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Post-column oxidation of purpald–aldehyde adducts at nickel electrodes

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Abstract

Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole), a chromogenic agent for the detection of aldehydes after TLC has been studied as a possible post-column derivatizing agent for improving the detectability of aldehydes after HPLC separation. In the first step of a two step reaction, Purpald forms a colorless adduct with aldehydes. Subsequent oxidation of the colorless adduct by air or other means yields a highly colored compound in the second step. The oxidized Purpald–aldehyde adduct is deep purple in color and has an absorption maximum at 550 nm. An on-line post-column electrochemical reactor has been investigated as a possible alternative to air or chemical oxidation for the analysis of aldehydes using HPLC. The method was shown to form colored adducts in bulk solution and on-line using electrochemical oxidation at nickel electrodes for formaldehyde, acetaldehyde, and propionaldehyde. There was a marked increase in reaction time in bulk solution as the molecular mass of the aldehyde increased. Thus, when performing chromatography using on-line electrochemical oxidation the sensitivity of the method decreased significantly as the aldehyde molecular mass increased. Reactor design, electrode configurations, electrode potentials, and optimum reaction conditions are described. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

1.1. Oxidation mechanism at Ni electrodes

The use of nickel electrodes in alkaline medium has been cited in numerous papers for the anodic oxidation of carbohydrates [1–4], sugars and sugar degradation products [5,6], and amines and alcohols [7]. It has been shown by Fleischman [8] and others that when a nickel electrode contacts an alkaline solution, its surface is spontaneously covered by a layer of Ni(II) hydroxide that can be subsequently oxidized to a Ni(III) oxide/hydroxide layer. The character of the Ni(III) oxide/hydroxide layer is strongly dependent on the applied potential, the pH, and the temperature [4]. Once formed, the Ni(III) surface acts as a strong oxidant and can oxidize organic compounds to give Ni(II) hydroxide on the electrode surface, which can be subsequently re-oxidized by an applied potential to give an analytical signal [9]. Fleischmann and his colleagues have proposed the following mechanism for the catalytic oxidation of an organic compound at a Ni electrode.

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$$Ni(OH)_2 \xrightarrow{fast} NiO(OH) + e^- + H^+$$

$$NiO(OH) + \text{organic compound} \xrightarrow{slow} Ni(OH)_2 +$$

product

For this work, rather than utilizing a Ni electrode for amperometric detection of organic molecules, the Ni(III) surface was investigated as a possible bulk oxidizing agent for the determination of aldehydes using HPLC with spectrophotometric detection. The detection was based upon the oxidation of aldehyde derivatives of the compound 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole, commercially known as Purpald, to form highly colored adducts.

Purpald has been used for a number of years to visualize carbohydrates on plates after thin-layer chromatography, and as a reagent for carbonyl group analysis [10]. An example of the reaction between Purpald and formaldehyde is shown in Fig. 1.

In the reaction, the triazole (I) condenses with an aldehyde to form an unstable, oxygen labile, intermediate (II) [11]. Oxidation of this intermediate leads to the formation of a purple, intensely colored, bicyclic, heterocyclic, 6-mercapto-3-substituted-s-triazole[4,3-b]-s-tetrazine (III). This highly conjugated
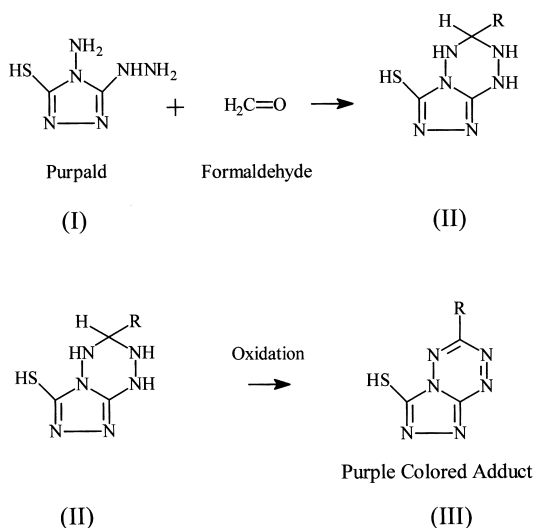

Fig. 1. Reaction of Purpald with aldehydes to give a purple colored adduct.

system allows for spectroscopic examination in the visible wavelength region of 520–550 nm.

If the reaction is done in open air, free diffusion allows sufficient O_2 into the reaction system to perform the oxidation of the intermediate. Under chromatographic conditions, however, O_2 cannot freely diffuse into the closed system. As a result, an oxidation method other than O_2 is required if this reaction is to be used for chromatographic analysis of aldehydes. While chemical oxidizing agents such as hydrogen peroxide might be utilized in a post-column reaction system, this paper describes one possible alternative: the use of an electrochemical reactor.

The advantage of a post-column electrochemical reactor for the oxidation is that it allows mechanical simplification of the post-column reaction system and thereby helps minimize band-broadening since a separate pump and reagent lines are not required for adding a chemical oxidizing agent. Also, even though this method was shown to work with relatively high sensitivity for formaldehyde, its main purpose was as a demonstration of the feasibility of using a post-column electrochemical reactor for modification of analytes to improve detectability in diverse situations. This method would be useful in those situations specifically requiring analysis of formaldehyde in the presence of other analytes and other types of aldehydes (e.g., environmental analysis of air, water, or other materials), or for analysis of non-trace amounts of the higher-formula-mass aldehydes.

1.2. Aldehyde analysis by HPLC

Most of the derivatization methods that have been used for GC, GC–MS, MS, and HPLC analysis of aldehydes and ketones are based on formation of the more classical types of carbonyl derivatives such as phenylhydrazones and dinitrophenylhydrazones, as well as oximes. The dinitrophenylhydrazones have been found useful for chromogenic derivatization of aldehydes before HPLC analysis [12–14].

Many current methods of analysis also utilize 2,4-dinitrophenylhydrazine (DNPH) with electrochemical detection. Chiavari and Bergamini [15] describe a method where carbonyl compounds in acid rain were derivatized with DNPH. HPLC analy-

sis of aldehydes has also been described using 3-methylbenzothiazolone as a derivatizing agent with electrochemical detection at a glassy carbon electrode [16,17]. Analysis of atmospheric formaldehyde as its lutidine derivative followed by HPLC with spectrophotometric detection has been performed by the same author [18]. Also three methods of reductive amination have been investigated by Mann and Grayeski [19] for derivatization of aldehydes and ketones using 3-aminofluoranthene as a chemiluminescence agent.

In work done by Del Nozal et al. [10], Purpald was used as a post-column derivatizing agent for monosaccharides. The reagent phase contained 4000 ppm Purpald dissolved in 2 M NaOH. The monosaccharides were separated using an Aminex HPX-87P cation-exchange column at 85°C. A 20-m mixing coil provided the time for the reaction to go to completion. The coil was kept at 90°C in a constant temperature water bath. Immediately after the heated bath the eluent was cooled in an ice bath. The derivatized product was detected at 550 nm using a UV-visible spectrophotometer. A 0.04 M H_2O_2 solution served as the oxidant. The optimum concentration for the NaOH was found to be 1 M and the optimum concentration of the Purpald to be 0.01 M. The temperature was also critical for the reaction time.

2. Experimental

2.1. Solvents and reagents

All chemicals and solvents used were of the highest purity available. HPLC-grade acetonitrile (Fisher Scientific, Pittsburgh, PA, USA) was filtered through nylon filters with a pore size of 0.45 μm (Micron Separations). Crystalline Purpald (Sigma, St. Louis, MO, USA) was used to prepare 0.050 M and 0.10 M solutions. Formaldehyde solution (37%) (Fisher Scientific) was diluted with acetonitrile to prepare a 0.010 M stock solution. Acetaldehyde, propionaldehyde, butyraldehyde, and valeraldehyde (Aldrich, Milwaukee, WI, USA) were diluted with acetonitrile to prepare individual solutions with concentrations of 0.010 M.

2.2. Electrochemical studies

Cyclic voltammetry studies were performed in quiescent solutions with a supporting electrolyte prepared containing 0.025 M Purpald, 0.50 M NaOH, 0.10 M sodium perchlorate, and 20% acetonitrile. A nickel electrode was prepared in the laboratory by embedding a 5 cm×6 mm nickel rod in an epoxy matrix. The surface was polished to a mirror bright finish. A platinum wire served as the auxiliary electrode. All potentials were measured with respect to a saturated Ag/AgCl reference electrode. A model CV-27 potentiostat (Bioanalytical Systems, West Layfayette, IN, USA) coupled to an Omnigraphic series 200 X-Y recorder was used to conduct all spectroelectrochemical and cyclic voltammetric experiments.

For spectroelectrochemical experiments, a sandwich-type of optically transparent electrochemical cell was constructed. Two quartz windows were held in place using two stainless steel faceplates of the type normally used to hold salt windows for infrared spectroscopic analysis. The optically transparent working and auxiliary electrodes were made from nickel microscreen with 80 wires/in. (Buckbee-Mears, St. Paul, MN, USA) and held against the window with a U-shaped piece of parafilm. Electrical contact to the electrodes was provided by copper foil. A 0.5-cm thick U-shaped piece of parafilm was used to separate the electrodes and provided a cavity with a volume of 750 μl and a path length of 5.0 mm.

2.3. Chromatographic studies

Two HPLC systems were utilized in the course of this study, but both were arranged in the same configuration. After chromatographic separation of the aldehydes, the Purpald reagent was mixed with the column eluent and passed into the electrochemical cell. Various lengths of delay coils were utilized before and after the electrochemical cell to optimize reaction conditions as described in the text.

Preliminary studies to evaluate types of electrochemical reactors were done using a System Gold HPLC (Beckman Instruments, Fullerton, CA, USA), which consisted of two Model 110B solvent delivery modules, an Altex 210A injection valve with a 20 μl

sample loop, and a Model 168 diode array detector. A BAS CV-27 (Bioanalytical Systems) was used to apply the potential for the three electrode reactor studies and a Heath-Schlumberger electrophoresis power supply (Heath, St. Joseph, MI, USA) was used to apply potential for two electrode studies. In the two electrode studies, current was monitored using a digital multimeter.

Later work using the three-electrode electrochemical reactor and on optimization of the chromatographic conditions was done using a Model SIL-9A Autosampler (Shimadzu Scientific, Columbia, MD, USA), a Model 2300 isocratic pump (ISCO, Lincoln, NE, USA) connected by a tee joint to a Model 600 ternary pump and a Model PDA 990 photodiode array detector (Waters Division of Millipore, Milford, MA, USA). The chromatographic column was a 250 mm×4.6 mm, 5 μm Adsorbosphere UHS C_{18} (Alltech Associates, Deerfield, IL, USA), which was maintained at ambient temperature. Potentials were applied to the electrochemical cell using a BAS CV-1B (Bioanalytical Systems). All potentials were

measured with respect to a saturated Ag/AgCl reference electrode.

The mobile and reagent phases were prepared and filtered daily. Both were saturated with helium during operation. A 60:40 mixture of aqueous 0.20 M sodium perchlorate and acetonitrile, respectively, served as the mobile phase. The reagent phase was prepared by dissolving Purpald at a concentration of 0.05 M in 1.0 M NaOH. The flow-rate for both phases was maintained at 0.50 ml/min. A 3-m length of 0.25 mm I.D. Flexon tubing (Alltech Associates) knitted through a plastic screen served as a mixing coil. A hotplate was used to maintain an elevated temperature water bath.

2.4. Electrochemical reactor designs

Three types of electrochemical reactors were investigated in the course of this work. Two of them were two electrode devices with no reference cell and the third was a three electrode device with a Ag/AgCl reference.
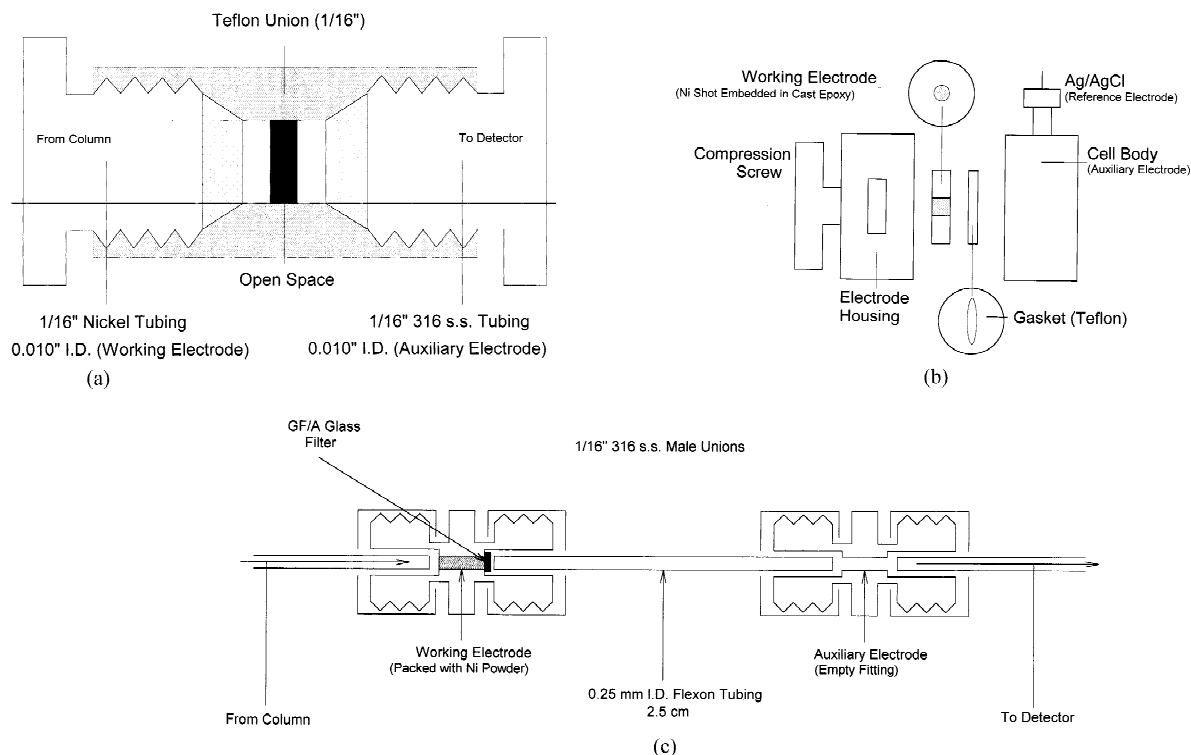

Fig. 2. Electrochemical reactors with nickel working electrode: (a) simple two electrode design; (b) two electrode packed cell configuration (20); (c) three electrode modified commercial electrode (21). '=inch; s.s.=stainless steel.

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The first of the two electrode reactors was very simplistic in design and is shown in Fig. 2a. It was constructed by connecting a 10-cm length of 1/16 in. O.D.×0.25 mm I.D. nickel 200 tubing (Alltech Associates), which acted as the working electrode, to a 10-cm length of 1/16 in. O.D.×0.18 mm I.D. 316 stainless steel, which acted as the auxiliary electrode (1 in.=2.54 cm). The ends of the tubing were joined by 0.87-in. Delrin flangeless nuts, Tefzel ferrules, and a Teflon union fitting. When the connection was made caution was taken so as not to allow the ends of the tubing to come in contact to prevent short circuiting of the cell.

The design of the second electrochemical reactor, shown in Fig. 2b, had been previously described in a paper by Mike [20]. Two 316 stainless steel male connectors were placed in series and connected with a 2.54-cm length of 1/16 in. O.D.×0.25 mm I.D. Flexon tubing. The electrode material was gently packed inside one connector in a cavity measuring approximately 1 mm×5 mm. A 1/16 in. diameter× 0.81 mm thick 316 stainless steel frit with a 2-μm porosity was placed inside the connector followed by nickel powder. The electrode was repacked daily to ensure consistent results.

The three electrode electrochemical reactor was commercially available as part of a Waters 400 series electrochemical detector (Waters Division of Millipore) but was modified as described and shown in Fig. 2c [21]. The cell body was constructed of 316 stainless steel, which served as the auxiliary electrode. A Ag/AgCl reference electrode was fit inside of a Teflon insert in the body of the electrode, with a Vycor frit at the bottom as an ion junction. The working electrode was changed from the commercially available glassy carbon to a nickel one constructed in the laboratory. The working electrode was made by embedding a 0.4 cm diameter piece of 99% pure Ni shot in an epoxy resin matrix. After curing of the resin, the electrode was polished to a mirror bright finish. A gasket with a thickness of 50 μm separated the working and auxiliary electrodes.

3. Results and discussion

3.1. Electrochemical characterization

When a nickel electrode is immersed in a solution of 1 *M* NaOH the surface becomes spontaneously converted with a passivating film of Ni(III) hydroxide [22,23]. Consecutive cyclic voltammograms for a freshly polished nickel electrode were performed to verify this. A linear sweep in the anodic direction on the initial pass showed a peak at +0.43 V versus Ag/AgCl. This was due to the oxidation of $Ni(OH)_2$ to NiO(OH) and its reduction back to Ni(II) [24]. The charge under the peak indicated the coverage was only 1 or 2 layers thick. On subsequent passes the wave gradually shifted to a less positive potential of +0.38 V versus Ag/AgCl, with an increase in peak height indicating an absorbed species on the surface. As the potential was cycled the charge under the peak stabilized indicating a steady state at the electrode surface. The peaks in the steady state voltammogram corresponded to the one electron reversible reaction between $Ni(OH)_2$ and NiO(OH).

The addition of Purpald and formaldehyde to the supporting electrolyte converted the anodic peak into a wave, while the cathodic peak remained in almost the same position prior to adding the aldehyde. This type of behavior was indicative of the fact that organic compounds are oxidized by the NiO(OH) formed on the electrode surface via a mechanism where direct electron transfer from the electrode surface to the reacting species did not occur. Fleishman et al. found this to be true for oxidation of a large group of alcohols and amines at nickel oxide electrode surfaces [7]. The potentials at which all of these organic compounds underwent oxidation were identical and were equal to the potential at which Ni^{+2} was oxidized to Ni^{+3}. During these studies similar observations were made for the Purpald–aldehyde adducts addressed in this study. It is assumed that they would apply to other types of Purpald–aldehyde adducts as well.

During the experiments, a purple color indicative of the Purpald–aldehyde reaction product was observed at the solution/electrode interface during and after the studies performed with Purpald and formaldehyde in the solution. The color continued to develop with successive cycling between potentials indicating that the nickel oxide electrode did indeed act as an oxidant for this reaction.

3.2. Spectroelectrochemical characterization

Initial investigations using spectroelectrochemical

techniques showed the oxidation of the Purpald–formaldehyde adduct to be very rapid at the electrode surface with an applied potential of 0.50 V vs. Ag/AgCl. It was noted, however, that as the molecular mass of the aldehyde in the reaction mixture was increased, the reaction time to form a colored adduct was significantly increased as well, as shown in Table 1. For the experiment, color was defined as that point where the observed absorbance was greater than 0.05 A.U.

When the classification test for aldehydes was performed using Purpald, as outlined by Durst and Gokel [25], the time required for color development was less than 5 min for all of the compounds listed above. One important factor to consider in explaining this difference in reactivity, is that for color development for Purpald–aldehyde adducts in bulk solution, the oxidation step occurs by interaction with dissolved oxygen which, while diffusion limited in amount, would be in relatively abundant supply in the solution and would be readily accessible to the oxidation reaction. In the electrochemical cell, however, it was necessary for the Purpald–aldehyde adduct to interact with the surface of the electrode in order for oxidation to occur. As such, the orientation and catalytic interaction with the surface of the nickel electrode became important factors to consider. It was considered early on that perhaps decreasing diffusion coefficients as the size of the

Table 1
Influence of molecular mass of aldehydes on reaction time with Purpald

Aldehyde	Reaction time to 0.05 A.U. (s)
Formaldehyde	<1
Acetaldehyde	83
Propionaldehyde	175
Butyraldehyde	420
Valeraldehyde	810
Aldosterone	>1200

Experimental conditions: solvent: 1:1 ratio of chromatographic mobile phase and reagent phase. The chromatographic mobile phase was 60% acetonitrile and 40% 0.20 M aqueous sodium perchlorate. The reagent phase was 1.0 M NaOH containing 0.050 M Purpald.
Aldehyde concentrations: 0.010 M each.
Working electrode: nickel grid, 80 lines/in.
Auxiliary electrode: nickel grid, 80 lines/in.
Reference electrode: saturated Ag/AgCl.
Wavelength: 550 nm.

molecules increased played a role in this observation, but since diffusion coefficients are a function of the square root of the formula mass, this would appear unlikely. Given the dramatic decrease in the intensity of the signal as formula mass increased, it seems likely that unfavorable steric or electronic effects develop from alkyl substitution, at least with this electrode.

Another possible factor contributing to the increase of reaction time with increasing size could be the difference in NaOH concentration used in these experiments compared to those used in the classification test. When the classification tests were originally performed, aqueous 1 M NaOH was used as the solvent for the Purpald and the aldehyde. The spectroelectrochemical studies performed here were closer to the conditions that were used in subsequent chromatographic experiments, where the reagent phase containing the 1 M NaOH and 0.05 M Purpald was diluted by one-half when it was mixed with the mobile phase at the exit of the column. It had been noted in this laboratory, as well as by Del Nozal et al. [10] that the concentration ratio between the aldehyde containing sample and the Purpald reagent solution was critical for maximum color development.

It was also noted during these experiments that at relatively high potentials (>1.0 V), the presence of an intensely golden yellow colored product with absorbance maxima at 294 and 368 nm was noted and the purple color of the Purpald–aldehyde adduct did not form. This was postulated to be due to oxidation of Purpald to a non-reactive species since it occurred in all solutions containing Purpald, regardless of the presence or absence of an aldehyde. Such oxidation must occur by a mechanism different than the indirect oxidation observed at lower potentials, most likely by direct electron transfer at the electrode surface.

3.3. Optimizing reaction conditions for a flowing system

The electrochemical studies indicated that oxidation of the Purpald–aldehyde adducts could be accomplished when using reasonable potentials applied at a nickel electrode surface. In order to optimize the reaction conditions for this process to

be used as a detection method for HPLC, changes in the detector response were measured versus changes in electrochemical reactor design, hydroxide ion concentration, flow-rate, and the reaction time and temperature.

Three reactors in different configurations were examined. While all three reactor designs were able to oxidize the Purpald–formaldehyde adduct, none were able to oxidize other types of Purpald–aldehyde adducts as easily.

Increasing sophistication of the flow cell resulted in an increased oxidation of the Purpald adduct, as evidenced by an increased absorbance at 500 nm in the detector. The combination of the relatively large working electrode surface and presence of a reference electrode for potential control in the three electrode design resulted in that design having the largest response and this design was used in subsequent studies. The maximum peak absorbances at 500 nm for each of the three designs are shown in Table 2. For each reactor, it was required that a different potential be applied to the working electrode in order to oxidize the Purpald adduct. It can also be seen from Table 2, that for the two reactors without a reference electrode, this meant application of a relatively high potential across the electrochemical cell in order to obtain an appropriate oxidizing potential at the working electrode.

Chromatograms for formaldehyde, acetaldehyde, and propionaldehyde standards of equal concentration are shown in Fig. 3. The relative peak size
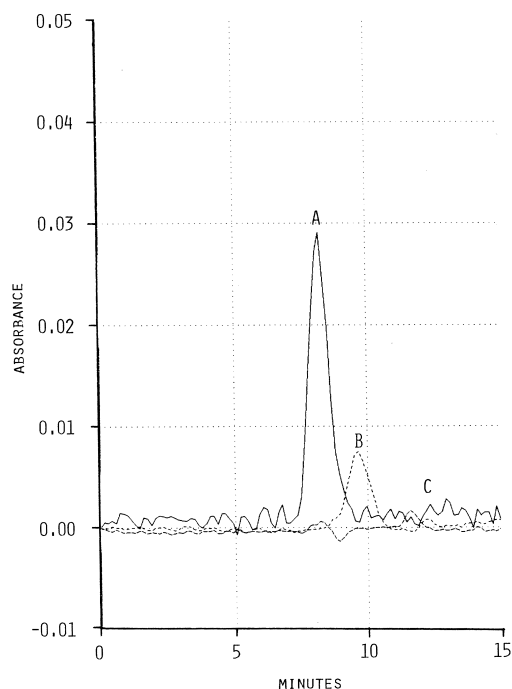

Fig. 3. Chromatograms of formaldehyde (A), acetaldehyde (B), and propionaldehyde (C) derivatives showing decreased signal with increasing molecular mass. Chromatographic column: 250 mm×4.6 mm Adsorbosphere UHS, C_{18}, 5-μm particles. Mobile phase: 60% acetonitrile and 40% 0.20 M aqueous sodium perchlorate, 0.50 ml/min. Reagent phase: 1.0 M NaOH containing 0.050 M Purpald, 0.50 ml/min. Temperature: ambient. Formaldehyde concentration: 0.010 mol/l. Working electrode: nickel; auxiliary electrode: stainless steel tubing; reference electrode: saturated Ag/AgCl. Applied potential: +0.50 V. Wavelength: 550 nm.

Table 2
Influence of reactor design on observed absorbance[a]

Working electrode	Applied potential	Reference electrode	Peak A.U. (500 nm)
Nickel tubing (1.59 mm O.D.; 0.25 mm I.D.; 0.67 mm wall thickness)	2.5 V	None	0.0086
Nickel packed powder (tubular reactor, 1 mm diameter; 5 mm long)	50 V	None	0.110
Nickel flat surface (4 mm diameter)	0.50 V	Ag/AgCl	0.220

[a] The applied potential represented the potential where maximum absorbance was seen for that reactor.
Experimental conditions: chromatographic column: 250 mm×4.6 mm Adsorbosphere UHS, C_{18}, 5-μm particles (Alltech Associates).
Mobile phase: 60% acetonitrile and 40% 0.20 M aqueous sodium perchlorate, 0.50 ml/min.
Reagent phase: 1.0 M NaOH containing 0.050 M Purpald, 0.50 ml/min. Formaldehyde concentration: 1×10^{-4} mol/l..

decreased significantly as the carbon chain length increased. This was consistent with the increasing reaction times with increasing formula mass seen in the spectroelectrochemical experiments.

Chromatograms for the oxidation of the Purpald–formaldehyde adduct using the three electrode design are shown in Fig. 4 at several potentials. Through cyclic voltammetry studies and in published work [1–9] the optimum anodic potential when working with nickel electrodes has been found to be $+0.50$ V versus Ag/AgCl. As seen in Fig. 4, increasing the potential in this system to $+0.75$ V showed no improvement in the signal. As noted earlier, when the potential was raised to $+1.10$ V the peak areas decreased. At such a high potential, it was likely that Purpald itself was oxidized making it unavailable for reaction to the colored product. This was consistent with the observation of the unreactive golden yellow colored product at high potentials seen in the spectroelectrochemical experiments.
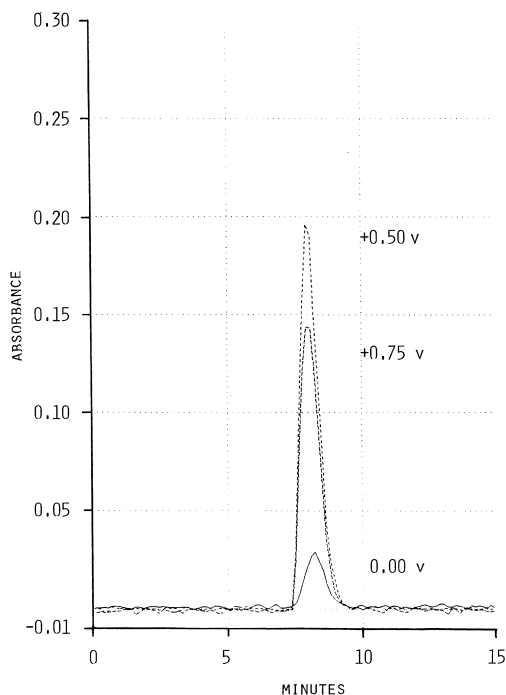
The one other variable that appeared to have the greatest effect on the detector response was the concentration of NaOH in the reactant stream. Initial investigations were conducted using a concentration of 1 *M* NaOH as reported in other studies. A ten-fold decrease in the NaOH concentration caused a significant loss in response regardless of the applied voltage. Del Nozal et al. [10] found the NaOH concentration to be a critical factor for maximum absorbance in bulk solution, and these results support his findings. In addition, lowering the concentration of the supporting electrolyte directly translated to a decrease in current. Once the NaOH concentration was restored to the original value the absorbance increased. Raising the concentration of the NaOH in

Fig. 4. Chromatograms of formaldehyde derivatives at various applied potentials vs. Ag/AgCl showing maximum color development at $+0.50$ V. Conditions as in Fig. 3.
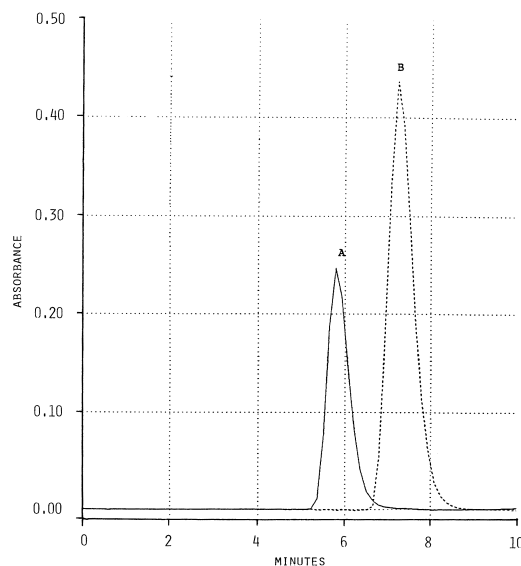

Fig. 5. Chromatograms illustrating the dependence of signal on flow-rate for formaldehyde derivatives. (A) was done using 0.65 ml/min mobile phase and 0.50 ml/min reagent solution. (B) was done using 0.50 ml/min mobile phase and 0.65 ml/min reagent solution. The dependence was stronger for mobile phase flow-rate than for reagent solution flow-rate. Chromatographic column: 250 mm×4.6 mm Adsorbosphere UHS, C_{18}, 5 μm particles. Mobile phase: 60% acetonitrile and 40% 0.20 *M* aqueous sodium perchlorate. Reagent phase: 1.0 *M* NaOH containing 0.050 *M* Purpald. Temperature: ambient. Formaldehyde concentration: 0.010 mol/l. Working electrode: nickel; auxiliary electrode: stainless steel tubing; reference electrode: saturated Ag/AgCl. Applied potential: $+0.50$ V. Wavelength: 550 nm.

the reagent phase for this particular system was not an option since it significantly increased the viscosity of the reagent phase, which in turn resulted in increased backpressure in the system. The increased pressure caused the electrochemical reactor to leak.

Also critical in determining response of the detector was the ratio at which the reagent stream was mixed with the mobile phase. For example, peak areas for a 20 μl sample of 0.01 *M* formaldehyde solution were about 290 000 to 300 000 integration units at an eluent flow of 0.5 ml/min. Increasing the mobile phase flow to 0.65 ml/min decreased the peak area by almost one-half, as shown in Fig. 5. Increasing the reagent flow to 0.65 ml/min had little effect on the observed peak areas. These observations were again attributed to the fact that NaOH concentration had a significant effect on the course of the reaction.

The only other concentration variable in the reagent stream was the concentration of Purpald. Decreasing the Purpald concentration below 0.050 *M* showed little change in the absorbance at 550 nm, while increasing it to 0.10 *M* created excessive

gassing in the detector and getting reliable results was impossible.

Increasing the reaction temperature increased detector response slightly. This trend was demonstrated in Fig. 6 for formaldehyde and acetaldehyde, respectively. A 30°C rise in temperature had little effect on the signal for the formaldehyde adduct, however there was a significant increase observed for the acetaldehyde adduct. Overall, the maximum signal for the formaldehyde adduct was about 50 times greater than that observed for the acetaldehyde adduct when the concentrations of both formaldehyde and acetaldehyde solutions were 0.010 *M*. This can also be seen in Fig. 6 where the peak area for the formaldehyde adduct at room temperature with no voltage applied was three times greater than acetaldehyde and seven times greater than propionaldehyde, both at 60°C with an applied potential of +0.50 V and a concentration of 0.010 *M* each. As the molecular mass of the aldehydes increased the detector response decreased drastically. These findings support the spectroelectrochemical study findings.
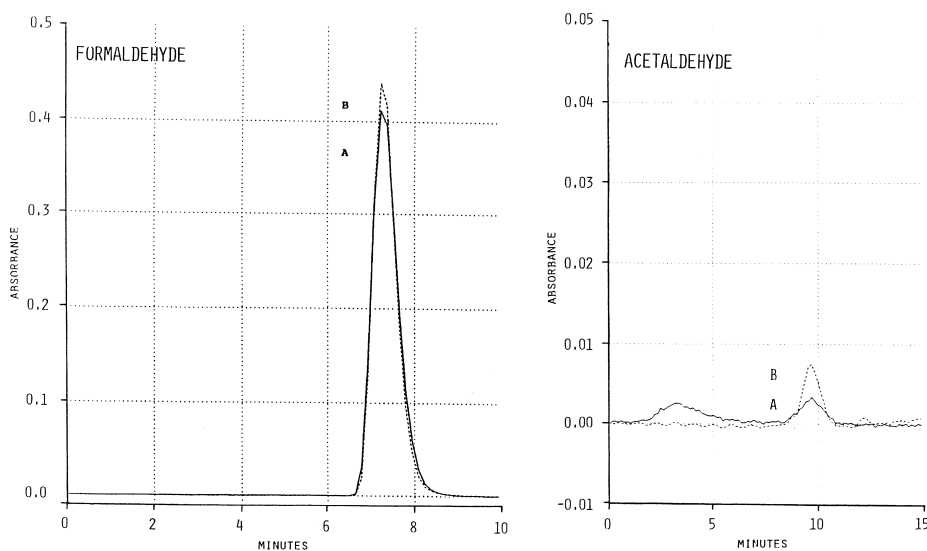

Fig. 6. Chromatograms of formaldehyde and acetaldehyde derivatives showing a slight dependence of temperature on color development. The dependence is greater for acetaldehyde than for formaldehyde. For the formaldehyde derivatives, (B) was at 50°C and (A) was at 21°C. For the acetaldehyde derivatives, (B) was at 60°C and (A) was at 30°C. Chromatographic column: 250 mm×4.6 mm Adsorbosphere UHS, C_{18}, 5-μm particles. Mobile phase: 60% acetonitrile and 40% 0.20 *M* aqueous sodium perchlorate, 0.50 ml/min. Reagent phase: 1.0 *M* NaOH containing 0.050 *M* Purpald, 0.50 ml/min. Formaldehyde concentration: 0.010 mol/l. Working electrode: nickel; auxiliary electrode: stainless steel tubing; reference electrode: saturated Ag/AgCl. Applied potential: +0.50 V. Wavelength: 550 nm.

4. Conclusion

It was apparent that formaldehyde reacted rapidly with Purpald to produce the 6-mercapto-3-substituted-s-triazole(4,3-b)-s-tetrazine using oxidation at the Ni(II) oxide/hydroxide electrode. The same was not true, however, for aldehydes of higher molecular mass. While they did eventually form highly colored complexes, the time required for this to occur was too long for convenient chromatographic analysis.

In order for this method to be of practical importance for the analysis of aldehydes of higher molecular mass, increasing the amount of the colorless intermediate formed in the first step of the derivatization reaction and improving the accessibility of the species to be oxidized to the electrode surface will be necessary. Increasing the temperature and the length of the mixing coil will likely improve the first step of the reaction. Improving the accessibility of the colorless adduct to the oxidizing surface of the electrode is more problematic, however. Some improvement would be seen from an increase in the temperature, but the most significant improvement would come from increasing the surface area of the electrode. These options could not be explored with the current design of the electrochemical reactor.

Increasing the length and increasing the temperature of the delay coil after the electrochemical cell resulted in increased pressure within the cell. The increased pressure caused leaks between the gasket and the stainless steel cell body, leaks at the reference electrode seal, and cracked the Vycor frit of the reference electrode. A change in the length of the mixing coil from 3 m to 4 m resulted in an increase in backpressure of 300 p.s.i. (1 p.s.i. = 6894.76 Pa). Increases in pressure were also seen when the 3 m coil was knitted through a plastic screen as compared to looping in a circle. Knitting the mixing coil was essential to prevent band broadening and to maximize mixing.

Re-designing the electrochemical cell should alleviate this problem. Schieffer [26,27] has described a thin-layer amperometric detector design that is simple and uses a cation-exchange membrane to separate the reference electrode from the working electrode. Johnson and Larocelle [28] used a coulometric detector in conjunction with forced-flow liquid chromatography. The design is also simple and low cost.

A design detailed by Goto [29] appears to be the simplest and totally eliminates the use of Vycor frits or ion-exchange resins to separate the working electrode and reference electrode.

References

[1] R.E. Reim, R.M. Van Effen, Anal. Chem. 58 (1986) 3203.
[2] M. Goto, H. Miyahara, D. Ishii, J. Chromatogr. 515 (1990) 213.
[3] J.M. Marioli, P.F. Luo, T. Kuwana, Anal. Chim. Acta 282 (1993) 571.
[4] T. Ueda, R. Mitchell, F. Kitamure, J. Chromatogr. 592 (1992) 229.
[5] W. Buchberger, K. Winsaur, C. Breitwierser, Fresenius Z. Anal. Chem. 315 (1983) 518.
[6] A. Stitz, W. Buchberger, Fresenius Z. Anal. Chem. 339 (1991) 55.
[7] M. Fleishman, K. Korinek, D. Pletcher, J. Chem. Soc., Perkin Trans. 2 (1972) 1396.
[8] M. Fleischman, K. Korinek, D. Pletcher, J. Electroanal. Chem. 31 (1971) 39.
[9] A. Stitz, W. Buchberger, Electroanalysis 6 (1994) 251.
[10] M.J. Del Nozal, J.L. Bernal, V. Hernandez, L. Toribio, R. Mendez, J. Liq. Chromatogr. 16 (1993) 1105.
[11] R.G. Dickinson, N.W. Jacobsen, J. Chem. Soc., Chem. Commun. (1970) 1719.
[12] D. Knapp, Handbook of Analytical Derivatization Reactions, Wiley, New York, 1979.
[13] C. Polo, J. Ally, J. Liq. Chromatogr. 10 (1987) 1165.
[14] G. Chiavari, G. Torsi, A.M. Asmundsdottir, Ann. Chim. 82 (1992) 349.
[15] G. Chiavari, C. Bergamini, J. Chromatogr. 318 (1985) 427.
[16] G. Chiavari, C. Laghi, G. Torsi, J. Chromatogr. 475 (1989) 343.
[17] G. Chiavari, M.C. Facchini, S. Fuzzi, J. Chromatogr. 378 (1987) 459.
[18] G. Chiavari, M.C. Facchini, S. Fuzzi, J. Chromatogr. 333 (1985) 262.
[19] B. Mann, M.L. Grayeski, J. Chromatogr. 386 (1987) 149.
[20] J.H. Mike, B.L. Ramos, T.A. Zupp, J. Chromatogr. 518 (1990) 167.
[21] Waters 460 Electrochemical Detector Operator Manual, Waters Associates, Milford, MA, 1st ed., 1986
[22] B.E. Conway, M.A. Sattar, D. Gilroy, Electrochim. Acta 14 (1969) 677.
[23] M.A. Sattar, B.E. Conway, Electrochim. Acta 14 (1969) 677.
[24] J.M. Marioli, P.F. Luo, T. Kuwana, Anal. Chim. Acta 282 (1993) 571.
[25] H.D. Durst, G.W. Gokel, J. Chem. Educ. 55 (1978) 206.
[26] G. Schieffer, Anal. Chem. 52 (1980) 1995.
[27] G. Schieffer, Anal. Chem. 53 (1981) 126.
[28] D. Johnson, J. Larochelle, Talanta 20 (1973) 959.
[29] M. Goto, Y. Koyangi, D. Ishii, J. Chromatogr. 208 (1981) 262.