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Automatic Flow System with Voltammetric Detection for Diacetyl Monitoring during Brewing Process

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Diacetyl can be determined by adsorptive stripping voltammetry after derivatization with *o*-phenylenediamine. The method may be applicable to the determination of diacetyl in different foods, being a good alternative to other analytical methods. In this work an on-line automated analytical system for diacetyl determination in beer is described. A hanging mercury drop electrode voltammetric flow detector was used, and the analyte was determined without the traditional deoxygenation procedure. The method was successfully applied to the determination of diacetyl during beer fermentation and in the final product. The automation strategy used was based on a flow network similar to those used in multicommutated flow systems, with a pervaporation unit used for diacetyl separation. The developed system was tested in real conditions in the monitoring of brewing processes. The results obtained were similar to those obtained with the usual GC−ECD methodology in the 5−600 ppb range. The analytical rate of the developed method is about 12 determinations/h.

KEYWORDS: Diacetyl determination; beer; flow analysis; cathodic stripping voltammetry; automation; quinoxalines

INTRODUCTION

Diacetyl is a compound formed by oxidative decarboxylation of α-acetolactate, a product of amino acid (valine and leucine) anabolic metabolism. During fermentation the high levels of α-acetolactate in fermenting beer suggest an imbalance in the regulation of α-acetohydroxy acids production and their use for final amino acid production in yeast, leading to accumulation in the yeast and afterward an excretion to the beer. In the final stages of fermentation yeast reabsorbs the α-acetolactate from beer.

Diacetyl is a strong smelling compound that evokes a buttery aroma. Above a certain concentration, it imparts a negative effect on beer aroma, and therefore its level must be controlled during the fermentation and maturation periods. In lagers its concentration is generally slightly lower than 100 ppb, but it is usually higher in stouts and ales (*1*).

Diacetyl determination is one of the most important analyses for the brewing industry, as it is essential not only in several quality assurance procedures, but also for logistical and economic reasons as it indirectly assesses the end of the fermentation/maturation stages, called "diacetyl rest". The diacetyl level and its fermentation/maturation profiles are very important for brewers, as these profiles can provide an important tool for process control and can be used for evaluation of wort composition, and they can even assess eventual problems related to pitching and fermentation (*2, 3*). As diacetyl is a critical compound for beer flavor quality, its analysis is common within breweries as part of inspection and testing plans during brewing as well as in final product "audition" plans. The diacetyl reduction capacity of yeast has been used recently for predicting yeast vitality (*4*). Diacetyl has also found application in the flavor stability field, as a marker for beer aging (*5*).

The traditional spectrophotometric determination of diacetyl in beer (*6*) is being replaced by GC−ECD in many laboratories. Despite the good quality of the results, the chromatographic method has some drawbacks (*7*): the equipment is expensive, and the analysis is relatively slow, with an output of 4−6 samples/h (but without results during the first hour). However, the major disadvantage of the spectrophotometric and chromatographic methods is that they are not suitable for use outside the laboratory and on-line in the "cellars".

Voltammetric diacetyl determination in beer, wine, and other foods, after *o*-phenylenediamine derivatization, has proven to have the advantages in simplicity, sensitivity, and selectivity when compared with other instrumental methods *(8−10)*.

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However, despite their high sensitivity in some specific applications, electrochemical techniques using the hanging mercury drop electrode (HMDE) are not widespread in laboratory situations. Three major reasons are pointed out as being the principal drawbacks of these techniques: the time-consuming inert gas purge to remove oxygen interference (*11*), the laborious sample handling (*11*), and the lack of a reliable HMDE flow cell.

In previous work it was shown that the interference of oxygen could be overcome in some situations by using high-frequency square wave voltammetry (*9*). With this approach an adsorptive stripping voltammetric flow system with no need for any deoxygenating process was developed (*12, 13*) from a modified Taylor's flow cell (*11*). The instrument is easily adapted to commercial voltammetric stands, overcoming the difficulties of other HMDE voltammetric flow detectors (*12*).

In this work an automated on-line flow system for the determination of diacetyl using voltammetric detection is proposed, using the reduction of 2,3-dimethylquinoxaline resulting from on-line diacetyl derivatization with *o*-phenylenediamine, and its application to the determination of diacetyl in beer is evaluated.

MATERIALS AND METHODS

Instrumentation. Voltammetric determinations were performed using an Autolab PGSTAT 10 Voltammetric System (EcoChimie, Utrech, Netherlands) controlled with a PC equipped with GPES 4.8 and Windows 98 software. A Metrohm 663VA voltammetric stand was used in the SMDE mode, with drop size set to the 2 position. The three-electrode potentiostatic system was completed with a glassy carbon auxiliary electrode and an AgCl/Ag (3 M KCl) reference electrode. The HMDE voltammetric flow cell was fabricated in our laboratory, and is described below.

Two peristaltic pumps were used, a Gilson Minipulls 2 for the sample flow network and a Gilson Minipulls 3 (Villiers Le Bel, France) for the analysis flow network. Tygon tubing with different internal diameters was used according to the flow rate needed. The flow network consisted of model 161T031 three way solenoid valves (Nresearch, W. Caldwell, NJ) and a 0.8 mm i.d. PTFE tube.

The homemade Perspex pervaporation module was similar to a model already described (*14*). A new support for the membrane and other construction details were introduced, allowing better performance in terms of resistance, reproducibility, improved flow, and stability of the receptor chamber. The membranes used were of PTFE with 5.0 μm pore size and 47 mm diameter, from Gelman Sciences (Ann Arbor, MI).

A digitally controlled thermal heating unit, Falc-Termobloc TD150 P3 (Falc Instruments S.r.l, Lurano, Italy), was used for pervaporation. Three holes were made in the aluminum block of the heater to hold up to three pervaporation modules.

A Pentium PC equipped with a PCL-711s interface card (American Advantech, San Jose, CA) was used to control the solenoid valves through a homemade power drive based in a ULN 2003 integrated circuit, as well as to control the peristaltic pumps. The software was developed using Visual Basic 3.0 (Microsoft).

A GPES 4.8 Software project was developed for automatic data acquisition, data saving, and signal processing, using signal trigger transmission through the P2 in the DIO48 module of the Autolab and controlled by the above software.

The gas chromatograph used for comparison studies was a Varian 3400 with a Varian electron capture detector, coupled to a Varian Genesis auto-sampler.

Reagents and Solutions. All chemicals used were of analytical grade. Ultra-purified water with conductivity less than 0.1 μS/cm was used for solutions preparation. Phosphate buffer was prepared from 0.1 M di-sodium hydrogen phosphate and adjusted to pH 7.00 with 1 M HCl.

The 0.005% *o*-phenylenediamine derivatization solution was prepared fresh daily in a 0.1 M phosphate buffer, pH 7.00, and kept in a dark PVC container during use.

Diacetyl and 2,3-pentanedione stock solutions (1×10^{-3} M) were prepared weekly from the commercial products and kept in the refrigerator. The solutions used for standard additions calibration were prepared the day they were used.

Flow System Design. The overall system can be divided into two parts (**Figure 1**): one part for the extraction of diacetyl from beer (at the pervaporation cell) and the other part for the determination (at the voltammetric flow cell). The direct voltammetric determination of diacetyl in beer is not possible because of matrix interference. To develop an on-line analytical method a separation step has to be employed. Pervaporation was used as the separation technique. In this technique there are two distinct chambers separated by a gas permeable membrane. In the lower chamber, included in the beer circuit (A), diacetyl is evaporated from beer, passes through the hydrophobic membrane to the upper chamber, included in the analytical circuit (B), where it is readily derivatized with *o*-phenylenediamine. After pervaporation and derivatization the upper chamber content is transported into the voltammetric flow detector.

The module maintenance includes cleaning procedures and a membrane change every 3−4 months. Teflon O-rings, employed between every individual piece, were used to seal the module. The module began to deform and the material started to degrade in a progressive way and had to be replaced after 29 months of work. The peristaltic tubes were replaced every 3 weeks to avoid excessive tube weakness.

The HMDE voltammetric flow cell developed (**Figure 2**), although adapted from the Taylor model (*11*), contains some important modifications. The cell is characterized by having an adaptation/injection head with a cavity containing the tip of the capillary and the mercury drop. This flow voltammetric detection is based on the use of high-frequency adsorptive striping square-wave voltammetry. With square-wave high frequencies oxygen interference is minimized, thereby avoiding the traditional time-consuming need for deoxygenation of the solutions (*9*).

It must be stressed that the manifold and all the pumps must allow reproducible transport of the injected sample toward the cell. Also, the electrolyte concentration and flow must be constant during the adsorption step, a goal that was accomplished with the special construction of the cavity of the injection head. The detection cell also enabled an easy recovery of the mercury and the solutions, avoiding the problems related to the use of this kind of electrode.

As stated, the flow manifold was designed assuming two independent flow networks: one for beer pervaporation and the other for diacetyl determination. Two pervaporation cells were used in parallel (**Figure 1**). In the pervaporation flow network beer is pumped at a constant rate with a peristaltic pump. Valve V7 selects air or water flow during the cleaning step. Valves V8, V9, and V11 are used as return valves for air/water during pervaporation and for beer during the cleaning phase. Valves V10 and V12 establish the connection with the pervaporation modules, which work out of phase: when one is in the cleaning step, the other is in the middle of the pervaporation step.

By switching valve V1 it is possible to fill the upper chamber of the pervaporation modules with *o*-phenylenediamine or to pump the eluent. Valve V2 directs *o*-phenylenediamine to one or the other of the upper chambers. Valves V3−V4 and V5−V6 work always simultaneously and synchronously, closing both the entry and exit of the upper chambers of each of the pervaporation modules during the pervaporation or allowing the eluent to pass through the upper chamber during the injection of the sample or during the cleaning and the filling with *o*-phenylenediamine. The valves were placed in propulsion flow mode, except valve V1, for the selection between *o*-phenylenediamine and the eluent that is working in the aspiration mode. Propulsion mode is preferred to avoid the formation of gas bubbles in the manifold.

The sequence of analytical procedures and its correspondent pathways are determined by the array and disposition of the valves as well as their switching timing diagrams. This can be controlled by simply actuating the right valves at the desired time to open the desired path. In the developed software, multistep routines were inserted to synchronize the valve status level, if on or off, the speed of the peristaltic

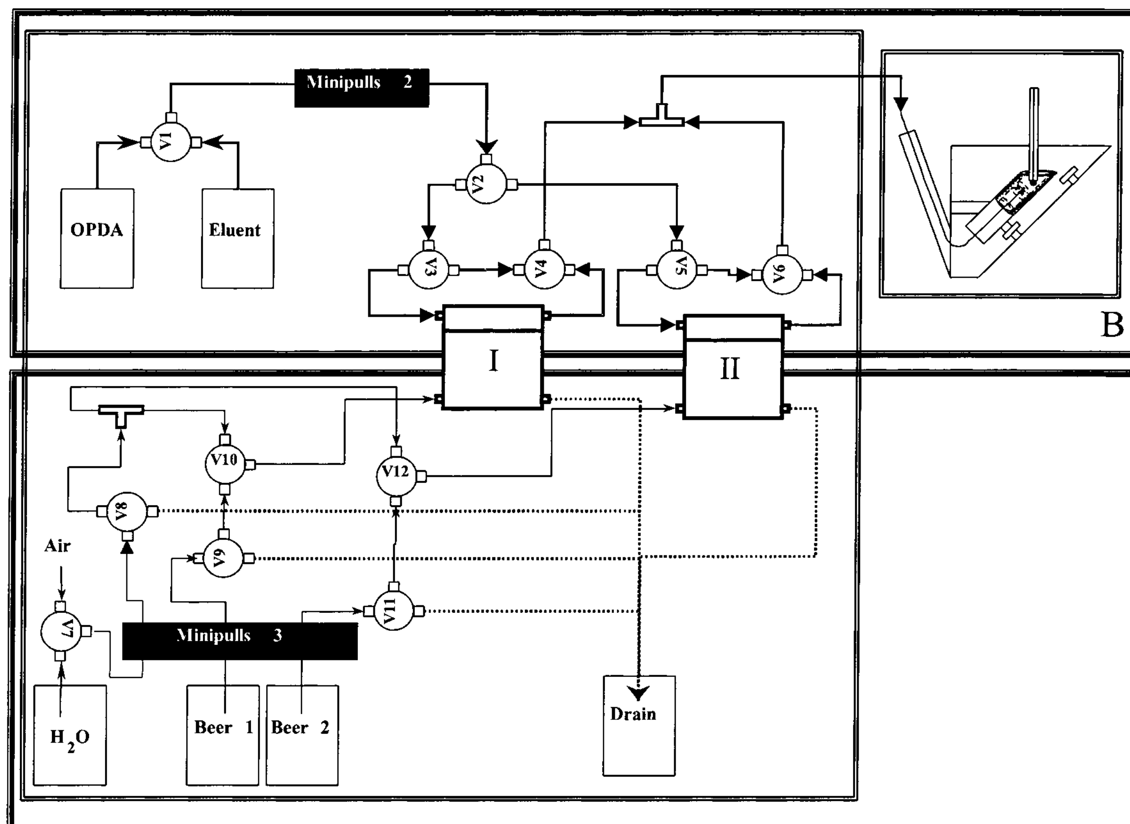

Figure 1. System diagram: A, beer pervaporation; B, diacetyl derivatization and detection; I, II, pervaporation cells, in parallel, working out of phase.

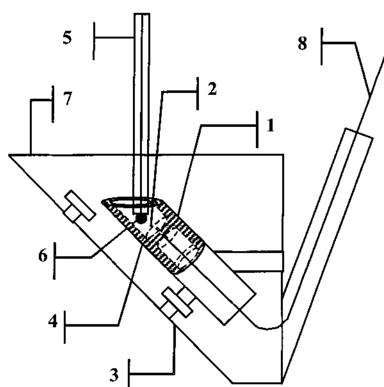

Figure 2. HMDE Flow Voltammetric Cell: 1, injector; 2, internal injector hole; 3, fitting for transport tube; 4, additional hole for mercury disposal; 5, capillar; 6, hanging mercury drop; 7, glass supporting cell; 8, transport tube.

pump, and the time interval desired to keep these before another synchronous step. The peristaltic pumps were always working in the same direction.

Each routine must comprise all the steps needed to perform a round of two analyses (the two different analytical and beer circuits for each pervaporation module) and at the end of each multistep routine the program restarts the routine for another round and so on. The control unit also enables a trigger signal for starting the GPES routine.

The first operation of the analytical procedure is the filling of the upper chamber with *o*-phenylenediamine. This chamber is then isolated, and the beer sample starts to pass through the lower chamber. Beer forms a thin continuous film at the bottom, facilitating diacetyl evaporation. Pervaporated diacetyl passes through the membrane and is readily derivatized with *o*-phenylenediamine at the upper chamber. At the end of pervaporation, the beer circuit is cleaned with water and air and the content of the upper chamber is transported to the detector. The tube between the pervaporation module and the flow cell must be short to avoid high dispersion of the injected analyte. This product of the reaction between diacetyl and *o*-phenylenediamine is adsorbed at the HMDE and determined by cathodic stripping voltammetry. Between injections, the flow cell is automatically cleaned by the eluent (phosphate buffer) that is permanently passing through it. A new mercury drop is formed for each determination. As the pervaporation of diacetyl is the limiting step of the overall process, lasting about 9 min versus the 2 or 3 min needed for the detection and cleaning of the flow cell, up to 3 pervaporation modules working out of phase can be used to increase the rate of analysis.

Sample Preparation and Reference Method. The samples from fermentation or maturation beer and nonpasteurized beer, when received in the laboratory, were immediately filtered twice through a paper filter to avoid most of the yeast entering the beer manifold where it could cause problems (dirt or clogging). Although in fermentation/maturation beer there is no significant level of free diacetyl, there is a “diacetyl potential”. In fact, its precursor, α-acetolactate, is usually excreted by yeast into beer, and therefore its conversion into diacetyl is necessary prior to the analysis. If the sample is to be analyzed for free diacetyl determination only, without any conversion of precursors, the beer sample may be directly aspirated into the developed analytical system and the analytical procedure can be started immediately. If the analysis is for total diacetyl a pre-conversion step is needed as in other conventional techniques. In the method developed, conversion was obtained by heating the beer sample for a period of 25 min at 90 °C in a closed vessel.

In the pasteurized final product samples all the α-acetolactate is converted into diacetyl. This means that in the case of pasteurized beer there is no need for pre-conversion. The final product only needs to be decarbonated before entering the beer manifold.

The samples for GC−ECD (control samples) were submitted to the same preparation as their voltammetric analogues before being analyzed.

Traditionally, diacetyl has been determined in beer using the spectrophotometric method adopted by the European Brewing Convention (EBC). More recently, an EBC gas chromatography method has been used instead. To assess the validity of the results obtained by voltammetry, beer samples were also analyzed by gas chromatography,

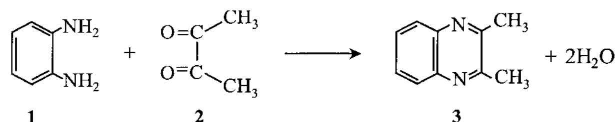

Figure 3. Derivatization reaction of diacetyl with *o*-phenylenediamine: 1, diacetyl; 2, *o*-phenylenediamine; 3, 2,3-dimethylquinoxaline.

using a chromatograph with electron capture detector, taking 110−120 min for each determination.

RESULTS AND DISCUSSION

The objective of this work was to develop a system that can be used in the usual conditions existing in cellars where it can be installed for on-line analysis of beer coming directly from the fermentation vessels, and analyses can be performed with minimal human interference and maintenance.

Development of the automatic analytical flow system was made using a strategy similar to those for multicommutated flow systems (*15*). A multi-valve automatic manifold has proven to be the most suitable for use in a continuous or semi-continuous flow network, involving consecutive cleaning, separation, and detection procedures.

The selectivity of the process was obtained using a beer pervaporation unit for diacetyl separation (*16*), its reaction with *o*-phenylenediamine in the receptor chamber, and the subsequent detection at the HMDE (*13*).

This system was developed in three steps. First, the flow voltammetric conditions were optimized for the determination of standard solutions of diacetyl without the inclusion of any separation step. Then, the pervaporation module was added to the system, and the new variables were studied including some adjustments of the flow detection and the consideration of interferences. Finally, the complete on-line diacetyl determination system was applied to the determination of diacetyl both in fermentation/maturation and in final beer product and the results were compared with those obtained using the GC−ECD reference method.

A good linearity was obtained in the voltammetric determination of diacetyl without pervaporation using a flow system. The relationship between peak current intensity, Ip (in nA), and diacetyl concentration, C (in ppb), can be expressed by the equation $\mathrm{Ip} = 2.92 + 1.75C$, with a correlation coefficient $R = 0.994$. Diacetyl is previously derivatized with *o*-phenylenediamine to form 2,3-dimethylquinoxaline, as can be seen in **Figure 3**. The derivatized solution is then loaded into a 500-μL loop of a 4-way injection valve and injected in a 0.1 M pH 7 phosphate buffer eluent. This eluent is passing continuously through the voltammetric cell.

This system was used for the determination of diacetyl in beer distillates using the standard additions method. The procedure involved the distillation of 100 mL of beer with the recovery of 25 mL of distillate as recommended in the EBC-Analytica for spectrophotometric determination of vicinal diketones (*6*). A total of 5 mL of beer distillate was diluted to 25 mL with a 0.125 M phosphate buffer, pH 7 and 0.00625% *o*-phenylenediamine, including the addition of different amounts of standard diacetyl, and the final solution was injected into the eluent. Results are shown in **Figure 4**. A concentration of 35.5 ppb of vicinal diketones was found in solution, corresponding to a concentration of 142 ppb in the initial beer sample.

The study of the detection process using pervaporation was made. The dynamic conditions around the HMDE are dependent on the injector head design but also on the properties of the eluent, including its flow rate. A better sensitivity, but a poorer

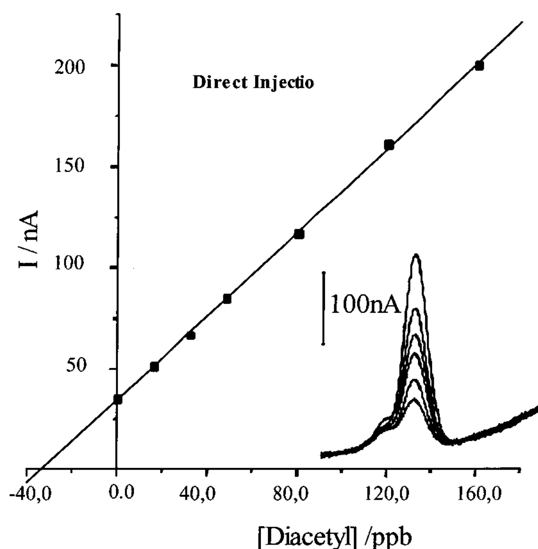

Figure 4. Standard additions curve for determination of vicinal diketones in beer. Derivatization off-line of beer distillate; square wave frequency 300 Hz.

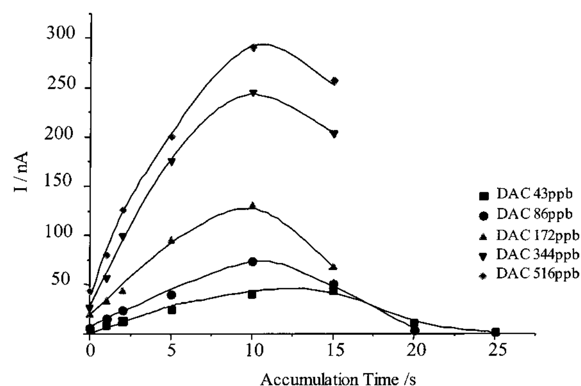

Figure 5. Effect of accumulation time in voltammetric flow signal for different diacetyl concentrations using the pervaporation module.

reproducibility, was obtained by increasing the flow rate. A flow rate of 1.33 mL/minute was adopted as a good compromise between these two opposing tendencies.

The *o*-phenylenediamine concentration was also evaluated using a derivatization solution of 0.005% in *o*-phenylenediamine. For lower concentrations the derivatization of diacetyl was too slow. Higher concentrations are not recommended because of interference of *o*-phenylenediamine at the electrode.

Adsorptive voltammetry was used because it was found that the analyte accumulates at the electrode prior to the voltammetric stripping scan, leading to an increase in sensitivity. In **Figure 5** the signal increase with adsorption time can be seen for different concentrations of diacetyl. After about 10 s the signal starts to decrease possibly due to the interference of *o*-phenylenediamine and its degradation products or to redissolution of the adsorbed analyte in the flowing eluent. As can be seen in **Figure 6**, calibration curves are not linear for higher accumulation times, so it was decided 5 s would be used, as with this accumulation time the problem was minimized.

As previously stated, oxygen interference can be eliminated in the determination of 2,3-dimethylquinoxaline if high square-wave frequencies are used. A square-wave frequency of 500 Hz was adopted because of broadening peaks and poorer baselines detected at higher frequencies.

Pervaporation time was also studied. This time is associated with the volume of beer that passes through the lower chamber

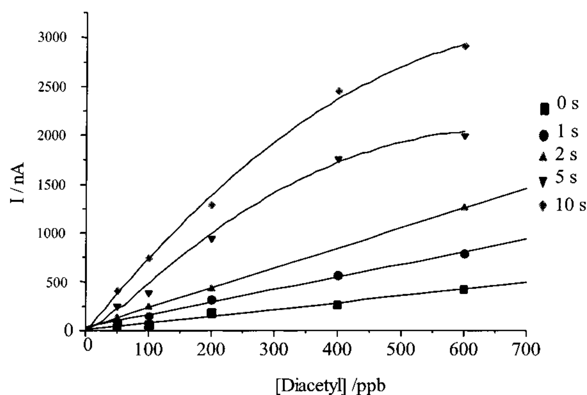

Figure 6. Calibration curves for diacetyl standard additions in water using different accumulation times. Experiments made using the pervaporation module.

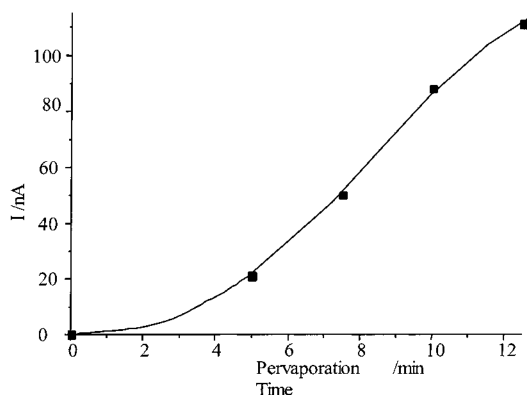

Figure 7. Effect of pervaporation time in the voltammetric signal of 2,3-dimethylquinoxaline resulting from the pervaporation of a beer.

of the pervaporation module (to maintain a laminar flow, a flow rate of 0.4 mL/min was assumed). **Figure 7** shows that there is a linear variation of the voltammetric signal for pervaporation times between 5 and 10 min. The signal is small for lower pervaporation times because some time is needed for the diacetyl to evaporate, pass through the separation membrane, and be derivatized in the upper chamber. For higher pervaporation times, some saturation of the derivatizing solution starts to occur. A pervaporation time of 9 min was adopted allowing an easy detection of the diacetyl concentrations that exist normally in beer samples (10 to 200 ppb). Lower pervaporation times can be used if samples have higher diacetyl content.

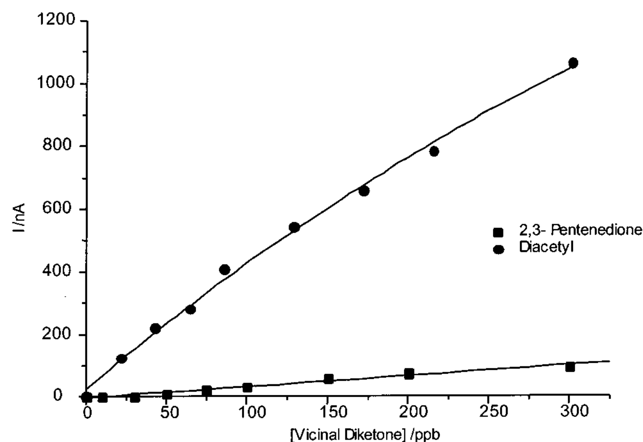

Figure 9. Voltammetric signals for additions to beer of diacetyl and 2,3-pentanedione, using the flow voltammetric pervaporation system developed in the optimized conditions.

The boiling point of diacetyl is 88 °C. From the results shown in **Figure 8**, it was decided to use a pervaporation temperature of 90 °C. Despite the higher peaks obtained at higher temperatures, these are not recommended because they cause a faster degradation of the pervaporation membrane, formation of bubbles in the upper chamber, and beer foam in the lower chamber; an additional problem is the pervaporation of 2,3-pentanedione (boiling point 100−112 °C) a compound that also exists in beer and that interferes in the determination of diacetyl.

Oxygen is the main cause of interference in any voltammetric reduction at the HMDE. This interference could be eliminated by using a fast square-wave scan, because the species to be determined (2,3-dimethylquinoxaline) is adsorbed at the electrode and its reduction is reversible. Ethanol is another interference to consider, as a slight decrease of sensitivity was observed in its presence, a situation that was already found in wine determinations (*12*). Nevertheless, this decrease does not affect the results in a significant way if the method of standard additions to beer samples is used.

2,3-Pentanedione is another carbonyl compound that exists in beer and that can interfere in the determination of diacetyl. In fact, although its concentration is generally lower than that of diacetyl, it also reacts with *o*-phenylenediamine, with formation of a quinoxaline which is reduced at the same potential as 2,3-dimethylquinoxaline. Nevertheless, 2,3-pentanedione is less volatile than diacetyl and only a small fraction of it is pervaporated at 90 °C, as shown in **Figure 9**.

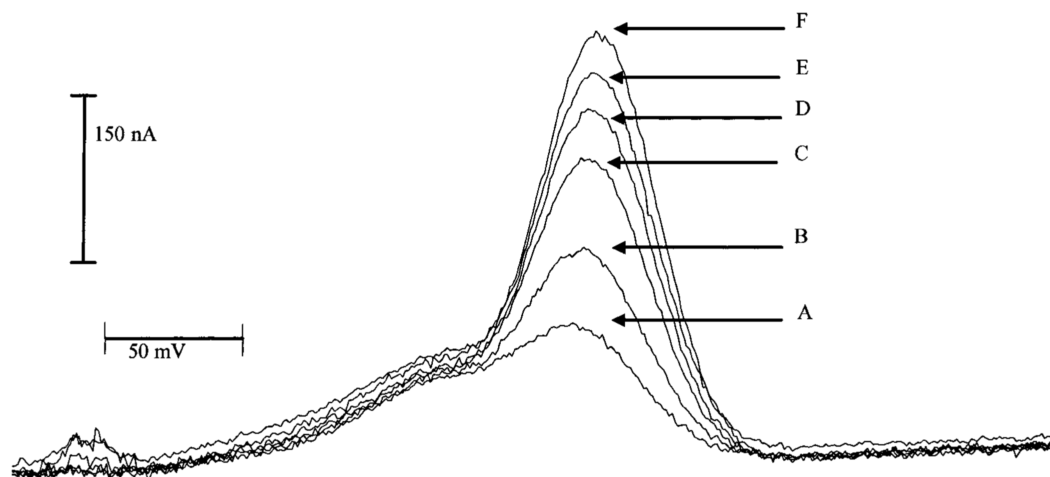

Figure 8. Effect of increasing the pervaporation temperature in the voltammetric signal of 2,3-dimethylquinoxaline resulting from pervaporation of an aqueous solution of 1×10^{-6} M (86 ppb) of diacetyl: A, 60 °C; B, 70 °C; C, 80 °C; D, 90 °C; E, 95 °C; F, 100 °C.

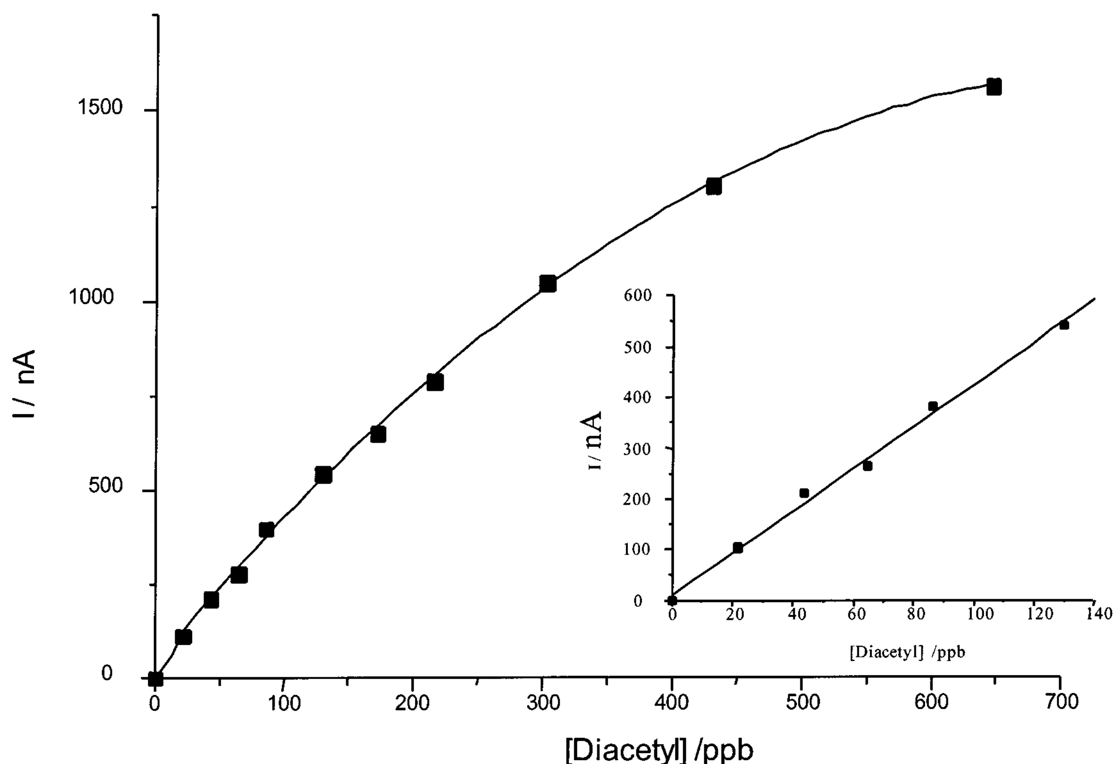

Figure 10. Diacetyl calibration curves using voltammetric flow detection coupled to a pervaporation unit.

APPLICATION TO THE ON-LINE ANALYSIS OF DIACETYL IN BEER SAMPLES

In the analysis of diacetyl in beer, sample volumes of 2500 μL at a flow rate of 1.33 mL/min were used, and the beer flow rate was 0.39 mL/min. As acceptor solution, a phosphate buffer 0.1 M, pH 7 and 0.005% *o*-phenylenediamine was used. The pervaporation/derivatization time was 9 min. The adsorption time at the electrode was 5 s (starting 22 s after injection), and the pervaporation temperature was 90 °C.

A square-wave voltammetric mode at 500 Hz frequency with $E_{ac} = -0.6$ V, $E_i = -0.6$ V; $E_f = -1.0$ V; $E_{step} = 1.05$ mV; and $E_{ampl} = 4.95$ mV was used. The 663VA stand was set to a drop size of 2. Although the operating range of the method can be varied by modification of some analytical parameters, the optimized conditions cover the concentrations of diacetyl that are important in brewing practice. With a limit of determination of 10 ppb of diacetyl and an upper determination limit of 800 ppb, the method is suited to both fermentation and maturation control as well as for diacetyl control in the final product.

In the case of low diacetyl levels, application of the standard additions method is recommended. Nevertheless, negative deviations to linear calibration were observed for diacetyl concentrations higher than 150 ppb, as can be seen in **Figure 10**, and the use of the calibration curve method is advisable in this case.

It is important to note that the sensitivity of the method for diacetyl determination is different in beer and in water. For this reason, it is recommended that the calibration curves used in the determination of the higher diacetyl contents be obtained using a "low diacetyl beer" as the blank solution, to which standard additions are made.

Automatic Flow System Versus GC−ECD Reference Method. The results obtained with the automatic flow system were compared with those obtained using the brewing industry reference method. The same samples, with the same pre-conversion treatment, were analyzed by both the developed system and by GC−ECD. The study involved 75 different samples, mainly samples at the end of fermentation/maturation, as well as final product and fermentation samples. Only two samples were collected well before the end of fermentation. Although noncontrollable differences may exist in GC−ECD because of the high-temperature headspace process, the methods correlate very well in the 5−600 ppb range. Regression analysis of the results shows a good correlation between both methods, with a slope of 1.01, a correlation coefficient of 0.993, and a standard deviation of 4.1 ppb between methods, with an axis intersection of 1 ppb.

With the separation procedure being the more time-consuming step of the system, two parallel pervaporation units with out-of-phase injection were used with the same voltammetric flow cell, allowing a sampling frequency of 12 determinations/h. Because of the short detection time, a third pervaporation unit could be used, with a corresponding increase in sampling frequency.

The method can also be used in the determination of "free diacetyl" as it can be applied without a previous conversion of the diacetyl precursors. This is an advantage over the GC−ECD which requires a heating period for headspace formation during which there are precursors converted into diacetyl. Besides providing a good alternative to other methods, the analytical system proved to be reliable during several months of use in the laboratory, opening the possibility of being applied to the production environment; thus, affording direct monitoring of diacetyl in beer fermentation as a possibility.

LITERATURE CITED

(1) Hammond, J. R. M., Rose, A. H., Harrison, J. S., Eds.; *The Yeasts, Vol. 5 − Brewer's Yeasts*, 2nd ed.; Academic Press Inc. Ltd.: London, 1993; pp 7−67.

(2) Moll, M. *Beers and Coolers*; Intercept Ltd.: Andover, England, 1991; pp 191−193.

(3) Master Brewers Association of the Americas. *The Practical Brewer*, 2nd ed.; Impressions Inc.: Madison, WI, 1977; pp 297−298, 332.
(4) Boulton, C. A.; Box, W. G.; Quain, D. E.; Molzahn, S. W. Vicinal Diketone Reduction as a Measure of Yeast Vitality. *MBAA − Technol. Quart.* **2001**, *38*, 89−93.
(5) Bravo, A.; Scherer, E.; Madrid, J.; Herrera, J.; Virtanen, H.; Rangel-Aldao, R. Identification of α-dicarbonyl compounds in aged beers: their role in beer aging process. *Proceedings 28th European Brewing Convention Congr.* Budapest; Fachverlag Hans Carl: Nürnberg, Germany, 2001; pp 602−611.
(6) European Brewing Convention. Vicinal Diketones in Beer: Spectrophotometric Method − Method 9.24.1. In *Analytica-EBC*, 5th ed.; Verlag Hans Carl: Nürnberg, Germany, 1998.
(7) European Brewing Convention. Vicinal Diketones in Beer: Gas Chromatographic Method − Method 9.24.2. In *Analytica-EBC*, 5th ed.; Verlag Hans Carl: Nürnberg, Germany, 1998.
(8) Rodrigues, J. A.; Barros, A. A.; Rodrigues, P. G. Differential pulse polarographic determination of α-dicarbonyl compounds in foodstuffs after derivatization with *o*-phenylenediamine. *J. Agric. Food Chem.* **1999**, *47*, 3219−3222.
(9) Barros; A. A.; Rodrigues, J. A.; Almeida, P. J.; Rodrigues, P. G.; Fogg, A. G. Voltammetry of compounds confined at the hanging mercury drop electrode surface. *Anal. Chim. Acta* **1999**, *385*, 315−323.
(10) Rodrigues, J. A.; Barros, A. A.; Machado Cruz, J. M.; Ferreira, A. A. Determination of diacetyl in beer using differential-pulse polarography. *J. Inst. Brew.* **1997**, *103*, 311−314.
(11) Taylor, L. R. Voltammetry Made Easy. *Chemtech* **1994**, *24*, 38−44.
(12) Rodrigues, J. A.; Barros, A. A.; Almeida, P. J.; Rodrigues, P. G.; Fogg, A. G. Flow injection square wave cathodic stripping voltammetric determination at a hanging mercury drop electrode of rapidly reduced compounds - Determination of diacetyl in wine as 2,3-dimethylquinoxaline. *Anal. Chim. Acta* **2001**, *449*, 119−127.
(13) Barros, A.; Rodrigues, J.; Almeida, P.; Guido, L.; Rodrigues, P.; Santos, J.; Machado Cruz, J. M.; Ferreira, A. A. Application of voltammetry in beer analysis. *Proceedings 28th European Brewing Convention Congr.*, *Budapest*; Fachverlag Hans Carl: Nüurnberg, Germany, 2001; pp 773−782.
(14) Mattos, I. L.; Luque de Castro, M. D.; Valcárcel, M. Pervaporation: an integrated evaporation/gas-diffusion approach to analytical continuous separation techniques. *Talanta* **1995**, *42*, 755−763.
(15) Lapa, R. A. S.; Lima, J. L. F. C.; Reis, B. F.; Santos, J. L. M. Continuous sample recirculation in an opened-loop multicommutated flow system. *Anal. Chim. Acta* **1998**, *377*, 103−110.
(16) Izquierdo-Ferrero, J. M.; Fernández-Romero, J. M.; Luque de Castro, M. D. On-line Flow Injection-Pervaporation of Beer Samples for the Determination of Diacetyl. *Analyst* **1997**, *122*, 119−122.

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