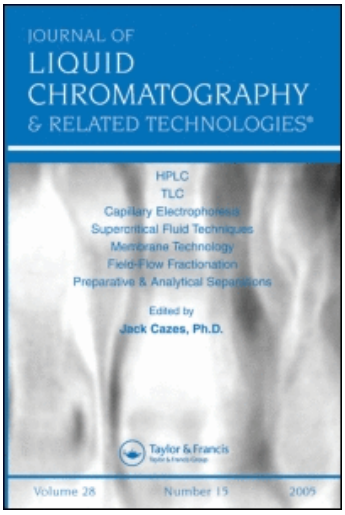

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THE APPLICATION OF A METALLIC COPPER ELECTRODE TO POTENTIOMETRIC DETECTION OF REDUCING SPECIES IN ION CHROMATOGRAPHY

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ABSTRACT

The use of a metallic copper electrode for potentiometric detection of reducing species such as ascorbic acid, hydrazine and hydroxylamine is reported. Detection of these species is based on the increase in electrode potential resulting from reduction of cupric ions to cuprous ions at the electrode surface, with the potential increase occurring because of the higher standard potential of Cu^{+}/Cu^{o} in comparison to that of Cu^{2+}/Cu^{o}. Ascorbic acid and its oxidation product, dehydroascorbic acid, may be determined using a Vydac 302 IC anion-exchange column with 20 mM sodium tartrate at pH 6.2 as eluent, whereas hydrazine and hydroxylamine may be determined using a Nucleosil 10SA cation-exchange column with 2 mM citrate and 2 mM ethylenediamine at pH 4.5 as eluent. Calibration plots showed a linear relationship between electrode potential and low concentrations of injected species, while at higher concentrations, a logarithmic relationship was followed. Detection limits were in the range 2-10 nmol for the species examined.

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INTRODUCTION

Potentiometry is the electrochemical technique which has been least applied to detection in high performance liquid chromatography, ion chromatography or flow injection analysis. This may be attributed to the facts that potentiometric detection methods are often slow in their response and may be too selective for general chromatographic use. For some time, we have been studying the utility of a metallic copper electrode as a detector for the above mentioned techniques.

Our previous studies [1-4] have shown that detection with this electrode can be based on three different mechanisms which produce a change in the electrode potential. Detection of copper complexing species such as amino acids [1], other organic acids [2] and many inorganic anions [3] utilizes a direct dependence of the copper electrode potential on the concentration of these species. Alternatively, detection of strongly oxidising species such as chlorate, bromate and iodate [3] is based on oxidation of the metallic copper electrode by eluted solutes. Finally, indirect detection of species which do not complex copper, such as certain organic acids [2], inorganic anions [3] or metal ions [4], is based on creation of a vacancy in an eluent stream which contains a copper complexing ligand.

The present study involved an investigation of a reductive mode of electrode function, wherein eluted solutes participated in reduction reactions at the surface of the metallic copper electrode. Test species selected for this study were ascorbic acid, hydrazine and hydroxylamine, all of which are strong reductants.

EXPERIMENTAL

The HPLC equipment and instrumentation for potentiometric detection was the same as previously used [2,3]. The columns used were a Nucleosil (Macherey-Nagel, Dueren, West Germany) Type 10 SA (300 x 4.0 mm I.D.), a Zorbax NH_2 (Du Pont, Wilmington, DE, U.S.A.) (250 x 4.6 mm I.D.) and a Vydac (Separations Group, Hesperia, CA, U.S.A.) Type 302 IC 4.6 (250 x 4.6 mm I.D.).

The reagents used were: L-ascorbic acid and (+)-tartaric acid (Tart) from Ajax Chemicals (Sydney, Australia), dehydroascorbic acid from Pfalz and Bauer

(Stamford, CT, U.S.A.), hydrazine sulphate and ethylenediamine (En) from BDH (Sydney, Australia) and citric acid and hydroxylammonium sulphate from Merck (Darmstadt, F.R.G.). All solutions were freshly prepared in distilled and deionised water. Eluents were filtered through a 0.45 um membrane filter and degassed before use. The pH of each eluent was adjusted with sodium hydroxide or nitric acid.

RESULTS AND DISCUSSION

Detection of ascorbic acid

Ascorbic acid is a weak acid (pKa_1 = 4.04, with dissociation of the second proton occurring on a very strongly alkaline media [5], a moderate reducing agent (E^o = 0.39 V [6]) whose reductive strength depends on acidity and a weakly commplexing ligand towards copper(II) ions [5]. It reduces cupric to cuprous ions and this has been utilized for titrimetric determination of copper(II) in the presence of reagents which complex or precipitate cuprous ions, such as thiocyanate, bromide or chloride [7]. It has also been reported that cupric ions strongly catalyse autooxidation of ascorbic acid [8].

Detection methods for ascorbic acid in liquid chromatography have included UV absorption, both with and without pre-column derivatisation, e.g. [9], chemiluminescence [10] and amperometry with a graphite paste electrode [11] or an alumina modified glassy carbon electrode [12]. Because both ascorbic acid and its oxidation product, dehydroascorbic acid (pKa_1 = 9.1 [13]), are weak acids and their redox process involves hydrogen ions, the equilibrium constant for reduction of cupric ions by ascorbic acid will depend significantly on pH. In view of this, the potential change of a metallic copper electrode will also be influenced by pH.

Detection of ascorbic acid was studied using an amino column with an eluent containing tartrate, which weakly complexes cupric ions. In the studied pH range of 3.5-5.5, from 19-98% of total ascorbic acid exists in the anionic form (HL^-). In order to ascertain whether the peak recorded for ascorbic acid resulted from an indirect mechanism caused by a vacancy of tartrate in the eluent concomitant with elution of ascorbic acid, nitrate was injected under the same conditions as used for ascorbic acid. Nitrate does not form a copper complex and is detected by the above indirect or "vacancy" method, with the

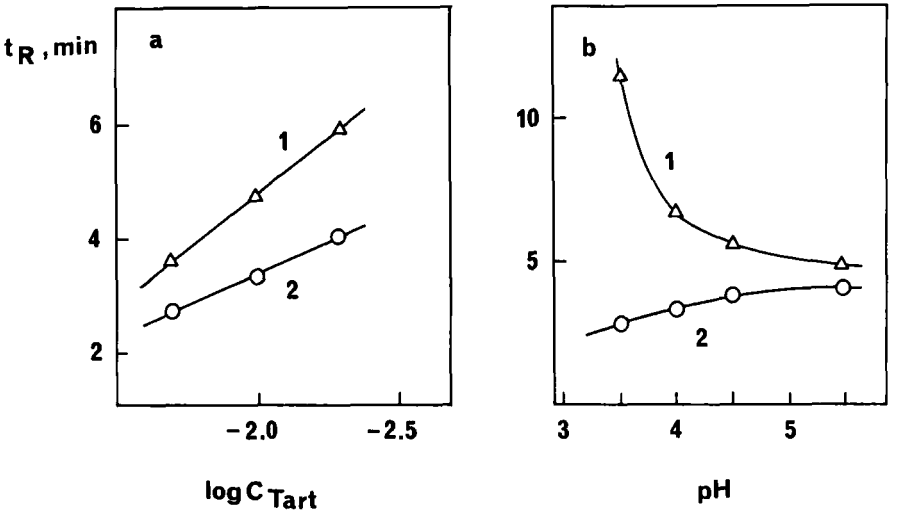

Fig. 1. Relationships between retention times and total tartrate concentration at pH 4.5 (a), or pH at 5 mM tartrate concentration (b) for nitrate (1) and ascorbic acid (2). Column, Zorbax NH_2; flow rate, 2 mL/min. 1 μmol of each species was injected.

observed electrode change resulting from an increase in the concentration of copper ions at the electrode surface caused by the decreased tartrate concentration accompanying elution of nitrate. As such, the peak direction for nitrate should be opposite to that produced if ascorbic acid reduces cupric ions to metallic copper at the electrode surface, causing a decrease in the cupric ion concentration. This relationship between the peak directions of ascorbic acid and nitrate was not observed in practice and both solutes exhibited a positive peak. This can be explained in terms of the higher standard potential for the couple Cu^+/Cu^o (E^o = 0.52 V) compared to that of Cu^{2+}/Cu^o = 0.34 V). when ascorbic acid causes partial reduction of cupric ions to cuprous ions, an increase in the electrode potential can be expected to occur, leading to formation of a positive peak.

When the pH was held constant, decreasing the total concentration of tartrate in the eluent caused increases in retention time for both nitrate and ascorbic acid (Fig. 1(a)). On the other hand, when pH was varied using a

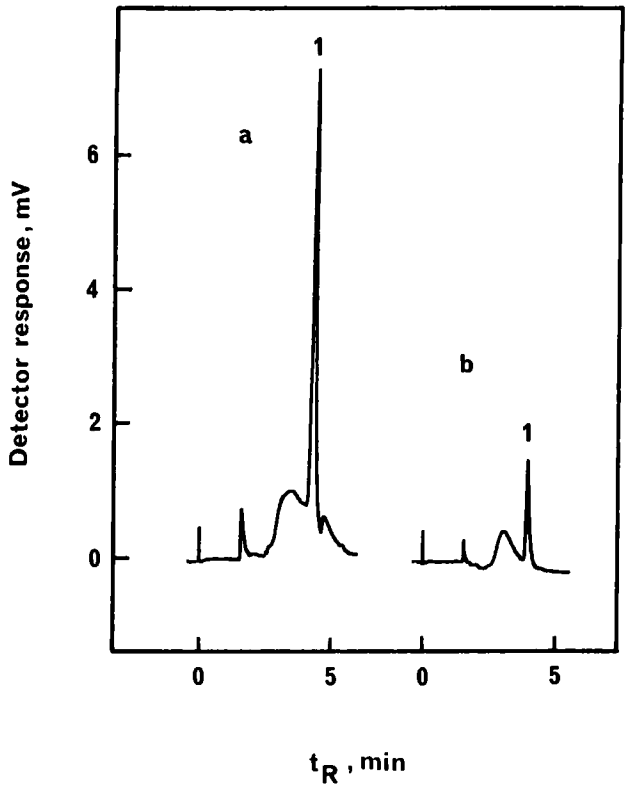

Fig. 2. Peaks recorded for ascorbic acid (1) using a Zorbax NH_2 column and 5 mM sodium tartrate at pH 4.0 as eluent. Injected amounts: 0.5 μmol (a) and 25 nmol (b). Flow rate: 2 mL/min.

constant total tartrate concentration, both solutes behaved differently (Fig. 1 (b)). A decreease in pH increases the fraction of protonated tartrate in the eluent and hence should cause an increase in retention time; this was observed for nitrate. In contrast, ascorbic acid showed a slight decrease in retention which can be attributed to a decrease in fraction of total ascorbic acid present as anionic ascorbate. It is noteworthy that the peak for ascorbate was accompanied by a broad "system peak" (Fig. 2). A similar peak has been observed by other authors [14-16] in ion-exchange chromatography.

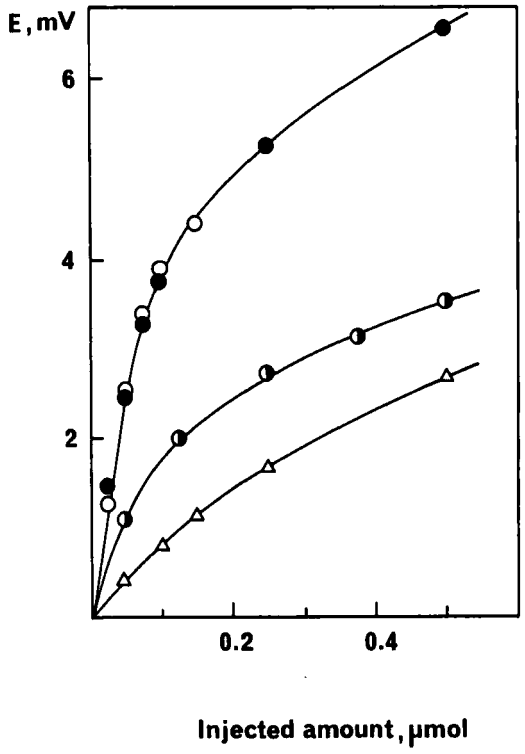

Fig. 3. Calibration plots obtained for nitrate at pH 4.0 (▲) and ascorbic acid at pH 3.5 (o), 4.0 (●) and 4.6 (◑) with a Zorbax NH_2 column using 5 mM sodium tartrate as eluent. Flow rate: 2 mL/min.

Detector sensitivity was also strongly dependent on eluent pH and over the pH range 3.5-5.5, the sensitivity of ascorbic acid detection increased with decreasing pH (Fig. 3). Calibration data for nitrate shown on the same Figure indicate that electrode potential changes for indirect detection of nitrate are much smaller than those for ascorbic acid. For the range of injected amounts of ascorbic acid tested, the calibration relationship was non-linear except for small injected amounts (up to 50 nmol). A semilogarithmic calibration plot was linear for larger injected amounts of ascorbic acid. Taking into account a random noise level of about 0.06 mV, the detection limit for ascorbic acid calculated for potential change of three times the random noise level was 2 nmol of ascorbic acid in the injected sample, which corresponds to injection of 1 ml of 0.4 ppm ascorbic acid.

Dehydroascorbic acid does not exhibit the strong reducing properties of ascorbic acid and there are no data available regarding complex formation between dehydroascorbic acid and metal ions [17]. In addition, previous studies on the reaction between ascorbic acid and cupric ions have not considered the formation of such a complex [18]. We have observed a reproducible negative peak for dehydroascorbic acid when an ion-exchange column was used (Fig. 4), which strongly suggests that this solute complexes cupric ions. Fig. 4 also contains a small peak corresponding to oxalate which is a by product of oxidation of dehydroascorbic acid [19]. We have previously shown that oxalate may be sensitively detected with the metallic copper electrode [2].

Detection of hydrazine and hydroxylamine

Hydrazine and hydroxylamine are much stronger reductants than ascorbic acid ($E^{o}_{N_2H_4,N_2}$ = -0.25 V; $E^{o}_{NH_2OH,N_2}$ = -1.94V [20]). Both compounds form complexes with cupric ions, however hydrazine forms the stronger complex (log β_1 = 6.67 [5]). In the reaction of hydroxylamine with cupric ions, the final result is the oxidation of hydroxylamine, however it has been postulated that temporary complexation reactions are also involved to some extent [21]. With both hydrazine and hydroxylamine, the participation of hydrogen ions in their redox equilibria means that the sensitivy of detection of these species should be strongly dependent on pH.

The determination of hydrazine is of practical importance and when HPLC separation has been used, colorimetrioc detection with post column derivatisation [20] and amperometric detection [23] have been employed. Taking into consideration the protolytic properties of hydrazine and hydroxylamine ($pKa_{N_2H_5^+}$ = 8.06; $pKa_{NH_3OH^+}$ = 6.00 [24]), both compounds would be protonated at pH values less than 5 and under these conditions, should be suited to cation-exchange separation.

A Nucleosil 10SA column was applied to this separation using citric acid and ethylenediamilne (En) as eluent. In this mixture, the ethylenediammonium cation serves as the ion-exchange competing ion, while citrate acts as a buffering agent and provides the weak complexation of cupric ions necessary to produce a stable baseline with the metallic copper electrode [25]. The retention time of the hydroxylammonium cation showed the expected decrease with increasing concentrations of En at a fixed pH, whereas only very small changes in retention

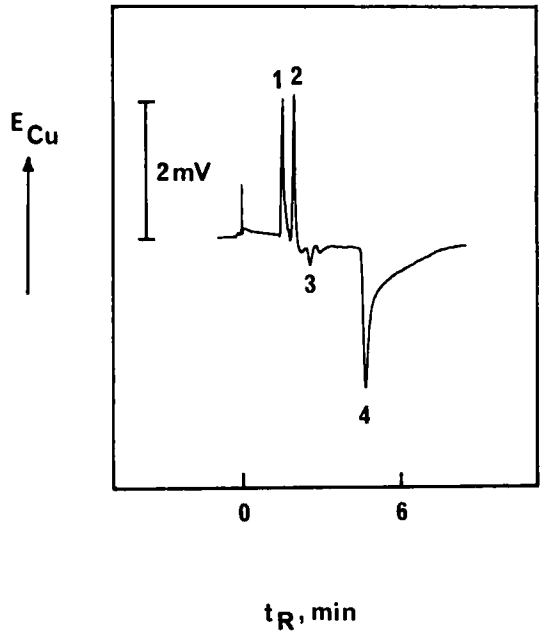

Fig. 4. Chromatogram obtained for a mixture containing 1 μmol each of ascorbic acid (2) and dehydroascorbic acid (4) using a Vydac 302 IC column with 20 mM sodium tartrate at pH 6.2 as eluent. Flow-rate: 2 mL/min. Other peaks: 1-void, 2-oxalate.

were observed for hydrazonium ion (Fig. 5(a)). In addition, hydrazonium ion coeluted with a system peak which showed constant retention at a given flow rate, regardless of changes in eluent composition. Increased concentrations of citrate in the eluent led to a decrease in the retention time for hydrazine, yet had no effect on the retention time of hydroxylamine. These observations suggest a basic difference in the retention mechanisms applicable to these two species and this was further indicated by differences in the effect of pH on retention time (Fig. 5(b)). Typical chromatograms obtained at two different En concentrations are shown in Fig. 6.

Further study is required to resolve the anomalous effects of several parameters on the retention times of ascorbate and hydrazonium in ion exchange separations. One explanation in the case of hydrazonium ion is that the column

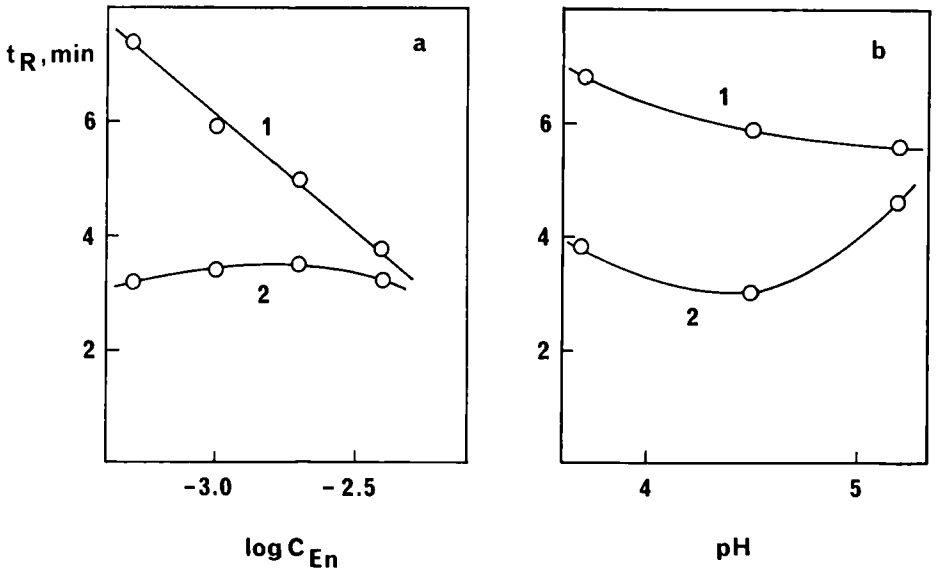

Fig. 5. Relationships between retention time and total ethylenediamine concentration (a) or pH (b) obtained for hydroxylammonium (1) and hydrazonium (2) ions using a Nucleosil 10SA column at a flow rate of 2 mL/min. Other experimental conditions: a - 2mM citrate at pH 4.5, b - 2 mM citrate and 1 mM En.

employed had been previously used for separations of metal ions which may have modified the stationary phase surface. This factor could exert considerable influence on retention in view of the disparity between complexation reactions of hydrazine and hydroxylamine. When the column was washed with triethylenetetramine to remove adsorbed metal ions, broader peaks were obtained for hydrazonium and hydroxylammonium cations at the same retention times previously observed. When the concentration of En was changed, the peaks for both solutes showed the same shift in retention time.

Separation of hydrazine and hydroxylamine was possible at pH values less than 5, En concentrations less than 2 mM and at a citrate concentration of 2 mM. Under these conditions however, severe overlap of hydrazonioum with the system peak occurred. This can be improved by increasing the citrate concentration in the eluent, but this results in a reduction in detection sensitivity caused by

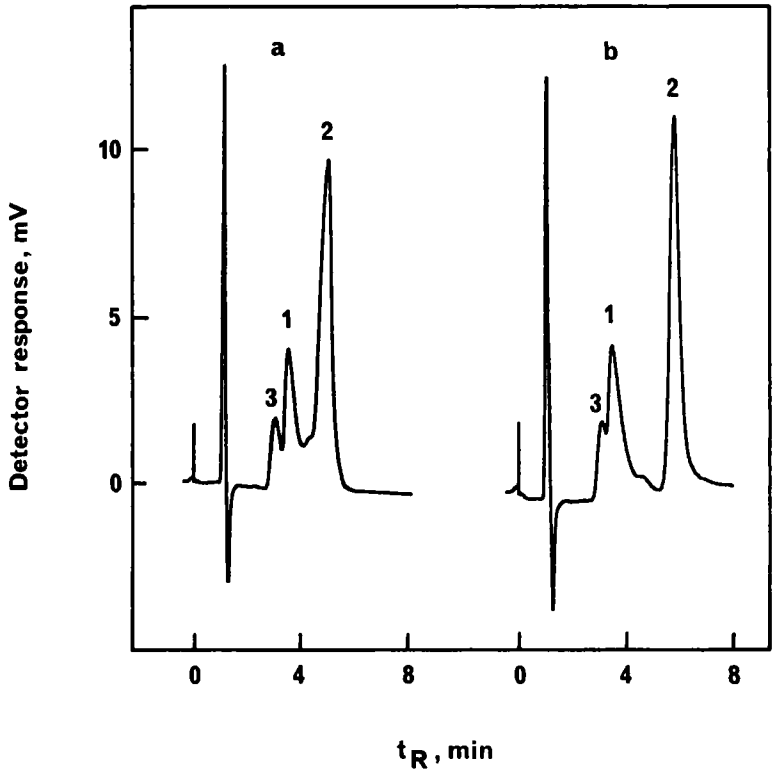

Fig. 6. Chromatograms obtained for a mixture containing 0.25 μmole each of hydrazine sulphate and hydroxylamine sulphate using a Nucleosil 10SA column with 2 mM citrate and 2 mM (a) or 1 mM (b) ethylenediamine at pH 4.5 as eluent. Flow rate: 2 mL/min. Injected volume: 50 μL. Peak identities: 1-hydrazonium ion, 2-hydroxylammonium ion, 3-system peak.

stronger complexation of cupric ions which renders their reduction more difficult.

The sensitivity of detection showed the expected dependence on pH, with an increase in eluent pH from 3.7 to 5.2 producing a two-fold increase in the sensitivity for hydrazonium and a ten-fold increase for hydroxylammonium ions. Although this trend could be expected to continue at higher pH values, further studies were not undertaken because of poor separation of the species (as

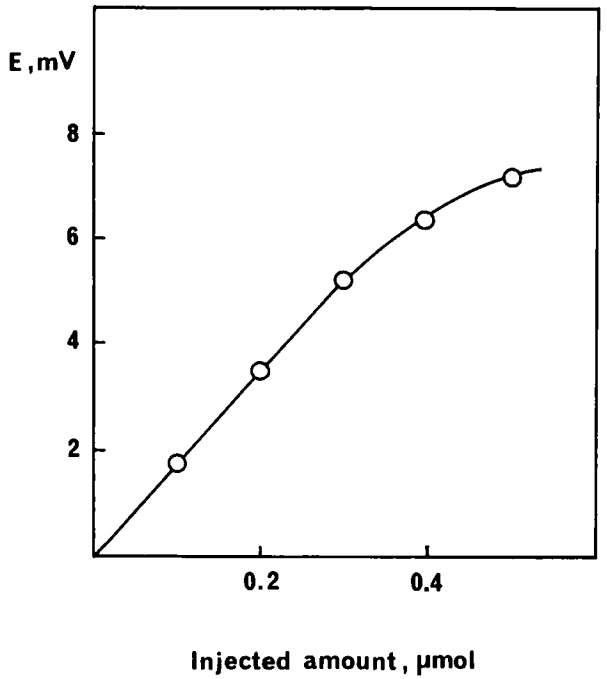

Fig. 7. Calibration plot obtained for hydroxylammonium ion using a Nucleosil 10SA column with 2 mM citrate and 2 mM En at pH 4.5 as eluent. Flow rate: 2 mL/min.

indicated by Fig. 5(b)) and a decrease in the degree of protonation of hydroxylamine above pH 5.2. A calibration plot for hydroxylammonium ion is shown in Fig. 7 and the detection limit calculated in the same manner used previously for ascorbate was 10 nmol, corresponding to a 1 ml injection of 0.3 ppm hydroxylamine. A similar detection limit can be expected for hydrazine, although quantitative interpretation of data for this solute is complicated by overlap with the system peak.

CONCLUSIONS

The aim of this study was to investigate the applicability of potentiometric detection with a metallic copper electrode to reducing species. The results

obtained confirm the possibility of this application, however fuller theoretical interpretation of observed potential changes is complex in that it involves simultaneous redox, acid-base and complexation equilibria. Under the conditions used, detection limits of the order of a few nanomoles were obtained for the reducing species studied.

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