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An air-driving FI device with merging zones technique for the determination of formaldehyde in beers

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Abstract

An air-driving flow injection device with merging zones technique is proposed for the rapid determination of formaldehyde in pale beers based on its catalytic action on the redox reaction between Victoria Blue B and potassium bromate in phosphoric acid medium. The reaction is monitored spectrophotometrically by measuring the decrease in absorbance of Victoria Blue B at the maximum absorption wavelength of 618 nm. Only 160 μL reagents and 40 μL sample solutions are needed. Formaldehyde in the range 8–700 ng mL^{-1} can be determined at a rate of about 50 samples h^{-1}, the RSD for the determination of 20 ng m L^{-1} formaldehyde is 2.1%. Due to appropriate consideration of the beer matrix, formaldehyde in beers can be determined directly and satisfactory results were obtained.

Keywords: Air-driving FI; Merging zones technique; Catalytic determination; Formaldehyde

1. Introduction

Flavor and stability, an important quality criterion for beer, has long been a concern to the brewing industry. During storage, beer quality is gradually decreased and the production of stale flavor, formation of haze and browning occurs (Guido et al., 2004). Formaldehyde, which can enhance the flavor and reduce the undesirable formation, plays an important role in beer. The formaldehyde in beer results from two ways: one is natural production during fermentation; the other is artificial addition. Its adverse health effects are embodied by acute and chronic irritation and the inheritance of its toxicity. The American Conference of Governmental Industrial Hygienists (ACGIH) recognizes formaldehyde as a suspect human carcinogen. Maximal permission of formaldehyde in beers and wines has been regulated in most countries. Various methods have been developed for the determination of formaldehyde including chromatogram (Kim & Kim, 2005; Luo, Li, Zhang, & Catharina, 2001; Medvedovici, David, Sandra, & David, 1999; Oliva-Teles, Paiga, Delerue-Matos, & Alvim-Ferraz, 2002; Ones, Lister, Johnson, & Terry, 1999), electrometry (Korpan et al., 2000; Norkus, Pauliukaite, & Vaskelis, 1999; Parham & Zargar, 1998; Yang, Li, & Zhang, 2001; Zhang, Wang, & Li, 2000; Zhang, Zhang, & He, 2002), fluorimetry (Helaleh, Kumemura, Fujii, & Korenaga, 2001; Motyka & Mikuška, 2004; Pinheiro, de Andrade, Pereira, & de Andrade, 2004; Sakai et al., 2002; Zhan, Li, Zhu, Zheng, & Xu, 2000), spectrophotometry (Gayathri & Balasubramanian, 2000; Gigante et al., 2004; Teixeira et al., 2004; Zhou, Zhong, Tan, Li, & Hou, 2005) and other methods (Rivero & Topiwala, 2004; Feng, Liu, Zhou, & Hu, 2005). Chromatographic methods have been shown to provide adequate sensitivity, however, chromatographic methods are slow and cannot be easily adopted for routine analysis for expensive instruments; fluorimetric methods are always subject to interferences from some carbonyl compounds in beers. Only one method (Burini & Coli, 2004) was reported for the determination of formaldehyde in spirits, which has low sensitivity

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and time-consuming derivatization procedure. Flow injection catalytic spectrophotometric methods are attractive for its sensitivity, easy control and simple instruments, though there reported such methods (Zhang, Yan, & Yue, 2004) for the determination of formaldehyde, but all were not suitable for the determination in beers. The need for a specific and sensitive, yet simple and reliable, method for the determination of formaldehyde in beers is therefore clearly recognized.

In this study, a fast sensitive automated procedure capable of excellent precision for the rapid determination of formaldehyde in beers is proposed, which is based on its catalytic action on the redox reaction between Victoria Blue B (CI No.44045) and potassium bromate in phosphoric acid medium. Compared with the previous work (Zhang et al., 2004), owing to the adoption of air-driving flow injection device and merging zones technique, the sensitivity and analysis speed is distinctly improved; and the consumption of reagents and sample is greatly decreased, which is very important for samples not easy to acquire; above all, the proposed method can be applied to determining formaldehyde in beers without distillation preprocess due to appropriate consideration of the beer matrix.

2. Experimental

2.1. Apparatus

A schematic diagram of the flow injection system is shown in Fig. 1. A model IFIS-C intellectual flow injector (Xi'an Ruike Electron Equipment Corporation, China) was used to set up the flow injection system. A model 501 thermostat (Shanghai Experimental Apparatus Factory, China) was employed to keep the reaction temperature. A model 722 grating spectrophotometer (Shanghai Analytical Instruments Factory, China) with an 18 μL flow cell (light path, 10 mm) was used to measure the absorbance of indicator at 618 nm. The results were recorded by an automatic balance recorder (Shanghai Dahua Instrument Factory, China). Except for the pump tube (Tygon), PTFE tubing (0.9 mm i.d.) was used throughout the manifold.

2.2. Reagents

All chemicals used were of analytical reagent grade or higher. Doubly deionized water was used throughout.

Formaldehyde stock solution ($10\ \mu g\ mL^{-1}$) was prepared by diluting 0.1 mL of 37% formaldehyde solution (Merck) to 2 L with water and was standardized by the iodimetry (Cui, Wang, & Wang, 1997). Working standard solutions were prepared freshly by appropriately diluting the stock solution.

Victoria Blue B (VBB) solution ($5 \times 10^{-4}\ mol\ L^{-1}$) was prepared by first dissolving 0.253 g VBB in 1 L ethanol.

Phosphoric acid solution ($1.0\ mol\ L^{-1}$) from the 85% reagent and potassium bromate solution ($1.0\ mol\ L^{-1}$) were prepared by dissolving the required amount of reagents.

Model-717 resin (Xi'an Electric Power Resin Plant, particle diameter 0.3–1.2 mm) was soaked in threefold volume of saturated sodium chloride solution for 24 h and then washed with vast water; the resin was then first washed by 5% hydrochloric acid until the effluent did not reacted with ammonium thiocyanate, and secondly washed by 5% sodium hydroxide until the COD (chemical oxygen demanded) of the effluent remained constant, the last processing step was to wash the resin with water until the effluent remained pH 7.0. The processed resin was finally filled into a mini-column (5 cm in length and 2 mm in diameter).

2.3. Procedure

The flow system is shown in Fig. 1. In this system R_1 (VBB solution) and R_2 (potassium bromate in phosphoric acid solution) were online mixed and then filled the reagent loop L_1, meanwhile, S (sample) was driven through a anion exchange column A and the sampling loop L_2 in sequence, at this time, the valve was at filling position (Fig. 2a); and then the valve switched to the injecting position (Fig. 2b), solution zones in both L_1 and L_2 were driven by C (clear

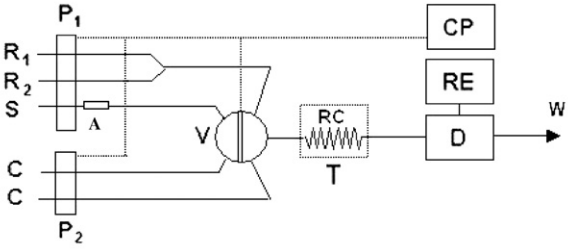

Fig. 1. Schematic diagram of the FIA system for the determination of formaldehyde. S, sample; C, air as carrier; R_2, potassium bromate in phosphoric acid solution; R_1, VBB solution; P_1, P_2, peristaltic pumps; V, injection valve; A, anion exchange column; RC, reaction coil; T, thermostatic water bath; D, detector; CP, computer controlling system; RE, recorder; W, waste.

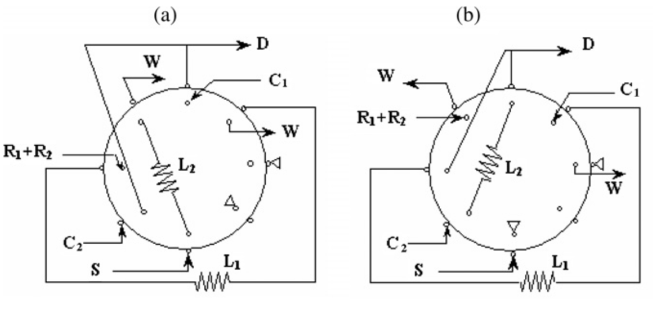

Fig. 2. Configuration of the injection valve for Fig. 1. (a, filling position; b, injecting position). L_1, reagent loop; L_2, sampling loop; △, block; R_1, R_2, C, S, D, W, the same as in Fig. 1.

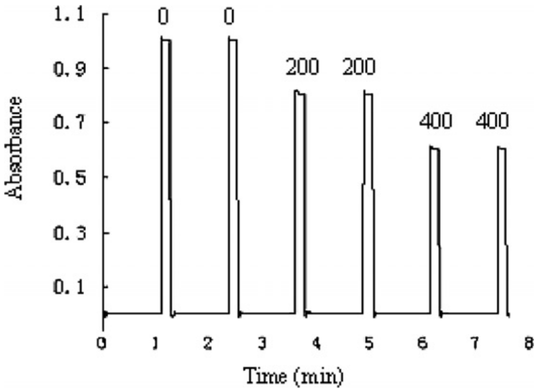

Fig. 3. Typical signals for the determination of formaldehyde. Numbers on the peaks refer to the corresponding formaldehyde concentration (ng mL^{-1}).

air) to mix with each other, and then the merged zones reacted in the reaction coil (RC) immersed in a thermostatic water bath for stopped-flow time as programmed before passing into the flow cell. Then the reaction zone was directed towards the detector (D) and the absorbance was measured at the maximum absorption wavelength of VBB (618 nm). When blank solution was measured, the absorbance was taken as A_0, and likewise formaldehyde solution as A. The difference $\Delta A = A_0 - A$ was used as the analytical parameter for the determination of formaldehyde. Typical signals for the determination of formaldehyde were shown in Fig. 3.

3. Results and discussion

3.1. Configuration designs and flow injection system optimization

In many cases the diffusion in flow injection is undesirable for its leading to the greater reagents consumption or reduction in sensitivity. In this work, a technique of merged solution zones driven by air was presented, as was shown in Figs. 1 and 2, reaction solutions were driven by air instead of solutions, which drastically eliminated the diffusion of the solutions, hence, extraordinarily less amount of reagents could suffice compared with former report, and the adherence of the indicator to the reaction coil diminished due to only low concentration of the dye was needed. Taking the potential interferences of anions from sample into consideration, an anion exchange column A was connected in the sampling flow to online remove the interferences.

Manifold parameters studied were volume of L_1 and L_2, flow rate, length of the reaction coil and stop time. The concentrations used in these experiments were as follows: S, 300 ng mL^{-1} formaldehyde; R_2, 0.20 mol L^{-1} potassium bromate in 0.10 mol L^{-1} phosphoric acid solution; R_1, 5.0×10^{-5} mol L^{-1} VBB; flow ratio of R_2 to R_1, 2. The reaction temperature was 70 ± 0.1 °C.

Large ratio of the volume of L_2 to that of L_1 can reduce the dilution of the sample and thus provide high sensitivity, 160 and 40 μL was chosen for L_2 and L_1, respectively, as a compromise between sensitivity and flow stability.

In order to merge sample zone and reagent zone effective, the flow ratio of C_2 to C_1 must remain the same with that of the volume of L_2 to L_1.

The sensitivity for the determination of formaldehyde obviously depended on the reaction time. Lower flow rate and longer reaction coil gave longer residence time, which could produce bigger ΔA, and consequent higher sensitivity, however, tailing were observed and sampling rate was slowed down with longer reaction coil or low flow rate. The best results were obtained by stopping the pump for a given period of time when the merged zone was located in the reaction coil. The optimum parameters chosen were as follows: reaction coil length, 50 cm; the injecting process comprised three steps: first, 4 s with 0.8 ml min^{-1} for C_1 and 3.2 ml min^{-1} for C_2; second, 60 s for stop flow; third, 10 s with 0.24 ml min^{-1} for C_1 and 0.96 ml min^{-1} for C_2. The timer was programmed such that 4 s after injection the reaction zone was stopped in the reaction coil for 60 s and then passed into flow cell for measurement. The total residence time was 74 s and the sampling rate was about 50 samples per hour.

3.2. Influence of reaction temperature

The influence of temperature on the sensitivity was studied in the range of 40–80 °C. Temperature has a very large effect on the reaction rate. The reaction rates of the catalyzed and uncatalyzed reaction increased with increasing temperature, the higher temperature, the more pronounced effect for the former, thus higher sensitivity (ΔA) could be obtained at higher reaction temperatures; on the other hand, a temperature of over 80 °C gave poor reproducibility because the air bubbles appeared inevitably. Therefore, a reaction temperature of 70 °C was maintained by placing the reaction coil in a thermostatic water bath.

3.3. Optimization of reagent concentrations

The effect of reagent concentrations was tested in the optimized flow system. The effect of KBO_3 concentration on obtaining a constant and maximum sensitivity was investigated. The reaction rates of the catalyzed and uncatalyzed reaction increased with increasing bromate concentration in R_2. However, the effect was more pronounced for the former up to 0.22 mol L^{-1}, and above which for the latter. As such a potassium bromate concentration of 0.22 mol L^{-1} was chosen in subsequent work.

Experiment results demonstrated that the catalytic reaction could proceed only in strongly acidic media. Between sulfuric and phosphoric acids, the higher and more constant sensitivity could be obtained in a phosphoric acid medium. The optimum concentration of H_3PO_4 was in the range 0.10–0.20 mol L^{-1}, under or above which the sensitivity for formaldehyde decreased slightly. Therefore, $0.15\,\mathrm{mol\,L^{-1}}$ H_3PO_4 in R_2 was chosen for further experiments.

VBB is the indicator for monitoring the catalytic decolorization reaction. Ethanol was selected as the solvent of VBB, which reduced the adherence of the indicator to the reaction coil and thus provided higher analysis speed due to faster restoration of the baseline. Meanwhile, the effect of ethanol content, the dominating component of beer matrix, on absorbance of the indicator was investigated, as was shown in Fig. 4, the absorbance of VBB increased with the percentage (V/V) of ethanol in reaction solution which located in the reaction coil, whereas, the absorbance exhibited a plateau when the value was above 3%, thus, when flow ratio of R_2 to R_1 was set at 2 and VBB was dissolved in 100% ethanol, considering the common content of ethanol in light beers (2–10%), the percentage of ethanol in reaction coil was bound to fall upon the plateau. As such, ethanol, the dominating component of beer matrix, had no effect on the formaldehyde determination due to appropriate pre-addition of ethanol to the system. Lower concentration of VBB in R_1 can obtain slightly higher sensitivity but narrower linear range for formaldehyde determination, very high concentration produced very large values in absorbance, which would result in a large reading error. $4.0\times10^{-5}\,\mathrm{mol\,L^{-1}}$ VBB was chosen as a compromise between sensitivity and linear range with the blank absorbance being 1.000.

3.4. Calibration graph, detection limit and precision

Under the chosen experimental conditions, the difference in absorbance (ΔA) varied linearly with the concentration of formaldehyde ranging from 8 to $700\,\mathrm{ng\,mL^{-1}}$ and fitted the equation

$$\Delta A = 0.001C + 0.002 \quad \gamma = 0.9997$$

where C was the formaldehyde concentration expressed in $\mathrm{ng\,mL^{-1}}$. The detection limit calculated from three times the standard deviation of the blank was $3\,\mathrm{ng\,mL^{-1}}$. Relative standard deviation of 11 replicate determinations of $20\,\mathrm{ng\,mL^{-1}}$ of formaldehyde was 2.1%.

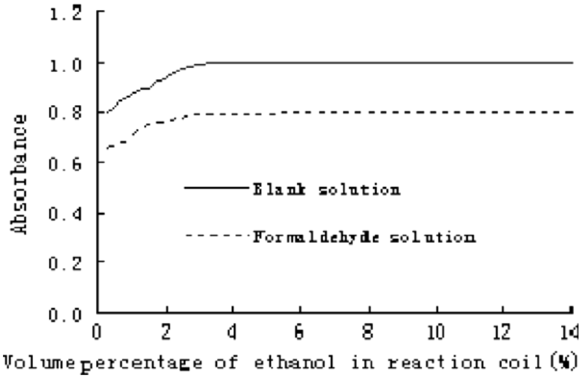

Fig. 4. The effect of ethanol content on the absorbance of the indicator.

Table 1
Tolerance limit of foreign species in the determination 100 ng $\mathrm{mL^{-1}}$ of formaldehyde

Foreign species	Tolerated ratio
K^+, Na^+, Mg^{2+}, Cu^{2+}, Al^{3+}, SO_4^{2-}, NO_3^-, Fe^{2+}, Zn^{2+}, Pb^{2+}, NH_4^+, Cl^-, CO_3^{2-},	10000
SO_3^{2-}, Mn^{2+}, Fe^{3+}, Ca^{2+}, formic acid, acetic acid, aldehyde, formate, acetate, glucose, fructose	2000
Oxalic acid, aspartic acid, glutamic acid, tartaric acid, citric acid, lactic acid	800
Gallic acid, vanillic acid	500
Br^-, CN^-, SCN^-	50
NO_2^-	$\leqslant 1$

3.5. Interference of foreign species

To study the selectivity of the proposed method, the effect of foreign species potential in beers on the determination of $100\,\mathrm{ng\,mL^{-1}}$ of formaldehyde was tested. The tolerance limit was defined as the concentration at which the species caused an error less than 5%. The results are summarized in Table 1. As can be seen, a large number of cations, anions and some organic species have no considerable effect on the determination of formaldehyde. Some anions such as NO_2^- do interfere and the interfering anions were successfully removed from the solution by passing it through a column containing anion exchange resin. So the system was expected to be useful in the formaldehyde determination in beers.

4. Applications

The proposed method was applied to the determination of formaldehyde in several Chinese brands of beers (June of 2005), including Qindao beer, Light Landmark beer, Light Hans beer, Snow beer, Yanjing beer. All were pale beers.

All samples were 10-fold diluted and degassed with ultrasonic-assistant sodalime-absorbing reduplicate flow procedure at 4 °C as usual before the determination so as to remove dissolved carbon dioxide, additionally, a gas–liquid separating device (PTFE membrane) coupled with self-made flow cell in the detector can remove trace gas accidentally produced.

The results which were compared with those obtained by iodimetry are given in Table 2. To examine the recovery, known amounts of formaldehyde were added to the samples. The results are also listed.

The direct determination results were also compared with those obtained by determining absorbing solution after sample distillation, there was a deviation below 8%, it indicated that direct determination is acceptable.

When the method was directly used for formaldehyde determination in black beers and turbid beer samples, absorbance of samples matrix at 618 nm could not be neglected, so the method can only be directly applied to those samples which have no or negligible absorbance at 618 nm without or with appropriate dilution, i.e. pale

Table 2
Results for the determination of formaldehyde in beer samples of different brands

Brands	This method[a] (ng/mL)	RSD (%)	Reference method[b] (ng/mL)	Added (ng/mL)	Found[c] (ng/mL)	Recovery (%)
Qindao	358	1.8	346	300	288	96
Landmark	420	2.3	434	300	311	104
Hans	234	1.3	218	300	280	93
Snow	369	1.9	356	300	283	94
Yanjing	417	1.8	425	300	314	105

[a] Average of six determinations.

[b] Obtained by standard method (Cui et al., 1997).

[c] Average of six determinations.

beers. For black beers and turbid beer samples, the formaldehyde determination can be performed only after distillation and subsequent absorption.

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The results answer for the determined samples, not for the brands. The research was supported by Doctor Innovation Foundation of Shaanxi Normal University, China.

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