffusion method. Red color stains were observed on the membrane surface which may have led to clogging of membrane pores and hence underestimation of the sulfite content.

4. Conclusion

We have successfully developed a new flow-based method for determination of total sulfite suitable for quality control of white wine. Since its first report in 2006, this is the first application of the membraneless vaporization technique for the determination of sulfite. Dissolution of the evolved volatile SO_2 gas into the water acceptor leads to change in the conductivity of the acceptor which was measured using a C4D flow cell. Our method offers direct analysis of samples. This method fulfills all the four priorities of 'Green Analytical Chemistry'. The method significantly reduces use of reagents. Only pure water is used as the acceptor solvent. Only a small amount of common acid is used for on-line acidification of the sample. Membrane is no longer used and therefore there is no non-degradable waste. Our method is automated, reducing labor consumption.

It has also been demonstrated that improvement of the sensitivity of the MBL-VP flow system can be achieved by increasing the surface area of the acceptor solution but without increasing its volume. This was conveniently accomplished by introducing a second acceptor cone in the vaporization unit. In the new unit one reservoir is used for the donor plug and two reservoirs for holding two separate plugs of acceptor solution. The two acceptor plugs are measured sequentially and the sum of the two signals employed for analysis. By doing so, we could effectively improve the sensitivity of the MBL-VP flow system 3.8 fold without heating or additional aeration. The precisions (% RSD) were also significantly improved.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors acknowledge the financial supports from the Thailand Research Fund for the following grants: RSA5580021 (for DN) and IRN59W0007 (chaired by Prof. Skorn Mongkolsuk), TRG5880170 (for TM). Equipment was partially supported by the Office of Higher Education Commission and Mahidol University under the National Research Universities Initiative, chaired by Prof. Manat Pohmakotr and by Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education. We would like to thank scholarship from the Development and Promotion of Science and Technology Talents Project (for NC) and scholarship for international graduate students of the Faculty of Science, Mahidol University (for WA). The work is also partially supported by RA scholarship from the Faculty of Graduate Studies, Mahidol University (for WA). The authors would like to thank Prof. Prapin Wilairat for the editing work. We would like to thank Dr Jirayu Sitanurak, Ms Saichon Sumantakul, Ms Jutamanee Rattana and Ms Kamolchanok Kotakanok for their photography in all art production.

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Elsevier Editorial System(tm) for Talanta
Manuscript Draft

Manuscript Number:

Title: Concurrent determination of ethanol and total sulfite in white wine using on-line membraneless gas-liquid separation flow system

Article Type: Research Paper

Keywords: Membraneless vaporization; Concurrent determination; Ethanol; Sulfite; Paired emitter-detector diodes; Contactless conductivity detector

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Abstract: This work presents the use of a single membraneless vaporization unit (MBL-VP unit) in a flow system for concurrent determination of ethanol and total sulfite in white wine. The flow system comprises a MBL-VP unit with three cone-shaped reservoirs and two in-house detectors, a paired emitter-detector diodes (PEDD) and a capacitively coupled contactless conductivity detector (C4D). Wine sample is first acidified in the flow system. Then a 200-□µL of the acidified sample is delivered to the donor reservoir of the MBL-VP unit in which the two acceptor reservoirs contain 200-µL of the gas acceptor reagents, viz. acidic permanganate solution and deionized water. Vaporization of ethanol and SO2(g) (converted from sulfite) from the donor into the acceptor reservoirs is carried out for 15 s. The acceptor solutions are then sequentially transferred by an air carrier stream for separate detection at the PEDD and C4D detectors. Decolorization of the permanganate solution from the reduction reaction with absorbed ethanol gives an increase in the PEDD signal, whereas dissolution of SO2(g) in the water acceptor leads to an increase in conductivity detected by the C4D. Linear calibrations were obtained in the range of 5.0-15.0 % (v/v) for ethanol (ΔV = ((4.0±0.03)×10-2)x-((1.85±0.35)×10-2): r2 = 0.999) and 10-200 mg L-1 for sulfite (ΔV = ((0.64±0.015)×10-2)x-((3.25±1.64)×10-2): r2 = 0.999). The analysis is rapid with total analysis time of 2.5 min. Percentage recoveries were 81-104% and 88-110% for ethanol and sulfite, respectively. The method was successfully validated with gas chromatography and iodometric titration for the determination of ethanol and sulfite, respectively.

Opposed Reviewers:

Novelty statement

1. This is the first use of a single MBL-VP unit for concurrent determination of two analytes. One of which is volatile (ethanol) and the other is non-volatile (sulfite).

2. Automated flow system is developed for dual determination of ethanol and sulfite in white wine.

3. Paired emitter-detector diode (PEDD) is presented for monitoring decolorization of permanganate in the detection of absorbed ethanol vapor.

Highlights

1. Single membraneless vaporization unit is presented for concurrent and rapid determination of ethanol and sulfite in wine with in-house and low-cost detectors including ‘paired emitter-detector diode’ (PEDD) and ‘capacitively coupled contactless conductivity detector’ (C4D), respectively.

2. Non-toxic reagents, viz. permanganate solution and deionized water were used as gas acceptor liquids.

Concurrent determination of ethanol and total sulfite in white wine using on-line membraneless gas-liquid separation flow system

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Abstract

This work presents the use of a single membraneless vaporization unit (MBL-VP unit) in a flow system for concurrent determination of ethanol and total sulfite in white wine. The flow system comprises a MBL-VP unit with three cone-shaped reservoirs and two in-house detectors, a paired emitter-detector diodes (PEDD) and a capacitively coupled contactless conductivity detector (C4D). Wine sample is first acidified in the flow system. Then a 200-μL of the acidified sample is delivered to the donor reservoir of the MBL-VP unit in which the two acceptor reservoirs contain 200-μL of the gas acceptor reagents, *viz.* acidic permanganate solution and deionized water. Vaporization of ethanol and $SO_{2(g)}$ (converted from sulfite) from the donor into the acceptor reservoirs is carried out for 15 s. The acceptor solutions are then sequentially transferred by an air carrier stream for separate detection at the PEDD and C4D detectors. Decolorization of the permanganate solution from the reduction reaction with absorbed ethanol gives an increase in the PEDD signal, whereas dissolution of $SO_{2(g)}$ in the water acceptor leads to an increase in conductivity detected by the C4D. Linear calibrations were obtained in the range of 5.0-15.0 % (v/v) for ethanol ($\Delta V = ((4.0\pm0.03)\times10^{-2})\ x - ((1.85\pm0.35)\times10^{-2})$: $r^2 = 0.999$) and 10-200 mg L^{-1} for sulfite ($\Delta V = ((0.64\pm0.015)\times10^{-2})\ x - ((3.25\pm1.64)\times10^{-2})$: $r^2 = 0.999$). The analysis is rapid with total analysis time of 2.5 min. Percentage recoveries were 81-104% and 88-110% for ethanol and sulfite, respectively. The method was successfully validated with gas chromatography and iodometric titration for the determination of ethanol and sulfite, respectively.

Keyword: Membraneless vaporization, Concurrent determination, Ethanol, Sulfite, Paired emitter-detector diodes, Contactless conductivity detector

1. Introduction

Quality control is very essential for wine production since there is a big market worldwide for wines. Among various parameters of interest in quality control, ethanol and total sulfite content are important. Ethanol content in wine affects the wine sensory sensation [1]. It also influences the viscosity, body and flavor [2]. In relation to health and safety issue, there has been demand for maintaining the wine alcohol content to 9 - 13% (v/v) [2]. Taxation of alcoholic beverages in many countries is also based on ethanol content of the products [3]. Hence, there is always a need for of a suitable and reliable method for analyzing ethanol content in wine products as part of quality assurance and taxation.

Densitometric-based methods such as pycnometry and hydrostatic balance are classical methods for determination of alcohol content in wine. These methods employ pre-distillation of alcohol prior to the densitometric measurement [4]. Since, distillation process is tedious and time consuming, there have been other alternative methods developed for quantitative analysis of ethanol in wine and alcoholic beverage such as gas chromatographic methods [5-7], high-performance liquid chromatographic method [8], enzymatic methods [9-12], biosensor methods [13-16], chemical sensor method [17], infrared methods [18, 19] and ^{1}H NMR method [20].

Flow analysis methods, with some means of spectrometric detection of ethanol, have been proposed for automation [21-31]. For colourless or very dilute samples, direct introduction of the sample in the flow system is possible without interference in the measurement of the reduction of dichromate by ethanol [21]. For application to both coloured and colourless samples on-line gas-liquid separation devices were employed in flow methods for separating the ethanol from wines or beverages prior to spectrometric detection [22-30]. These on-line gas separation devices include those which employ porous membrane gas-diffusion units [22-25] and

pervaporation units [26]. For gas-diffusion and pervaporation, ethanol vapour diffuses through the pores of the membrane from the donor liquid (sample) and dissolves in the acceptor liquid. Detection of ethanol in the acceptor can be spectrometric detection of the reduction of dichromate [22-23, 26] or schlieren detection of the acceptor zone with dissolved ethanol [24-25].

Membraneless on-line gas-separation in flow system can also be employed at a much lower cost than membrane-based analyses [27-30, 32]. For ethanol, two configurations of membraneless gas separation devices have been presented [27, 30]. A membraneless gas-diffusion unit with two parallel grooves connected by a headspace was presented in 2006 for ethanol analysis in alcoholic drinks including wine [27]. This design is suitable for continuous flow system. For significant reduction in reagent consumption and employing complete automation, a cone design for donor and acceptor reservoirs was later presented [30]. Another arrangement for separating ethanol from wine samples is the 'single-drop micro-extraction' method [31]. A 20-µL drop of the dichromate reagent is hung inside the syringe barrel above the sample for collection of ethanol vapour prior to sending the reagent drop for spectrometric detection. In our opinion, this technique can be classified as a membraneless gas diffusion method or more accurately as a membraneless extraction method.

Similar to certain types of food and drinks, sulfite is used in wine production generally as a preservative due to its inhibition of bacterial growth and as an antioxidant to stabilize flavor and colour of the wine [33]. However sulfite is known to cause asthma and allergies in certain group of population [34, 35]. According to the World Health Organization (WHO), the acceptable daily intake of sulfite (expressed as SO_2 equivalent) is 0.7 mg kg^{-1} body weight [36]. The level of sulfite in food and beverages, including wine, must be strictly controlled to levels as

set by the regulation of each country [37]. According to the European regulation 1169/2011 [38], wine products containing more than 10 mg L^{-1} of sulfite, calculated as SO_2 equivalent, must be declared on the labels of the wine products.

In view of regulations, as well as health concern, quantification of sulfite in wine is needed in winery. The standard AOAC method which employs an optimized Monier-Williams method [39] requires many steps with conversion of sulfite to SO_2 gas, refluxing, purging and trapping of the gas before acid-base titration. Since the AOAC method is laborious and the method is prone to error due to loss of the gas, there have been other alternative methods developed for determination of sulfite in wine. From the electroactive properties of sulfur compounds, various electrochemical methods have been utilized for sulfite in wines [40-46]. Spectrometric detection [47, 48], luminescent detection [49, 50] as well as pH ISFET detection [51] have also been employed.

Flow injection (FI) analysis has been widely employed as a method for automating the analysis of sulfite [52]. In many FI systems [47-52], gas diffusion unit was incorporated into the flow system for selective detection of $SO_{2(g)}$ that was formed by acidification of the sample. Gaseous $SO_{2(g)}$ diffused from the donor (sample) through the membrane and dissolved in the acceptor solution. Detection of the dissolved SO_2 can be done in various ways, *e.g.*, spectrometric detection of a pH indicator [47] or spectrometric detection based on de-coloration of malachite green reagent [48], luminescence detection [48, 49] or pH detection by ion-sensitive field-effect transistor (ISFET) [51]. The gas-diffusion unit does have some advantages particularly allowing for on-line interface of SO_2 separation, trapping and detection. Nonetheless, there are some drawbacks of using a membrane, such as contamination and clogging of the memebrane pores [30]. Alternative method is to carry out membraneless extraction using

'membraneless extraction module' (MLEM) prior to voltammetric detection [44]. However MLEM does not offer on-line interface and therefore it is not suitable for flow analysis.

In this work, we incorporated a membraneless gas separation device with three cone-shaped reservoirs to a flow system for concurrent determination of ethanol and sulfite in white wine. This membraneless device with three cones was recently presented for analysis of total sulfite in white wine [53]. In this previous work, one cone was used for holding the donor plug of acidified sample (200 µL). The other two cones contained 200-µL plugs of deionized water as acceptor of the generated $SO_{2(g)}$. The conductivity of the two acceptors were employed for the determination of sulfite in the donor sample. Addition of the second acceptor cone increased the surface area of the trapping liquids leading to improvement in the sensitivity of the analysis. For this work, the same unit design was employed for concurrent analysis of ethanol and sulfite. We used one of the cones for holding the donor sample (acidified wine). However, the second and the third cones were separately used for holding 200-µL aliquot of a solution of acidic permanganate and deionized water as acceptors for ethanol and sulfur dioxide, respectively. After allowing the acceptors to trap ethanol and $SO_{2(g)}$, the liquids were withdrawn from the unit and sent to the respective detectors. In-house detectors, comprising a paired emitter-detector diodes (PEDD) [54, 55] and a capacitively coupled contactless conductivity detector (C4D) [53, 56-58] were employed. PEDD detects the de-coloration of the permanganate solution caused by reduction reaction with the dissolved ethanol. Permangamate is a strong oxidizing agent [59] for ethanol and is less toxic than dichromate reagent. C4D detects the conductivity change when $SO_{2(g)}$ dissolved in the water acceptor. To the best of our knowledge, this is the first time that ethanol and sulfite were concurrently determined by on-line gas-liquid separation flow analysis.

Use of a single membraneless vaporization (MBL-VP) unit for simultaneous gas diffusion and trapping allows rapid determination of both analytes to be accomplished within 2.5 min.

2. Experimental

2.1 Chemicals and reagents

All chemicals in this work were analytical reagent grade and solutions were prepared in deionized Milli-Q® water (resistivity 18.2 MΩ·cm). Stock standard sulfite solution was prepared by dissolving Na_2SO_3 (Merck Millipore, Germany) in 100.0 mL of 0.1% (w/v) Na_2EDTA (Fisher Scientific, UK) to give *ca.* 10,000 mg L^{-1} sulfite solution. The accurate concentration of this stock solution was determined by titration with standardized iodine solution. For calibration sulfite standard solutions were freshly prepared from the 10,000 mgL^{-1} sulfite stock solution by aliquoting appropriate volume to give a series of sulfite standards containing 0.1% (w/v) Na_2EDTA, 0.4 mol L^{-1} NaOH (Merck Millipore, Germany) and 10 % (v/v) ethanol. The concentration range of the sulfite standards was 10-200 mg L^{-1}. Working standards for ethanol (5-15 %(v/v)) were prepared by diluting absolute ethanol (99.5% (v/v) Merck Millipore, Germany) with a solvent comprising 0.1% (w/v) Na_2EDTA, 0.4 mol L^{-1} NaOH and 100 mg L^{-1} sulfite.

The optimized acceptor solution for ethanol (0.8 mmol L^{-1} permanganate) was prepared by dissolving 0.032 g of potassium permanganate (Ajax Chemicals, Australia) in 25 mL of 4.0 mol L^{-1} H_2SO_4 (Lab-Scan, Thailand) and diluted ten-fold with 4.0 mol L^{-1} H_2SO_4. The acceptor solution for sulfite was deionized (DI) water.

2.2 Preparation of wine samples

Seven white wine samples were purchased from local supermarkets in Bangkok. An aliquot of 25.00 mL of a wine sample was transferred into a 50.00 mL volumetric flask. To this sample, 5.00 mL of 4 mol L^{-1} NaOH and 1.00 mL of 5% (w/v) Na_2EDTA were added. Deionized water was then added to the mark. The sample was mixed thoroughly and left to stand for approximately 15 min before analysis of ethanol and sulfite content using the developed method and the comparison methods [60, 61].

Recovery study was carried out by preparing a sample as described above but with the addition of ethanol and sulfite at concentrations of 2.5% (v/v) ethanol and 25 mg L^{-1} sulfite, respectively.

2.3 MBL-VP unit with 3 cone shaped reservoirs

The MBL-VP unit previously presented in 2013 [30] with 2 cone shaped reservoirs was modified by adding a third cone reservoir as shown in Fig. 1a. The MBL-VP unit was constructed from an acrylic block using milling technique. Fig. 1b is the top-view showing the dimensions of the cone-reservoirs and the MBL-VP base. The dimensions of the entire unit are shown in Fig. 1a. The volume of each cone reservoir is 300 μL. However, the volume of liquid delivered into the cone was set at 200 μL to avoid overflowing. The lid is a screw-top lid allowing the volume of headspace above the reservoirs to be varied from 4.8 to 40 mL. A vent tubing (1.0 mm i.d., 20 cm long) was fitted to the unit to prevent excessive build-up of pressure inside the headspace.

2.4 Flow system and operation

The design of the flow system employed in this work is shown in Fig. 2. The system is divided into two sections comprising the flow injection analysis (FIA) section and the sequential injection analysis (SIA) section, respectively. The MGC-MPV LMPro (version 5.2) software was used for controlling all the electrical components. The FIA unit is used for sample on-line acidification and transportation of the acidified sample into the MBL-VP unit. The SIA section is used for handling of the liquid acceptor zones into and out of the MBL-VP unit. PTFE tubing (1.0 mm i.d., VICI, Switzerland) is employed for all the flow lines.

As shown in Fig. 2, the FIA section comprises two peristaltic pumps, PP1 and PP2 (Ismatec, Switzerland), a 6-port injection valve IV (model 5020, Rheodyne, USA) with a 200 μL sample loop and a 4-port switching valve SV (model V-101D, Upchurch Scientific, USA). Tygon^{TM} pump tubes were used for PP1 and PP2. The SIA section comprises two sets of SIA with syringe pumps SP1 and SP2 (Hamilton-MVP, Japan), each fitted with a 5.00-mL syringe barrel, selection valves SLV1 and SLV2, PTFE holding coil (1.5 mm i.d., 188 cm in length) and the PEDD and C4D detectors.

Construction of the two detectors was carried out as described in previous works ([55] for PEDD and [53] for C4D). For the PEDD, two green LEDs (525 ± 35 nm, Kingbright, Taiwan) were used with a 10 mm flow-through cuvette cell. LabVIEW 8.0^{TM} software was used for recording the PEDD signal. This cell was employed for measuring ethanol in the acceptor solution. The C4D flow cell is a PEEK tubing (1 mm i.d., 1.6 mm o.d., 15 cm length) with two silver painted electrodes. An AC potential (20 V_{pp}, 20 kHz) is fed into one electrode from a

function generator (GW Instek, SFG-2104, Taiwan). The AC current flowing between the two electrodes is monitored at the second electrode. The current is amplified and rectified by a custom built electronics unit (Bangkok High Lab Co., Ltd., Thailand). The final DC voltage output, proportional to the AC current, is recorded by a signal recorder (e-corder 210, eDAQ, Australia). This cell was used for the determination of sulfite dissolved in the water acceptor.

The flow system in Fig. 2 is operated according to the optimized sequence in Table 1. A precise volume of the liquid acceptors (200 µL of acidic permanganate and deionized water) is introduced simultaneously into the acceptor reservoirs A1 and A2 using the two SIA modules. At the same time as these acceptor aliquots are introduced into the cones A1 and A2, the streams of sample and 1.5 M H_2SO_4 solution are merged and mixed employing pump PP1 of the FIA unit. This acidified stream of sample flows into the 200-µL IV loop. This 200-µL plug of acidified sample is then injected into an air carrier stream and drawn into the donor cone D of the MBL-VP unit using the peristaltic pump PP1. After vaporizing and diffusion from the donor for 15 s, the two acceptor solutions are simultaneously withdrawn from cones A1 and A2 to the PEDD and C4D detectors, respectively, again using the two SIA modules. At the same time, the pump PP2 and the switching valve SV of the FIA section are operated to withdraw the spent 200-µL sample from the donor cone D to waste W2. PP2 is left on for 90 s for evacuating the residual gas from the headspace of the MBL-VP unit to waste W2. This operating cycle takes 2.5 min. Before starting the next cycle, a cleaning cycle is operated using the same procedure as shown in Table 1 but with the sample bottle in the FIA section replaced by a bottle of deionized water.

For cleaning the flow lines in the SIA sections, 200 µL volumes of permanganate solution and deionized water are delivered into cones A1 and A2. After the cleaning process, analysis of the next sample is carried out according to the procedure in Table 1.

3. Results and discussion

3.1 Signal profile and zone sequence

The flow system in Fig. 2 was set-up and used for preliminary tests. Initial optimization was carried out to obtain conditions giving reasonable signal profiles of ethanol and sulfite.

The first test was carried out using standard ethanol solution (see Section 2.1) or a negative control solution (100 mg L^{-1} sulfite, 0.1% (w/v) Na_2EDTA and 0.4 mol L^{-1}NaOH) as the donor. The procedure in Table 1 was employed. Although sulfite signal at the C4D was not recorded in this experiment, cone A2 was loaded with the sulfite acceptor (200 µL of deionized water) for ensuring that the system was operated at constant pressure inside the MBL-VP unit. The negative control solution was first introduced into the donor cone D of the MBL-VP unit, in which a 200 µL zone of the permanganate acceptor (0.8 mmol L^{-1} $KMnO_4$ in 4.0 mol L^{-1} of H_2SO_4) was held in cone A1. After 15 s, the acceptor zone was pushed through to the PEDD for signal recording. The second cycle was then operated following the procedure in Table 1 using 10 % (v/v) ethanol standard solution. We obtained signal profiles for the negative control and standard ethanol as shown in Fig. 3a. The figure also shows the sequence of the air, acceptor and water plugs from acceptor cone A1 as they reside in the SIA holding coil HC1 (see Fig.2). It should be noted that the signal (V, in volt) from the PEDD decreases linearly with increasing absorbance of a sample ([55]). Thus for the ethanol determination, decrease in the permanganate concentration in the acceptor A1 leads to higher PEDD signal. As expected, the height of the blank ($HA1_B$) is smaller than the height for 10% (v/v) ethanol ($HA1_S$) since the reduction reaction of $MnO_4^-{}_{(aq)}$ by absorbed ethanol vapor leads to decrease of the purple color in the acceptor zone and subsequent

increase in the PEDD signal. We also tested the flow system with a series of ethanol standard solutions (2.5 - 12% (v/v) ethanol). The difference in the signal heights between the standard and the negative control was used for the constructing of the calibration plot. A linear calibration was satisfactorily obtained from this preliminary work [$((HA1_S - HA1_B), \Delta V) = ((3.32\pm0.07)\times10^{-2})$ %(v/v) ethanol + $((0.21\pm0.5)\times10^{-2})$: $r^2 = 0.999$].

A second experiment was carried out using the same flow system in Fig. 2 for a series of sulfite standard solutions (25-200 mg L^{-1}, see Section 2.1). The operating procedure in Table 1 was used. In an operating cycle, 200-µL aliquot of deionized water was delivered into the acceptor cone A2 for 15 s to trap the SO_2 gas. Although the ethanol signal was not recorded in this experiment, cone A1 was loaded with 200-µL aliquot of the permanganate acceptor. Fig. 3b shows the sequence of the air, acceptor and water plugs from acceptor cone A2 as they reside in the SIA holding coil HC2 (see Fig.2) and examples of signal profile of the negative control sample (10% (v/v) ethanol, 0.1%(w/v) Na_2EDTA and 0.4 mol L^{-1}NaOH) and a 100 mg L^{-1} sulfite standard solution. The signal ($HA2_B$) for the negative control sample was not different to the signal of the deionized water. Compared to the blank signal, $HA2_B$, the signal for the 100 mg L^{-1} standard $HA2_S$ was 7.5 times greater (note the comment concerning the scale for $HA2_B$ in Fig.3b) indicating there was a significant amount of SO_2 gas converted from sulfite trapped by the water acceptor. It was found that the calibration of sulfite was also linear over the concentration range of the standards employed: $((HA2_S - HA2_B), \Delta V) = ((0.65\pm0.02)\times10^{-2})$ mg L^{-1} sulfite – $((3.81\pm1.50)\times 10^{-2})$: $r^2 = 0.999$.

3.2 Investigation of effect of ethanol and sulfite contents on acceptor solutions

Our method employs a separate series of standard solutions for ethanol and sulfite, respectively. However the presence of other volatile compounds in a sample can affect vaporization efficiencies of each volatile species. Most wines contain ethanol from 7 to 21 % (v/v) [60] and the amount of ethanol will affect the sensitivity of the sulfite analysis [53]. We investigated, using the flow system in Fig. 2, standard sulfite solutions containing various amount of ethanol from 0 – 20 % (v/v), respectively. It was found (data not shown) that the sensitivity of analysis increased as the ethanol content was raised from 0 to 5%, but remained constant for ethanol concentration $\geq$ 5 % (v/v). We therefore prepared the sulfite standards in 10 % (v/v) ethanol.

We also investigated the effect of sulfite on the vaporization of ethanol. We did not observe (data not shown) any significant change in the PEDD signal for a 10% (v/v) ethanol standard with addition of sulfite from 25 to 200 mg L^{-1}, respectively.

3.3 Optimization

3.3.1 Reagent concentration

For the system in Fig. 2 measurement is performed after a fixed time, not at the equilibrium state. Thus concentrations of the reactants, $KMnO_4$ and H_2SO_4, employed for the ethanol analysis are crucial. Analysis using a series of ethanol standard solutions (5-15 % (v/v)) were carried out for various concentrations of $KMnO_4$ and H_2SO_4 of the acceptor solution.

The results in Fig. 4a show that the sensitivity of the ethanol analysis increased when the concentration of $KMnO_4$ was raised from 0.2 to 0.6 mmol L^{-1}, reaching a constant value at 0.8

mmol L^{-1} permanganate. Thus for the routine procedure the concentration of $KMnO_4$ in the acceptor solution was set at 0.8 mmol L^{-1}. The oxidation of ethanol by permanganate also requires acidic condition. The results in Fig. 4b show that that the sensitivity of analysis increased with increasing acid concentration, especially when the concentration of H_2SO_4 was increased from 3 to 4 mol L^{-1}. For optimal sensitivity, permanganate was prepared in 4 mol L^{-1} H_2SO_4.

The concentration of H_2SO_4 in the reagent stream in the FIA section (Fig. 2) was also optimized. The role of H_2SO_4 is important for conversion of the so-called 'sulfite preservatives' to SO_2 gas. In the optimization study, a sulfite standard solution at 100 mg L^{-1} was merged to mix with a stream of solution of H_2SO_4 at various concentrations using the flow injection (FIA) system in Fig. 2. The results in Fig. 4c clearly show that without the acid, *i.e.* merging the standard sulfite with a stream of deionized water, the C4D signal was low (~0.32 volts). The signal increased approximately 1.75 times when using 1 mol L^{-1} H_2SO_4. However when employing 2 mol L^{-1} H_2SO_4 the signal observed was not significantly different, indicating sufficient excess of acid was already achieved with 1.5 mol L^{-1}. We therefore chose 1.5 mol L^{-1} H_2SO_4 in the reagent stream of the FIA section in Fig.2 to ensure sufficient excess acid.

3.3.2 Volume of donor, acceptor and headspace

The volumes of donor and acceptor solutions were optimized. As shown in Fig. 1, each cone has a diameter of 12 mm and a height of 8 mm. The calculated volume of the cone is just over 300 µL. The donor volume was thus varied from 100 to 350 µL. Although 300 µL is the capacity of the cone it was found that a cone could accommodate a slightly larger volume of a

liquid. When loading 350 µL of liquid we observed a slight concave shape of the liquid surface above the cone which remained stable. Unlike the previous work [30], we did not employ aeration of the donor sample and therefore there was neither the problem of instability of liquid surface at the top of the cone nor overflowing.

The effect of the volume of donor was studied using samples containing both ethanol and sulfite. We operated the flow system in Fig. 2 using the optimized acceptor solutions as described previously with the acceptor volumes fixed at 200 µL. A standard solution containing 10% (v/v) ethanol and 100 mg L^{-1}sulfite in 0.1% (w/v) Na_2EDTA and 0.4 mol L^{-1}NaOH was loaded into cone D. The procedure in Table 1 was modified in order to introduce various volumes of this standard into cone D. The results are shown as bar graphs in Fig. 5a and Fig. 5b for ethanol and sulfite, respectively. The solid purple bars in Fig. 5a are the PEDD signals obtained for the donor standard solution at various volumes. The larger the bar graphs the smaller is the absorbance of permanganate solution, *i.e.* larger amount of ethanol vapor was adsorbed by the acceptor solution, leading to greater decolorization of the permanganate. The signals (purple striped bar) for samples without ethanol (*i.e.* negative ethanol control) are not different indicating that none of the constituents in the negative control contributed to the decolorization of the permanganate. The difference in signal heights (ΔV) between the ethanol standard and negative control are also shown in Fig.5a. As expected, when we increased the donor volume from 100 to 350 µL, ΔV for ethanol significantly increased from 0.17 to 0.52 V.

Fig. 5b shows the results for the sulfite measurements where the bar graphs are the signals from the C4D detector. The larger the signal means the greater the amount of SO_2 adsorbed in the deionized water acceptor. The signals for the negative control sulfite samples are almost constant indicating that other volatile components in the sample (*e.g.* ethanol) have little effect

on the conductivity of the water. Increasing the donor volume leads to increase in the sulfite detection. In this work, the donor volume of 200 µL was selected as it provided sufficient sensitivity for wine analysis.

The operating procedure in Table 1 was also modified to investigate the effect of the acceptor volume. The volumes of acceptor solutions in cone A1 (acidic permanganate) and in cone A2 (deionized water) were varied from 100 to 350 µL. The donor volume was fixed at 200 µL. Results in Fig. 5c and Fig. 5d show that the difference in signal (ΔV) between the standard solution and the negative control decreased as the acceptor volumes were increased, both for ethanol and sulfite. This decrease is due to dilution effect. Using a fixed donor volume and fixed diffusion time, there would be the same quantity of the volatile gases adsorbed by the acceptor solutions. This amount of gas would become more diluted as it dissolves into increasing acceptor volume from 100 to 350 µL, respectively. Although a volume of 100 µL gave the largest signal we did not choose this volume since it was sometimes observed that breakage of the acceptor zone occurred during the SIA procedure. Therefore 200 µL was selected as the optimum acceptor volume for both the permanganate solution and deionized water.

The headspace volume is also another factor that may affect the sensitivity of the analysis. Measurements were carried out using a standard mixture containing 10% (v/v) ethanol and 100 mg L^{-1}sulfite in 0.1% (w/v) Na_2EDTA and 0.4 M NaOH. Operation of the flow system in Fig. 2 followed the procedure in Table 1. The donor volume and acceptor volumes were fixed at 200 µL. Signals for ethanol and sulfite of the standard and negative controls were measured for headspace volumes from 4.8 to 17.5 mL, respectively. Results (data not shown) show that there was no significant change in the observed signal with headspace volume. Large headspace volume would however require longer suction time to remove the residue sample gases. But

higher imprecision in the measurements (1.0 - 3.24% RSD) were observed for both ethanol and sulfite for volumes less than 11 mL. This may be due to a buildup of pressure within the headspace. We therefore chose 11 mL as the optimum headspace volume, as at this volume the %RSD was less than 1 for both ethanol (0.92 %RSD) and sulfite (0.95 %RSD), respectively.

3.3.3 Diffusion time

Diffusion time is defined as the time selected for vaporization of ethanol and sulfite from the donor reservoir and diffusion across the headspace to the acceptor reservoirs A1 and A2, respectively. Measurement of the diffusion time commences at the completion of the the loading of the donor into reservoir D (step 6 in Table 1). As expected, the sensitivity of the ethanol analysis improved when increasing the diffusion time from 10 to 30 s (see Table 2). The sensitivities for sulfites were comparable for diffusion times of 10 and 15 s but improved as the diffusion time was increased from 15 to 60 s. However the linear working range decreased with increased diffusion time. A diffusion time of 15 s was selected since it provided sensitivity of analysis, linear working range and analysis times suitable for wine samples.

3.4 Analytical feature

Using the optimum condition, calibration curve is linear in the range of 5.0-15.0 % (v/v) for ethanol (*e.g.*, $\Delta V = ((4.0 \pm 0.03) \times 10^{-2})$ % (v/v) ethanol $- ((1.85 \pm 0.35) \times 10^{-2})$: $r^2 = 0.999$)) and 10- 200 $mgL^{-}1$ sulfite (*e.g.*, $\Delta V = ((0.64 \pm 0.015) \times 10^{-2})$ mgL^{-1} sulfite $- ((3.25 \pm 1.64) \times 10^{-2})$: $r^2 = 0.999$), respectively. These working ranges are suitable for the levels of ethanol and sulfite found

in most white wines. The system provides a lower limit of quantitation (LLOQ = 3SD of intercept/slope) of 0.26 % (v/v) ethanol and 7.68 mg L^{-1} sulfite, respectively. The system also provides satisfactorily good precisions with %RSD less than 1 for both ethanol (%RSD = 0.24 - 0.92, n = 6) and sulfite (%RSD = 0.11 - 0. 95, n=6), for analysis of a standard mixture containing 10% (v/v) ethanol and 100 mg L^{-1} sulfite. The method provides rapid analysis with sample throughput of 24 samples h^{-1} (analysis time = 2.5 min).

3.5 Wine analysis and validation

This proposed method was applied to the determination of ethanol and sulfite contents in seven samples of white wine as shown in Table 4. Ethanol contents obtained from our method were compared with a gas chromatographic method with flame ionization detector (GC-FID). Sulfite contents were conpared with an iodometric titration method. According to paired *t*-test, ethanol contents obtained from our method are not significantly different to the contents as obtained from the GC-FID method (t_{stat} = 1.24, $t_{critical}$ = 2.45 at P = 0.05). Similarly for the sulfite analysis, there is no significant difference between the results obtained from our method and the iodometric method (t_{stat} = 0.40, $t_{critical}$ = 2.45 at P = 0.05).

We also analyzed four samples of red wines. However the results were significantly different from values obtained using the comparison method. For the ethanol analysis, the values obtained from our method were slightly greater than that using GC-FID method, ranging from 2.9 to 10.1%. As for the sulfite data, results from our method did not agree well with the results from the iodometric method. There was also no clear trends in the differences between the two methods. These results suggest that volatile matrices of red wine are more complicated than in

white wines. Further investigation is needed for analysis of red wines using this developed method.

4. Conclusion

We present development of a flow system suitable for quality control in production of white wine. The system offers concurrent determination of two important components required to be reported on the labels of wine, *viz.* ethanol and total sulfite. A membraneless vaporization unit with three cones was employed for simultaneous vaporization of ethanol and sulfur dioxide (produced from acidification of sulfite). Vaporization and diffusion inside the membraneless unit allows detection of the two volatile compounds adsorbed in selective acceptor liquids without interference from the wine matrices. The method is green chemistry employing environmentally friendly gas acceptors, *i.e.*, permanganate solution and deionized water for trapping vapors of ethanol and sulfur dioxide, respectively. This is also the first use of a PEDD for monitoring the decolorization of permanganate by ethanol. Sulfite is determined based on the change in conductivity of the water acceptor by adsorbed SO_2 using a C4D detector.

Acknowledgements

The authors acknowledge financial support by the matching fund from the Thailand Research Fund and Srinakharinwirot University (Grant No. TRG5880170 given to TM), and by the Thailand Research Fund (Grant No. MRG5480092 given to NR). Equipment was partially supported by the Office of Higher Education Commission and Mahidol University under the National Research Universities Initiative, chaired by Prof. Manat Pohmakotr and by the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education. We would like to thank the scholarship from the Development and Promotion of Science and Technology Talents Project for PK. We would like to thank Miss Kamolchanok Kotakanok for her help in the production of the graphical abstract.

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Table 1 Operation procedure of the MBL-VP unit in conjunction with the two flow systems.

Table 2 Effect of diffusion time on the sensitivity of ethanol and sulfite analysis.

Table 3 Determination of ethanol and total sulfite in white wines by the developed method and by comparison methods.

Table 1 Operation procedure of the MBL-VP unit in conjunction with the two flow systems.

Step	FIA section					SIA section				
	Action	PP1	PP2	IV position	SV position	Action at SP1	SP1[a] (Flow rate)[b]	Action at SP2	SP2[a] (Flow rate)[b]	Duration (s)
1	Draw out vapor from MBL-VP unit	OFF	ON	Load	To W2	• Aspirate 1,500 μL of H_2O(1) into syringe	↓ (12)	• Aspirate 1,500 μL of H_2O(2) into syringe	↓ (12)	7.5
2	Draw out vapor from MBL-VP unit	OFF	ON	Load	To W2	• Aspirate 165μL air into HC1 • Changing SLV1 position	↓ (3)	• Aspirate 165 μL air into HC2 • Changing SLV2 position	↓ (3)	3 3
3	Mixing of sample and H_2SO_4 into 200 uL injection loop by PP1	ON	OFF	Load	To W2	• Aspirate 200 μL $KMnO_4$ into HC1 • Changing SLV1 position	↓ (3)	• Aspirate 200 μL H_2O(3) into HC2 • Changing SLV2 position	↓ (3)	4 3
4	Mixing of sample and H_2SO_4 into 200 uL injection loop by PP1	ON	OFF	Load	To W2	• Dispense 365μL of air and $KMnO_4$ zone to A1 reservoir	↑ (3)	• Dispense 365 μL of air and H_2O(3) zone to A2 reservoir	↑ (3)	7 3
5	Inject donor zone to rest in reservoir D	ON	OFF	Inject	To D reservoir	• $KMnO_4$ zone rest in • A1 reservoir	hold	• H_2O(3) zone rest in • A1 reservoir	hold	15
6	Sample held in reservoir D	OFF	OFF	Load	To D reservoir	• Gas diffusion process for A1:	hold	• Gas diffusion process for A2:	hold	15
7	Sample held in reservoir D	OFF	OFF	Load	To D reservoir	• Draw the following zone from A1 into HC1: (130 μL HP air+200 μL $KMnO_4$+165μL air in line) • Changing SLV1 position	↓ (3)	• Draw the following zone from A2 into HC2: (130 μL HP air+200 μL $KMnO_4$+165 μL air in line) • Changing SLV2 position	↓ (3)	10 3
8	Draw out donor solution and vapor from MBL-VP unit	OFF	ON	Load	To W2	• Send the following zone to PEDD: (130 μL HP air+200 μL $KMnO_4$+165μL air in line) and clean tube line to W4	↑ (1.8)	• Send the following zone to C4D: (130 μL HP air+200 μL $KMnO_4$+165 μL air in line) and clean tube line to w6	↑ (1.8)	16.5
9	Draw out vapor from MBL-VP unit	OFF	ON	Load	To W2	-	hold	-	hold	60

Total analysis time 2.50 min

[a] (↓) syringe piston down; (↑) syringe piston up. [b] flow rate with a unit of mL min^{-1}

PP1, PP2: peristaltic pump1 (2 mL min^{-1}), peristaltic pump2 (10 mL min^{-1}). IV: injection valve. SV: switching valve. SLV: selection valve D: donor reservoir. A: acceptor reservoir. SP: syringe pump. W1, W4, W6: waste. HC: holding coil. HP: headspace. PEDD: Paired Emitter-Detector Diodes Detector. C4D: Capacitively Coupled Contacless Conductivity Detector.

Table 2 Effect of diffusion time on the sensitivity of ethanol and sulfite analysis[a].

Diffusion times/s	Ethanol			Sulfite			Analysis time/ min
	Working range/ %(v/v)	calibration curve	r^2	Working range/ mg L^{-1}	Calibration curve	r^2	
10	2.5-17	$y = (2.9\pm0.43)\times10^{-2}x + (3.73\pm0.49)\times10^{-2}$	0.999	25-200	$y = (0.7\pm0.015)\times10^{-2}x - (6.23\pm1.87)\times10^{-2}$	0.999	2.42
15	1.0-15	$y = (3.9\pm0.67)\times10^{-2}x - (0.49\pm0.57)\times10^{-2}$	0.999	10-200	$y = (0.64\pm0.015)\times10^{-2}x - (3.25\pm1.64)\times10^{-2}$	0.999	2.50
30	0.5-12	$y = (4.5\pm1.1)\times10^{-2}x - (0.40\pm0.73)\times10^{-2}$	0.998	10-150	$y = (1.1\pm0.01)\times10^{-2}x - (1.83\pm0.86)\times10^{-2}$	0.999	2.75
60		-		5-75	$y = (1.8\pm0.024)\times10^{-2}x - (2.6\pm1.03)\times10^{-2}$	0.999	3.25

[a]Standard ethanol and sulfite were prepared separately. All ethanol standards contain sulfite at 100 mg L^{-1}. All sulfite standards contained 10% (v/v) ethanol.

Table 3 Determination of ethanol and total sulfite in white wines by the developed method and by comparison methods.

Wine sample	Ethanol contents/ % (v/v)		Sulfite contents/ mgL^{-1}	
	Proposed method (mean ± SD)	GC-FID method [60] (mean ± SD)	Proposed method (mean ± SD)	Iodometric titration method [61] (mean ± SD)
1	7.4 ± 0.2	7.2 ± 0.1	97.2 ±1.1	95.1 ± 0.4
2	6.5 ± 0.1	6.4 ± 0.2	106.7 ± 1.3	107.9 ± 1.3
3	10.7 ± 0.0	10.3 ± 0.1	87.8 ± 1.3	87.8 ± 0.8
4	8.3 ± 0.2	7.8 ± 0.1	106.6 ± 1.4	107.1 ± 0.8
5	7.3 ± 0.6	7.1 ± 0.1	99.2 ± 3.5	100.7 ± 2.9
6	10.4 ± 0.6	10.8 ± 0.2	103.4 ± 1.6	101.5 ± 1.4
7	13.3 ± 0.3	13.3 ± 0.0	105.2 ± 4.6	107.9 ± 0.0

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Fig. 1 Schematic diagram of the three-cone MBL-VP unit. (a) Side view of three-dimensional MBL-VP unit and (b) top view of the MBL-VP unit.

Fig.2 Schematic illustration of the flow manifold for simultaneous analysis of ethanol and sulfite. The system consists of a FIA section and a SIA section connected to the membraneless vaporization (MBL-VP) unit. SP1, SP2: syringe pump; PP1, PP2: peristaltic pump; IV: injection valve; SV: switching valve; D: donor reservoir; A1, A2: acceptor reservoir; SP: syringe pump; W1-W6: waste; MC1, MC2: mixing coil; HC: holding coil; PEDD: Paired Emitter-Detector Diodes Detector; C4D: Capacitively Coupled Contactless Conductivity Detector.

Fig. 3 Examples of signal profiles obtained (a) from PEDD for permanganate measurement from acceptor A1 (0 and 10% (v/v) ethanol) and (b) from C4D for water conductivity from acceptor A2 (0 and 100 mg L^{-1}sulfite).

Note: C4D signals for 0 mg L^{-1} sulfite are displayed at 1.5 times greater than the actual voltage.

Fig. 4 Results of optimization of reagent concentration. (a) [$KMnO_4$] in permanganate acceptor, (b) [H_2SO_4] in permanganate acceptor and (c) [H_2SO_4] in FIA reagent.

Fig. 5 Bar graphs of the effect of volume of donor on (a) PEDD signal and (b) C4D signal and the effect of acceptor volume on (c) PEDD signal and (d) C4D signal. The PEDD data are for standard ethanol (10% (v/v)) and its negative control (0% (v/v)). The C4D data are for the standard sulfite (100 mg L^{-1}) and its negative control (0 mg L^{-1}).

Figure
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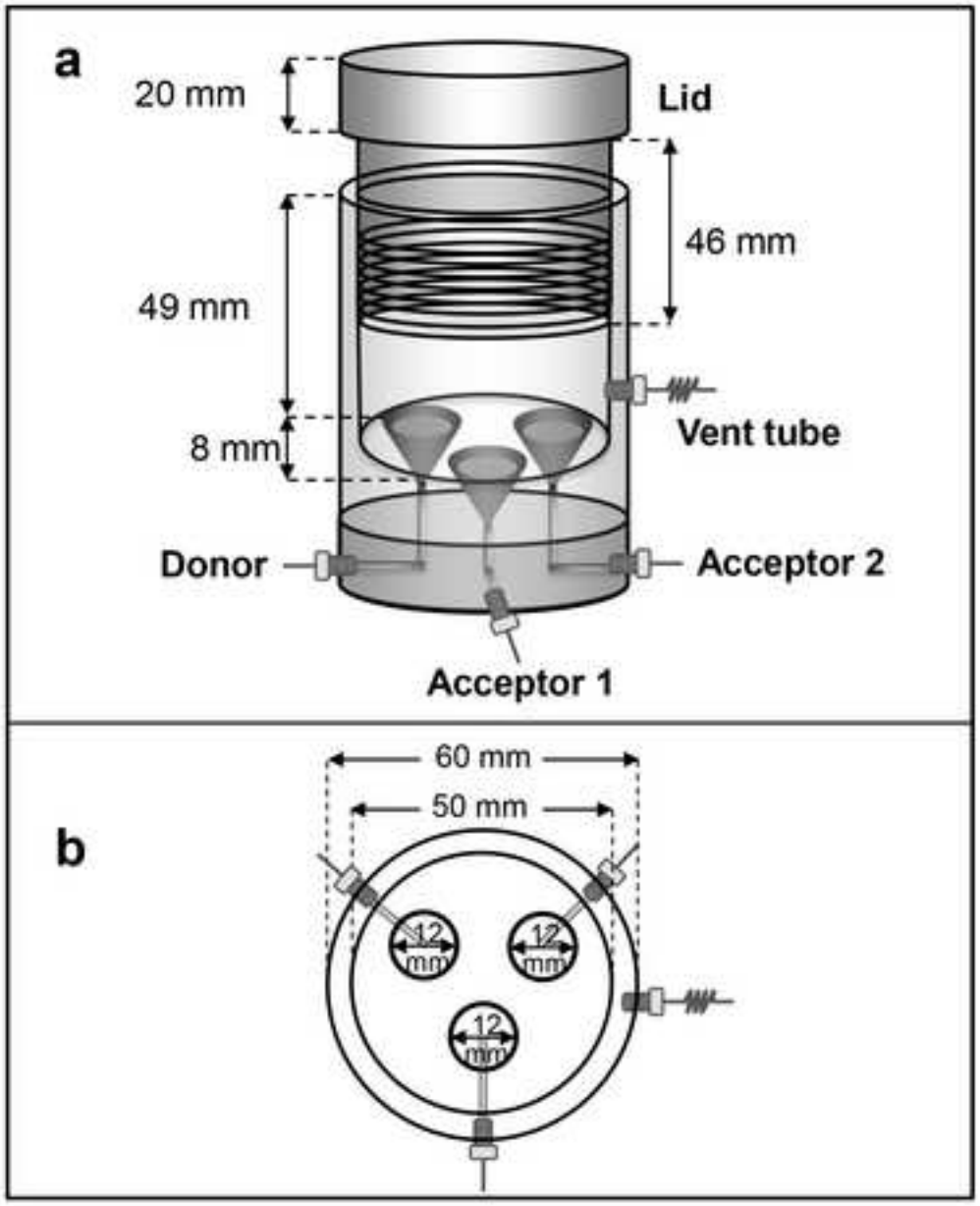

Fig.1

Figure

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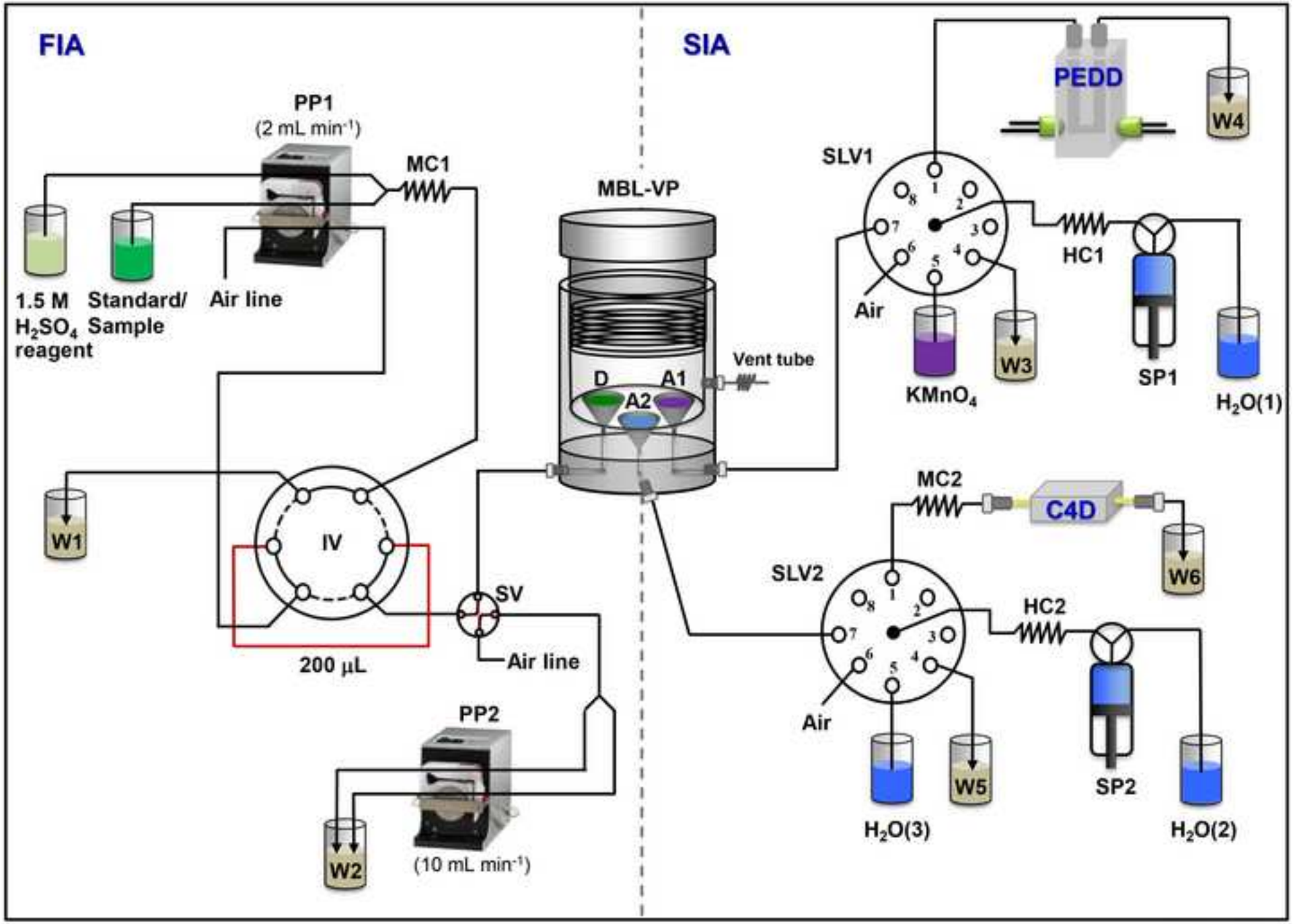

Fig. 2

Figure 3

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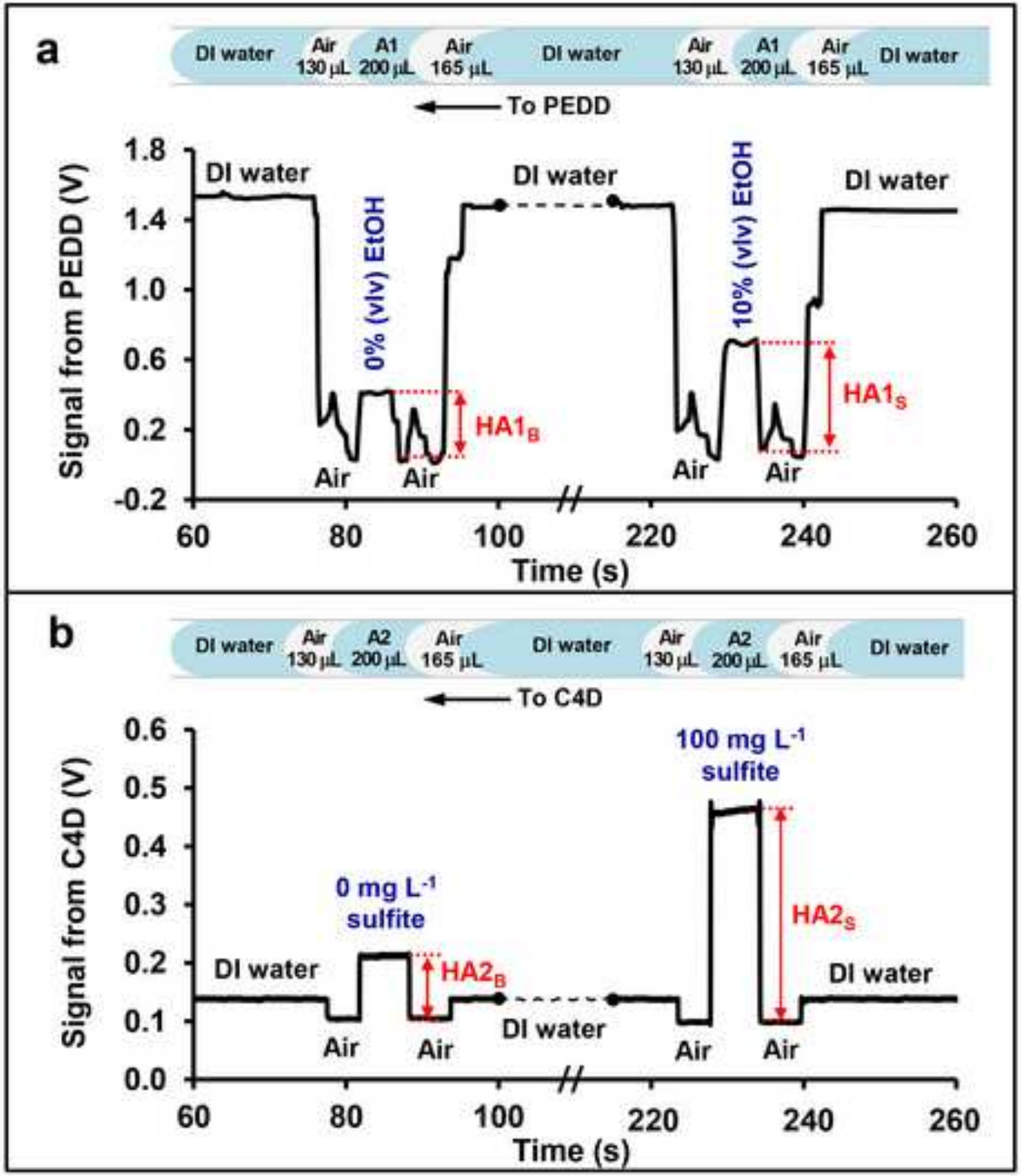

Fig.3

Figure 4

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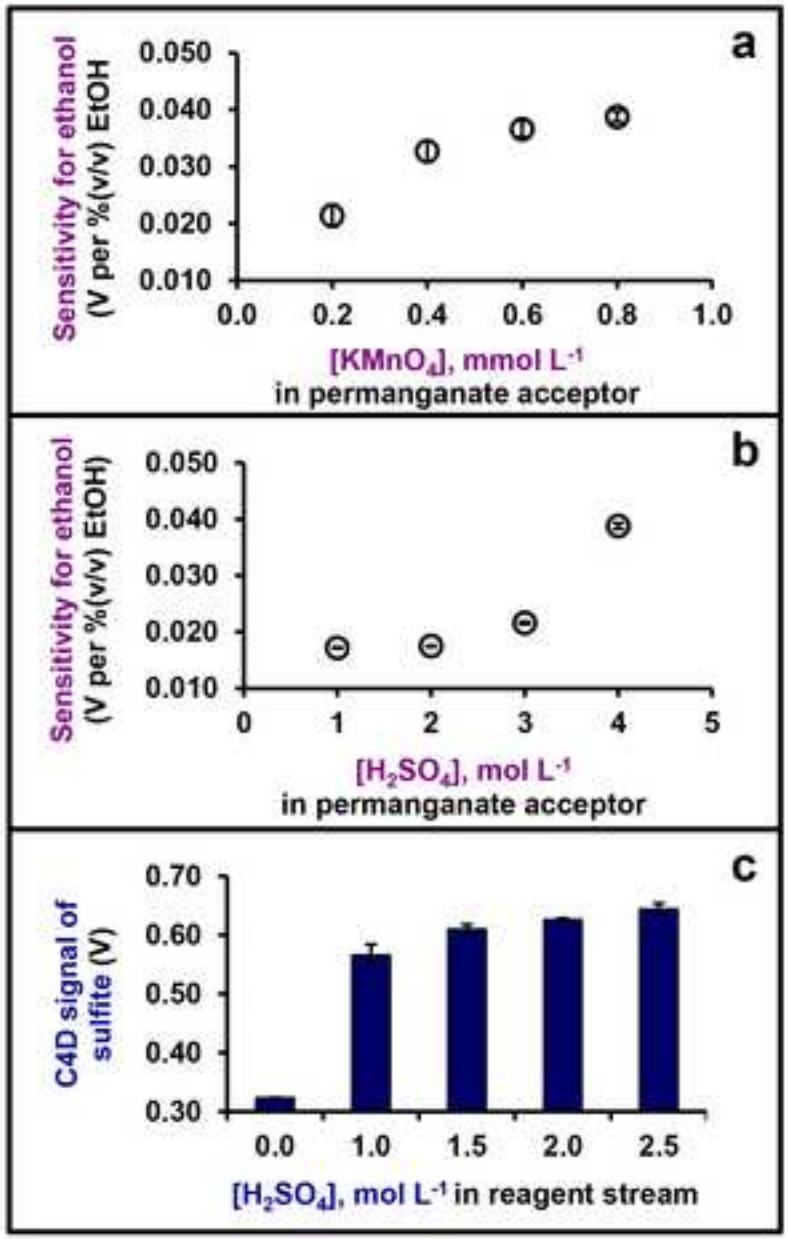

Fig.4

Figure 5

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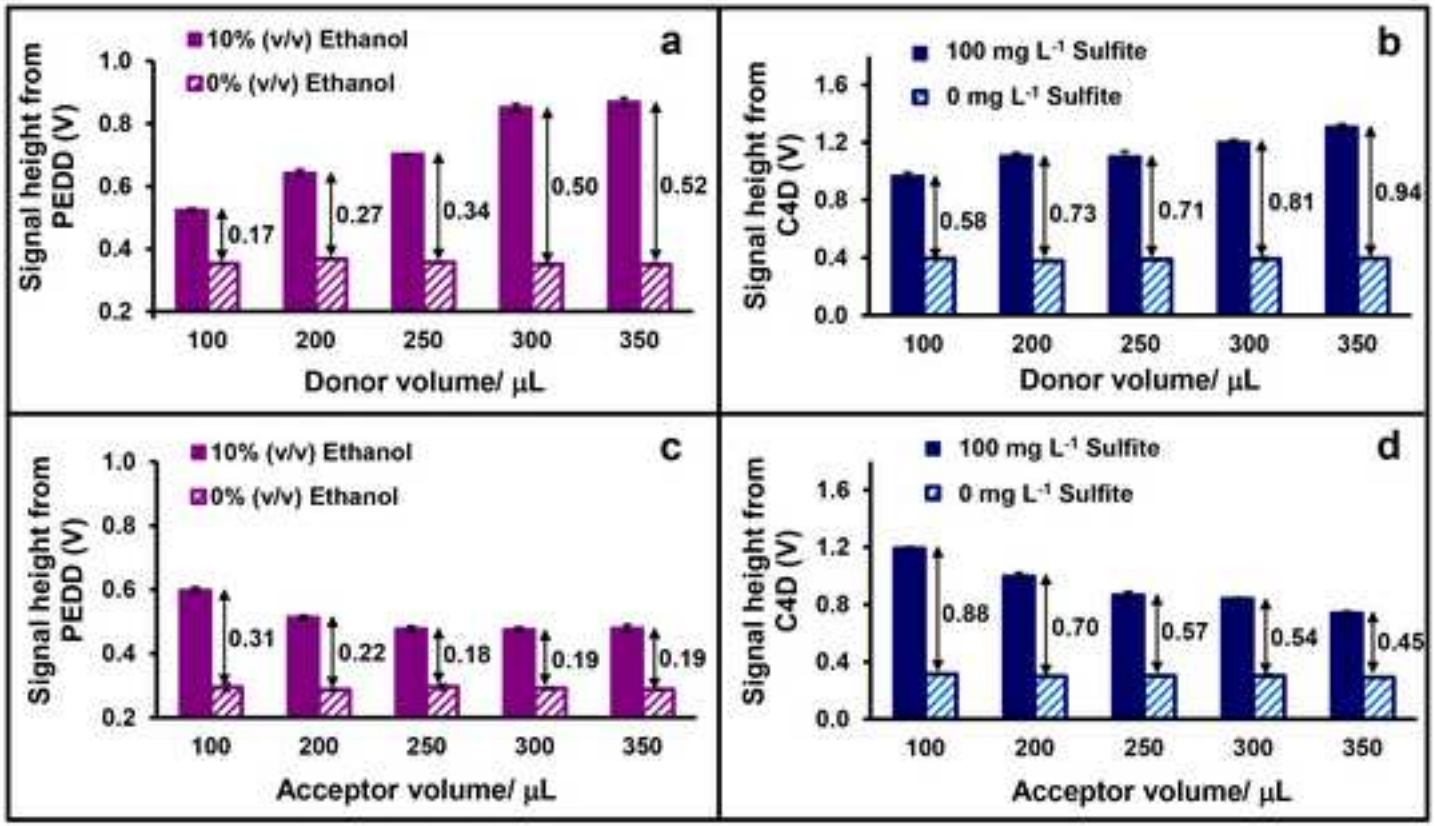

Fig.5