able, it may be useful for the processors to use soybeans that lack L-1. In the case of bread making soybean lipoxygenase is effective as a bleaching agent, it increases the mixing tolerance, and it improves dough rheology (Frazier, 1979; Faubion and Hoseney, 1981). Use of soybean genotypes without lipoxygenase-1 may therefore be undesirable in bread making.

Greater knowledge of chemical and biochemical differences of seeds of different soybean genotypes will likely enhance the capacity of soybean derivatives to increase the nutritional and organoleptic properties of processed food products.

ACKNOWLEDGMENT

The technical assistance and advice by Drs. P. A. Reynolds, B. P. Klein, and C. Chaven are gratefully acknowledged.

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Received for review September 3, 1981. Accepted March 15, 1982. This research was supported by the Illinois Agricultural Experiment Station, University of Illinois, and a grant from the Illinois Soybean Program Operating Board.

# Degradation Kinetics of Betanine in Solutions As Influenced by Oxygen

Ernest L. Attoe and Joachim H. von Elbe*

The degradation kinetics of betanine were determined for nitrogen-flushed solutions. Trace levels of residual oxygen were found to influence pigment degradation unless a large molar excess of betanine was present. In the absence of oxygen, betanine stability was greatly enhanced and degradation occurred by a 0.5 reaction rate order. An activation energy of 30.7 ± 1.0 kcal/mol was calculated for this reaction.

Determination of stability of quality factors in foods requires an understanding of rate orders and the parameters that influence them. First-order reactions are most common, but other rate orders are often encountered. If first-order kinetics were used to describe a reaction proceeding by a different rate order, major errors can result. When materials are subject to oxidation, the level of oxygen present may affect the rates of destruction.

The oxidative deterioration of fats and oils is well characterized. The kinetics and mechanisms of oxidation for organic materials in aqueous solution have not been as thoroughly explored. Numerous pigments, vitamins, and flavor compounds are susceptible to reaction with oxygen in solution. In this study, the role of oxygen on the degradation rate of betanine in solution was explored.

Betanine is the primary pigment of red beets. Three carboxyl groups, a quaternary amine, and a glucose moiety impart hydrophilic character to the conjugated chromophore. The sensitivity of beet pigments to oxygen has long been known (Vilece et al., 1955; Habib and Brown, 1956). More recently, the effects of various parameters on betanine stability have been researched. In a study of the effects of pH, temperature, light, and air exposure of betanine solution at pH 7.0, oxygen appeared to increase the rate of degradation by 15% at 15 °C (von Elbe et al., 1974). Pasch and von Elbe (1979) measured betanine degradation rates at 75 °C as influenced by organic acids, multivalent cations, antioxidants, and sequestrants. These authors found that both $Fe^{2+}$ and $Cu^{2+}$ at 100 ppm increased rates of betanine loss while 10 000 ppm of citric acid or EDTA prolonged half-life values by 1.5 times. These results are consistent with an oxidative mechanism. The effectiveness of the antioxidants $\alpha$-tocopherol and ascorbic acid in this study could be attributed to the high level of oxygen that was maintained. Bilyk et al. (1981) found that both ascorbic acid and isoascorbic acid stabilize betanine when used at levels greater than 0.1%.

In the presence of sufficient oxygen, betanine loss in solution follows first-order kinetics. Saguy et al. (1978) observed first-order kinetics with an activation energy of 20.4 kcal/mol for heated betanine solutions. Beet juice in unsealed vials showed similar pigment stability with an

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0021-8561/82/1430-0708$01.25/0 

activation energy of 19.2 kcal/mol for betanine (Saguy, 1979).

Experiments to limit oxygen during degradation first indicated that oxygen had relatively little effect on the rate of reaction order of betanine loss (von Elbe et al., 1974). Further studies in this laboratory produced differing and unpredictable kinetic data during degradation in low oxygen systems. The present investigation was undertaken to elucidate the role of oxygen in betanine degradation.

## MATERIALS AND METHODS

A mixture of betanine and isobetanine was purified from beet juice. Gel filtration and adsorption column chromatographies were used as described earlier (Attoe and von Elbe, 1981). Additional purification was achieved by preparative high-performance liquid chromatography on C-18 columns (Waters Associates). Eight-milliliter portions of highly concentrated pigment solution from the adsorption column were injected via a 10-mL loop of a Waters Associates U6K injector. An eluant consisting of methanol and 0.005 M phosphate buffer (17:83 v/v) at pH 2.75 was delivered at 9 mL/min. The betanine and isobetanine fractions were combined, partially lyophilized, and stored frozen to serve as a pigment source.

**Sample Preparation and Reaction Conditions.** Appropriate amounts of frozen pigment concentrate were thawed and dispersed in a 0.1 M phosphate–acetate buffer at pH 5.0. Pigment concentrations in solution were determined by applying Beer's law. Light absorbance was measured at 537 nm and a 1% absorptivity ($E^{1cm}_{1\%}$) equal to 1120 was assumed (Piattelli and Minale, 1964).

A variety of reaction vessels and seals were surveyed to find an air-tight system permitting nitrogen flushing. Two milliliter glass vials fitted with sleeve-type rubber stoppers were found to be most suitable. The insertion of 25-gauge needles through the stopper allowed gas exchange with subsequent resealing of the vial. Nitrogen flushing was accomplished by bubbling oxygen free grade $N_2$ (Linde Division, Somerset, NJ) through the solution. Between 95 and 99% of the dissolved oxygen was removed within 2 min as measured by oxygen electrode and chemical analysis. Further nitrogen flushing had little effect on oxygen levels.

Betanine concentrations were adjusted to range between 10 and 700 ppm. Vials were filled and sealed, allowing for a small headspace when nitrogen flushing was employed. No headspace was allowed for air-saturated solutions. These solutions were stirred and allowed to equilibrate with $O_2$ at 25 °C before sealing. Temperatures of pigment solutions were maintained in a temperature-controlled water bath. Most of the data were obtained at a temperature of 65 °C. More limited data were collected at temperatures of 40, 55, and 75 °C.

**Determination of Dissolved Oxygen.** The efficiency of oxygen removal and the maintenance of oxygen-free environments were monitored with a galvanic cell oxygen electrode (Johnson et al., 1964). Oxygen concentrations were determined from the response of a 10-mV chart recorder with the range bracketed by air-saturated and sulfite-saturated solutions. Levels of dissolved oxygen in air-saturated solutions were estimated from APHA (1975). Verification of oxygen determinations as measured by the electrode was made by a chemical colorimetric analysis (CHEMetrics, Inc., Warrington, VA). In the range of 0–1 ppm of dissolved oxygen, the two methods differed by no more than 0.1 ppm.

**Pigment Analysis.** Samples were removed from the water bath after appropriate intervals and refrigerated. Overnight storage prior to analysis was used to allow pigment regeneration to come to completion (von Elbe et al., 1981). After quantitative dilution, the samples were analyzed by HPLC under isocratic conditions for retained pigment (Schwartz and von Elbe, 1980).

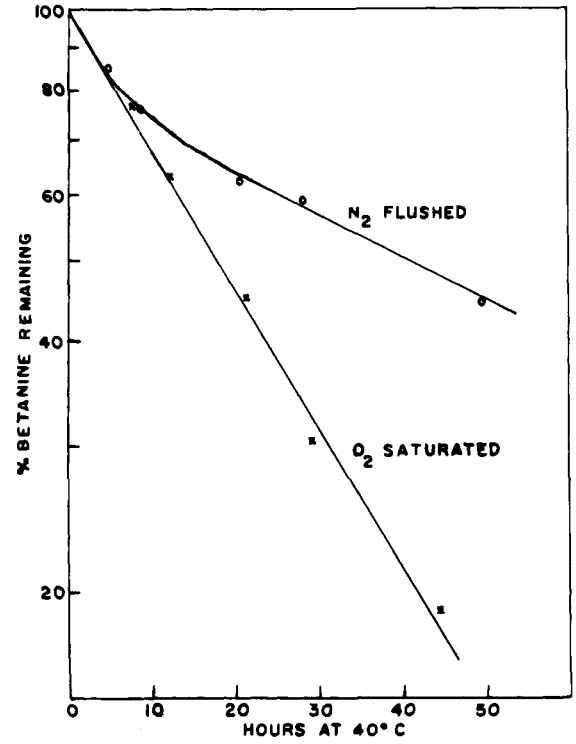


**Figure 1.** Degradation rates of betanine in solution at 40 °C, pH 5.0, in the presence and absence of oxygen. The initial betanine concentration was 0.027 mM (15 ppm). Data points represent the average of duplicate betanine determinations.

## RESULTS AND DISCUSSION

Quantification of betanine was based on the amount of betanine and isobetanine remaining. The total of these two pigments was used because significant isomerization occurred during the prolonged heating periods of this study. Since the isomers have identical stabilities, the use of total pigment avoided the problem of estimating how much betanine isomerized as opposed to actual degradation.

The thermal stability of red beet pigments has usually been determined in model systems containing a molar excess of oxygen over pigment. Under these conditions, pseudo-first-order reaction rate kinetics are observed for betanine while the level of oxygen in solution remains relatively constant. These data produce a straight line when log betanine concentration remaining is plotted against time, allowing the calculation of first-order rate constants and half-life values. Figure 1 shows the rate of degradation at 40 °C of an oxygen-saturated system containing 15 ppm of betanine at pH 5.0. The degradation rate was found to be essentially independent of initial betanine concentration and level of $O_2$, as long as the concentration of oxygen remained in excess of pigment concentration. This condition was met for all samples containing less than 80 ppm of betanine in an aqueous solution that had been air saturated at room temperature.

Initial attempts to determine betanine degradation kinetics in an oxygen-free system resulted in a deviation from the first-order rate as is illustrated by the $N_2$-flushed samples of Figure 1. The rate decreased rapidly during the first 20 h. Beyond 20 h a slower degradation prevailed. Pigment concentrations in the samples were 0.027 mM while dissolved oxygen levels were approximately 0.20 and

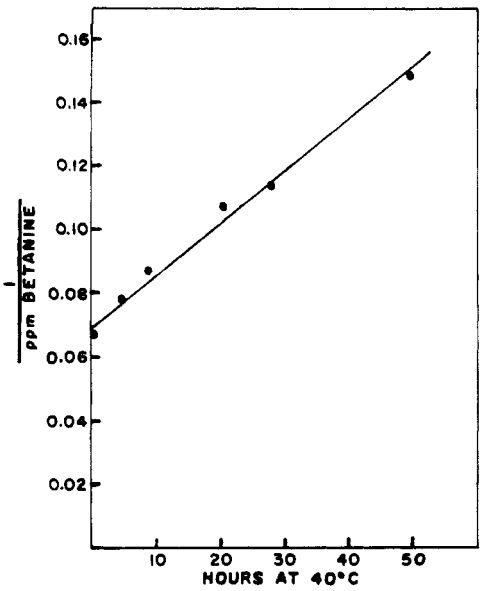


**Figure 2.** Degradation rate (reciprocal concentration vs. time) of betanine in solution at 40 °C, pH 5.0, of the nitrogen-flushed samples in Figure 1.

0.01 mM for the air-saturated and nitrogen-flushed samples, respectively. A severalfold excess of oxygen over pigment in the air-saturated samples would be expected to maintain a pseudo-first-order loss of betanine in which oxygen depletion does not slow the rate. However, the 0.01 mM residual oxygen of deoxygenated samples could be expected to influence the degradation of 0.027 mM pigment until no oxygen remains. This depletion of dissolved oxygen during betanine degradation, as measured by the oxygen electrode, is reflected by the slowing of betanine loss. An initial rapid oxidative rate is followed by a slower loss in the absence of oxygen.

The consecutive degradations in the presence and absence of dissolved oxygen may give the false appearance of a second-order loss with respect to betanine. Figure 2 represents the transformation of the data from the $N_2$-flushed samples of Figure 1 to second-order coordinates (reciprocal of concentration vs. time). This is a strictly empirical phenomenon that occurred when residual oxygen reacted in a molar ratio between 1:2 and 1:5 with initial pigment levels. Higher oxygen to pigment ratios led to a betanine loss following approximate first-order reaction kinetics, while lower oxygen to pigment ratios resulted in kinetics approaching those of the oxygen-free system to be discussed subsequently. The relation is only coincidental and does not reflect the reaction mechanism. It is noteworthy that degradations of anthocyanins (Adams, 1973), ascorbic acid (Singh et al., 1976), and folic acid (Ruddick et al., 1980) showed similar deviations from first-order kinetics in oxygen-limited model systems. Low levels of oxygen may have affected these reactions in a similar manner. However, different reaction mechanisms would complicate this comparison.

The influence of trace amounts of residual oxygen on the rate of betanine degradation in an oxygen-free system was expected to be lessened if the pigment concentration in the samples was increased. Samples containing betanine concentrations far greater than the approximate 0.01 mM residual oxygen should approach the degradation kinetics of a sample free of oxygen. Degradation studies were made with initial pigment levels in the range of 15–725 ppm (0.027–1.32 mM). Figure 3 shows the effects of increasing pigment levels on the rate of betanine loss at 65 °C when first-order reaction kinetics are assumed. The slope of

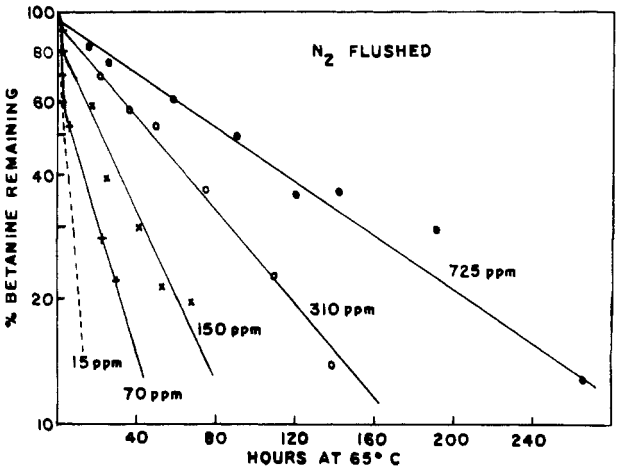


**Figure 3.** Degradation rates of betanine in solution flushed with nitrogen at 65 °C, pH 5.0. Initial betanine concentrations were 15, 70, 150, 310, and 725 ppm. Data points represent the average of duplicate betanine determinations. The broken line represents an extrapolation beyond 50% of pigment degraded.

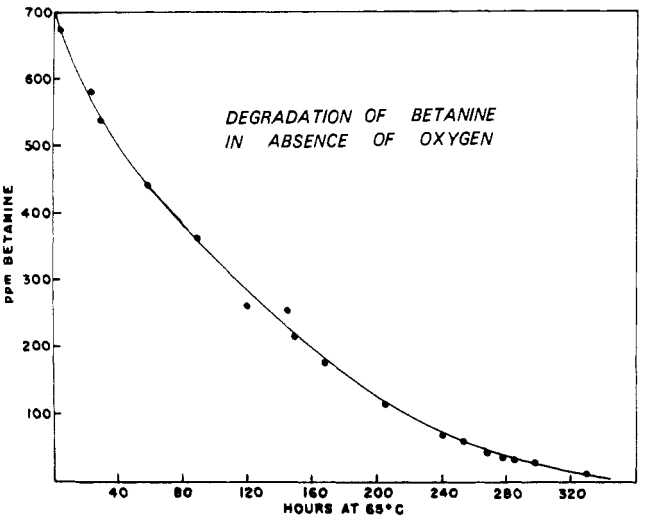


**Figure 4.** Degradation rate of betanine in solution at 65 °C, pH 5.0, in the absence of oxygen. Data points from the 725-, 310-, and 150-ppm reaction series (Figure 3) were transcribed, omitting the influence of residual oxygen.

successive degradation lines decreases with increasing betanine concentration. These data indicate that the degradation of betanine without significant dissolved oxygen does not follow first-order rate kinetics. The reactions, at each concentration level, appear to be linear over the observed range, but different slopes negate a first-order model. Furthermore, the lines appear to intercept the concentration axis somewhat below the 100% initial level, indicating that some pigment is still lost by a rapid oxidation with residual $O_2$. The first-order kinetic bias was removed from the data by transcribing these data points to linear coordinates (concentration vs. time, Figure 4). In this transformation, the early data points at each concentration level, which may have been influenced by residual oxygen, were omitted. Joining the separate degradation curves at equivalent pigment concentrations allowed the degradation to be viewed over a wide concentration range. From the degradation reaction for samples containing 725 ppm, data points between concentration levels of 670 and 220 ppm were used. From the samples initially containing 310 ppm, data points between 220 and 50 ppm were taken. The lowest levels of from 50 to 11 ppm were obtained from the series having an initial concentration

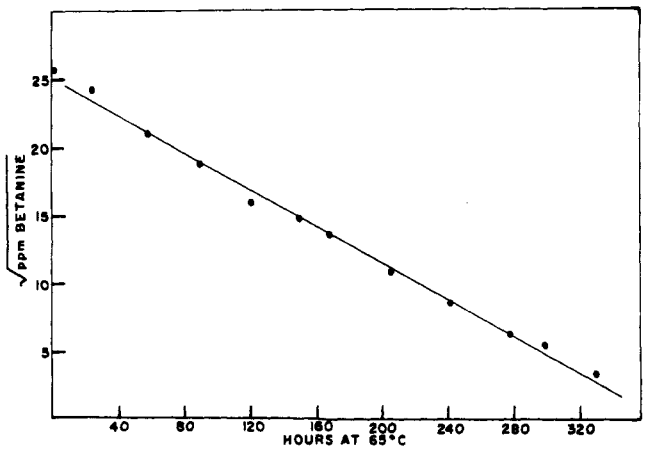


**Figure 5.** Degradation rate of betanine in solution at 65 °C, pH 5.0, in the absence of oxygen, plotting (concentration)$^{1/2}$ vs. time, $n = 0.55$.

of 150 ppm of betanine. The total time for these losses to occur was 138 h + 130 h + 61 h = 329 h. The smooth curve thus derived (Figure 4) would suggest a uniform effect of concentration on rate.

A reaction order and rate constant were determined for the degradation of betanine in the absence of oxygen at 65 °C. When log reaction rate is graphed against log concentration, the slope of the resulting line indicates the order of the reaction (Castellans, 1971). A reaction order of 0.55 was obtained by using regression analysis of the data in Figure 4. Since this value is in the proximity of a 0.5-order reaction, when experimental error is taken into consideration, the reaction rate was assumed to be proportional to the square root of betanine concentration. Figure 5 demonstrates that a 0.5-order model adequately describes the data. Plotting the square roots of the concentrations given in Figure 4 vs. time produces the linear relationship of Figure 5. A correlation coefficient of –0.998 was determined. The following rate equation for the degradation of betanine in the absence of oxygen is proposed:

$$\frac{d[\text{betanine}]}{dt} = -k[\text{betanine}]^{1/2}$$

At 65 °C, $k$ was calculated to be approximately 0.006 $mM^{1/2}\ h^{-1}$. Similar data were obtained at 55 and 75 °C, resulting in $k$ values of 0.0017 ± 0.0002 and 0.026 ± 0.003 $mM^{1/2}\ h^{-1}$, respectively. An activation energy of 30.7 kcal/mol (±1 kcal/mol) was calculated by using these rate constant values. This value is higher than the approximate 20 kcal/mol reported by Saguy (1979) and others for betanine loss in the presence of oxygen. The higher activation energy may reflect a different degradation mechanism in the absence of oxygen.

The effect of oxygen on the degradation of betanine was further demonstrated in an experiment in which a relatively high betanine concentration was allowed to react with a smaller molar concentration of oxygen, causing its depletion. Later during the reaction, additional oxygen was introduced, again causing the rapid pigment loss characteristic of an oxygenated system. The loss of betanine in the presence and absence of dissolved oxygen is illustrated in Figure 6. The data points represent a series of 2-mL vials filled with a 1.1 mM betanine solution saturated with oxygen at 20 °C (0.29 mM $O_2$). As shown, a rapid pigment loss occurs during the first hour in the presence of molecular oxygen. As oxygen is depleted, the degradation rate decreases. The change in rate occurs at a point where the molar concentration of pigment depleted

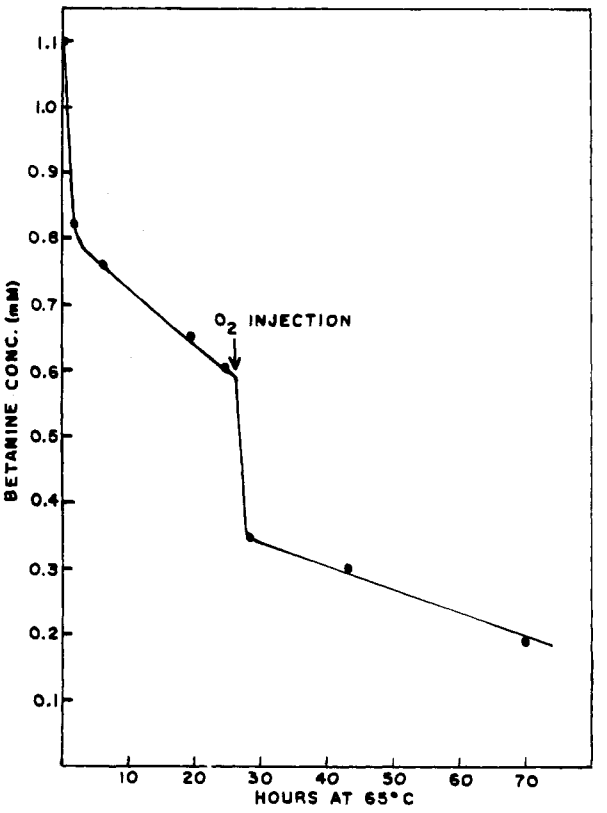


**Figure 6.** Degradation rate of betanine in solution at 65 °C, pH 5.0, in the presence and absence of dissolved oxygen. Initial betanine oxygen concentrations were 1.1 and 0.29 mM, respectively. Fifty microliters of additional oxygen was added after the original oxygen level was depleted.

was equal to the initial molar concentration of oxygen. This result suggests a 1:1 molar reactivity. The degradation thereafter follows a rate characteristic of betanine degradation in the absence of oxygen. Fifty microliters of $O_2$ was injected at the point indicated. Rapid oxidation again removes both oxygen and pigment until the oxygen is depleted.

## CONCLUSIONS

Betanine degrades by a pseudo-first-order rate in the presence of an excess of oxygen. A large excess of betanine over dissolved oxygen produced a much slower degradation rate that could best be described by a 0.5 reaction rate order. The activation energy for the loss of betanine in the absence of oxygen was calculated to be 30.7 ± 1 kcal/mol. Determinations of betanine stability need to consider both betanine and oxygen levels in a system. When oxygen is limited, betanine may no longer degrade by a first-order reaction rate.

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Received for review December 23, 1981. Accepted April 5, 1982. This research was supported by the College of Agricultural and Life Sciences, the University of Wisconsin—Madison, and by the U.S. Department of Agriculture.

# High-Performance Liquid Chromatographic Separation with Triple-Pulse Amperometric Detection of Carbohydrates in Beverages

Scott Hughes[1] and Dennis C. Johnson*

A new amperometric technique was applied at a miniature Pt electrode in a flow-through electrochemical detector for the chromatographic determination of several carbohydrates in selected samples of beverages. Detection limits for dextrose were approximately 4.6 μg/mL in a 100-μL sample, which is more than 100× lower than that reported for conventional refractive index detection.

The analysis of food systems for carbohydrates has been an important practice for many years. Recently, the determinations of individual sugars in complex mixtures has been facilitated by development of relatively efficient liquid chromatographic columns, and these analyses have become more routine (Shaw et al., 1980; Conrad and Palmer, 1976; Johncock and Wagstaffe, 1980). Determination of carbohydrates by high-performance liquid chromatography (HPLC) is characterized by better resolution than that obtained by traditional chemical methods, is less costly than enzymatic methods, and involves no derivatization as is required for gas chromatographic methods. Since carbohydrates exhibit only weak photometric absorbance in the UV–visible region of the electromagnetic spectrum, the measurement of refractive index (RI) has served as the standard method of chromatographic detection. The detection limits for RI detection are not sufficiently low for many applications, and an improvement in sensitivity for detection of carbohydrates is desired.

Amperometric detection in HPLC with glassy carbon and carbon paste electrodes has been successfully applied for many easily oxidized or reduced organic compounds; however, these electrodes exhibit no response for carbohydrates. A surface-catalyzed anodic oxidation of polyalcohols and carbohydrates is obtained at Pt electrodes; however, the faradaic response at a constant applied potential is transitory, decaying to virtually a zero value within a few seconds. The faradaic response is concluded to result from oxidation of hydrogen atoms produced by the surface-catalyzed dehydrogenation of the adsorbed organic molecules. The hydrocarbon products of the dehydrogenation remain adsorbed on the electrode surface, thereby inhibiting adsorption of unreacted molecules and the resulting anodic current. The fouling hydrocarbon products can be oxidatively cleaned from the electrode surface presumably as $CO_2$, with simultaneous formation of oxide on the Pt surface, if the electrode potential is stepped to a large positive value corresponding approximately to the anodic breakdown of the aqueous solvent. The surface oxide is subsequently reduced by stepping the potential to a negative value corresponding approximately to the cathodic breakdown of the solvent. Molecules of the organic analyte are again adsorbed and are detected following a subsequent step of the potential to a more positive value but not so large as to cause formation of surface oxide.

A triple-pulse potential waveform (see Figure 1) has been designed for detection of carbohydrates in 0.1 M NaOH which automatically processes the electrode potential through the sequence of values for detection ($E_1$ = −0.4 V), oxidative cleaning ($E_2$ = +0.8 V), and reduction with adsorption ($E_3$ = −1.0 V) (Hughes et al., 1981; Hughes and Johnson, 1981). The anodic faradaic signal is sampled approximately 173 ms after the application of $E_1$, and this signal is retained in a sample-hold circuit which is outputed continuously to a strip chart recorder. The result of applying the wave form is maintenance of a high and uniform electrode activity, making possible the reproducible anodic detection of numerous organic compounds. The time period for execution of the waveform is 590 ms, which is sufficiently short to permit virtually continuous monitoring of the effluent stream for HPLC.

The faradaic signal has been determined to be proportional to the fractional surface coverage of the electrode by the adsorbed organic molecules (Hughes et al., 1981). By use of the model of adsorption according to the Langmuir isotherm, the peak current ($I_p$) is given by

$$I_p = -nFA\Gamma_{RH,max}\left(\frac{k_2k_1}{k_{-1}}\right)C_p/\left[1+\left(\frac{k_1}{k_{-1}}\right)C_p\right]$$

where $n$ = number of electrons transferred per molecule, $F$ = the Faraday constant, $A$ = electrode area, $\Gamma_{RH,max}$ =

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