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Analysis of small amines in pharmaceuticals by capillary ion electrophoresis with conductivity detection

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Abstract

Capillary ion electrophoresis (CIE) with direct conductivity detection is a simple, fast, and versatile method for analysis of ions. In this study CIE with conductivity detection was used to analyze for hydroxylamine which is often used in the synthesis of pharmaceutical intermediates and drug substances. The conductivity response of hydroxylamine was investigated by using different buffer types and buffer concentrations. Other small amines were also studied with this method and were correlated with the molecular weight of the amines. The response of hydroxylamine increased significantly with MES–glycylglycine buffer as compared to MES–histidine buffer (LOD from 10 to 1 ppm). The detection of trace level of hydroxylamine in a drug substance proved to be possible with this method. The developed method was also tested for the linearity range, reproducibility, and selectivity with several small amines. The method developed in this study was demonstrated as a sensitive and reliable method for detection and quantification of small amines in pharmaceutical substances. © 2000. Dupont Pharmaceutical Company. Published by Elsevier Science B.V. All right reserved

Keywords: Capillary ion electrophoresis; Hydroxylamine; Small amines; Pharmaceuticals; Response

1. Introduction

Hydroxylamine is used routinely as a reducing agent in photography, synthetic and analytical chemistry. It is often used as a raw material for synthesis of pharmaceutical intermediates and final drug substances. Quantitative determination of low levels of hydroxylamine is very important because it is moderately toxic [1].

Existing methods for analyzing low level of hydroxylamine include gas chromatography with pre-column derivatization [2], HPLC with UV (210 nm) detection [3], and recently ion chromatography with conductivity and amperometric detection [4,5]. However, the recent development of various techniques of capillary electrophoresis (CE) has also brought about its application to ions which is rapidly gaining practical importance. Many capillary ion electrophoresis (CIE) methods for analysis of inorganic ions and some small amines were developed in recent years [6–9].

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The CIE technique with direct conductivity detection explored in this study is a simple, universal and sensitive technique. It separates and detects ions based on their ionic mobilities. Detected signals correspond to time-dependent conductivity changes, whereby the signal reflects the conductivity difference between the sample ion and the background electrolyte. As compared with IC, CIE offers more efficient separation, shorter analysis time, greater versatility and much lower mobile phase consumption. The great versatility offered by CIE provides analysis of both anions and cations within the same run, where as a specific column must be dedicated to analysis of either anions or cations in IC. The potential limitations of this technique are likely its reproducibility of migration time and accuracy for quantification, since the conductivity response by CIE is directly related to mobility of the ion and the peak symmetry. The peak symmetry by CIE generally depends on the mobility of the co-ion in electrolyte. The faster the mobility of the analyte in comparison with the co-ion, the more the analyte fronts. Conversely, the slower the analyte is compared to the co-ion, the more the analyte tails [10]. The advantages and disadvantages of these two techniques (IC vs. CIE) have been reviewed in many monographs and reviews [11–13].

The aim of this study was to: (1) develop an efficient and sensitive CIE method for analysis of hydroxylamine in a drug substance; (2) to investigate the ruggedness and reproducibility of the method, including linearity, reproducibility for trace level detection; (3) to extend the study to other small amines.

2. Experimental

2.1. Equipment

The separations were performed on a Crystal CE 300 with a Crystal 1000 conductivity detector from Thermo CE (Boston, MA). The detector output was interfaced to a Hewlett Packard Chemstation for LC software program (version A.04.02.) on a Compaq Deskpro computer.

The ConCap™ I fused silica capillaries (50 μm I.D. × 375 μm O.D. × 60 cm) were purchased from Thermo CE and used in this study. The ConTip I conductivity sensor for the detection consisted of a platinum electrode. The capillary end was precisely connected to the conductivity sensor by a stainless steel connector, which was described in detail by Haber et. al [14].

2.2. CIE conditions

The temperature for the capillary and conductivity cell was set at 35°C. The voltage through the capillary was set at 25 kV. The detector cell voltage was adjusted with an auto cell voltage setting to obtain a stable background. Pressure injection was used for sample injection at 25 mbar for 12 s. The capillaries were preconditioned before use and whenever a new buffer was used. The conditioning of the capillary which exposes a fresh layer of silica is the key for maintaining constant migration times in CE. Preconditioning procedure consists of a three-step rinse program, which includes a one-minute rinse with D.I. water purified by a Milli-Q system (Millpore Corp., Bedford, MA) at 2000 mbar pressure, a five-minute rinse with 1 M NaOH at 2000 mbar pressure, and a 1-min final rinse of the D.I. water. The capillary was then rinsed with running buffer before the actual run. Conditioning vials were filled at different heights in order to avoid carry over of the conditioning solution into the run buffer. The buffer vial was filled with the run buffer a few millimeters lower than the neck of the vial, the water was filled to the neck of the vial, and the 1 M NaOH was filled one half to three quarters of the vial. The specific height of the different solutions prevents sample contamination. A post-conditioning of the capillary was done at the end of the day to flush out any remaining sample which may undergo interactions with capillary inner wall from a prolonged exposure. The post-conditioning program consists of a 1.5-min rinse of the running buffer at 2000 mbar.

If the capillary was not going to be used for more than one day, the capillary was rinsed with water, 1 M NaOH, and then water for storage. Also the electrolyte bottle in the detector com-

partment was filled with D.I. water and a conductivity cell rinse was performed until the background conductance displayed 1 µs or less. The electrolyte was filtered with a MilliCup™-HV 0.45 µm filter unit from Millipore Corp. (Bedford, MA). Samples were filtered with Millex®-LCR filter units (0.5 µm × 25 mm ID) which were purchased from Millipore Corp. (Bedford, MA).

2.3. Reagents and sample preparation

Glycylglycine was purchased from Aldrich Chemicals (Milwaukee, WI). MES(2-[*N*-Morpholino]ethanesulfonic acid), and histidine were purchased from Sigma Chemical Co. (St Louis, MO). Hydroxylamine hydrochloride, hydrazine hydrochloride, methylamine, ethylamine hydrochloride, diethylamine hydrochloride, triethylamine hydrochloride, propylamine hydrochloride, ethanolamine hydrochloride, diethanolamine hydrochloride, triethanolamine hydrochloride, and tetrabutyl ammonium chloride were purchased from Aldrich (Milwaukee, WI).

All the buffer solutions were prepared daily in plastic flasks and filtered with the 0.45 µm MilliCup-HV filter units from Millipore Corp(Bedford, MA). All the amine samples were also prepared with D.I. water in plastic vessels, because some amines were found to be absorbed onto the glass surface.

Table 1
The effect of concentration of MES–histidine buffer on the response of hydroxylamine[a]

Buffer concentration (MES–histidine) (mM)	Conductivity response (µs/ppm)
10–10	282.9
30–30	22.7
60–60	13.4

[a] Capillary: fused silica, 50 µm I.D. × 60 cm. Voltage: 25 kV. Temperature: 35°C. Detection: direct conductivity. Injection: 25 mbar, 12 s.

3. Results and discussion

3.1. Effect of buffer concentration on sensitivity of hydroxylamine

The initial run buffer for the analysis of hydroxylamine consisted of 30 mM MES–30 mM histidine [8]. However, the method did not give desired sensitivity for hydroxylamine. Therefore, the concentration of the MES/histidine buffer was adjusted to increase the sensitivity. Table 1 shows the conductivity responses of 25 ppm hydroxylamine obtained with MES–histidine buffer at concentrations of 10–10 mM, 30–30 mM, and 60–60 mM. The response of hydroxylamine increased significantly as the buffer concentration decreased, especially from 30–30 to 10–10 mM. This result was in agreement with the expectation because histidine is a cationic buffer in the neutral pH range. Therefore, the higher the MES–histidine concentration, the higher the conductivity background, and hence the lower the analyte's response.

The detection limit of hydroxylamine using 10 mM MES–10 mM histidine was 10 ppm in solution. However, this level of sensitivity was not high enough for our purpose of detecting residual hydroxylamine in a drug substance. Therefore, the buffer was optimized to increase the sensitivity of hydroxylamine determination by CIE.

3.2. Response of hydroxylamine using MES/glycylglycine versus MES/histidine buffers

Glycylglycine was chosen to replace histidine in the run buffer since glycylglycine is a zwitterion peptide ($pK_2 = 8.1$) with a low conductivity at neutral pH. The conductivity background of 30 mM MES–30 mM glycylglycine decreased to 1.5 µS compared with 18 µS with 30 mM MES–30 mM histidine buffer. This buffer (30 mM MES–30 mM glycylglycine) was used to measure the responses of hydroxylamine and several other amines. The results were compared with 30 mM MES–30 mM histidine buffer. As shown in Fig. 1, the responses of hydroxylamine, hydrazine, ethanolamine, 1*S*,2*R*-ephedrine using MES–glycylglycine buffer were significantly higher than

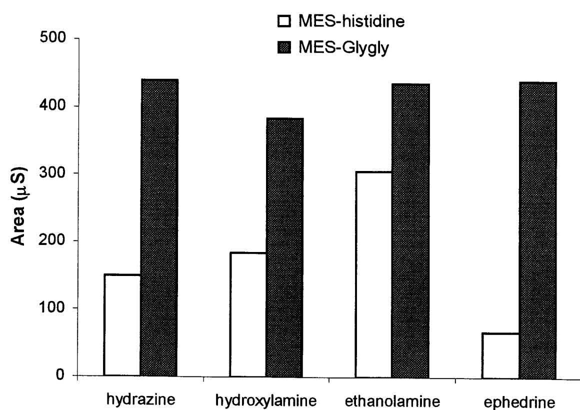

Fig. 1. Comparison of responses of amines using 30 mM MES–30 mM histidine as eletrolyte vs. 30 mM MES–30 mM glycylglycine.

those using MES–histidine buffer. The detection limit for hydroxylamine using 30 mM MES–30 mM glycylglycine buffer reached 1 ppm as compared with 10 ppm with 30 mM MES–30 mM histidine buffer.

The above MES–glycylglycine buffer also gave stable baselines and suitable migration times (2–4 min) for the amines tested in this study.

3.3. *Effect of molecular weight of small amines on sensitivity*

A few small amines (ammonium, hydroxylamine, hydrazine, methylamine, propylamine, benzylamine, tetrabutylammonium, ethanolamine, diethanolamine, triethanolamine) were analyzed by CIE using 30 mM MES–30 mM glycylglycine as buffer to explore the relationship between conductivity response and molecular weight of the amines, since the response by CIE is related to an analyte's conductivity which in turn depends on the analyte's charge-to-mass or charge-to-size ratio. Our results indicated that conductivity response by CIE not only related to the analyte's molecular weight, but also to the analyte's structure. Among the amines with different functional groups, there was no correlation observed between the response and molecular weight. How-

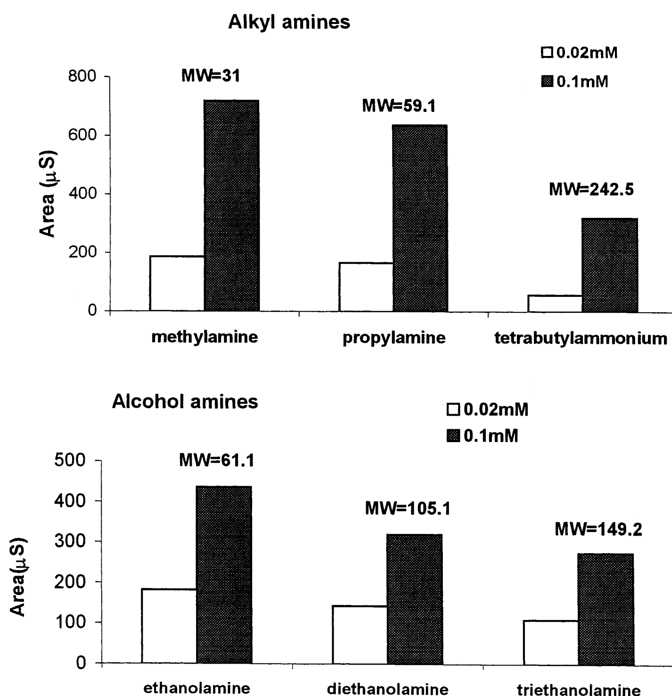

Fig. 2. The effect of molecular weight of amines at different concentrations (0.02 and 0.1 mM) on responses by CIE.

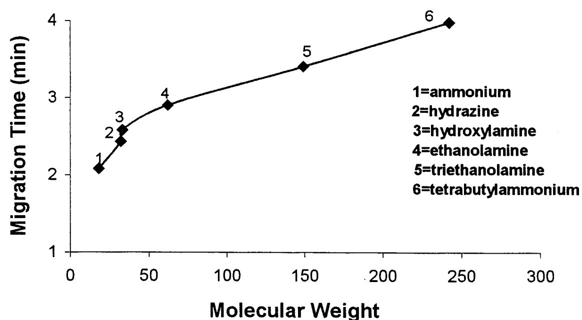

Fig. 3. The effect of molecular weight of amines on their migration times by CIE.

ever, as shown in Fig. 2, the responses of amines within the same group (alkyl or alcohol amines) decreased as the molecular weight increased. The migration time also increased with the increased molecular weight of the analytes as expected (Fig. 3).

3.4. Linearity

The linearity between the response and concentration of amines was investigated for several amines in the concentration range of 0.05–0.5 mM (Fig. 4). All of the amines showed good linearity ($R^2 > 0.99$) using 30 mM MES–30 mM glycylglycine buffer.

Table 2
Separation of alkyl-, alcohol-, and other small amines by CIE[a]

	Migration time (min)
Alkyl amines	
Methylamine	2.16
Ethylamine	2.43
Propylamine	2.59
Butylamine	2.68
Benzylamine	2.79
Alcohol amines	
Ethanolamine	2.50
Diethanolamine	2.74
Triethanolamine	2.89
Other amines	
Hydrazine	2.20
Hydroxylamine	2.38
Norephedrine	2.98
Ephedrine	3.01

[a] Capillary: fused silica, 50 μm I.D. × 60 cm. Buffer: 30 mM MES–3 0mM glycylglycine. Voltage: 25 kV. Temperature: 35°C. Detection: direct conductivity. Injection: 25 mbar, 12 s.

3.5. Reproducibility

The reproducibility of this method was also investigated with hydroxylamine as analyte. Six injections of 1 ppm hydroxylamine gave a RSD of 2.1% and an RSD of 0.33% for 25 ppm hydroxylamine. It was found that a lower RSD was obtained by replenishing the run buffer between the runs. RSD decreased to 1.6% for six injections

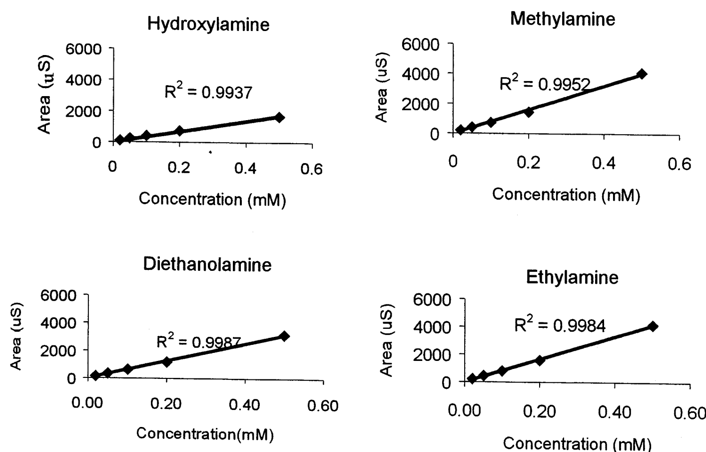

Fig. 4. The linearity of amine responses by CIE with 30 mM MES–30 mM glycylglycine buffer.

of 1 ppm hydroxylamine by replenishing the run buffer after each two injections. The buffer replenishment presumably decreased ion depletion in the capillary.

3.6. Selectivity of amines

To test the applicability of the method, a separation of a mixture consisted of five alkyl amines,

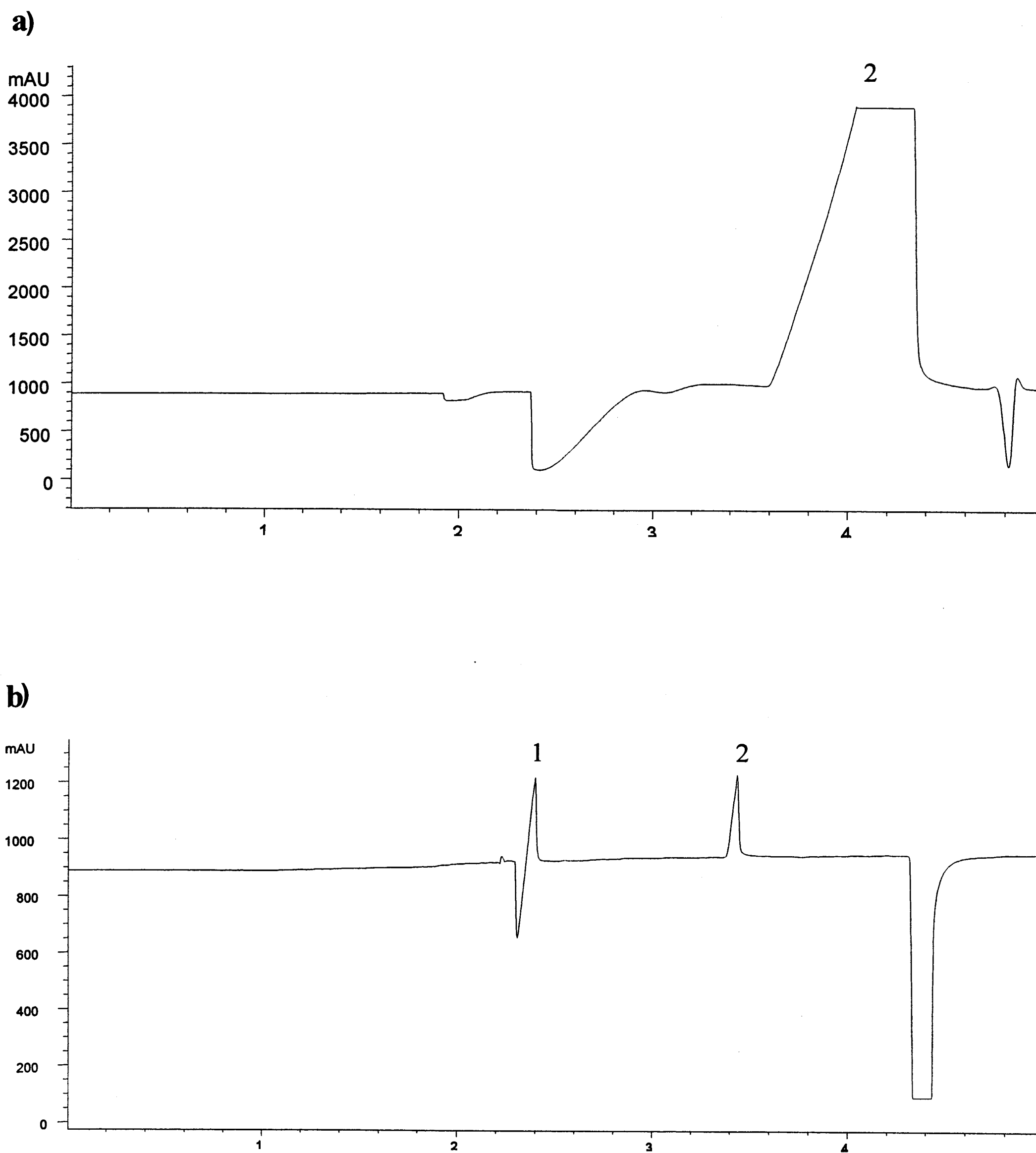

Fig. 5. (a) Analysis of residual hydroxylamine in drug substance before a Sep-Pak C_{18} extraction. (b) Analysis of residual hydroxylamine in drug substance after a Sep-Pak C_{18} extraction.

three alcohol amines, and four other small amines was performed by CIE using 30 mM MES–30 mM glycylglycine as buffer. As shown in Table 2, all of the amines within the same group were baseline resolved in three minutes with this method.

3.7. Analysis of hydroxylamines in drug substance

Hydroxylamine is a common reagent used in pharmaceutical synthesis. The CIE method developed in this study was employed to analyze residual hydroxylamine in an active drug substance. However, hydroxylamine coeluted under the large peak of the drug substance due to the fact that the drug substance was also an amine. A Sep-Pak C_{18} cartridge was successfully used to extract the drug substance. Fig. 5a shows the detection of residual hydroxylamine in drug substance before a Sep-Pak C_{18} cartridge extraction. Fig. 5b shows the detection of residual hydroxylamine in drug substance after a Sep-Pak C_{18} cartridge extraction.

4. Conclusions

The sensitivity of hydroxylamine determination by CIE increased ten times (LOD from 10 to 1 ppm) by changing the run buffer from MES–histidine to MES–glycylglycine. Sensitivity of hydroxylamine determination by CIE decreased as buffer concentration increased. The results on other small amines showed that the response of those amines also decreased as the molecular weight of the amines increased due to their decreased charge-to-mass ratios and ionic mobilities. The method developed in this study which used 30 mM MES–30 mM glycylglycine buffer proved to be a sensitive and reliable method for quantification of hydroxylamine and other small amines in pharmaceutical drugs with great linearity over a wide concentration range, reproducible peak area and migration time, and acceptable selectivity for separation of small amines.

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