Chemiluminescent Methods in Alcoholic Beverage Analysis

M. J. Navas* and A. M. Jiménez

Department of Analytical Chemistry, Faculty of Pharmacy, University of Seville, 41012 Seville, Spain

Chemiluminescent (CL) techniques employed in alcoholic beverage analysis are summarized. Specific applications to wine, beer, brandy, and tequila are described, and the determination of sulfur compounds, phosphate, L-malate, glycerol, fatty acids, phenolic compounds, and urate is included. Possibilities and limitations of the various CL detection systems are evaluated.

Keywords: *Chemiluminescence; beer analysis; wine analysis; alcoholic beverage analysis*

INTRODUCTION

Chemiluminescence (CL) is the production of electromagnetic radiation (UV, visible, or IR) by a chemical reaction between at least two reagents in which an electronically excited intermediate or product is obtained and subsequently relaxes to the ground state with emission of a photon or by donating its energy to another molecule which then luminescences. CL emission can be characterized by the four parameters of color, intensity, rate of production, and decay of intensity, and the reaction conditions have a significant effect on the progress of the chemiluminescence. The intensity of light emission depends on the rate of the chemical reaction, the efficiency of production of the excited state, and the efficiency of light emission from the excited state. In recent years, CL has become a powerful analytical tool for sensitive and selective detection of chemical species. The advantages of CL for quantitative analytical applications include superior sensitivity, low detection limits, wide linear dynamic ranges, and speed of response, but it has some disadvantages such as poor reproducibility and long observation times, although not all the reactions are slow. In recent years numerous reports have been published about the importance of the CL methods in analytical chemistry (García Campana et al., 1997; Barnett and Lewis, 1996, Bowie et al., 1996) and their applications in the determination of a great variety of compounds, for example, in the drug (Calokerinos et al., 1995), food (Navas and Jiménez, 1996), agricultural (Jiménez and Navas, 1997), environmental (Jiménez et al., 1997; Navas et al., 1997) and clinical (Kricka, 1994) fields. Also, some bibliographic reviews have appeared about analytical techniques applied to alcoholic beverages (Illet, 1995; Stefani, 1995; Scollary, 1995).

We have attempted to expose in this work chemiluminescent methods as employed in alcoholic beverage analysis over the past few years, emphasizing the numerous advantages and the great possibilities that these techniques show in the quality control of these products. This work has been structured in three parts including wine analysis, beer analysis, and applications of CL to other alcoholic beverages. In each paragraph we comment on the principal determinations shown in the bibliography consulted, and the results are summarized in Tables 1−3.

APPLICATIONS OF CL ANALYSIS TO WINE

1. Determination of Antioxidant Capacity of Wine. During the course of a lifetime the human body is continuously exposed to potentially harmful oxidative stress, which contributes to the pathology of oxidative diseases, such as cardiovascular diseases, cancer, inflammation, and brain dysfunction (Simonetti et al., 1997). Recently, flavonoids and related phenolics have gained increasing attention for their antioxidant role, which may contribute to explaining the protective effect of vegetable-rich diets on coronary heart disease. Indeed, flavonoids and related phenolic acids are present in fruits, vegetables, and some beverages, being an integral part of the human diet. Red wine is a rich source of flavonoid antioxidants.

Whitehead et al. (1992) have developed a sensitive enhanced chemiluminescent assay for determining the total antioxidant capacity of biological fluids. A light emission can be produced by mixing the chemiluminescent compound luminol with an oxidant (hydrogen peroxide) and an enhancer phenol (*p*-iodophenol) in the presence of the enzyme horseradish peroxidase. The light emission can be detected in a conventional luminometer and depends on the constant formation of free radical intermediates of *p*-iodophenol and luminol. Therefore, samples containing radical-scavenging antioxidants interfere with light emission from the glowing chemiluminescent reaction. When all of the added antioxidants are consumed (oxidized) in the reaction, light emission resumes. The period of light suppression is then compared with a standard curve created by adding the water-soluble tocopherol analogue trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) to calculate the antioxidant activity of the test sample (μmol of trolox eqiv/L). This technique is very reproducible (within- and between-batch coefficients of variation for serum samples are 1.7 and 5.0%, respectively), as well as being cheaper and requiring less technical expertise than previously reported antioxidant assays.

Maxwell et al. (1994) have applied the last method to the determination of the antioxidant activity of red wine in the serum. This study demonstrated that ingestion of red wine is associated with increased antioxidant activity in serum. After ingestion of red

* To whom correspondence should be addressed (e-mail, navas@fafar.us.es).

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Table 1. Analysis of Wine

analyte	CL method	ref
	Determination of Antioxidant Capacity of Wine	
polyphenols	light suppression of luminol	Whitehead et al. (1992, 1995) Maxwell et al. (1994)
urate in serum	light suppression of luminol	Day and Stansbie (1995)
	Sulfur Products	
SO_2	oxidation of disulfitomercurate complex with cerium(IV) in acidic solution	Burguera and Burguera (1988)
sulfite	suppression of luminol CL	Huang et al. (1992)
sulfite	CL reaction between Na_2CO_3 and Cu(II) with sulfite	Lin and Hobo (1996)
	Determination of Other Analytes	
phosphate	reaction with luminol	Fujiwara et al. (1996)
L-malate	reaction with luminol	Kiba et al. (1995)
glycerol	reaction with luminol	Kiba et al. (1996)

Table 2. Analysis of Beer

determination	CL method	ref
	Deterioration of Beer Quality	
staling beer	CL produced after heating beer	Kaneda et al. (1990b)
flavor stability and effect of sulfite	CL produced after heating beer	Kaneda et al. (1991a)
oxidative reactions during beer pasteurization	CL produced after heating beer	Kaneda et al. (1994b)
beer quality during storage	CL producing after heating beer	Kaneda et al. (1994c)
influence of pH on beer flavor	CL producing after heating beer	Kaneda et al. (1997)
role of active oxygens during flavor staling	reaction with 2-methy-6-phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one	Kaneda et al. (1991b)
	Determination of Fatty Acids	
linoleic and linolenic acids	reaction of active oxygens with luminol	Kobayashi et al. (1993a−c)
trilinolein hydroperoxides	reaction with luminol	Kobayashi et al. (1994)
	Detection of Sulfur Compounds	
volatile sulfur compounds	SCD	Stenroos et al. (1994)
volatile sulfur	SCD	Derkensen et al. (1996)
sulfite	CL produced after heating beer	Kaneda et al. (1996)
diketopiperazine cyclo(Met-Pro)	SCD	Gautschi et al. (1997)
	Determination of Phenolic Acids	
p-coumaric acid	enhanced CL emission of luminol	García Sánchez et al. (1995)

Table 3. Analysis of Other Alcoholic Beverages

analyte	CL detection	ref
	Brandy	
sulfur compounds	sievers CL detector	Nedjma and Maujean (1995) Nedjma and Hoffman (1996)
	Tequila	
sulfur compounds	sievers CL detector	Benn and Peppard (1996)

wine serum antioxidant activity rose rapidly to reach a peak after 90 min, before a gradual decline. Furthermore, the increases of the antioxidant activity are of a magnitude that has been associated with significant inhibition of LDL oxidation in vitro.

Using a chemiluminescent assay of serum antioxidant capacity (SAOC), Whitehead et al. (1995) have studied the effects in normal individuals of ingesting red wine. Sodium perborate in the presence of horseradish peroxidase produces reactive oxygen species, and luminescence is produced if luminol is present. If *p*-iodophenol is also present, the light output is considerably enhanced and remains relatively constant for several minutes. If certain known antioxidants are added to the reaction, the light is suppressed until the antioxidant has been consumed, after which the light returns. The length of time of suppression of light is proportional to the quantity of antioxidant added. Adding to the chemiluminescent reaction 20 μL of a 1:500 dilution of the red wine given to the study subjects demonstrated its powerful antioxidant capacity. The flavonoid quercitin and three nonflavonoid polyphenols (hydroquinone, gallic acid, and 1,2,3-trihydroxybenzene) were studied for their antioxidant capacity. To the luminescent reaction was added 20 μL of a 50-fold dilution of a 10 mg/100 mL solution in 100 mL/L ethanol, and the reaction was monitored. All of these substances showed antioxidant activity. Despite the differences in the response of the individual polyphenols, in combination they gave a curve very similar in shape to that produced by red wine. The authors showed by enhanced chemiluminescence method that the red wine consumption results in an acute increase in serum total antioxidant capacity.

An antioxidant present at relatively high concentration in human serum is urate, an end product of purine metabolism. Day and Stansbie (1995) report changes in serum urate concentrations after consumption of port and wine, which suggests that polyphenolics may not be the only species that result in increased antioxidant activity after the consumption of alcoholic beverages. The total antioxidant capacity of the port wine was determined by the enhanced chemiluminescent method of Whitehead et al. (1992). A significant correlation was found between the increase in serum total antioxidant capacity and the increase in serum urate concentration.

2. Sulfur Detection. Before the wine is commercialized, this chemical parameter should be controlled both for its toxicity and for the changes of the organoleptic and chemical characteristics caused in the product before its consumption. The determination of sulfur products in wines is difficult because of their volatility and their very low olfactory perception levels, which require the use of highly sensitive detectors. Sulfur dioxide is widely used as a wine preservative due to its antiseptic power and antioxidation effect; moreover,

international regulation establishes a limit of the total SO_2 present in wines, which compels its control (Araújo et al., 1998). Numerous classical methods and standard methods are available for the determination of sulfur dioxide in wines, but none is very suitable for modern routine usage. Burguera and Burguera (1988) have described a flow-injection system for the determination of sulfur dioxide in young white wines based on the chemiluminescence emission resulting from the oxidation of the disulfitomercurate complex with cerium(IV) in an acidic solution and in the presence of riboflavin sulfate, used to sensitize the reaction. The procedure is simple and reproducible. Under the recommended conditions, the calibration graph was linear over the range 5.0−300 mL/L sulfur dioxide. The relative standard deviations for 10 and 100 mg/L sulfur dioxide, obtained from six replicate runs, were 3.2 and 2.5%, respectively. Results are in good agreement with those obtained by the spectrophotometric pararosaniline method, but unfortunately, reproducible results were not obtained with red wines.

A sensitive and rapid assay principle for sulfite determination was developed by Huang et al. (1992) based on the suppression of luminol chemiluminescence. The typical CL reaction of luminol catalyzed by horseradish peroxidase is quantitatively suppressed by sulfite and EDTA amplified the response.

It was found that EDTA enhanced the effect of sulfite on CL but had no effect on the CL when sulfite was absent. The CL reaction was monitored with ease and rapidity in a flow-through analysis system, but it was subject to interferences from some unknown factors when it was directly applied to the determination of sulfite in wines. For wine analysis, a flow-injection analysis system with a gas diffusion cell based on this principle was established. In the FIA system, the free sulfite could be detected as the suppression of CL corresponded to the concentration of SO_2 that was produced from the sample reacted directly with sulfuric acid and diffused through the gas diffusion cell; the total sulfite could be detected as the suppression of CL corresponded to the concentration of SO_2 that was produced from the sample reacted with sodium hydroxide and sulfuric acid sequentially and then diffused through the gas diffusion cell. The detection limit was considered to be at the millimolar level, and the relative standard deviation was <4% ($n = 5$) for each point in the measurable range. The method has been demonstrated to be fairly reproducible and specific toward sulfite. A direct determination without any extraction would be another advantage of this method. The authors have used a titration method as reference; the results obtained for free sulfite determination by FIA are always higher than those given by titration, and for total sulfite, the results obtained by FIA are always lower than those obtained by titration.

A simple flow-injection system with chemiluminescent detection for selective determination of sulfite is described by Lin and Hobo (1996). The light accompanying the reaction between sodium carbonate and copper(II) mixture solution with sulfite is detected. The method was applied to the determination of micro amounts of sulfite in wine. Because sulfite in wine is partly bound to other components such as acetaldehyde and anthocyanin pigments in wine, for the determination of the total sulfite concentration in wine samples, the sulfite must be liberated from its complexes before analysis. Chemiluminescent intensity is proportional to the concentration of sulfite in the range of $(1.0 \times 10^{-6})-(5 \times 10^{-4})$ M. The limit of detection is 5×10^{-7} M, and the relative standard deviation is 4.6% for the 5×10^{-6} M sulfite solution in nine repeated measurements. Based on the calibration graphs, the results agreed well with those obtained by a titration method with iodine.

3. Determination of Phosphate. A flow-injection chemiluminescence method has been proposed by Fujiwara et al. (1996) for sensitive determination of phosphate, after separation by ion chromatography (IC) in rice wine. The postcolumn detection system involved formation of heteropolyacid in a H_2SO_4 medium before the CL reaction with luminol in an NaOH medium. The detection limit for phosphorus(V) was 1 μg/L. This method, incorporating the advantages of FI into the sensitive CL assay, provided a rapid and reproducible method for the determination of phosphate. Linear calibrations were obtained for the analyte, and the linear dynamic range was 1−1000 μg/L for P(V). The relative standard deviation found using five replicate injections of a mixed standard containing P(V) was 3.7%. The analytical results indicated that the CL system is suitable as a sensitive detector for IC and the potential CL interference from metal ions in the samples could be eliminated sufficiently by IC. To verify the results for phosphorus(V), the authors have obtained measurements by coupled plasma atomic emission spectrometry (ICP-AE). The data were consistent with the those obtained by the proposed method.

4. Determination of L-Malate. L-Malic acid affects the sensory characteristics of wines, and its content is associated with microbial changes during aging and handling. A flow-injection system with a coimmobilized malate dehydrogenase/reduced nicotinamide adenine dinucleotide (NADH) oxidase reactor and a chemiluminometer is described by Kiba et al. (1995) for the determination of free L-malate in wine. NADH oxidase (NAOD) was used as a trapping enzyme, which catalyzes the oxidation of NADH with the production of hydrogen peroxide in the presence of molecular oxygen as an electron acceptor ($NADH + O_2 + H^+ = NAD^+ + H_2O_2$). The NAOD was coimmobilized with L-malate dehydrogenase (MDH) onto poly(vinyl alcohol) beads. The NADH produced by the MDH reaction is removed by the NAOD reaction with concomitant formation of hydrogen peroxide. The H_2O_2 produced was detected chemiluminometrically via a luminol−hexacyanoferrate(III) reaction. The calibration graph was linear from 3×10^{-7} to 2.5×10^{-4} M, and the detection limit was 8×10^{-8} M. The method gave precise and reproducible results.

5. Determination of Glycerol. Glycerol, which is a byproduct of alcohol fermentation, affects the sensory characteristics of wine, especially richness of taste. A chemiluminometric flow-injection method has been proposed by Kiba et al. (1996) for the determination of glycerol in wine. The method describes a system with a coimmobilized glycerol dehydrogesase (GDH)/NAOD reactor. A simple chemiluminometric FI system for the determination of glycerol can be achieved by coupling a coimmobilized GDH/NAOD reactor with a luminol chemiluminescence assay for the detection of H_2O_2, since the chemiluminometric reaction was carried out in media buffered at pH 10−11. In the present system, the enzymatic reaction and the chemiluminometric reaction were performed in the same buffered solution.

GDH and NAOD were coimmobilized on poly(vinyl alcohol) beads. This method was applied to the determination of glycerol in wines without any pretreatment procedure, except for dilution. The calibration graph was linear in the range $(3 \times 10^{-7})-(3 \times 10^{-4})$ M, and the detection limit was 7×10^{-8} M. The method gave precise and reproducible results, and the authors concluded that the system is useful for the sensitive measurement of glycerol in wine.

APPLICATIONS OF CL TECHNIQUES TO BEER ANALYSIS

1. Deterioration of Beer Quality. During storage, beer quality gradually decreases. The deterioration of beer quality is known to result from oxidative changes in beer, and the production of stale flavor occurs. Stale flavor was attributed to aldehyde formation in beer. Radical reactions are thought to play an important role during beer oxidation, and it has been demonstrated that free radicals were produced in beer and reduced quality. Kaneda et al., an investigation group of Brewing Research Laboratory in Sapporo, have developed several CL analyses for investigating problems in brewing science (1994a) and for quality control in breweries.

CL formation occurs during a series of radical reactions by activated oxygen, such as peroxide anion, hydrogen peroxide, singlet oxygen, hydroxyl radical, and hydroperoxy radical. Free radicals are generated during beer storage, and they oxidize several components, such as isohumulones and unsatured fatty acids, to aldehydes and ketones which could be responsible for the beer's stale flavor. The CL-producing reaction probably plays an important role in the early stage of this radical reaction. When fresh beer was incubated at 60 °C (Kaneda et al., 1990a), the CL intensity of beer remained constant for the first 30 min. After that, it increased, reached a maximum in 50−70 min, and then gradually decreased. The CL of oxidized beer had maximum emission intensities at 450, 480, 510, 550, and 580 nm. Many researchers have conjectured that excited carbonyl compounds have a maximum emitting intensity at 440 nm or from 520 to 530 nm, and singlet oxygen has many peaks such as 480, 520, 580, and 635 nm; therefore, it is possible that these compounds may be responsible for the CL production in beer. CL analysis should provide useful information in the study of oxidative deterioration of beer. Kaneda et al. have studied the evaluation of staling beer (1990b), the flavor stability and the effect of sulfite on flavor staling in several types of beers (1991a), the oxidative reactions during beer pasteurization (1994b), the relationship between chemiluminescence production and the deterioration of beer quality during storage (1994c), and the importance of the control of finished beer pH and the influence of pH on beer flavor (1997). The authors concluded that CL analyses can be effectively applied not only to investigating problems in brewing science but also for quality control in breweries. CL measurements could become popular analytical tools along with absorbance and fluorescence measurements.

The role of active oxygens during flavor staling in packaged beer was studied by chemiluminescence analysis by Kaneda et al. (1991b) using 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (CLA) which can only react with active oxygens such as $^1O_2$ and O_2^- and shows a marked luminescence. The maximum CLA luminescence intensities of the beers were reached sooner with increasing storage times, but their levels were almost the same. On the basis of the results presented, the authors have proposed pathways for the deterioration of beer flavor.

2. Determination of Fatty Acids. Fatty acids in wort and beer are important because they affect several beer qualities and yeast metabolism. Some fatty acids have a high flavor potential. Especially, linoleic and linolenic acids have received great attention because their oxidative degradation may lead to the formation of a characteristic aging flavor. The enzymatic or autoxidation of these fatty acids results in the formation of their hydroperoxides as primary products.

Linoleic and linolenic acid hydroperoxides in malt, mash, or wort were determined by Kobayashi et al. (1993a) with high sensitivity and high selectivity by chemiluminescence−high performance liquid chromatography (CL−HPLC). The principle of the method is as follows: Hydroperoxides separated by the HPLC column are mixed with luminescent reagent, and the chemiluminescence produced in the reaction is detected. Hydroperoxides react with heme peptide (cytochrome *c* or microperoxidase) and produce peroxy radical or oxidants which produce active oxygen ($2ROO^\bullet \rightarrow {}^1O_2 + ROH + RC{=}O$). The active oxygens oxidize luminol or isoluminol under alkaline conditions and generate chemiluminescence. A good linear relationship between the concentration of standard hydroperoxides and their peak areas was obtained by the CL−HPLC method. The detection and determination limits of linoleic and linolenic acid hydroperoxides were 10 and 1 pmol, respectively. Based on the results so far, it was shown that the CL−HPLC method is the most useful analysis of linoleic and linolenic acid hydroperoxides in malt, mash, or wort. Using this method, Kobayashi et al. (1993b,c) have clarified the lipid oxidation mechanism during mashing. They suggested that lipoxygenase and lipase in malt play an important role in the production of linoleic and linolenic acid hydroperoxides during mashing. The authors have also indicated that lipid oxidation occurs mainly during mashing and lipoxygenase oxidation takes place more dominantly than autoxidation.

Lipid hydroperoxides such as trilinolein hydroperoxides in barley, malt and mash were analyzed by Kobayashi et al. (1994) using the same CL-HPLC method and the authors report on the behavior of lipid hydroperoxides during mashing and discuss the mechanism of lipid oxidation. They confirm that lipoxygenase and lipase in malt contribute to lipid oxidation and that their proposed mechanism for lipid oxidation is acceptable. Free fatty acid hydroperoxides are unstable and degraded or transformed into several carbonyls and acids during mashing, some of these compounds are thought to survive into the finished beer and affect beer quality.

3. Detection of Sulfur Compounds. Nauseous volatile sulfur products such as hydrogen sulfide (H_2S), carbonyl sulfide (COS), sulfur dioxide (SO_2), thiols (RSH), sulfides (RSR), polysulfides (RS_nR, $n = 2, 3,...$), or thioesters (RCOSR") are found in beers. Most of these compounds are the cause of olfactory problems and in some cases organoleptic defects, because of their nauseous nature and their very low perception levels. However, some of them (with medium and high boiling points) may have a positive influence. Analysis of H_2S in an aqueous matrix at parts per billion levels is even more problematic. The techniques used for isolation of volatile sulfur compounds can directly influence the

accuracy, precision, and sensitivity of the above-mentioned procedure.

The subcommittee of American Society of Brewing Chemists (Stenroos et al., 1994) recommends a gas chromatographic method employing chemiluminescence for the detection of volatile sulfur compounds in beers, demonstrating that the detector gives a linear response and has better sensitivity and selectivity than other currently available methods. Based on the collaborative study, repeatability coefficients of variation of 5.7−8.1% and reproducibility coefficients of 8.1−25% can be expected for beers with dimethyl sulfide levels up to 160 μg/L.

Recently, the use of a membrane extraction device with a sulfur chemiluminescence detector (SCD) for the analysis of volatile H_2S in beer has been reported by Derksen et al. (1996). The principle of the SCD detection is:

$$\text{sulfur products} \xrightarrow{H_2/\text{air}} SO + H_2O + \text{other products}$$

$$SO + O_3 \rightarrow SO_2^* + O_2$$

$$SO_2^* \rightarrow SO_2 + h\nu$$

The membrane consists of a silicone tube, purged with nitrogen and placed directly in the beer. The sulfur compounds that permeate through the walls of the tube are collected cryogenically and thermally desorbed onto a capillary gas chromatography column for analysis. Replicate analysis of a single sample shows a coefficient of variation of <5%, whereas replicate analysis of multiple samples from the same batch gave a coefficient of variation of approximately 10%. Recovery of sulfur compounds is linear over the range normally expected in beer.

Sulfite is usually considered to prevent the flavor staling of beer, and some breweries have used it for the stabilization of beer quality. The sulfite is produced by yeast during fermentation, survives in the finished beer, and inhibits beer oxidation during storage, acting as an antioxidant. Kaneda et al. (1996) have developed a sensitive determination method for aldehyde-bisulfites in beer to clarify the existing state of sulfite in beer. When fresh beer was placed at 60 °C, chemiluminescence was immediately produced. The emission intensity was found to remain constant for 30 min. After that, it increased and reached a maximum after approximately 60 min. When the beer was stored at 37 °C for 2 days, the production of CL was accelerated. The CL intensity reached a maximum at an earlier stage, and the maximum CL intensity increased. When free sulfite or the acetaldehyde bisulfite was added to beer, the CL production was significantly inhibited. The CL intensities of beers with sulfites remained constant and did not increase, and the maximum CL intensities were not observed. It has been shown that the CL is produced via the free radical reactions of beer components during beer storage and that the oxidation process in beer and the staling degree of beer flavor can be evaluated from the CL producing pattern. It was shown that the acetaldehyde-bisulfite as well as the free sulfite inhibited the free radical reactions in beer. The authors have concluded that sulfite produced during fermentation mainly contributes to the flavor stability in beer by a radical-scavenging activity rather than by a masking activity for aging off-flavor.

Diketopiperazines (DKPs) corresponding to cyclic dipeptides have been identified in beer by Gautschi et al. (1997). DKPs are presumably formed during the normal manufacturing processes associated with beer production in which first malt and then wort are exposed to elevated temperatures for prolonged periods of time. Beer was diluted and extracted with dichloromethane. These beer flavor extracts were analyzed by gas chromatography employing various methods of detection: (i) mass spectrometry, (ii) flame ionization detection, (iii) sulfur-specific chemiluminescence detection (SCD), and (iv) nitrogen/phosphorus detection in nitrogen-specific mode. Further analysis of the extract by capillary GC employing sulfur-specific chemiluminescence detection indicated that one of these peaks in addition contained sulfur. The individual compound identified by GC−SCD was cyclo(Met-Pro), being present only at trace levels or not at all.

4. Determination of Phenolic Acids. Phenolic acids are a group of numerous organic compounds which have one or more hydroxyl functions and a carboxylic acid function at the benzene ring. These compounds increase or decrease the chemiluminescence of the luminol−H_2O_2−horseradish peroxidase system. Interest in the phenolic constituents of beers is generally centered on those polyphenols implicated in clouding phenomena; however, recently the incidence of various phenolic compounds on the flavor, stability, and organoleptic characteristics of beers has been studied. *p*-Coumaric acid (4-hydroxycinnamic acid) is a phenolic acid, and García Sánchez et al. (1995) have described a study of the enhaced chemiluminescence emission over a short period of time during the luminol−H_2O_2−horseradish peroxidase reaction using a stopped-flow technique and monitoring the initial rate between 0 and 0.08 s. They have determined *p*-coumaric acid in beers without the need for chromatographic separation; a calibration graph was prepared, and it was linear in the range 0−12.5 nM for *p*-coumaric acid. The midrange precision of the method, expressed as relative standard deviation, was 8%.

APPLICATIONS OF CL ANALYSIS TO OTHER ALCOHOLIC BEVERAGES

1. Analysis of Brandies. A quantitative analytical method was developed by Nedjma and Maujean (1995) for the detection of sulfur compounds with the static headspace technique using a Sievers chemiluminescence detector (SCD). The authors have evaluated the capacity of the SCD 355 and have improved the analytical conditions using the static headspace technique. Several new pieces of information have originated from this work: (i) the linearity and repeatability of the SCD 355 and the reproducibility of the sample preparation; (ii) the importance of the liquid-to-gas volume ratio, which yields optimum detection at a value of 4; (iii) the role of the ethanol content; (iv) the addition of salt which has no effect on the release of thiols from the liquid phase; and (v) the work temperature of 25 °C (even though increasing temperatures enhance the response much more than a change in the above parameters). The method gives a linear response over the concentration range 10−100 μg/L with a repeatability or reproducibility error of less than 5%. The results presented illustrate the high performance of the SCD 355. It is more stable than SCD 350 with time and results in higher reliability in terms of repeatability and repro-

ducibility. Moreover, it exhibits a linear response over a wide concentration range, in contrast to the FPD instrument.

Nedjma and Hoffmann (1996) have presented results concerning the formation of symmetrical and unsymmetrical dialkyl trisulfides in hydroalcoholic media via the reactivity of H_2S with thiols (methanethiol and ethanethiol) in the presence of copper(II). The authors have employed a gas chromatograh coupled to a chemiluminescence detector (SCD 355 from Sievers). The results obtained with cognac brandies show that after 24 h at room temperature, the reaction of H_2S in the presence of copper(II) with methanethiol leads to dimethyl disulfide and trisulfide. Reaction with ethanethiol under the same conditions leads to diethyl disulfide and trisulfide.

2. Analysis of Tequila. Tequila is an alcoholic distillate from a fermented mash derived principally from Agave tequilana Weber ("blue" variety) with or without additional fermentable substances, distilled in such a manner that the distillate possesses the taste, aroma, and characteristics generally attributed to tequila and bottled at greater than or equal to 80 proof (40% v/v alcohol). Benn and Peppard (1996) extracted the tequila using dichloromethane to characterize the tequila flavor. The extract obtained, which represented approximately 0.03% v/v of the original product, was analyzed by gas chromatography employing various methods of detection, one of them being sulfur chemiluminescence detection (SCD) which revealed the presence of dimethyl disulfide, dimethyl trisulfide, and 4-methyl-5-vinylthiazole, as well as numerous other, unidentified sulfur-containing constituents.

CONCLUSIONS

As previously mentioned, CL measurements can be used for alcoholic beverage samples, and with the aim of improving sensitivity of determination, the use of these reactions is interesting. In addition, CL reaction can be monitored with ease by using a sensitive light-detecting instrumentation, being relatively inexpensive and very simple. This paper has examined several CL techniques applied to beer, wine, and other alcoholic beverages. We claim that with the observation of the light suppression of luminol by some antioxidant compounds, we can determine the antioxidant capacity of wine. The reaction of luminol has been employed also in the determination of other compounds such as phenolic acids and fatty acids in beer and sulfite in wine. The SCD detector is, perhaps, the most employed detector of sulfur compounds in alcoholic beverages, but there are other CL reactions with good results as we have previously mentioned. It is possible to control the deterioration of beer quality by the CL produced after heating beer, and it can become an analytical alternative to other techniques such as fluorescence or spectrophotometry. Therefore, we can conclude that the CL reactions provide a reliable procedure to analyze and control alcoholic beverages, and the CL techniques can be used for routine analysis in laboratories.

LITERATURE CITED

Araújo, A. N.; Couto, C. M. C. M.; Lima, J. L. F. L.; Montenegro, M. C. B. S. M. Determination of SO_2 in wines using flow injection analysis system with potentiometric detection. *J. Agric. Food Chem*. **1998**, *46* (1), 168−172.

Barnett, N.; Lewis, S. Tripping the ligh fantastic. *Anal. Eur*. **1996**, March/April, 28−33.

Benn, S. M.; Peppard, T. L. Characterization of tequila flavor by instrumental and sensory analysis. *J. Agric. Food. Chem*. **1996**, *44* (2), 557−566.

Bowie, A. R.; Sanders, M. G.; Worsfold, P. J. Analytical applications of liquid-phase chemiluminescence reactions. A review. *J. Biolumin. Chemilumin*. **1996**, *11*, 61−90.

Burguera, J. L.; Burgera, M. Determination of sulfur dioxide in young white wines by flow injection with chemiluminescence detection. *Anal. Chim. Acta* **1988**, *214*, 429−432.

Calokerinos, A. C.; Deftereos, N. T.; Baeyens, W. R. G. Chemiluminescence in drug assay. *J. Pharm. Biomed. Anal*. **1995**, *13*, 1063−1071.

Day, A.; Stansbie, D. Cardioprotective effect of red wine may be mediated by urate. *Clin. Chem*. **1995**, *41* (9), 1319−1320.

Derksen, A.; Laurens, J.; Torline, P.; Axcell, B. C.; Rohwer, E. Quantitative analysis of volatile sulfur compounds in beer using a membrane extraction interface. *J. Am. Soc. Brew. Chem*. **1996**, *54* (4), 228−233.

Fujiwara, T.; Kurahashi, K.; Kumamaru, T.; Sakai, H. Luminol chemiluminescence with heteropoly acids and its application to the determination of arsenate, germanate, phosphate and silicate by ion chromatography. *Appl. Organomet. Chem*. **1996**, *10*, 675−681.

García Campana, A. M.; Baeyens, W. R.; Zhao, Y. Chemiluminescence detection in capillary electrophoresis. *Anal. Chem. News Features* **1997**, February 1, 83A−88A.

García Sánchez, F.; Navas Díaz, A.; González García, J. A. Study of the enhanced chemiluminescence from the luminol-horseradish peroxidase-hydrogen peroxide-p-coumaric acid system at very short times: stopped flow selective determination of p-coumaric acid in beers. *Anal. Chim. Acta* **1995**, *310*, 399−406.

Gautschi, M.; Schmid, J. P.; Peppard, T. L.; Ryan, T. P.; Tuorto, R. M.; Yang, X. Chemical characterization of diketopiperazines in beer. *J. Agric. Food Chem*. **1997**, *45* (8), 3183−3189.

Huang, Y. L.; Kim, J. M.; Schmid, R. D. Determination of sulfite in wine through flow-injection analysis based on the suppression of luminol chemiluminescence. *Anal. Chim. Acta* **1992**, *266*, 317−323.

Illett, D. R. Aspects of the analysis, role, and fate of sulfur dioxide in beer-a review. *Technol. Q. − Master Brew. Assoc. Am*. **1995**, *32* (4), 213−221.

Jiménez, A. M.; Navas, M. J. Chemiluminescent methods in agrochemical analysis. *CRAC* **1997**, *27* (4), 291−306.

Jiménez, A. M.; Navas, M. J.; Galán, G. Air analysis: Determination of ozone by chemiluminescence. *Appl. Spectrosc. Rev*. **1997**, *32*, 141−149.

Kaneda, H.; Kobayashi, N.; Takashio, M.; Tamaki, T. Contribution of sulfite to flavour stability of beer. *Proc. Conv.- Inst. Brew. (Asia Pac. Sect.)* **1996**, 24, 116−119.

Kaneda, H.; Kobayashi, N.; Tsuchiya, Y.; Munekata, M.; Koshino, S. Some applications of chemiluminescence analysis to brewing. *J. Am. Soc. Brew. Chem*. **1994a**, 52 (4), 163−168.

Kaneda, H.; Kano, Y.; Kamimura, M.; Osawa, T.; Kawakishi, S. Evaluation of beer deterioration by chemiluminescence. *J. Food. Sci*. **1990b**, 55 (5), 1361−1364.

Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Koshino, S. Free radical reactions in beer during pasteurization. *Int. J. Food Sci. Technol*. **1994b**, *29*, 195−200.

Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Koshino, S. Role of beer components on chemiluminescence production during beer storage. *J. Am. Soc. Brew. Chem*. **1994c**, *52* (2), 70−75.

Kaneda, H.; Kano, T.; Kamimura, M.; Osawa, T.; Kawakishi, S. Detection of chemiluminescence produced during beer oxidation. *J. Food Sci*. **1990a**, *55* (3), 881−882.

Kaneda, H.; Kano, Y.; Kamimura, M. A study of beer staling using chemiluminescence analysis. *J. Inst. Brew*. **1991a**, *97*, 105−109.

Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Koshino, S. Role of active oxygens on deterioration of beer flavor. *Eur. Brew. Conv. Proc. 23rd Cong*. **1991b**, 433−440.

Kaneda, H.; Takashio, M.; Tamaki, T. Influence of pH on flavour staling during beer storage. *J. Inst. Brew*. **1997**, *103*, 21−23.

Kiba, N.; Inagaki, J.; Furusawa, M. Chemiluminometric flow-injection method for determination of free l-malate in wine with co-immobilized malate dehydrogenase/NADH oxidase. *Talanta* **1995**, *42*, 1751−1755.

Kiba, N.; Azuma, N.; Furusawa, M. Chemiluminometric method for the determination of glycerol in wine by flow-injection analysis with co-immobilized glycerol dehydrogenase/NADH oxidase. *Talanta* **1996**, *43*, 1761−1766.

Kobayashi, N.; Kaneda, H.; Kano, Y.; Koshino, S. Determination of fatty acid hydroperoxides produced during the production of wort. *J. Inst. Brew*. **1993a**, *99*, 143−146.

Kobayashi, N.; Kaneda, H.; Kano, Y.; Koshino, S. The production of linoleic and linolenic acid hydroperoxides during mashing. *J. Ferment. Bioeng*. **1993b**, *76* (5), 371−375.

Kobayashi, N.; Kaneda, H.; Kano, Y.; Koshino, S. Lipid oxidation during wort production. *EBC Cong.* **1993c**, 405−412.

Kobayashi, N.; Kaneda, H.; Kano, Y.; Koshino, S. Behavior of lipid hydroperoxides during mashing. *J. Am. Soc. Brew. Chem*. **1994**, *52* (4), 141−145.

Kricka, L. J. The clinical and research potential of bioluminescence and chemiluminescence in medicine. In *Biolumin. Chemilumin., Proc. Int. Symp., 8th*; Campbell, A. K., Kricka, L. J., Stanley, P. E., Eds.; Wiley: Chichester, U.K., 1994; pp 171−178.

Lin, J.-M.; Hobo, T. Flow-injection analysis with chemiluminescent detection of sulfite using Na_2CO_3−$NaHCO_3$−Cu^{2+} system. *Anal. Chim. Acta* **1996**, *323*, 69−74.

Maxwell, S.; Cruickshank, A.; Thorpe, G. Red wine and antioxidant activity in serum. *Lancet* **1994**, *344*, 193−194.

Navas, M. J.; Jiménez, A. M. Review of chemiluminescent methods in food analysis. *Food Chem*. **1996**, *55*, 7−15.

Navas, M. J.; Jiménez, A. M.; Galán, G. Air analysis. Determination of nitrogen compounds by chemiluminescence. *Atmos. Environ*. **1997**, *21*, 3603−3608.

Nedjma, M.; Maujean, A. Improved chromatographic analysis of volatile sufur compounds by the static headspace technique on water-alcohol solutions and brandies with chemiluminescence detection. *J. Chromatogr. A* **1995**, *704*, 495−502.

Nedjma, M.; Hoffmann, N. Hydrogen sulfide reactivity with thiols in the presence of copper (II) in hydro alcoholic solutions or cognac brandies: Formation of symmetrical and unsymmetrical dialkyl trisulfides. *J. Agric. Food Chem*. **1996**, *44*(12), 3935−3938.

Scollary, G. R. Analytical chemistry and oenology: a productive blend of science. *Chem. Aust*. **1995,** *62* (12), 10−11.

Simonetti, P.; Pietta, P.; Testolin, G. Polyphenol content and total antioxidant potential of selected italian wines. *J. Agric. Food Chem*. **1997**, *45* (4), 1152−1155.

Stefani, R. Metals in wines. *Lab. 2000* **1995**, *9* (6), 50−55.

Stenroos, L.; Bates, M.; Burmeister, M.; Cerwinka, M.; Davis, N.; Hutte, R.; Lashley, R.; Lewandowski, R.; Nakagiri, H.; Sakuma, S.; Williamson, S.; Crumplen, R. Dimethyl sulfide in beer by chemiluminescence detection. *J. Am. Soc. Brew. Chem*. **1994**, *52* (4), 191−193.

Whitehead, T. P.; Thorpe, G. H. G.; Maxwell, S. R. J. Enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Anal. Chim. Acta* **1992**, *266*, 265−77.

Whitehead, T. P.; Robinson, D.; Allaway, S.; Syms, J.; Hale, A. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem*. **1995**, *41* (1), 32−35.

Xue-Xin, Q.; Yue-Ying, G.; Yamada, M.; Kobayashi, E. 7,7,8,8-Tetracyanoquinodimethane chemiluminescence sensitized by rhodamine B on surfactant bilayer membrane assemblies for determination of sulfide by a flow-injection method. *Talanta* **1989**, *36* (4), 505−508.

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