*Journal of Chromatography*, 518 (1990) 167–177
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 635

# Electrochemical enhancement of high-performance liquid chromatography–UV detection for determination of phenylpropanolamine

J. H. MIKE*, B. L. RAMOS and T. A. ZUPP
*Department of Chemistry, Youngstown State University, Youngstown, OH 44555-0001 (U.S.A.)*
(First received February 13th, 1990; revised manuscript received June 14th, 1990)

## ABSTRACT

A common difficulty for many high-performance liquid chromatographic (HPLC) analyses is the inadequate detectability of analytes in the chromatographic eluent. One of the most common detection modes for HPLC, UV absorption, is also among the most problematic in this regard. A fast, simple and inexpensive solution to this problem is accomplished through an application of spectroelectrochemistry. A post-column electrochemical reactor placed before the UV detector was used to oxidize phenylpropanolamine (PPA), a common drug with a very low UV absorptivity, to species the UV absorptivities of which were significantly higher. The net effect of the oxidation was a significant enhancement of detector sensitivity for PPA. Electrochemical and spectroelectrochemical data, reactor design characteristics and application of the system to the analysis of PPA in dosage forms are presented.

## INTRODUCTION

Inadequate sensitivity of detection when using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection is a commonly encountered problem. The problem arises because UV detection has two conditions that must be met to give good, sensitive detection and reliable quantitation: (1) a suitably strong chromophore must be present in the analyte(s), and (2) the chromophore must absorb within a useful wavelength range of the detector. If these conditions are not met other modes of detection, such as fluorescence, if the analyte(s) can be derivatized to fluorescent compounds, or electrochemistry, if the analyte(s) are accommodating to amperometric detection, may be used.

Other modes of detection also require the availability of suitable detection equipment. If the equipment is unavailable, or budgetary limitations preclude such costly purchases, it may be possible to derivatize the analyte(s) to enhance their UV absorption properties using dansylating agents [1] or 3,5-dinitrobenzoyl chloride [2,3]. However, such reagents inevitably require additional manipulations that are often time consuming and complex and may represent a significant investment of labor and cost.

Phenylpropanolamine (PPA), a widely analyzed drug, represents this type of problem. The molecule has a low molar absorptivity at the wavelengths commonly

0021-9673/90/$03.50 

used for UV detection (*i.e.*, >230 nm). Additionally, it has been reported that PPA is an unsuitable analyte for electrochemical detection for HPLC analysis [4] nor is it inherently fluorescent. PPA can be oxidized, however, to the highly UV-absorbing compound benzaldehyde using sodium periodate. This is the basis for an analytical determination of PPA in pharmaceutical mixtures using UV spectrophotometry [5]. This process has found analytical use as a pre-column derivatization for HPLC analysis of PPA as well [6]. Electrochemical oxidation has not been reported however.

We demonstrate here a fast, simple and economical method that is useful for enhancement of the absorption properties of PPA in liquid chromatographic eluents. In addition, the method may be generally useful for enhancing the absorption properties of other molecules.

A post-column electrochemical reactor has been developed using common and readily available off-the-shelf hardware. Electrochemical oxidation of PPA generates products that are significantly more UV absorbing than PPA at wavelengths greater than 230 nm. This enhancement markedly improves the detectability of PPA using UV absorption. The method is simple and no additional pumps or reagents are required nor is pre-column derivatization. Time is saved and additional sample manipulations beyond normal sample preparation are unnecessary. The method is presented for the analysis of PPA in pharmaceutical mixtures.

## MATERIALS AND METHODS

### *Reagents and supplies*

Phenylpropanolamine · HCl was obtained from Sigma (St. Louis, MO, U.S.A.). Anhydrous sodium perchlorate, ACS reagent grade, was obtained from GFS (Columbus, OH, U.S.A.). Powdered nickel, platinum and copper (all Aldrich Gold Label grade, −100 mesh) and Darco G-60 powdered (100–325 mesh), high-purity, activated carbon, were obtained from Aldrich (Milwaukee, WI, U.S.A.). HPLC-grade acetonitrile and water were manufactured by J. T. Baker (Phillipsburg, NJ, U.S.A.). The male 316 stainless-steel connectors and Flexon tubing (0.25 m I.D.) were obtained from Alltech (Deerfield, IL, U.S.A.). The optical quartz split cell and holder used for spectroelectrochemical studies were obtained from NSG Precision Cells (Farmingdale, NY, U.S.A.). The gold-grid (4 wires/cm) electrode for the spectroelectrochemical cell was obtained from Buckbee-Mears St. Paul, MN, U.S.A.). Generic drug samples were obtained from a local drug store.

### *Electrochemical studies*

A Model CV-27 potentiostat (Bioanalytical Systems, W. Lafayette, IN, U.S.A.) was used for both cyclic voltammetric and spectroelectrochemical experiments. Cyclic voltammetry was performed in stirred solution using a working electrode of either gold, platinum or glassy carbon and auxiliary and reference electrodes of platinum and Ag/AgCl, respectively.

A spectroelectrochemical cell was constructed from a demountable, split quartz spectrophotometer cell with a path length of 1.0 mm. A gold-grid working electrode (4 wires/cm) was sandwiched between PTFE spacers and placed to cover approximately the lower two-thirds of the cell, which was glued together. A portion of the grid protruded from the cell to allow connection of the potentiostat. A reference electrode

(Ag/AgCl) was a length of narrow glass tubing fused to fritted glass that was suspended in the test solution above the light beam. The platinum auxiliary electrode was suspended at the same level as the reference. A Model 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA, U.S.A.) was used for data collection.

*Chromatographic studies*

Chromatographic experiments were performed using a Model LC/9533 high-performance liquid chromatograph (IBM, Danbury, CT, U.S.A.). The system consisted of a ternary gradient pump, a Model 7120 injector (Rheodyne, Cotati, CA, U.S.A.) fitted with a 20-$\mu$l loop, and an IBM Model LC/9523 variable-wavelength UV–VIS detector equipped with a high-pressure flow cell. To eliminate excessive bubbling in the flow cell, a backpressure regulator was packed after the detector to maintain a pressure of 1.4 MPa on the flow cell. All separations were done using an Econosphere, silica-based, octadecylsilane column (Alltech). The column was 250 × 4.6 mm I.D. and was packed with 5-$\mu$m particles. The mobile phase was water–acetonitrile–acetic acid (64.5:34.5:1) containing 0.2 mol/l sodium perchlorate at a flow-rate of 1.00 ml/min. All experiments were performed at ambient temperature.

The electrochemical cell was made from two 316 stainless-steel male connectors, serially placed and connected by a 2.54-cm length of 0.25 mm I.D. Flexon tubing. Flexon was chosen over PTFE because of its durability.

Potentials for the electrochemical cell were applied using a Heath–Schlumberg Model 17a (Heath, St. Joseph, MI, U.S.A.) constant-voltage electrophoresis-type power supply. It had a potental range of 0–400 V at up to 100 mA current. Current was monitored with a digital voltmeter. Electrical connections to the electrodes were shielded for safety.

*Electrode preparation*

The stainless-steel male connectors used for the reactor were not of the zero-dead-volume type. Inside the connectors there was a gap that measured approximately 1.0 × 5.0 mm into which the electrode material was packed. A plug of glass fiber was held in place in the bottom of the fitting by short tube and ferrule. Small portions of the electrode materials were tapped gently into the cell until the gap was filled. Packing the materials very tightly resulted in excessive backpressures that ruptured the Flexon lines. When using metal electrodes, the oxidation potentials were such that the electrodes had a limited lifetime due to solubilization. To insure an adequate amount of electrode material the electrodes were repacked every 2–3 days. A schematic representation of the electrochemical cell is shown in Fig. 1.

*Sample preparation*

Cold syrup samples were prepared for analysis by pipetting 5.00 ml of sample into a 50-ml volumetric flask and diluting to the mark using deionized water. Diet aid tablet samples were prepared by weighing ten tablets to obtain a weight per tablet. The ten tablets were crushed using a mortar and pestle and an amount of powder approximately equivalent to one tablet was weighed and dissolved in 25 ml of deionized water in a 100-ml volumetric flask. A volume of 35 ml of acetonitrile was added and the flask shaken for at least 2 h on a mechanical shaker. Deionized water

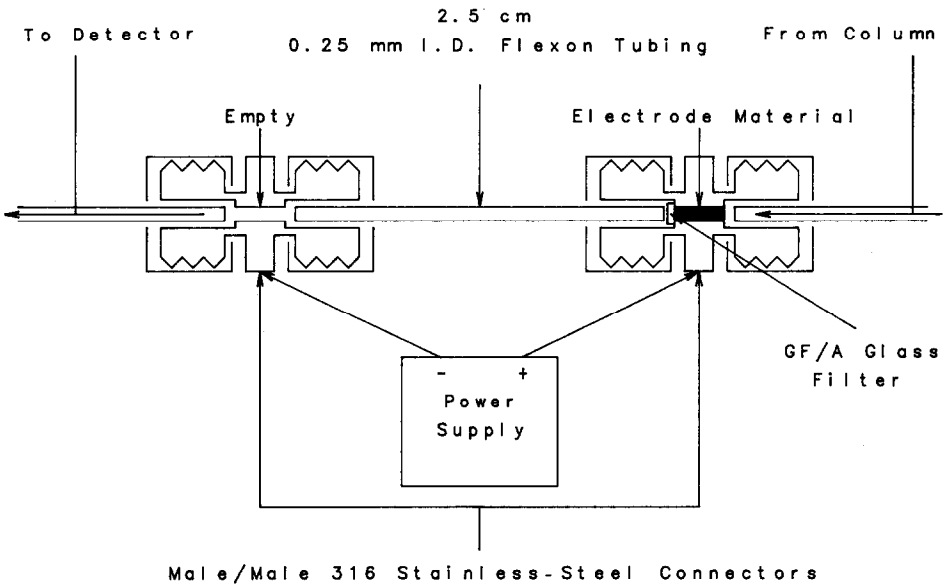



Fig. 1. Schematic representation of flow-through electrochemical cell.

was added up to the mark in the flask and the contents were thoroughly mixed. All solutions were filtered using a 0.45-$\mu$m nylon filter before injection.

## RESULTS AND DISCUSSION

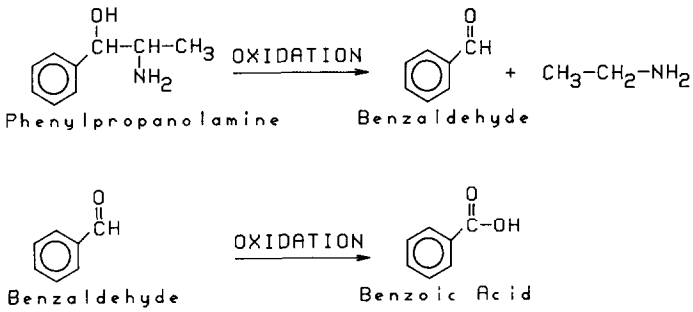
### *Oxidation of PPA*

For this study it was shown, using techniques such as cyclic voltammetry, spectroelectrochemistry and chromatographic peak collection, that PPA could be oxidized electrochemically. For cyclic voltammetry, scans were performed in the positive direction from 0 to 2.00 V and showed a distinct wave with a peak at +1.89 V. There was no reduction peak observed on the reverse scans. The oxidation peak was virtually identical with respect to peak voltage and shape for all electrodes tested, although later experiments demonstrated that the choice of electrode material as well as the reaction conditions were important factors to consider for optimization of the electrochemical reaction for maximum UV absorption.

In the reaction scheme shown below, it appeared that two possible products could result from the oxidation of PPA: benzoic acid or benzaldehyde, dependent upon the degree of oxidation.

Both benzaldehyde and benzoic acid are very strong UV absorbers above 230 nm while PPA is not. In ethanol, benzaldehyde has an absorption maximum at 250 nm ($\varepsilon$ = 15 000 l mol$^{-1}$ cm$^{-1}$) and benzoic acid at 230 nm ($\varepsilon$ = 10 000 l mol$^{-1}$ cm$^{-1}$) [7]. PPA was determined to have an absorption maximum at 258 nm ($\varepsilon$ = 190 l mol$^{-1}$ cm$^{-1}$) in the chromatographic solvent.

Investigations of the anodic oxidation reactions for evidence of formation of either benzaldehyde or benzoic acid were performed by spectroelectrochemistry.




Initially, the studies were done using a spectroelectrochemical photometer cell. Later studies involved collection of chromatographic peaks obtaind at various applied potentials, followed by spectrophotometry. In each case, as the potential was increased it was possible to see spectral changes occur that appeared consistent with the formation of the two products. The observed data are outlined below.

Initial efforts using the spectroelectrochemical cell appeared to point to formation of benzoic acid as the major anodic oxidation product. The spectra, shown in Fig. 2, exhibited a large peak at 230 nm that was attributed to benzoic acid formation. Some benzaldehyde appeared to be present and was detected as a barely discernible shoulder at around 252 nm on the much larger benzoic acid peak. Initial chromatographic studies, using a nickel electrode, were consequently performed by monitoring the chromatographic eluent at 230 nm. This resulted in only minor enhancement of the PPA chromatographic peak and a very high background absorbance. Monitoring at 252 nm gave a significantly greater enhancement, as well as a much lower background absorbance.

To examine this further, HPLC peak collection after on-line oxidation with the nickel electrode in the reactor cell was performed. Under these conditions, the major

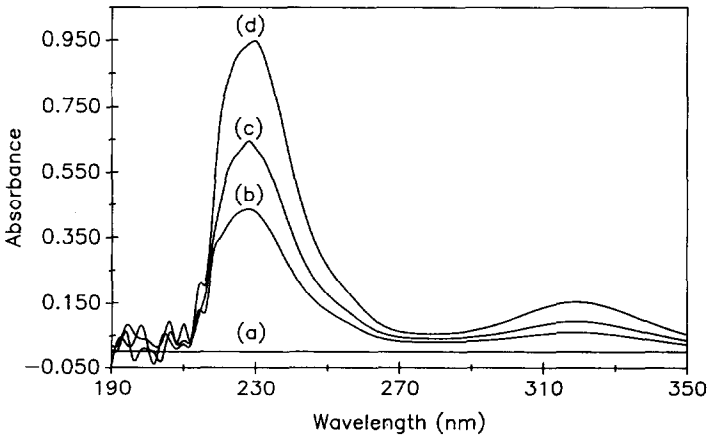



Fig. 2. Spectra obtained at: (a) = 0 s; (b) = 60 s; (c) = 120 s; (d) 180 s; electrochemical oxidation of PPA, 1 mg/ml, in chromatographic mobile phase using spectroelectrochemical photometer cell. Potential, 1,89 volts; gold-grid working electrode, Pt auxiliary electrode, Ag/AgCl reference electrode.

oxidation product appeared to be benzaldehyde, since an absorption maximum was observed at 252 nm. From these spectra there appeared to be little formation of benzoic acid, since as the potential was increased there was only a small increase in absorbance at 230 nm. This was difficult to confirm, however, since the mobile phase absorbed very strongly in this spectral region, with a cut-off close to 230 nm.

These results appeared to be at odds with one another. Oxidation at different electrodes may proceed by different mechanisms, however. It has been pointed out by other investigators [8] and observed in later experiments that gold, and noble metal electrodes in general, behaves much differently than do certain transition metal electrodes such as nickel. These experiments plausibly demonstrated that both benzaldehyde and benzoic acid may be formed as products of the electrochemical oxidation of PPA, but it was apparent from these and later data that both the solvent and the electrode played an important role in the electrochemical reaction. The results indicated clearly, however, that the chromatographic solvent system, with the reactor cell set up with a nickel electrode, would be an excellent choice for optimization of the electrode reaction for maximal yield of benzaldehyde, since benzaldehyde appeared to be the only major oxidation product in that system. Further studies are presently underway to elucidate the mechanisms for electrochemical oxidation of PPA at different electrodes and under different solvent conditions.

*Electrochemical cell design*

In the design of the flow-through electrochemical cell, a simple two-electrode system was adopted. Simplicity was a major consideration in the design of the cell since many laboratories may not have sophisticated machine-shop facilities or complex instrumentation. A two-electrode system was less expensive, easier to build and easier to maintain than a three-electrode system and required a simple power supply rather than a potentiostat. In addition, bulk oxidation of solutes without precise monitoring of the current was the only purpose for the cell and two-electrode cells have been used for such purposes [8,9].

The mobile phase was in large proportion non-aqueous resulting in a substantial resistance between the working and auxiliary electrodes. High potentials were required to obtain a sufficiently positive oxidation potential at the working-electrode surface. Such high potentials were beyond the limits of the potentiostats available in our laboratories. To produce these potentials it was necessary to use an electrophoresis-type power supply

The stainless-steel fittings did not contribute to the oxidation reaction. Potential applied to an empty electrode gave no detectable enhancement of absorption. In conjunction with this, the electrodes were inspected frequently. After approximately 8 months of using a single pair of fittings as the working and auxiliary electrodes, no observations of pitting or malformation were made in either fitting. The fittings were thus considered inert, serving as simple electrical conductors.

The order of placement of the working and auxiliary electrodes in the flow stream was important. Oxidation of PPA was observed with either orientation of the electrodes in the flow stream. However, the yield of product absorbing strongly at 252 nm was significantly greater when the working electrode was placed first, followed by the auxiliary electrode. The reason for this has not yet been determined but all subsequent experiments were done with the working electrode placed first.

With respect to the effects of the electrochemical reactor cell on chromatographic performance, as seen in Fig. 3, addition of the cell to the flowing system contributed very little to the total band broadening observed on the chromatograms. The major advantages of the chosen reactor design were thus the simplicity of the system as a whole and the null effects of the cell on chromatographic performance.

*Optimization of the electrode reaction*

Electrochemical oxidation of PPA under the chromatographic conditions presumably gave benzaldehyde as the primary product detected by monitoring absorbance at 252 nm. For optimization of the reaction, changes in absorbance at 252 nm were monitored as the electrode conditions were changed. The type of electrode material, the applied potential and the concentration of sodium perchlorate (supporting electrolyte) were all important variables for obtaining a maximal yield of benzaldehyde.

The electrode materials examined were powdered carbon, copper, platinum and nickel, all chosen primarily to represent a range of electrode types. As seen in Fig. 4, the yield of product formed at 252 nm increased with increasing applied potential. This

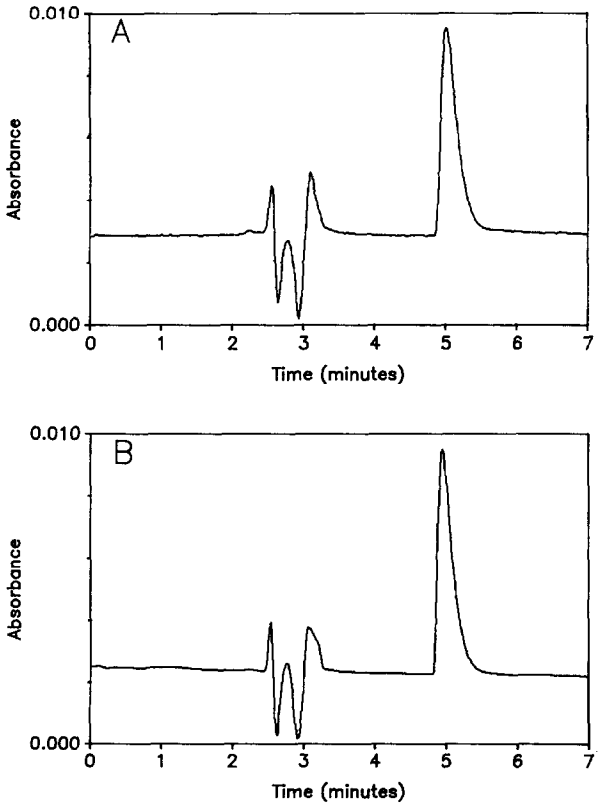



Fig. 3. Demonstration of the effect of addition of the electrochemical reactor to the HPLC system, 20 $\mu$g PPA injected. (A) Reactor in place, 258 nm; peak width at half height ($w_{\frac{1}{2}}$) = 0.23 min; (B) no reactor, 258 nm; $w_{\frac{1}{2}}$ = 0.22 min.

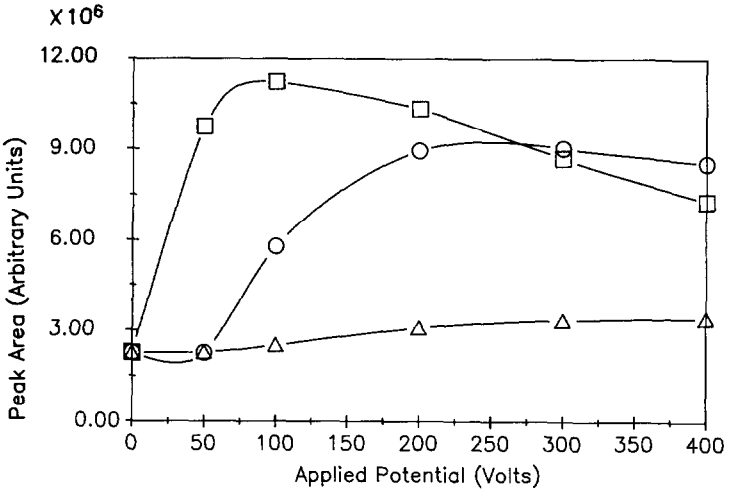



Fig. 4. Oxidation profiles for different electrode material in the electrochemical reactor, 20 μg PPA per 20 μl injection, 252 nm. ○ = Ni electrode; △ = C; □ = Pt.

was noted with all electrode materials except copper, but in differing degrees with each material. Copper was not suitable at any potential due to extremely high background absorbance from oxidized copper.

With a powdered carbon electrode, little increase in absorption at 252 nm was evident up to the 400-V limit of the power supply. This could have been due either to non-oxidation of PPA at the carbon electrode, or to almost exclusive formation of benzoic acid during the oxidation, which would have had shifted the absorption maximum.

When using a platinum electrode, oxidation to benzaldehyde occured at a relatively low potential. But, the absorbance maximum showed a fairly sharp peak that decreased significantly as the applied potential was increased. The decrease in absorption at higher potential was attributed to more complete oxidation of the analyte to benzoic acid with subsequent shifting of the absorbance maximum from 252 nm to 230 nm.

In this application, nickel electrodes appeared to perform the best. The absorbance enhancement climbed to a maximum and stayed there over nearly the entire range of potentials investigated. This behavior was attributed to the general manner in which certain metal electrodes, such as nickel or silver, function. Unlike noble metal or carbon electrodes, oxidation at these electrodes proceeds by an indirect electron transfer process. The electrode surface first becomes oxidized and this oxide subsequently passes electrons to the substrate. This mechanism has been observed in anodic oxidations of alcohols and amines using transition metal electrodes [8]. It has also been observed when using these electrodes, that oxidations of compounds from the same class all appear to occur at the same potential, that of the formation of the metal oxide. In the oxidation of PPA at the nickel electrode this was the presumable reason that the oxidation did not readily proceed past the benzaldehyde stage, but was nearly constant for the range of potentials investigated.

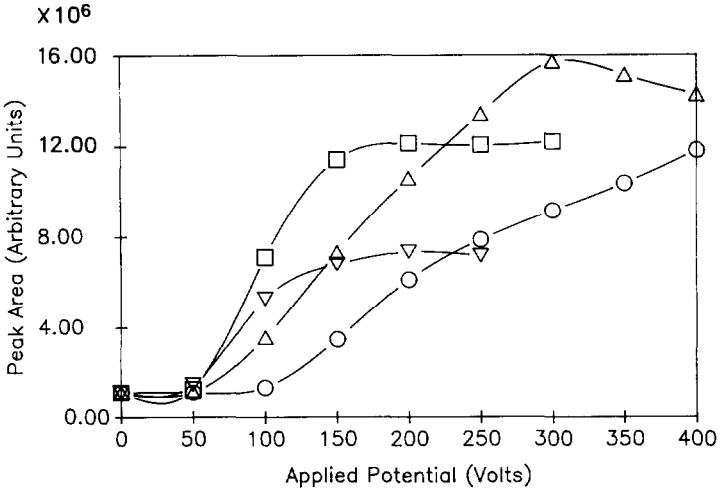



Fig. 5. Variation of oxidation profile with concentration of sodium perchlorate, 20 μg PPA per 20 μl injection, Ni electrode. ○ = 0.1 mol/l; △ = 0.2 mol/l; □ = 0.3 mol/l; ▽ = 0.5 mol/l.

Fig. 5 shows the dependence of the oxidation reaction on the concentration of the supporting electrolyte when monitoring at 252 nm for yield of benzaldehyde. At a given applied potential, the yield of benzaldehyde at first increased and then decreased as the electrolyte concentration was increased. The initial increase in peak areas was attributed to increased formation of benzaldehyde from more efficient charge transfer through the solution. Above 0.2 mol/l sodium perchlorate concentration, it appeared that product formation began to shift from benzaldehyde to benzoic acid. This shifted the absorbance maximum and decreased the peak areas observed at 252 nm.

*Quantitative analysis*

Reproducibility of injections when using the electrochemical cell was very good. A coefficient of variation of $\pm 3.302\%$ was determined for 9 replicate injections. To achieve this level of reproducibility required conditioning of the new electrode by the application of potential for approximately 15 to 30 min. Repeated injections performed prior to this equilibration period showed peaks heights 5–10% over the observed equilibrium enhancement, which gradually decreased to the equilibrium height. Presumably, formation of a stable oxide coat on the nickel surface was required to insure a reproducible oxidation mechnism. After the initial equilibration period, the electrode functioned reproducibly throughout its usable lifetime of 2–3 days.

For quantitative analysis of PPA, linearity was observed over the range of the calibration curve. A comparison of slopes from sensitivity curves, using identical standards for PPA and oxidized PPA demonstrated that the sensitivity of analysis was enhanced by addition of the electrochemical reactor. The line slope for unoxidized PPA was $1.07 \cdot 10^6$/(ng/ml) while that for oxidized PPA was $9.84 \cdot 10^6$/(ng/ml). Approximately a 10-fold improvement in sensitivity was observed.

In a comparison of the linearity of PPA and oxidized PPA over a much wider

TABLE I

QUANTITATIVE ANALYSIS OF PHARMACEUTICALS

Each point represents duplicate analyses; 252 nm; applied potential, 275 V; current, 2.60 mA.

| | Multicomponent liquid | | Diet aid | |
|---|---|---|---|---|
| | mg/5 ml | % of label claim | mg/tablet | % of label claim |
| | 24.8 | 99.2 | 74.8 | 99.7 |
| | 25.5 | 102.0 | 74.8 | 99.7 |
| | 24.9 | 99.6 | 75.4 | 100,5 |
| Mean | 25.1 | 100.3 | 75.0 | 100.0 |
| S.D. | 0.38 | 1.5 | 0.35 | 0.46 |
| Coefficient of variation (%) | 1.5 | 1.5 | 0.46 | 0.46 |

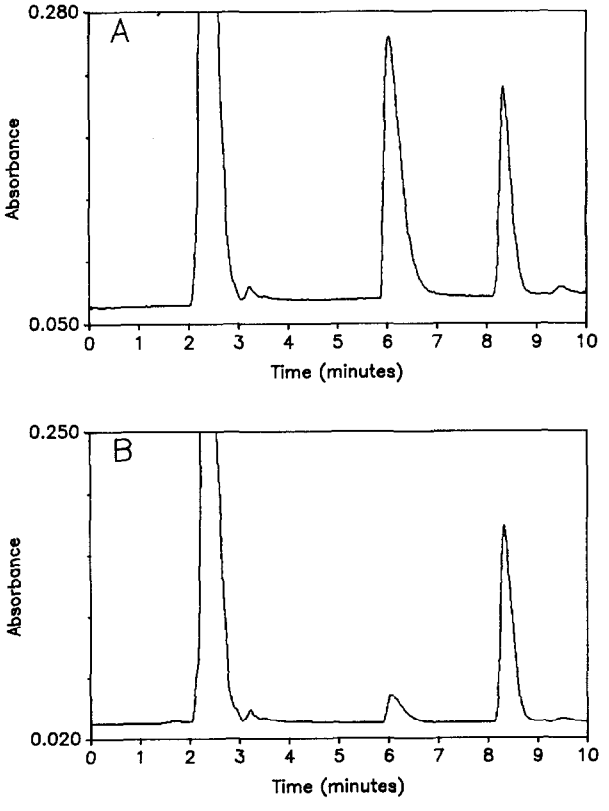



Fig. 6. Chromatograms from analysis of multicomponent cold liquid. (A) = 275 V applied potential, 2.52 mA, 252 nm; (B) = 0 V applied potential, 258 nm.

range of concentrations and under identical chromatographic conditions, neither PPA nor oxidized PPA displayed a large range of linearity with the IBM 9523 UV–VIS detector. Generally, if a detector exhibits a linear response, a logarithmic plot of response *vs.* concentration should have a slope of 1.00 [10]. In an experimental logarithmic plot of the detector response in each case, parallel lines with nearly equal slopes of 0.8907 and 0.9021, for oxidized and native PPA respectively, were obtained. Considering this, it is likely that the detector, not the electrochemical reactor, was the limiting factor for linearity.

Analysis of pharmaceutical samples displayed excellent agreement with labelled quantities (Table I). As an illustration of the enhancement, typical chromatograms are shown in Fig. 6, for an over-the-counter multicomponent liquid cold preparation, with the reactor off and with potential applied. From a comparison of peak areas, the enhancement obtained for this sample was 10-fold. Given this enhancement, application of this system to the analysis of PPA in biological systems, where detection much nearer to the detection limit is typical should thus prove advantageous.

## CONCLUSIONS

On-line electrochemical oxidation for enhancement of UV detectability represents an inexpensive and easy-to-construct system that has been applied to the analysis of PPA in pharmaceutical samples. The degree of detection enhancement in this instance was significant and no reagents, extraneous pumping systems or temperature control was required. The simplicity of the system was such that it should be readily and easily available to almost every laboratory.

Many compounds are oxidizable. The system should consequently be broadly applicable to many other compounds, pharmaceutical and non-pharmaceutical alike, even those that are already UV absorbers, to enhance their detectability. The potential exists, likewise, for use of such a system for enhancement of other modes of detection (*e.g.*, fluorescence, visible or electrochemical) in many applications.

## ACKNOWLEDGEMENT

This work was supported, in part, by a grant from the Youngstown State University Research Council, Grant 708.

## REFERENCES

1 R. W. Frei and J. F. Lawrence, *J. Chromatogr.*, 83 (1973) 321.
2 M. A. Carey and H. E. Perisinger, *J. Chrom. Sci.*, 10 (1972) 537.
3 J. F. Lawrence and R. W. Frei, *Chemical Derivatization in Liquid Chromatography*, Elsevier, Amsterdam, 1976.
4 I. Jane, A. McKinnon and R. J. Flanagan, *J. Chromatogr.*, 323 (1985) 191.
5 A. C. Nonzioli, *SAFYBI*, 15 (1975) 865; *C.A.*, 83 (1975) 209460h.
6 H. S. I. Tan and G. C. Salvador, *J. Chromatogr.*, 261 (1983) 111.
7 R. M. Silverstein, G. C. Bassler and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, Wiley, New York, 3rd ed., 1974, p. 250.
8 S. D. Ross, M. Finkelstein and E. J. Rudd, *Anodic Oxidation*, Academic Press, New York, 1975.
9 D. K. Kyriacou, *Basics of Electroorgaic Synthesis*, Wiley, New York, 1981.
10 I. A. Fowliss and R. P. W. Scott, *J. Chromatogr.*, 11 (1963) 1.