Comparison of a Piezoelectric Impedance Sensor-Based Flow-Injection System and a *N,N,N',N'*-Tetrakis-2-hydroxyethyl Ethylenediamine-Coated Quartz Crystal Microbalance for Determination of CO₂ in Wine and Beer

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A novel FIA system for CO₂ was constructed and compared with a *N,N,N',N'*-tetrakis-2-hydroxyethyl ethylenediamine (THEED)-coated quartz crystal microbalance (QCM) for the determination of CO₂ in wine and beer samples. This FIA method is based on the use of a gas-permeable membrane to separate CO₂ from a carrier stream of 0.5 mol/l phosphate buffer (pH 6.0) into a recipient stream of 10 mmol/l tris(hydroxymethylamino)methane (Tris) containing 1.0 mmol/l KCl and a piezoelectric impedance sensor to follow the conductance change occurring in the acceptor solution. Although the results for real samples obtained by the FIA, the QCM and the conventional titrimetric methods, respectively, agree well with each other, the FIA method offers the best precision. The FIA method is also characterized by its high throughput, promising sensitivity, excellent selectivity, low cost and easy manipulation, *etc.* The influence of some experimental parameters on the FIA's performance is discussed in detail.

Keywords Flow injection analysis, piezoelectric impedance sensor, quartz crystal microbalance, carbon dioxide, wine, beer

A piezoelectric quartz crystal (PQC) has been used as a microbalance in a vacuum or in the gas phase for measuring mass loading on the electrode surface of a crystal based on the famous Sauerbrey equation.¹ When it is used for liquid applications, it also responds to other factors, such as the conductivity, viscosity, density and permittivity of the liquid phase.^{2,3} Recently, several new types of piezoelectric sensors, *e.g.*, the series PQC (SPQC)⁴ and the electrode separated PQC⁵, have been developed for liquid uses. The SPQC sensor, which is constructed by connecting a vacuum-sealed AT-cut PQC and a conductivity electrode immersed in the liquid phase in series, responds only to the solution conductivity and permittivity; also the frequency stability and frequency-temperature coefficient are better than that of the normal PQC sensor, in which the crystal is in direct contact with the solution. Hence, SPQC is a non-mass effect-based device and could be called a piezoelectric impedance sensor (PIS). Such an impedance sensor has proven to be an ideal alternative to the conventional conductometry, because it can detect a slight change in the solution conductivity in the presence of an excess of a foreign electrolyte; also, the sensitivity and accuracy are even better than those obtained in the absence of a foreign electrolyte.^{4,6}

The applications of a mass or non-mass effect based PQC sensors, both in the gas and liquid phases, have almost exclusively utilized manual methods, which are

tedious and may be subject to a variety of errors, since any variation in the experimental conditions, even any small change in the position of the quartz crystal and in the distance between the leading wires, would alter the response of the sensors. Flow injection analysis (FIA) is unquestionably an excellent technique for automating the existing analytical methods. Therefore, we recently carried out much work on the combination of FIA with piezoelectric detection.⁷⁻¹⁰ In this study, a novel FIA system for CO₂ based on the use of a gas-diffusion unit and a piezoelectric impedance sensor was constructed and compared with a *N,N,N',N'*-tetrakis-2-hydroxyethyl ethylenediamine (THEED)-coated quartz crystal microbalance (QCM)¹¹ for determination of CO₂ in wine and beer samples.

The importance of the CO₂ measurement for these samples is due to the fact that CO₂ is responsible for the freshness of taste and enhances the fragrance. The measurement is usually carried out by the conventional manometric or titrimetric method, which is both time consuming and suffer from inadequate sensitivity and reproducibility. THEED has proven to be a satisfactory coating for the detection of CO₂.^{12,13} Recently, Gomes' investigations¹¹ showed that although QCM-THEED is better than the titrimetric method in terms of precision for the determination of CO₂ in wine, SO₂ interacts with THEED to a greater extent than does CO₂, and hence causes a serious interference. Also, the calibration graph had a limited validity probably due to a spreading of the liquid coating on the surface of the

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crystal. To overcome this limitation, the above-mentioned PIS-FIA method was proposed as an effective alternative to the above-mentioned QCM system. The proposed PIS-FIA method is characterized by its excellent precision and rapidity, promising sensitivity, desirable selectivity, low cost and easy manipulation.

Experimental

Apparatus

The proposed PIS-FIA system (Fig. 1a) comprised an LDB-M peristaltic pump (Zhejiang, China), a sampling equipment, including a Shimadzu SLC-6B system controller and an SIL-6B auto injector, and a gas-diffusion unit (made of two pieces of Plexiglas with a shallow groove of 125 mm length, 3 mm width and 0.1 mm depth in each piece). Further a PTFE gas-permeable membrane (pore size 0.02 μm , purchased from Jiangsu Electroanal. Instrumt. Factory, China) was placed between them before being screwed together. A flow-through detection cell (ca. 15 μl made of Plexiglas with

two platinum wires (diameter ca. 1 mm) was inserted oppositely and radially, which served as conductivity electrodes. These were connected in series with a vacuum-sealed PQC to make up the feedback circuit of an oscillator. All of the connections were made of PTFE tubes (0.7 mm i.d.).

The QCM-THEED system employed (Fig. 1b) was constructed on the basis of a minor modification to the set-up proposed by Gomes *et al.*¹¹ It consisted of a flow meter (Model LZB2, Shengyang Glass Instrum. Factory), a glass coil for stabilizing the N_2 stream, an improved wine cell with an injection port, through which the standards or samples were introduced with a syringe into the cell, a silica-gel column for drying the gas, a THEED-coated PQC and a detection cell, as described previously.

For both of the above-mentioned systems, the PQC used was AT-cut crystals (diameter 12.5 mm, Model JA-5, Beijing Factory No. 707) having silver electrodes (diameter 6 mm) on both sides, and driven by an integrated-circuit TTL oscillator (described previously).¹⁴ The oscillator was supplied with 5 V by a direct current voltage regulator, its oscillating frequency was recorded by using an Iwatsu SC-72001 universal frequency counter, and the data readings were collected and transferred to an IBM-compatible PC for further processing. The temperature was controlled by an air bath and a WMZK-1 temperature controller (Shanghai Medical Equipment Co.) at $25 \pm 0.1^\circ\text{C}$.

Reagents

Tris(hydroxymethylamino)methane (Tris), of biochemical-reagent grade, was obtained from Shanghai Biochemical-reagent Co. *N,N,N',N'*-Tetrakis-2-hydroxyethyl ethylenediamine (THEED), stationary phase for gas chromatography, was obtained from Shanghai Reagent Co. All other chemicals were of analytical-reagent grade or better quality. Doubly distilled/deionized water was used throughout. Standard solutions for CO_2 were made by mixing known amounts of NaHCO_3 with an excess of citric acid.

Procedure

PIS-FIA method: samples were analyzed as follows without any pretreatment, except for the simple dilution, if necessary. Standards and samples (120 μl) were injected sequentially into a carrier stream (1.5 ml/min) of 0.5 mol/l phosphate buffer (pH 6.0). The liberated CO_2 was transported across a PTFE gas-permeable membrane, captured by an acceptor stream (1.5 ml/min) of 10 mmol/l Tris containing 1.0 mmol/l KCl, and detected by the PIS detector. From a calibration graph based on the peak heights (the maximum frequency shifts), the contents for total CO_2 were obtained.

THEED-QCM method: the nitrogen flow was kept constant at 30 ml/min after obtaining a stable baseline; then a 10 ml standard or sample (pretreated with H_2O_2 as described by Gomes *et al.*¹¹) was introduced into the wine cell. The CO_2 liberated was dried on the silica-gel

Fig. 1 Schematic diagram of the PIS-FIA manifold (a) and THEED-QCM system (b) for the determination of CO_2 in wine and beer. DC, detection cell; PQC, AT-cut piezoelectric quartz crystal; O, IC-TTL oscillator; VR, voltage regulator; FC, frequency counter; PC, IBM-compatible personal computer. (a): P, peristaltic pump; S, sampling system; L, mixing tube (30 cm $\times$ 0.6 mm i.d.); GD, gas-diffusion cell; Pt, platinum conductive electrode; W, waste; C, carrier stream: 0.5 mmol/l phosphate buffer (pH 6.0); A, acceptor stream: 10 mmol/l Tris+1.0 mmol/l KCl. Flow rates are given in ml/min. (b): FM, flow meter; GC, gas coil; WC, wine cell; IP, injection port; SC, silica-gel column.

column, carried by the nitrogen flow into the detection cell, and detected by the THEED coated PQC.

Results and Discussion

Typical response curves

The typical response curve for the PIS-FIA method and the THEED-QCM method is illustrated in Figs. 2a and 2b, respectively. As can be seen, in both methods the injection of CO₂ standard results in a decrease in the oscillating frequencies of the detector. The proposed FIA method is obviously superior to the latter in terms of analytical speed and sensitivity. In addition, the response principles of these two methods are different from each other. The former is based on a non-mass effect, *i.e.*, the negative frequency shift is attributed to an increase in the conductivity of the acceptor solution due to dissociation of the CO₂ diffused from the donor stream. The latter is based on a mass-effect, *i.e.*, the signal is caused by an increase in the mass loading of the crystal due to an interaction between THEED and CO₂.

Fig. 2 Typical response curve of the PIS-FIA system (a) and THEED-QCM setup (b) caused by an injection of 120 μ l and 10 ml of 10 mmol/l CO₂, respectively.

Influence of the experimental parameters

The THEED-QCM method has been thoroughly investigated by Gomes *et al.*^{11,13} and Fatibello-Filho *et al.*¹², respectively. For the PIS-FIA method, the influencing factors should be considered to include those affecting the detector's performance and those affecting the gas-diffusion process.

It has been established that the mass transfer of a volatile analyte across a gas-permeable membrane is influenced by the temperature, the geometrical dimensions of the gas-diffusion cell, the membrane thickness, the pore size and porosity and the FIA parameters.^{15,16} It is much easier to change FIA parameters than to change other parameters mentioned above. Thus in this work preliminary tests were aimed at optimizing the FIA parameters. It was observed that decreasing the flow rate (particularly the acceptor flow rate, see Fig. 3) or increasing the sample volume would lead to an enhancement in sensitivity due to an increase in the mass transfer of CO₂ across the membrane, together with a decrease in the sampling rate and worse reproducibility due to an increase in dispersion. The FIA parameters illustrated in Fig. 1 were a compromise between the sensitivity, throughput and reproducibility. Although raising the temperature could also increase the sensitivity due to an increase in the gas-transfer efficiency, all experiments were performed at 25 $\pm$ 0.1 $^{\circ}$ C to control the noise level and the baseline shift within $\pm$ 1 Hz and 5 Hz/h, respectively.

According to Shen *et al.*⁴, for a dilute electrolyte solution, with the change in the permittivity being negligible and the other factors being kept constant, the relation between the frequency shift of the PIS detector (ΔF) and the conductance change (ΔG) is linear, and can be expressed as

$$\Delta F = \alpha \Delta G + \beta \quad (1)$$

where α is the slope, *i.e.*, the sensitivity, and β is the intercept. It should be mentioned that the sensitivity α is influenced by the conductance background (G_0), *e.g.*,

Fig. 3 Influence of the donor (1) or acceptor (2) flow rate on the sensitivity of the PIS-FIA system.

it can be improved by increasing G_0 over certain range. The composition of the acceptor stream can affect the FIA performance regarding both of these aspects, *i.e.*, in the conductance background (G_0), and in the dissociation degree of the CO_2 absorbed in it, and hence the conductance change ΔG . The influence of the acceptor composition was investigated using the same FIA manifold, except for the acceptor stream being H_2O , Tris and Tris/KCl, respectively. As shown in Fig. 4, the calibration graph obtained with the acceptor of Tris is obviously better than that obtained with H_2O in terms of the linearity and sensitivity; also for the former the sensitivity increases with increasing concentration of the added KCl until a maximum is reached at 1.0 mmol/l and subsequently decreases. The appropriate addition of an inert electrolyte, such as KCl, can significantly improve the sensitivity; this can be regarded as being a particular advantage over other FIA methods with the conventional conductometric detection, in which a high conductance background of the recipient solution may cause an unacceptable noise level.^{16,17} In addition, the non-linearity of the calibration graph obtained with water as the acceptor can be explained by the fact that the change in the solution conductivity is not linearly related to the concentration of the CO_2 trapped in the recipient solution, since carbonic acid is a weak acid.

Under the above-mentioned conditions, the influence of the cell constant of the detection cell was investigated. The cell constant was varied by changing the distance between the electrodes. Figure 5 shows that the sensitivity of the proposed FIA method increases with increasing the cell constant until a maximum is achieved at 1.37 cm, and then begins to decrease. This is because the influence of cell constant (k) includes two opposing aspects: when k increases the solution conductance (G) increases ($G=k\chi$, χ is the specific con-

Fig. 4 Calibration graphs obtained with various acceptor streams. A, H_2O ; B, 10 mmol/l Tris; C, 10 mmol/l Tris-0.1 mmol/l KCl; D, 10 mmol/l Tris-0.5 mmol/l KCl; E, 10 mmol/l Tris-1.0 mmol/l KCl; F, 10 mmol/l Tris-2.0 mmol/l KCl.

Fig. 5 Influence of the cell constant of the conductivity electrode on the sensitivity of the PIS-FIA system.

ductivity) and hence the sensitivity of the detector increases; meanwhile, the solution capacitance (C_s) also increases ($C_s=k\epsilon+C_p$, here ϵ is the dielectric constant and C_p the parasitic capacitance), which reduce the sensitivity.⁴

Selectivity

As mentioned above, one of the main drawbacks of the THEED-QCM method is the lack of selectivity especially due to the interference from SO_2 . To eliminate such an interference, the samples had to be pretreated with H_2O_2 , which made the method more cumbersome.

In the PIS-FIA method, although no pretreatment, except for simple filtration, was taken to the samples, a high selectivity was still available. This is because the gas-permeable membrane used in the FIA manifold offers an effective barrier for ionic species.^{15,18} Thus, the potential interference only arises from other acid gases that exist as their free forms in the donor stream, and can permeate through the membrane and then dissociate in the acceptor thus causing a conductance change. Based on the mass-balance equation, the percentage (P) of the free forms of some acid gases in an aqueous solution can be given by:

$$P(\%) = 100[\text{H}^+]/(K_a + [\text{H}^+]) \quad (2)$$

for a monoacid with a dissociation constant of K_a ,

$$P(\%) = 100[\text{H}^+]/(K_1 + [\text{H}^+] + K_1 K_2/[\text{H}^+]) \quad (3)$$

for a binary acid with its first and second dissociation constant being K_1 and K_2 , respectively.

Figure 6 demonstrates the computed results for P as a function of the solution pH. It can be seen that under a slightly acidic condition ($\text{pH}=5-6$), CO_2 mainly exists as its free form, which can diffuse across the membrane. Most of the other acid gases, *e.g.*, SO_2 , HAc , HCOOH , HF and NO_x , mainly exist as their hydrolytic

Fig. 6 Computed results for the percentage of the free forms of some acid gases as function of the solution pH.

states, which can not permeate through the membrane, and hence do not interfere with the CO_2 measurement. Although the free forms of H_2S and HCN are also of a high percentage, which means a relatively high gas-transfer efficiency across the membrane, it is extremely unlikely that these two substances would be present in wine and beer samples at levels sufficient to cause significant interference. Additionally, the difference in the diffusion coefficients in air and partitions between the aqueous and gaseous phase of the above substance would also result in different transfer degrees.

In the proposed method, a phosphate buffer (pH 6.0) was selected as the carrier stream. The pH of the donor

stream was thus similar to that of the original wine and beer samples, *i.e.*, the pH value was always in such a range where CO_2 mainly existed as its free form and other acid gases did not interfere with the determination. Since SO_2 is a frequently-used additive in the production of wine and beer, it was chosen to test the potential interference. As expected, it caused no significant interference, with a 5% error criterion, even when present in excess over CO_2 .

Applications and evaluation of the methods

The results for CO_2 in wine and beer obtained by the proposed PIS-FIA, the THEED-QCM and the conventional titrimetric methods, respectively, are presented in Table 1. It can be seen that although these three methods agree well with each other, the proposed method offers the best precision.

Table 1 Results for the determination of CO_2 in various samples obtained by different methods

Sample	Result (g/l) ^a		
	PIS-FIA	QCM-THEED	Titrimetric
White wine S1	0.473±0.007	0.463±0.023	0.481±0.026
White wine S2	0.859±0.011	0.846±0.035	0.852±0.041
White wine S3	1.18 ±0.014	1.21 ±0.038	1.16 ±0.036
White wine S4	0.257±0.006	0.253±0.015	0.261±0.018
Beer S1	5.47 ±0.045	5.53 ±0.149	5.39 ±0.151
Beer S2	3.59 ±0.038	3.72 ±0.097	3.61 ±0.114
Beer S3	4.56 ±0.042	4.73 ±0.120	4.53 ±0.127

a. Mean±SD ($n=3$).

Table 2 Comparison of the PIS-FIA method with the QCM-THEED method

Evaluation item	PIS-FIA method	QCM-THEED Method	
	this work	this work	Ref. 9
PQC's response principle	non-mass effect	mass effect	mass effect
Linear calibration range (mmol/l)	0.5 – 20.0	5.0 – 25.0	ca. 11 – 22
Calibration equation ^b obtained on 1st day	$\Delta F=2.5+51.7C$ ($n=10$, $r=0.9993$)	$\Delta F=135.8+12.3C$ ($n=7$, $r=0.9985$)	
2nd day	$\Delta F=2.1+51.9C$ ($n=10$, $r=0.9997$)	$\Delta F=103.6+10.2C$ ($n=7$, $r=0.9972$)	
Sample volume (ml)	0.12	10	10
Sampling rate (samples/h)	56	2	ca. 1 – 2
Detection limit (mmol/l)	0.01	0.3	
Precision (% RSD)	1.30 ^a	3.7 ^a	2.3
Interference from SO_2	negligible	significant	significant
Sample pretreatment with H_2O_2	unnecessary	necessary	necessary
Noise level (Hz)	±1	±3	
Baseline drift (Hz/h)	5	12	
PQC's life-span	long (over 1 year)	limited	limited

a. The average value obtained from Table 1. b. ΔF , the maximum frequency shift in Hz; C , the concentration of CO_2 in mmol/l; n , number of the standards, r , the correlation coefficient.

For an overall evaluation of the proposed method and the QCM method, they are compared in terms of other important items. From Table 2, it can be concluded that the PIS-FIA method is obviously advantageous over the THEED-QCM method in terms of most items, *e.g.*, the detection limit, sensitivity, calibration's validity, selectivity, through-put, reproducibility, sample volume, noise level, baseline shift, PQC's life span, as well as the manipulation, *etc.*

Additionally, it was previously shown^{7,8} that the PIS-FIA method is also advantageous over other detection modes employed in gas-diffusion FIA, such as the spectrophotometric^{15,19-21}, the conductimetric^{17,21-23} and potentiometric²⁴ methods in terms of cost, sensitivity or signal stability.

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