Determination of Sulfite in Beer Using Ion-Exclusion Chromatography and Pulsed Amperometric Detection[1]

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ABSTRACT

The use of ion-exclusion chromatography combined with electrochemical detection has been suggested for the analysis of total SO_2 in beer. However, direct amperometry is prone to errors resulting from the loss of detector sensitivity, which occurs as the working electrode becomes contaminated. The sequential analysis of calibration standards and samples is required to overcome these difficulties. Pulsed amperometry was investigated as a means of maintaining electrode integrity and detector sensitivity and thereby eliminating the need for the sequential analysis of standards and samples. Correlation coefficients of 0.997 or better were obtained in both pH 9 buffer and beer. Excellent spike recoveries of SO_2 added to beer were obtained. The small coefficient of variation and good agreement with the standard para-rosaniline method indicates that this ion-chromatographic method is both precise and accurate.

Keyword: Total SO_2

The introduction of the Monier-Williams method (9) for the analysis of total SO_2 in food products has enabled the brewing industry to monitor the SO_2 content of beer. However, the complexity, insensitivity, number of false positive samples, and long analysis times associated with this method prompted the search for alternative methods of analysis (12,17).

During the 1950s, serious efforts were devoted to the search for an alternative method for the analysis of SO_2 in beer (4,11). This progress eventually led to development by Stone and Laschiver (11) of the para-rosaniline method which was adopted in 1960 as the official ASBC method (Beer-21) for the analysis of total SO_2 in beer (2,3). The method was revised in 1975 and is currently the approved method (1).

In recent years, it has become apparent that a certain percentage of the population is hypersensitive to sulfite residues in food products. The adverse reactions suffered by these individuals prompted the appropriate U.S. health agencies to thoroughly examine the use of sulfiting agents in foods and to promulgate regulations for the use and labeling of sulfiting agents in food and alcoholic beverages (12,14,15,17).

The current status of the analysis of sulfite in beer was addressed during the ASBC symposium on sulfite in beer in 1987 (17). Several concerns with the para-rosaniline method, which has served the brewing industry well for the past three decades, were highlighted. Alternative methods currently in use within the industry were also discussed. It was suggested that perhaps some of these methods have been adopted to shorten analysis times, which is a requirement of many quality control laboratories, without due concern for specificity, sensitivity, and accuracy of the alternate methods (17).

Ion-exclusion chromatography with electrochemical detection for the analysis of sulfite in foods (7,8) was suggested as one alternative to the para-rosaniline method (17). It is well documented that normal amperometry is subject to the introduction of errors associated with the loss of detector sensitivity, which occurs as the working electrode becomes contaminated. In fact, a 1990 collaborative study of sulfite in food and beverages reported that the loss of detector sensitivity could be as much as 40% over an 8-hr period (6). The sequential analysis of calibration standards and samples is required to overcome these shortcomings of direct amperometry (6).

Pulsed amperometry, which maintains the integrity of the working electrode, has been successfully used for the analysis of carbohydrates (5,10), alcohols (5), and the simultaneous analysis of ascorbic acid and sulfite (16). In this manner, the need for the sequential analysis of calibration standards and samples has been eliminated. A precise and accurate ion-chromatographic method that combines ion-exclusion chromatography with pulsed amperometry was developed for the analysis of SO_2 in beer.

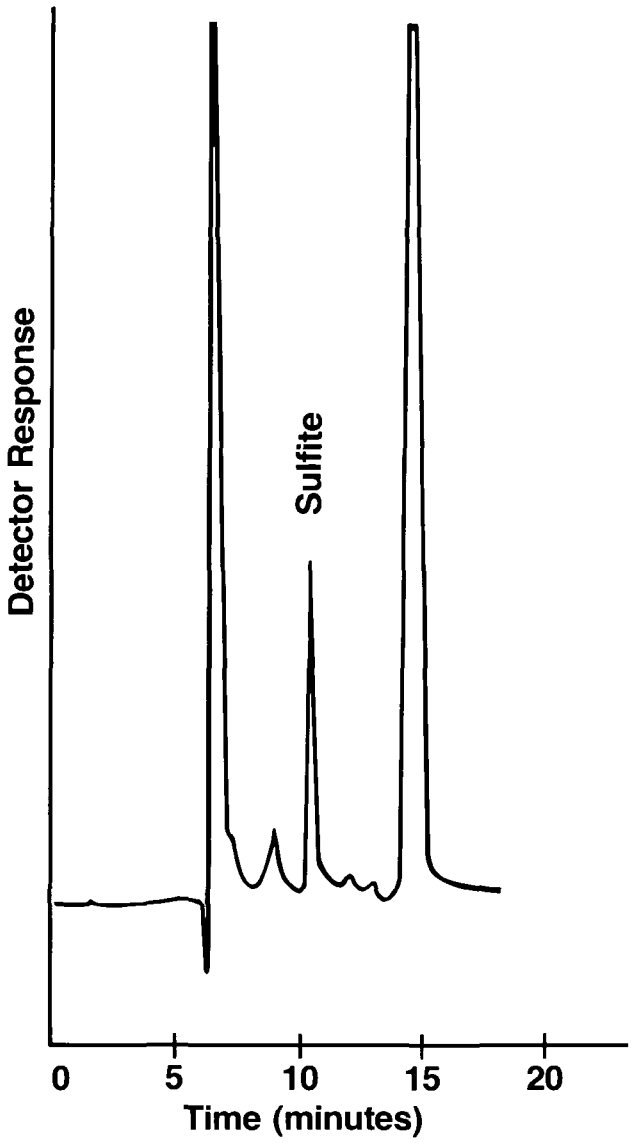

Fig. 1. Chromatogram of commercial 5% v/v alcohol beer containing 5.5 mg/L sulfite diluted 1:20 with pH 9 buffer.

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EXPERIMENTAL

A Dionex model 4000i ion chromatograph, a pulsed amperometric detector, a standard amperometric cell with a platinum working electrode versus an Ag/AgCl reference electrode, an autosampler, an HPICE-AS1 column and a Spectra Physics 4270 integrator for data handling were used throughout this study.

The optimized HPICE conditions were as follows: The eluant, 10 m*M* H_2SO_4, was prepared with deionized water and filtered using a 0.45-μm membrane. The flow rate was 1.0 ml/min. The standard amperometric cell was connected to a pulsed amperometric detector set at a range of 300 nA full-scale. A measuring potential of +0.70 V for 240 msec (E_1) and cleaning potentials of +1.25 V for 60 msec (E_2) and −0.10 V for 240 msec (E_3) were applied to the platinum working electrode. A 50-μl sample loop was used. The integrator attenuation was 1,024.

Reagents and Standards

All reagents were either Analar or certified ACS grade. A 1,000-mg/L standard SO_2 solution (Na_2SO_3, Fisher Scientific, Toronto, Ontario) was prepared daily in 20 m*M* Na_2HPO_4 (BDH, Toronto)/10 m*M* D-mannitol (BDH) buffer adjusted to pH 9 with 1.0*M* NaOH. The purity of the sodium sulfite was determined using the standard iodine-thiosulfate titration method (1). The phosphate was required for the determination of total SO_2 in the beer samples, whereas the mannitol was required to stabilize the SO_2 (6,7,16,17).

TABLE I
Determination of Precision of Method for Analysis of Total SO_2 in Beer (mg/L)

Sample	Trial 1	Trial 2
1	8.3	6.4
2	8.1	6.3
3	8.0	6.0
4	7.8	5.9
5	7.9	5.7
6	7.7	5.7
7	7.5	5.7
8	7.5	5.7
9	7.2	5.3
10	7.2	5.3
Range	7.2-8.3	5.3-6.4
Mean	7.7	5.8
D	0.3706	0.3651
cv	4.8%	6.3%

TABLE II
Determination of Total Random and Systematic Error of the Analysis of Total SO_2 in Beer

Sample	SO_2 (mg/L)
1	6.6
2	6.0
3	6.0
4	5.8
5	5.8
6	5.8
7	5.7
8	6.0
9	5.4
10	4.8
11	5.3
12	5.8
Range	4.8-6.6
Mean	5.8
D	0.4421
cv	7.7%

Preparation of Standards and Samples

A daily calibration standard solution (8.0 mg/L SO_2) was prepared by diluting 80 μl of the 1,000-mg/L standard solution to 10 ml with the above pH 9 buffer. This calibration standard was diluted 1:20 with the pH 9 buffer immediately before injection.

All beer samples were degassed by filtration through Whatman No. 4 paper and diluted 1:20 with pH 9 buffer immediately before injection.

RESULTS

The use of ion-exclusion chromatography with electrochemical detection for the analysis of sulfite in food was reported by Kim and Kim (7,8) and was suggested as an alternative for ASBC Beer-21 for the analysis of total SO_2 in beer (17).

Sulfite and ascorbic acid (including isoascorbic acid) are the two most common antioxidants currently in use in the food and beverage industry (13). This investigation was originally initiated to develop a rapid, semiquantitative method to detect the presence of added antioxidants in beer. A novel approach of pulsing a standard amperometric cell was successful in overcoming the loss of detector sensitivity associated with direct amperometry and provided a suitable rapid, semiquantitative method for the simultaneous analysis of ascorbic acid and sulfite in beer (16). Under these conditions, isoascorbic acid eluted at the same retention time as ascorbic acid and would therefore be included in the analysis. The precision of this method for both ascorbic acid and sulfite was reported previously (16).

In general, sulfiting agents are currently the antioxidant of choice in the brewing industry. The introduction of regulations for the use and labeling of sulfite in food and alcoholic beverages by the U.S. regulatory agencies (12,14,15,17), coupled with concerns about possible laboratory health risks associated with the ASBC para-rosaniline method (17), prompted further investigation of this ion-chromatographic method for the analysis of total SO_2 in beer.

To confirm the identity of the analyte, additions of 0–12 mg/L SO_2 were made to both pH 9 buffer and 5% v/v alcohol beer. Linear calibration curves with correlation coefficients of 0.997 or better were achieved. Recoveries of 95–105% of the theoretical amount of SO_2 added to beer indicated that the method

TABLE III
Comparison of Methods for Analysis of Total SO_2 in Beer (mg/L)[a]

	Ion-Chromatographic Method		Para-Rosaniline Method	
Sample	Analysis 1	Analysis 2	Analysis 1	Analysis 2
1	2.0	1.1	3	3
2	0.2	0.2	0	0
3	0.9	0.7	1	1
4	2.4	3.1	3	2
5	1.5	2.0	2	2
6	0.9	1.5	0	1
7	3.0	3.8	3	3
8	1.3	1.5	2	2
9	0.9	1.5	2	2
10	4.0	3.7	4	4
11	0.8	1.0	1	1
12	6.7	8.3	8	7
13	4.5	5.3	4	5
14	3.8	4.3	4	4
15	3.4	3.8	6	6

[a]Paired comparison Student's *t* test of the averages of the two methods: $t = 1.201$ and $df = 14$; for $df = 14$ at $P = 99.9\%$, $t = 4.140$. Analysis of variance test of the two methods: $F = 1.05$; for $df_1 = 29$, $df_2 = 29$, the critical value for F at 99% = 2.42. Therefore, the null hypothesis that the two methods are the same is not rejected.

was free of interferences. A typical chromatogram is illustrated in Figure 1.

The susceptibility of SO_2 to oxidation is a cause for concern. Therefore, care must be exercised to ensure that all samples are filtered and diluted without delay and injected into the ion chromatograph immediately after dilution (16). The precision of the method was determined by collecting 10 L of production 5% v/v alcohol beer in a 10-L stainless steel Cornelius soft drink dispenser under a CO_2 counterpressure. The vessel was partially immersed in an ice bath, and a standard restriction coil was used for sampling to prevent excessive foaming. At exactly 15-min intervals, the restriction coil was purged for 30 sec, after which a sample of approximately 50 ml of beer was collected. This sample was filtered, and after approximately 20 ml was collected, 0.5 ml was diluted to 10 ml with the pH 9 buffer. The diluted sample was then injected immediately into the ion chromatograph. A coefficient of variation (cv) of 4.8% was obtained for trial 1 (Table I). Although an excellent cv was obtained, the gradual decrease observed in trial 1 over the 3.5-hr analysis time may have resulted from temperature variations or minor changes in headspace composition that may have occurred as the beer was drawn off for analysis or sulfite oxidation. The beer was collected at 4°C and partially immersed in an ice bath for the remaining time of analysis.

To eliminate a temperature effect and assess the influence of minor changes in headspace volume and sulfite oxidation, this experiment was repeated by collecting 10 L of 5% v/v alcohol production beer in a similar 20-L container. The same sampling and analysis protocols were used. In this instance, the beer was collected 24 hr in advance to allow equilibration. The container was totally immersed in an ice bath for 4 hr before and throughout the entire analysis time. The results of trial 2 (Table I) produced a cv of 6.3%. The standard deviations obtained from both trials were virtually identical, suggesting that the modification made in trial 2 had no effect on the results. The cvs indicated that the precision of the method was acceptable. Therefore, the total error and accuracy of the method were evaluated.

Combined random and systematic error of the analysis, including bottle-to-bottle variations, were evaluated by analyzing 12 samples of normal 5% v/v alcohol beer. Individual bottles from a single purchased lot were analyzed consecutively. Immediately before injection, the samples were degassed by filtration and diluted 1:20 with pH 9 buffer. The samples were injected at exactly 20-min intervals. A cv of 7.7% was obtained (Table II).

The accuracy of the method was verified by comparing the results of the ion-chromatographic and the standard ASBC para-rosaniline methods. Fifteen cases of different Canadian beers were purchased, and from each case, two bottles were analyzed by the standard reference method and two by the ion-chromatographic method. Excellent agreement between the two methods was achieved (Table III). A paired comparison Student's *t* test of the averages of the two methods indicated that the null hypothesis that the two methods are equivalent (at the 99.9% confidence level) is not rejected. Analysis of variance of the two methods also indicated that the null hypothesis that the two methods are equivalent (at the 99% confidence level) is not rejected (Table III).

CONCLUSIONS

A reliable and accurate ion-chromatographic method for the analysis of total SO_2 in beer was developed. However, further work is required to shorten the analysis time and to obtain better stabilization of the SO_2 so that an autosampler could be used in a routine quality control role.

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