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Short Communication

Simultaneous analysis of hydroxylamine, N-methylhydroxylamine and N,N-dimethylhydroxylamine by ion chromatography

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Abstract

Hydroxylamine and N-alkylhydroxylamines have been separated and analyzed using ion chromatography with conductivity and amperometric detectors. Conductivity detection is simple, but lacks selectivity. Amperometric detection provides good selectivity at modest working electrode operating potentials. However, in order to realize the selectivity, it is necessary to pretreat the working electrode which results in a significant reduction of the detector operating potential. While both conductivity and amperometric detectors have the capability to quantify nanomole amounts of hydroxylamines (absolute amount injected), amperometric detection possesses superior selectivity and sensitivity.

1. Introduction

Hydroxylamine and N-alkylhydroxylamines belong to an important class of reducing agents which are routinely used in industrial and pharmaceutical processes. Hydroxylamine has been identified as an intermediate in many biological processes [1]. Generally, hydroxylamines are mutagenic in nature. Simple derivatives of hydroxylamine, for example, hydroxamic acids or oximes which are important industrial raw materials, can be determined as hydroxylamine after initial hydrolysis [1]. Quantitative determination of hydroxylamines is therefore, very important both in studies of biological processes and for industrial purposes. A survey of the analytical procedures that exist in the literature reveals that volumetric and spectrophotometric methods are commonly used for the analysis of hydroxylamines [1]. A GC procedure with pre-column derivatization has been reported for trace level determination of hydroxylamine [2]. HPLC methods with UV detection (210 nm) [3] and potentiometric detection [4] have also been reported. However, to our knowledge, simultaneous analysis of hydroxylamine and its derivatives (N-alkylhydroxylamines) has not been performed. In this paper, we report the utility of both conductivity and amperometric detection following ion chromatographic (IC) separation for the simultaneous determination of hydroxylamine and N-alkylhydroxylamines, as well as the

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selectivity and sensitivity aspects of both conductivity and amperometric detection schemes.

2. Experimental

2.1. Apparatus

Cyclic voltammetry experiments were performed using a Bioanalytical Systems (BAS) Model 100B instrument, glassy carbon working electrode, Pt counter electrode and Model RE-1 Ag/AgCl reference electrode. The chromatographic equipment used consisted of a Jasco Model 880 PU pump, Micromeritics Model 728 autosampler equipped with a Model 732 injector and 50-μl sample loop, Alltech Model 320 conductivity detector and PARC Model 400 electrochemical detector equipped with the BAS Model LC 17A thin-layer cell with a glassy carbon electrode. The post-column addition of base was achieved using a TimberLine Instruments reagent-delivery module, Model RDR-1. The chromatographic data were collected using a PE Nelson data system. All chromatography was performed using a 150 mm × 4.6 mm I.D. Alltech/Wescan Cation/R column. Unless otherwise stated, the mobile phase flow-rate was 1 ml/min. The post-column base (20 m*M* NaOH) was added at the rate of 1.5 ml/min.

2.2. Reagents

All solutions were prepared with Milli-Q water. The mobile phase for the chromatographic separation was prepared from Baker Ultrex-grade concentrated nitric acid by stepwise dilution to a final concentration of 10 m*M*. The mobile phase was filtered through a 0.45-μm nylon-66 membrane prior to use. The buffers used in the pH variation study were prepared according to Christian [5] using reagent-grade phosphate salts.

2.3. Electrode pretreatment

The glassy carbon electrode was polished three times with a 1-μm alumina slurry for 1 min and rinsed each time with Milli-Q water. The electrode was then sonicated using a Branson Model 1200 sonicator for 5 min in Milli-Q water to eliminate any adsorbed alumina particles. The electrochemical pretreatment itself consisted of holding the electrode at +1.75 V *vs.* Ag/AgCl in a pH 7 buffer solution for 5 min and then at −1.2 V *vs.* Ag/AgCl for 10 s.

3. Results and discussion

3.1. Chromatography of hydroxylamines

Reversed-phase separation of hydroxylamine and hydroxyurea has been reported with water as the mobile phase [6]. The reported k' for hydroxylamine was large enough to suggest that N-alkylhydroxylamines could indeed be separated by RPLC. However, in our hands k' values for unsubstituted *vs.* substituted hydroxylamines were not significantly different. Therefore, another mode of separation was deemed necessary.

Alkanolamines have been successfully separated by IC [6]. A similar strategy should be applicable for the separation of hydroxylamines. Fig. 1 shows the chromatogram obtained for the separation of three hydroxylamines using single-column IC. The N,N-dimethylhydroxylamine peak tails somewhat, probably due to the hydrophobic nature of the column packing. The peak for hydroxylamine is preceded by a shoulder which is due to trace amounts of sodium ions. In a typical analytical situation, if the sample contained a significant amount of sodium ions, then one would observe a co-elution of both hydroxylamine and sodium ions. In order for this separation to be useful analytically, it is imperative that the separation be improved or a selective detector be used. Attempts to improve the resolution between sodium and hydroxylamines were not at all successful. Hydroxylamines do not absorb in the useful UV region significantly. The absorption maximum is in the more noise prone 190–215 nm UV region which does not facilitate selective detection. Hydroxylamines have been reported to be electroactive [7], which

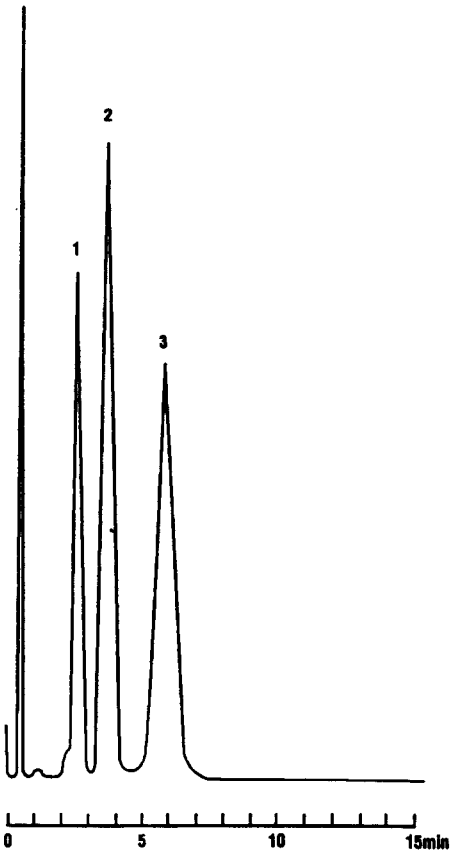

Fig. 1. Ion chromatogram of three hydroxylamines. Peaks: 1 = hydroxylamine $1 \cdot 10^{-4}$ *M*; 2 = N-methylhydroxylamine $2 \cdot 10^{-4}$ *M*; 3 = N,N-dimethylhydroxylamine $2 \cdot 10^{-4}$ *M*. Detector sensitivity 1 μS full scale.

raises the possibility of using LC with electrochemical detection (ED) for the selective detection of hydroxylamines.

3.2. Electrochemistry of hydroxylamines

N-Alkylhydroxylamines have been quantitatively determined by polarography [7]. There is very little information about the electrochemical behavior of hydroxylamines at solid electrodes such as glassy carbon which is commonly used in LC–ED. The electrochemical behavior of hydroxylamine at a glassy carbon electrode was first studied in 10 m*M* nitric acid, which was used as the mobile phase in the IC experiment. None of the hydroxylamines exhibited any electrochemical activity in 10 m*M* nitric acid. Organic oxidations are generally facile at alkaline pH conditions. The acidic pH (about 2.5) of the supporting electrolyte (10 m*M* nitric acid) might have inhibited the oxidation. To confirm this possibility, the effect of pH on the electrochemistry of hydroxylamine at the glassy carbon electrode was studied. It was found from the cyclic voltammetric (CV) studies that no significant electrochemical activity could be obtained until the pH of the supporting electrolyte was at or above pH 7. It was interesting to note that even at very alkaline pH levels, hydroxylamine underwent oxidation at substantial overpotentials. Similar results were obtained for N-methyl- and N,N-dimethylhydroxylamines. These observations indicated that in order to use ED for hydroxylamines, it becomes necessary to operate the electrochemical detector at very high potentials which would significantly decrease the detector selectivity and sensitivity by decreasing the *S/N* ratio. A simple electrochemical pretreatment has been shown to be very effective in enhancing the electrode response for many analytes [8]. Such a pretreatment has been successfully applied towards enhancement of LC–ED responses for several biologically important analytes in complex physiological matrices [9]. It is likely that a similar strategy could prove to be useful in enhancing the electrode response for hydroxylamines. Accordingly, the electrochemical behavior of hydroxylamines was studied at electrochemically pretreated electrodes. It was found that the electrochemical pretreatment of the electrode surface indeed resulted in increased electrochemical response. It was also observed that alkaline pH conditions were necessary to realize the diffusion-controlled oxidation of hydroxylamine. This observation is in agreement with the DME polarography work done by Iverson and Lund [7]. Fig. 2 shows the comparison of the electrochemical response for the three hydroxylamines at pH 9.0, before and after pretreatment. Clearly the electrode pretreatment does result in a reduction of the overvoltage needed to oxidize these species by 300–500 mV. It must be noted that the first scan in the CV exhibited a much higher response while the

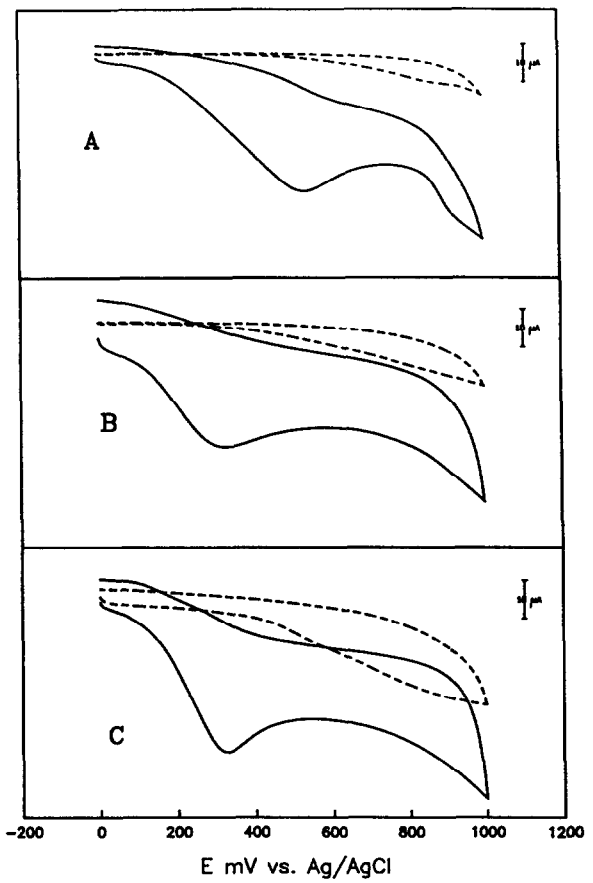

Fig. 2. Cyclic voltammograms of $2 \cdot 10^{-3}$ *M* of (A) hydroxylamine, (B) N-methylhydroxylamine and (C) N,N-dimethylhydroxylamine. Broken lines: untreated electrode response; solid lines: pretreated electrode response.

subsequent scans showed decreased response, possibly due to electrode surface fouling which is not likely to pose any problems in LC–ED. The electrochemical activity can be regained by polishing the electrode and reconditioning the surface electrochemically. These observations are very reproducible as long as the pretreatment procedure is followed as described in the Experimental section.

3.3. LC–ED of hydroxylamines

The electrochemical investigations described in the previous section indicated that in order to detect the hydroxylamines it is necessary to have the pH of the LC effluent at or above pH 7.0. However, the IC separation of the hydroxylamines requires 10 m*M* nitric acid which is obviously unsuitable for LC–ED. It is possible to achieve the neutral or alkaline pH of the effluent in two ways. The first method involves the use of chemical suppression of the acid. However, the resulting effluent has a low conductance rendering it almost useless for LC–ED. The second and simplest way to achieve the alkaline pH values is by post-column addition of a strong base to the effluent from the conductivity detector. It was determined by trial and error that addition of 20 m*M* sodium hydroxide to the conductivity detector effluent at a rate of 1.5 ml/min rendered the pH of the effluent to be around 10. Chromatograms obtained for a mixture of three hydroxylamines using LC–ED at both untreated and pretreated glassy carbon electrodes are provided in Fig. 3. In both cases, the electrode was maintained at 0.6 V *vs.* Ag/AgCl and the same hydroxylamine mixture was used to generate each chromatogram. At the modest working electrode potential used here, a very large re-

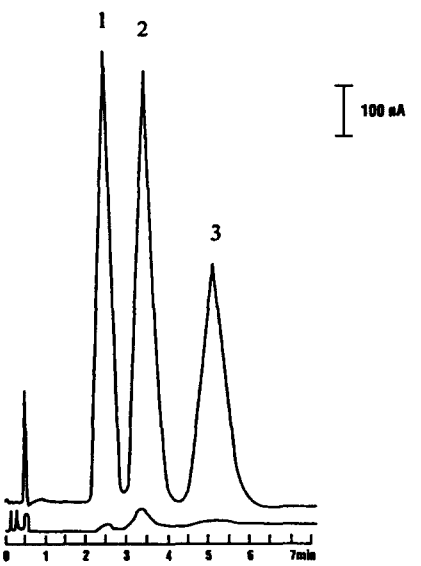

Fig. 3. Chromatograms of hydroxylamine mixture at 0.5 V *vs.* Ag/AgCl at (bottom curve) untreated and (top curve) electrochemically pretreated glassy carbon electrode. Peaks: 1 = hydroxylamine $1 \cdot 10^{-4}$ *M*; 2 = N-methylhydroxylamine $2 \cdot 10^{-4}$ *M*; 3 = N,N-dimethylhydroxylamine $2 \cdot 10^{-4}$ *M*.

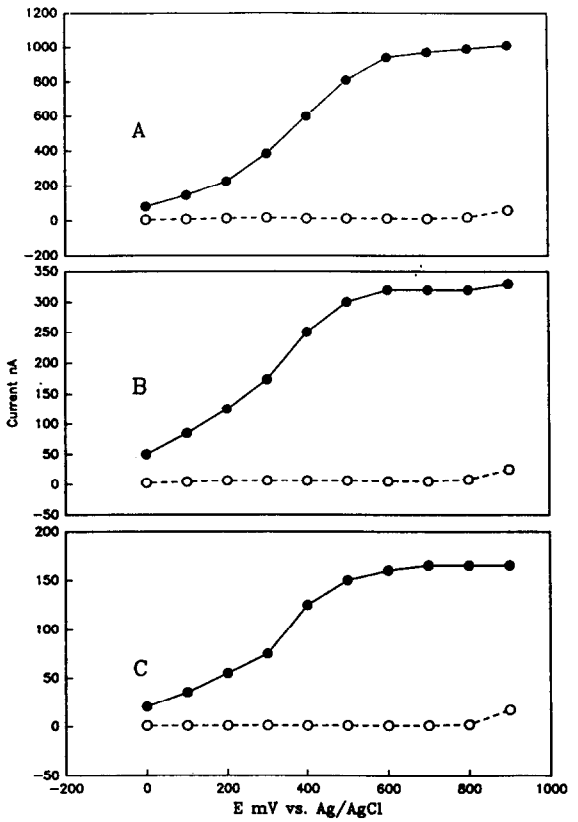

Fig. 4. Hydrodynamic voltammograms at untreated (○) and electrochemically pretreated (●) glassy carbon electrodes for equimolar mixture ($2 \cdot 10^{-4}$ *M*) of (A) hydroxylamine, (B) N-methylhydroxylamine and (C) N,N-dimethylhydroxylamine.

sponse was obtained for the pretreated electrode, while almost no signal was obtained at the untreated electrode. This behavior was exactly as expected based on the voltammetric data described in the previous section.

The hydrodynamic voltammograms (HDVs), peak current *vs.* potential profiles, for each of the three hydroxylamines at treated and untreated electrodes are shown in Fig. 4. These curves were obtained by plotting the detector response for a given concentration of each analyte at various applied electrode potentials under a given chromatographic condition. The HDV data for the untreated electrode was obtained first. The electrode was disconnected from the cell, pretreated as described in the Experimental section, reassembled and the HDV data was obtained. For the untreated electrode, an analytically useful signal could not be obtained even at 0.9 V. On the other hand, the pretreated electrode exhibited very well defined plateaus for all the three hydroxylamines in the vicinity of 0.6 V *vs.* Ag/AgCl. The calibration curves and detection limits for the three hydroxylamines were determined at 0.6 V. Calibration curves obtained over the concentration range examined were found to be linear with correlation coefficients greater than 0.99. The linear dynamic ranges in terms of the absolute amount injected were the following: hydroxylamine 0.05–2.5 nmol; N-methylhydroxylamine 0.25–5.0 nmol; and N,N-dimethylhydroxylamine 0.4–5.0 nmol. To the best of our knowledge, simultaneous analysis of hydroxylamines by HPLC techniques has not yet been reported. In the applications cited [3,4], no detection limits have been reported. The LOD value for hydroxylamine with conductivity detection reported in Table 1 com-

Table 1
Comparison of conductivity and electrochemical detector responses

Analyte	LC–conductivity detection		LC–ED	
	Linear range	LOD	Linear range	LOD
Hydroxylamine	1.0–25.0	0.5	0.05–2.5	0.01
N-Methylhydroxylamine	2.0–40.0	1.0	0.25–5.0	0.05
N,N-Dimethylhydroxylamine	2.5–50.0	1.0	0.40–5.0	0.08

All the amounts listed are absolute amounts injected on the column in nmol. LOD = Limit of detection (defined as the lowest detectable amount with *S/N* ratio of 3.0).

pares favorably with GC analysis with pre-column derivatization. The LOD value for hydroxylamine with ED is lower by at least an order of magnitude. The relative standard deviation of the response for all the three hydroxylamines for successive replicate injections ($n = 6$), was typically less than 5%, which supports the fact that surface fouling is not a problem in LC–ED. A comparison of the LC–ED detection limits with the conductivity detector is given in Table 1. The data demonstrate that ED does offer better sensitivity. It must be mentioned here that once the electrode surface has been pretreated, it can be used for over 10–12 h with no significant decrease in the signals for all the three hydroxylamines. Any signal change observed was within the 5% relative standard deviation and this observation is consistent with the work that has been previously reported [10].

One of our main interests in ED was prompted by the lack of selectivity associated with conductivity detection. The conductivity detector does not have the ability to quantify hydroxylamine accurately in the presence of excess sodium ions as evidenced by the shoulder in Fig. 1. The shoulder from the sodium ions is noticeably absent in the chromatograms obtained with the electrochemical detector (Fig. 3) which should not be surprising because of the electroinactive nature of sodium ions at the applied potential of 0.6 V *vs.* Ag/AgCl. In fact, we have observed in our laboratories that hydroxylamine can be quantified in the presence of excess of sodium ions using ED.

4. Conclusions

Our work has demonstrated that hydroxylamine and N-substituted hydroxylamines can be separated and analyzed by IC with both conductivity and ED schemes. Post-column addition of a strong base was deemed necessary to facilitate ED. The glassy carbon electrode must be electrochemically pretreated to obtain an LC–ED response. ED exhibits better selectivity and sensitivity than the conductivity detector although LC–ED requires the use of post-column reagent addition.

5. Acknowledgements

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