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Polarographic Method for the Determination of Propanedial (Malonaldehyde)

Sir: The dialdehyde, propanedial, OHC–CH_2–CHO, commonly known as malonaldehyde or malondialdehyde, is a compound present in many biological systems (*1*). Malonaldehyde in foods has generally been associated with oxidative rancidity (*2, 3*), although it has been detected in a range of unspoiled meats, fish, vegetable oils, orange juice, etc. (*4, 5*). Recently substantial interest in the significance of malonaldehyde in human health has been prompted by studies on its mutagenicity (*6, 7*) and the report that it is a carcinogenic initiator on mouse skin (*8*). Other reports have indicated that malonaldehyde is a reactive species and a rationale for its toxicity may arise from its interaction with nucleic acids and proteins (*9–15*). These reports, in combination, have subsequently led to implications that malonaldehyde is involved in many problems of medical significance (see ref *16–24* for example).

To date, identification of malonaldehyde has for the most part been made by complexing malonaldehyde with thiobarbituric acid (TBA) (e.g., ref 4, 25). However, in general the reliability of this method and modified forms has generally been less than desirable (*4, 25, 26*). Other methods based on chromatographic techniques have been employed (*27, 28*).

In an attempt to develop a simple, reliable, and rapid analytical method, a differential pulse polarographic method was investigated.

EXPERIMENTAL SECTION

(i) Standards. Standard solutions of malonaldehyde for polarographic determinations were prepared by hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) (Aldrich Chemical Co.) in 2 M hydrochloric acid.

Formation of malonaldehyde is quantitative over a short period of time. Prolonged heating, recommended when more dilute acid concentrations were used (*5, 10, 27, 29*), is unnecessary. Presence of malonaldehyde was confirmed by a mass spectrum of the compound in 2 M HCl, showing a parent peak with expected mass/charge ratio of 72. In 2 M HCl dilute solutions of malonaldehyde decomposed by less than 5% in 5 days.

(ii) Instrumentation. Two polarographic instruments (a and b below) were used.

(*a*) *PAR Model 174A Polarographic Analyzer (Princeton Applied Research Corp., NJ).* This instrument was used with a conventional dropping mercury working electrode, a platinum auxiliary electrode and a Ag/AgCl (saturated NaCl) reference electrode.

(*b*) *PAR Model 374 Microprocessor Controlled Polarograph.* This instrument was used with a static mercury drop electrode, Model 303, a platinum auxiliary electrode, and Ag/AgCl (saturated KCl) as the reference electrode.

For determination of malonaldehyde in 1 mL or less of human saliva or cervical mucus (*30*) Metrohm volumetric microcell EA 1102 was used.

Polarograms were recorded at ambient temperatures, typically (20 ± 1) °C, and all solutions were degassed with nitrogen prior to recording a polarogram. Mass spectra were obtained with a Finnigan 3000 series gas chromatograph mass spectrometer.

RESULTS AND DISCUSSION

In dc polarography, 5×10^{-3} M or more concentrated solutions of malonaldehyde give in 2 M HCl two waves with half-wave potentials approximately −0.98 V and −1.2 V vs. Ag/AgCl (saturated NaCl) (Figure 1). The half-wave potentials are drop time, concentration, and pH dependent. The second wave is accompanied by a maximum at longer drop times and is ill developed at lower malonaldehyde concentrations. For analytical purposes the process occurring at around −0.98 V is preferred. With increasing pH this wave is shifted to more negative potentials and at pH 4.4 in unbuffered solution it disappears, presumably assuming its basic structure (*31*) rather than acid form (pK_a = 4.46). At pH 3, a significant decomposition was observed to occur after a 72-h period. The wave in 2 M HCl has kinetic character, as proved by dependence on mercury column height and other tests.

For analytical purposes, the differential pulse polarographic method proved most useful. In 2 M HCl, using a conventional DME, the peak height was a linear function of concentration over the range from 3×10^{-5} to 1×10^{-3} M. At concentrations higher than 1×10^{-3} M, curvature of the peak height–concentration plot is observed. The detection limit is governed by the superimposition of the wave of hydrogen reduction. With background subtraction, use of the static mercury drop electrode, and the microprocessor controlled polarograph (Figure 2), the useful concentration range and the limit of detection were extended, and calibration curves for malonaldehyde were linear over the range 10^{-5} to 10^{-3} M.

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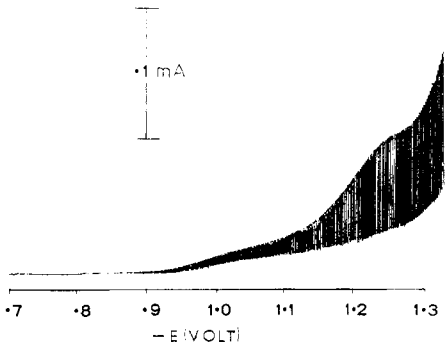

Figure 1. Dc polarogram: PAR 174 instrument with conventional dropping mercury electrode; concentration of malonaldehyde = 1.5 × 10^{-2} M in 2 M HCl; drop time = 0.5 s.

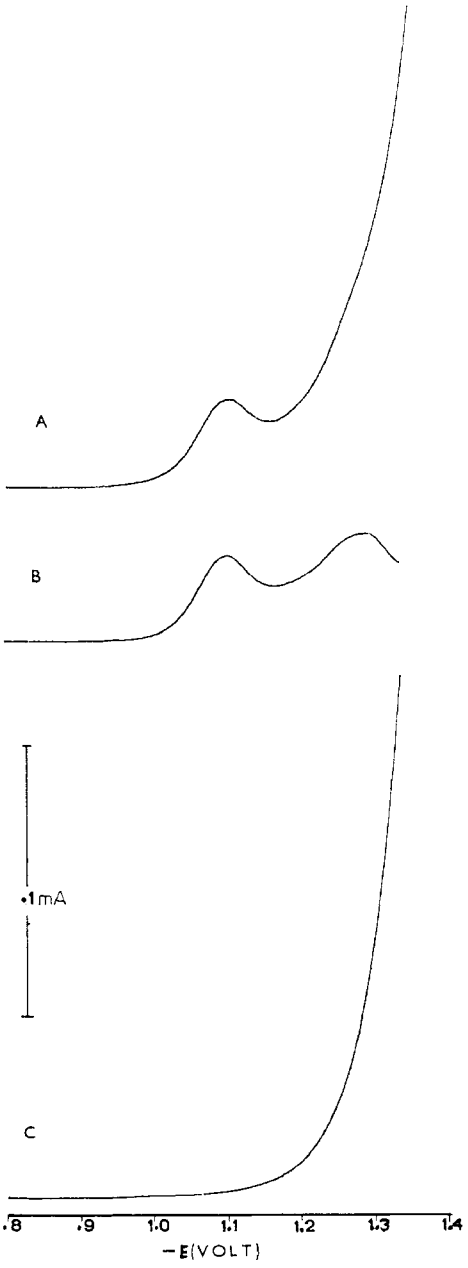

Figure 2. Differential pulse polarogram: PAR 374 instrument with 303 static mercury drop electrode; drop time = 0.5 s; concentration of malonaldehyde = 9 × 10^{-4} M in 2 M HCl; (A) without blank subtraction; (B) with blank subtraction; (C) blank.

To prove the possibility of application of the proposed polarographic method for analysis of foodstuffs, we carried out analyses of samples of vegetable oils after extraction of either the oil or its solution in toluene by 2 M HCl. Analysis of the aqueous layer showed a (100 ± 2)% recovery.

Possibility of analyses of biological materials was demonstrated for blood plasma (after treatment with 2 M HCl and centrifugation) and for human saliva and cervical mucus (*30*) after addition of 2 M HCl. In the latter two cases the total volume was less than 1 mL and the microcell (Experimental Section) was used. No interferences were encountered in any of these matrices.

No interference was observed for a fivefold weight excess of each of sodium chloride, potassium bromide, sodium iodide, cadmium sulfate, nickel sulfate, lead nitrate, maleic acid, fumaric acid, ascorbic acid, and methanol.

Equal weights of formaldehyde decreased the wave height by between 10 and 20%, but substantial further additions had no additional effect. Glutaraldehyde also decreased the peak height in a similar manner but by a factor of about 50%. Copper nitrate also diminished the wave height as did 2-aminopyridine and zinc nitrate gave a wave which at high concentration overlapped with that of malonaldehyde. Importantly, interference effects are generally small and the use of the method of standard additions can be used in an endeavor to eliminate such effects.

Decrease of the malonaldehyde wave after titration with sodium hydroxide to pH 4–5 should be used as an additional proof that wave measured in 2 M HCl corresponds to reduction of malonaldehyde.

It is currently doubtful whether the proposed method, similarly to other developed earlier, measures only free malonaldehyde or also that bound to proteins (*29*). As working hypothesis it is assumed that only free malonaldehyde and any naturally occurring species which can be hydrolyzed to malonaldehyde are measured. Further work is in progress in these laboratories aimed at understanding the principles of processes involved to explain inherent difficulties, still present, but perhaps not always fully recognized, in the determination of malonaldehyde and in correctly assigning its biologically important properties (*32*, *33*).

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Quantitative Surface Analysis of Copper–Nickel Alloys by Secondary Ion Mass Spectrometry

Sir: Secondary ion mass spectrometry (SIMS) is establishing a place for itself among surface analytical techniques (*1*) largely due to its extreme sensitivity (*2*) (10^{-6} monolayer for some metals and some molecules), the detectability of all elements as well as isotopic and isomer sensitivity (*3*), the ability to access working surfaces (*4*) such as catalysts and electronic devices, and sensitivity to molecular and crystal structure in the outer two to three atomic layers of a material (*5*, *6*). The ability to ionize and detect large involatile and thermally fragile molecules deposited on metal surfaces (*7*, *8*) and matrix-isolated organic species (*9*) has also been demonstrated. Quantitative analysis by SIMS has not been as fruitful as these other applications due to large variations in the secondary ion yield as a function of chemical environment, i.e., the so-called matrix effect (*10*). The purpose of this correspondence is to show that SIMS can provide precise quantitative surface analysis in cases where the absolute secondary ion yields are known. A series of CuNi alloys of known surface composition is used to illustrate this point.

The positive (negative) secondary ion intensity $I_{M_i}^{+(-)}$ [count/s] for isotope i of element M recorded during ion bombardment of a sample is related to the total yield of secondary ions, $S_{M_i}^{+(-)}$ [ions/ion], defined as the number of secondary ions of M_i emitted per incoming primary ion, according to (*11*)

$$I_{M_i}^{+} = j_0[M_i]\beta(M^+)T(E,R)S_{M_i}^{+} \quad (1)$$

In eq 1, j_0 is the primary ion flux [ions/s], $[M_i]$ is the atomic fraction of element M in the target, $\beta(M^+)$ is the detector efficiency, and $T(E,R)$ is the transmission of the ion optical system which depends upon the energy of the ion and resolution setting of the mass spectrometer. The above expression applies to atomic ions and represents a simplification of the complete secondary ion emission process for it neglects the angular dependence of secondary ion emission and cluster ion formation (*12–14*). With constant spectrometer parameters, eq 1 indicates that, to a first-order approximation, the ratio of secondary ion intensities of two elements M and N from a given sample can be expressed as

$$\frac{I_{M_i}^{+}}{I_{N_i}^{+}} \propto \frac{S_{M_i}^{+}}{S_{N_i}^{+}} \frac{[M_i]}{[N_i]} \quad (2)$$

S^+ is extremely sensitive (*15*) to the chemical environment of the specific element, e.g., an increase in S^+ of the order of 10^2–10^3 occurs upon oxidation or oxygen coverage of a clean metal surface. Benninghoven (*15*) has determined absolute secondary ion yields for many clean and oxygen-covered metals.

We have applied eq 2 to the I_{Cu}^{+}/I_{Ni}^{+} SIMS ratio obtained from a series of CuNi alloys for which the bulk composition was accurately known and the surface composition after Ar^+ bombardment had been determined (*16*) by Auger electron spectroscopy. The alloy samples were obtained from Tohoku University, Sendai, Japan, and the method of Auger analysis of the surface composition after Ar^+ bombardment has been published previously (*16*). Details of the design of the SIMS system and pumping facilities have been described elsewhere (*12*). The SIMS were acquired by pulse counting techniques using a 3-keV primary Ar^+ current of 10^{-8} A/cm^2. The samples were cleaned in the UHV system by heating to ~800 °C and then bombarding at room temperature with Ar^+ for periods of ~1 h. After such treatment, the SIMS yield of impurity ions was down in the background level.

The experimental I_{Cu}^{+}/I_{Ni}^{+} SIMS ratios for several CuNi alloy samples which had been pretreated by Ar^+ bombardment are plotted in Figure 1 as a function of bulk composition (upper abscissa) and surface composition as determined by Auger electron spectroscopy (lower abscissa) for similar bombarded samples. The Auger spectra show that the surface layers are enriched with nickel after extended Ar^+ bombardment. The I_{Cu}^{+}/I_{Ni}^{+} ratio was calculated by using the ion yields (*15*) for pure Cu and Ni ($S_{Cu}^{+} = 0.003$; $S_{Ni}^{+} = 0.006$) and the relationship 1 = ([Cu] + [Ni]) which, substituted in eq 2, yields

$$\frac{I_{Cu}^{+}}{I_{Ni}^{+}} \propto \left(\frac{1}{[Ni]} - 1\right) \Big/ 2 \quad (3)$$

where [Ni] is the atomic fraction of surface nickel. The plot of eq 3 in Figure 1 shows excellent agreement with the experimental data.

These results and their significance can be summarized as follows. (i) The agreement between the calculated and experimental data shows that the alloy surface concentrations determined by SIMS are consistent with those previously

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