Differential Pulse Polarographic Determination of α-Dicarbonyl Compounds in Foodstuffs after Derivatization with *o*-Phenylenediamine

J. A. Rodrigues,* A. A. Barros, and P. G. Rodrigues

Centro de Investigação em Química da Universidade do Porto, Departamento de Química da Faculdade de Ciências, U. P. Rua do Campo Alegre 687, 4169-007 Porto, Portugal

A differential pulse polarographic method has been developed for the indirect determination of diacetyl and other vicinal diketones in foodstuffs, based on the quantitative derivatization of the diketones with *o*-phenylenediamine to give quinoxalines. The method allows the identification of diacetyl and methylglyoxal in brandy, vinegar, wine, and butter samples. The polarographic reduction peaks of the quinoxalines formed are sufficiently separate, in some cases, to allow the simultaneous determination of these two compounds. The polarographic method is not affected by the presence of sulfite ion, probably because the *o*-phenylenediamine is capable of dislodging the α-diketones from their sulfite complexes.

Keywords: *Polarographic method; diacetyl; methylglyoxal; α-diketones; brandy; butter; vinegar; sulfite; derivatization; quinoxalines*

INTRODUCTION

α-Diketones are well-known fragrant products that result from the activity of microorganisms during fermentation processes (Yamaguchi et al., 1994), and they are usually present in fermented foods, such as wine, brandy, yogurt, cheese, vinegar, or butter. Diacetyl is one of the more common of these products, and its determination in foods is important because it contributes to their taste and organoleptic quality. For instance, in the brewing industry, diacetyl formation must be controlled during critical stages of the production of beer (Wainwright, 1973), due to its unpleasant butter-like taste. Methylglyoxal is also of biological relevance. Indeed, it is the substrate of an enzymic system involving glyoxalase I and glyoxalase II, which have been found to be widespread in all living organisms, without the system being well understood (Thornally, 1990).

The reaction with 2,4-dinitrophenylhydrazine is the basis of several methods of determination of α-diketones involving UV detection (Fung and Grosjean, 1981), including HPLC methods. The use of this reaction has, however, a tendency to produce high results, because aldehydes and ketones can interfere; also, the high temperatures required for the derivatization can induce changes in the sample. A more convenient HPLC method involves derivatization with *o*-phenylenediamine derivatives and UV detection of the resulting quinoxalines (McLellan et al., 1992). This method is specific for α-dicarbonyl compounds, and the changes in the sample are minimized as derivatization is accomplished at a low temperature and in aqueous solution. Gas chromatography is another method that has been applied to the determination of α-diketones, including diacetyl (Dupire, 1998). Although this technique cannot be directly applied to the determination of methylglyoxal, owing to the decomposition of this compound at the high temperatures needed in GC, a method has been described for the analysis of methylglyoxal by GC, after previous derivatization of this α-diketone with cysteamine (Hayashi and Shibamoto, 1985). Another problem is that α-diketones can complex quite easily with other compounds, such as sulfite, which decreases their vapor pressure when the GC method with headspace sampling is used (Verhagen et al., 1987).

The polarographic method that has been developed here is based on the reaction of α-dicarbonyl compounds with *o*-phenylenediamine (OPDA). The resulting quinoxalines are polarographically active (Wasa and Musha, 1967) and can be determined using differential pulse polarography. The method has been applied to the determination of diacetyl in beer with a detection limit of 5 ppb: this is a decade lower than that obtained using spectrophotometric detection (Rodrigues et al., 1997). Here the method has been developed further and has been applied to other foodstuffs. The combination of the selectivity of the derivatization reaction and the selectivity of the polarographic technique allows the determination of diacetyl and methylglyoxal to be made directly in some food samples without further treatment.

MATERIALS AND METHODS

Equipment. Differential pulse polarographic determination was made using a 646 VA processor and a 647 VA stand from Metrohm SA, Herisau, Switzerland. A three-electrode system was employed with a Metrohm multimode electrode (MME) as working electrode, a platinum wire as counter electrode, and a silver/silver chloride (3 M KCl) reference electrode.

Steam distillations were made using a conventional steam distillation system.

Reagents and Solutions. All chemicals used were of analytical grade. Deionized and distilled water was used for preparation of solutions.

Stock solutions of methylquinoxaline and dimethylquinoxaline (5×10^{-4} M) were prepared by dissolution of the compound in water. The *o*-phenylenediamine (OPDA) (Merck KgaA, Darmstadt, Germany) derivatization solution was prepared

* Author to whom correspondence should be addressed (fax 351-2-6082938; e-mail jarodrig@fc.up.pt).

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fresh daily by dissolution of the appropriate amount of this compound in the buffer solution used for derivatization. This solution was kept in a dark place. Suitable precautions were taken in using *o*-phenylenediamine as it is toxic and may also cause allergic reactions.

Stock solutions of methylglyoxal and diacetyl (0.1 M) were prepared from the commercial products obtained from Sigma-Aldrich S.A., Madrid, Spain.

Samples of brandy, butter, wine, and vinegar were directly purchased from a food store.

Recommended Procedures. *Brandy.* Aliquots of OPDA (25 mL, 0.05%) in 0.1 M phosphate buffer (pH 7) were added to the polarographic cell. An aliquot of the brandy (5 mL) was taken directly from the bottle and added to the cell. This solution was analyzed by differential pulse polarography.

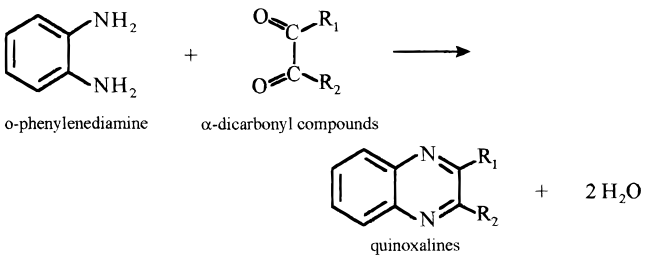
Butter. About 10 g of butter sample was accurately weighed and put into the distillation flask. Water (50 mL) was added, and this mixture was steam distilled until ~10 mL of distillate was obtained. The distillate was transferred quantitatively to a graduated flask, 5 mL of 0.25% OPDA in 0.5 M phosphate buffer (pH 7) was added, and the solution was diluted to 25 mL. The solution was transferred to the polarographic cell and was analyzed by differential pulse polarography.

Wine and Vinegar. Wine (25 mL) or vinegar (10 mL) was accurately measured and put into the distillation flask. In the case of vinegar, 10 mL of 1 M sodium hydroxide solution and 10 mL of 0.1 M phosphate buffer (pH 7) were added to neutralize the acetic acid, and in both cases the sample was steam distilled until ~10 mL of distillate was obtained. The distillate was quantitatively transferred to a graduated flask, 5 mL of 0.25% OPDA was added, and the solution was diluted to 25 mL. The solution was transferred to the polarographic cell and analyzed by differential pulse polarography.

Polarographic Conditions. The differential pulse polarographic determinations were performed in deoxygenated solutions (by bubbling nitrogen for 10 min) using the following polarographic conditions for the potential scan: $t_{drop} = 1$ s; pulse amplitude = −20 mV; scan rate = 6 mV s^{-1}. The quantitation was made using the standard addition method, by adding diacetyl or methylglyoxal standard solutions directly to the polarographic cell. After each standard addition of diacetyl, nitrogen was bubbled through the solution for 3 min before the application of the potential scan. In the case of methylglyoxal nitrogen was passed for 5 min before scanning.

RESULTS AND DISCUSSION

Polarographic Determination of α-Diketones. The polarographic method used in the determination of diacetyl and methylglyoxal involves their derivatization with *o*-phenylenediamine, accordingly to the following scheme:



This reaction takes place in aqueous medium, at room temperature, and the conversion of the α-dicarbonyl compounds into their corresponding quinoxaline derivatives is quantitative over a broad pH range (pH 1−10). The times required for the derivatization of diacetyl and other similar α-dicarbonyl compounds generally present in fermented products are given in Table 1 for two different pH values.

Electrochemical sensitivity is poor in acidic media (Strier and Cavagnol, 1957), and the best sensitivity is obtained with pH 7 phosphate buffer solution. The use of alkaline conditions is not recommended as slow decomposition of the α-diketones can occur (Rodriguez-Mellado and Ruiz, 1986). Depending on the sample being analyzed, a prior distillation step is required to eliminate interferences, such as certain α-dicarbonyl compounds. Only diacetyl and 2,3-pentanedione are quantitatively recovered in the distillation step (see Table 2).

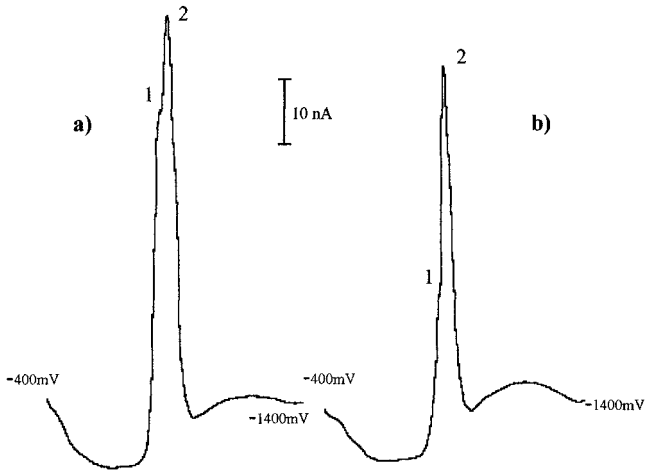


Figure 1. Polarographic analysis of an aqueous solution of 2 × 10^{-6} M diacetyl and 2 × 10^{-6} M methylglyoxal: (a) direct analysis; (b) analysis after simple distillation. Peak 1 refers to methylquinoxaline (derivatized methylglyoxal), and peak 2 refers to dimethylquinoxaline (derivatized diacetyl).

Table 1. Time Required for the Quantitative Derivatization of 10^{-5} M Solutions of α-Dicarbonyl Compounds Similar to Diacetyl, at Room Temperature, Using OPDA as the Derivatizing Agent

compound	derivatization product	A[a]	B[a]
glyoxal (R1 = R2 = H)	quinoxaline	25 min	32 min
methylglyoxal (R1 = H; R2 = CH_3)	2-methylquinoxaline	<4 min	<4 min
diacetyl (R1 = R2 = CH_3)	2,3-dimethylquinoxaline	<60 s	<60 s
2,3-pentanedione (R1 = CH_3; R2 = CH_2CH_3)	2-ethyl-3-methyl quinoxaline	<60 s	<60 s

[a] Derivatizing conditions: A, 0.1 M HCl; B, 0.1 M phosphate, pH 7.

Table 2. Distillation Recovery of α-Dicarbonyl Compounds

compound	recovery	compound	recovery
glyoxal	none	diacetyl	quantitative
methylglyoxal	partial	2,3-pentanedione	quantitative

It should be noted that the polarographic results reported for diacetyl are, in fact, strictly for diacetyl plus 2,3-pentanedione (i.e., for total vicinal diketones, as their corresponding quinoxalines are polarographically indistinguishable). This is not a serious problem, however, because diacetyl has a taste threshold 10 times lower than that of pentanedione and its concentration is, generally, much higher. In the case of the quinoxalines derived from diacetyl and methylglyoxal, there is a separation of the polarographic peaks (Figure 1), allowing their simultaneous determination, depending on the relative concentrations. After distillation (Figure 1b), the peak of methylquinoxaline is negligible, confirming that methylglyoxal is partly lost during distillation.

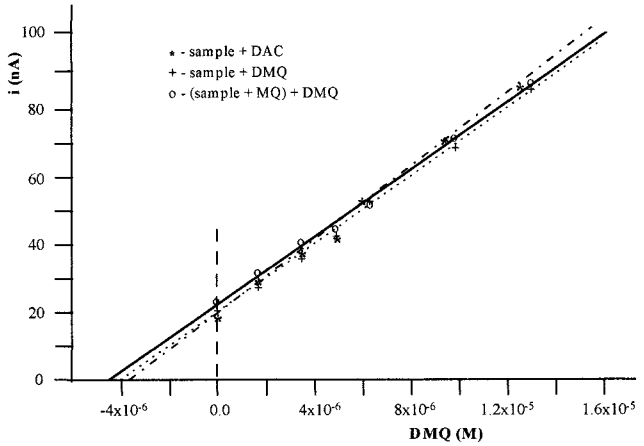


Figure 2. Use of standard additions in the polarographic determination of diacetyl in brandy. Standards added: (∗) diacetyl (derivatized in the polarographic cell); (+, o) dimethylquinoxaline (a previous addition of 1.4×10^{-5} M methylquinoxaline was made in representation o).

Determination of Diacetyl and Methylglyoxal in Brandy. As the brandy matrix is quite simple, it was possible to determine the compounds directly, with no need for any cleanup procedure. The sample of brandy was added directly into the polarographic cell containing buffer solution and the derivatization agent, and the determination of the two α-diketones was made using standard additions.

The application of the method for diacetyl is depicted in Figure 2. Similar results are obtained using standards of diacetyl (∗) or standards of the correspondent quinoxaline (+), proving that the derivatization reaction is complete. As a consequence, for the quantification of diacetyl, standard additions of dimethylquinoxaline can be used, simplifying the experimental procedure. Furthermore, the interference of methylglyoxal is practically negligible, due to the partial separation of the polarographic peaks of the quinoxalines involved (Figure 3). In this figure some of the polarograms used in the preparation of Figure 2 are shown, which illustrate the conclusions just presented. Similar results were obtained for methylglyoxal.

Using the method described, amounts of 2.1 ± 0.3 ppm for diacetyl and 1.9 ± 0.3 ppm for methylglyoxal (average of three determinations) were obtained in the analysis of a brandy sample.

Determination of Diacetyl in Butter. In the analysis of diacetyl in butter an initial step for the extraction of the compounds from the solid sample was obviously required. Steam distillation was used for the separation of the diacetyl from the matrix. The efficiency of extraction was tested by adding known amounts of diacetyl to the butter sample and verifying that >90% was recovered in the distillate. Using the procedure developed, a concentration of diacetyl of 2.5×10^{-6} M in the distillate was determined (Figure 4), corresponding to an amount of 0.5 ppm in the butter. As no signal for methylglyoxal was found, the amount of this compound in the butter sample is negligible.

Determination of Diacetyl and Methylglyoxal in Vinegar and Wine. Direct analysis of vinegar and wine was possible, by simple addition of 250 μL of vinegar (1 mL in the case of wine) to 25 mL of buffer in the polarographic cell. In both cases only a single large peak was found, at a potential similar to that of methylglyoxal (Figure 5a). Using standard additions, a preliminary value of 35 ppm for the amount of methylglyoxal in vinegar (10 ppm for wine) was obtained, a value several times larger than the real one obtained by other methods. This high result can be explained by the interference of α-dicarboxylic sugars structurally similar to methylglyoxal that are also present in vinegar and wine and which also react with OPDA to form quinoxalines (Morita et al., 1985).

To determine the amount of methylglyoxal in wine and vinegar, it was necessary to carry out a steam distillation prior to the polarographic analysis. As we can see in Figure 5b, the peak obtained in the analysis of 10 mL of vinegar at the potential of methylglyoxal (left peak) has a magnitude similar to that of Figure

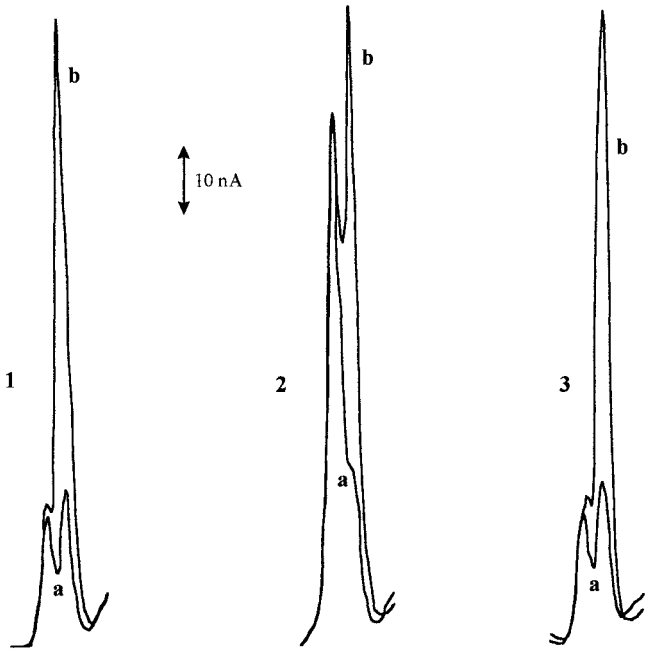


Figure 3. Polarograms obtained in the determination of diacetyl using standard additions: (1a) derivatized brandy; (1b) addition of 1.3×10^{-5} M dimethylquinoxaline; (2a) derivatized brandy + 1.4×10^{-5} M methylquinoxaline; (2b) addition of 1.3×10^{-5} M dimethylquinoxaline; (3a) derivatized brandy; (3b) addition of 1.3×10^{-5} M diacetyl. Initial potential = −550 mV; final potential = −900 mV.

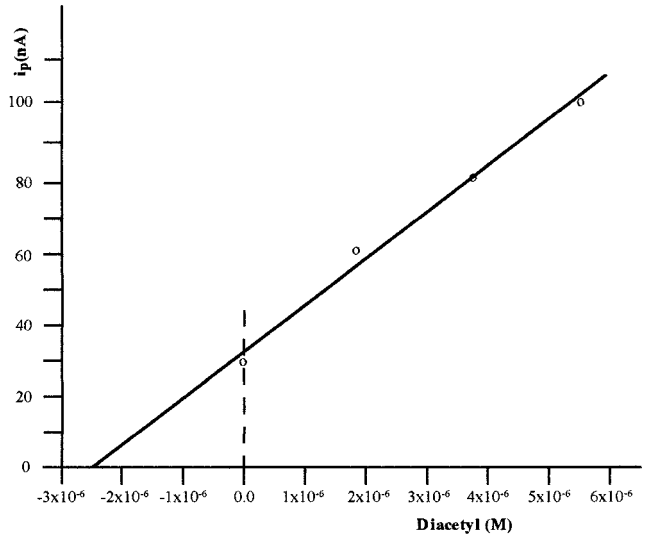


Figure 4. Peak currents obtained in the DP polarographic determination of diacetyl in butter using standard additions. Standards of diacetyl were added directly to the polarographic cell containing the butter distillate and the derivatizing agent.

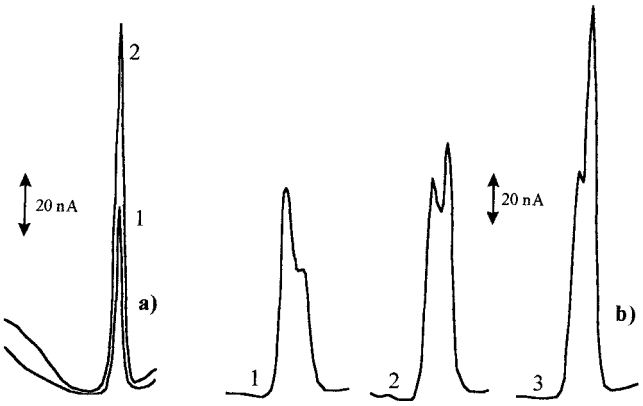


Figure 5. Polarograpic determination of methylglyoxal and diacetyl in vinegar: (a) direct analysis of 250 μL of vinegar (methylglyoxal added: 1, 0 ppm; 2, 30 ppm); (b) analysis after steam distillation of 10 mL of vinegar (diacetyl added: 1, 0 ppm; 2, 0.81 ppm; 3, 1.62 ppm).

5a. However, the amount of vinegar is 40 times larger than that used in the direct analysis. This means that the interfering α-dicarbonyl sugars have been fully removed by distillation. Assuming a 40% recovery of methylglyoxal in distillation (the value derived from that obtained in water), we can estimate that the amount of methylglyoxal in vinegar is ~2 ppm, a value similar to that obtained with other methods.

Steam distillation has an even more important role in the case of the determination of diacetyl. In fact, in Figure 5a there is no sign of the diacetyl peak; indeed, its peak must be completely masked by the presence of the large amount of α-dicarboxylic sugars and methylglyoxal. Nevertheless, using steam distillation, as the interferences are severely reduced and diacetyl is completely recovered, its determination becomes possible, as can be seen in Figure 5b, using the method of standard additions; a value of 1.4 ± 0.3 ppm was obtained for the concentration of diacetyl in the vinegar sample used, and a value of 0.17 ± 0.03 ppm was obtained for the concentration of diacetyl in the wine sample used.

Interference of Sulfite Ion. In the determination of diacetyl low results were reported in the presence of sulfite ion, either using gas chromatographic headspace analysis or by organoleptic perception, due to complexation of the compound with sulfite ion (Verhagen et al., 1987). No interference by sulfite ion was observed in the polarographic method developed, probably because *o*-phenylenediamine was able to dislodge diacetyl from its complex with sulfite. In acidic media the sulfite itself would be an interference in the polarographic analysis (EG&G Princeton Applied Research, 1980). However, as the supporting electrolyte chosen for the determination of the derivatized diacetyl is pH 7 phosphate buffer (see Table 2), no interference of sulfite would be expected in polarographic analyses in these conditions.

Any effects of the presence of sulfite ion on the stability of the solutions of α-dicarbonyl compounds or on the rate of the derivatization reaction with *o*-phenylenediamine were also investigated. No changes in the stability of the solution were observed. However, it was found that the presence of sulfite accelerates slightly the reaction between glyoxal and OPDA. In the presence of 50 ppm of sulfite ion the time necessary for the quantitative derivatization of a 10^{-5} M solution of glyoxal was reduced from 32 to 20 min.

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