Separation of Morpholine, *N*-Methylmorpholine and *N*-Methylmorpholine-*N*-oxide by Indirect UV Absorption Capillary Electrophoresis[†]

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A UV absorption bipolar capillary electrophoresis system was built on the basis of on-column detection. As an application of capillary electrophoresis, analysis of inorganic anions such as Cl^- , SO_4^{2-} and SO_3^- is demonstrated. The separation of morpholine (M), *N*-methylmorpholine (NMM) and *N*-methylmorpholine-*N*-oxide (NMMO) using indirect UV absorption technique is discussed as well. Chromate buffer of 10 mM was used to provide absorption of the mobile phase additive in indirect detection and precise pH control of the carrier electrolyte. The effect of buffer pH on the separation of M, NMM and NMMO is illustrated.

Keywords Indirect absorption, UV-VIS capillary electrophoresis

Recently capillary zone electrophoresis (CZE) has become important in the field of clinical and biological chemistry due to its excellent separation efficiency, detection capabilities, relatively easy sample handling requirements. The application of CZE is now extending to environmental analyses not only for hazardous organic compounds but also for inorganic ions.

Many detection methods can be applied to capillary electrophoresis, such as UV-VIS absorption, fluorescence, electrochemical detection, mass spectrometry, and so on. Among them, UV-VIS absorption detector is widely used due to its diversity and flexibility for the detection of various kinds of samples. Although most organic and biological materials strongly absorb ultraviolet and/or visible light, so they can be easily detected in CZE, some analytes such as inorganic cations and anions do not absorb at all or only very weakly absorb the light in the range. Thus an indirect UV absorption technique, in which the analyte displaces a mobile phase additive in the eluted band, has been used successfully.¹⁻⁷ The displacement causes a decrease in the signal, because the concentration of the mobile-phase additive is lower in the eluted bands when compared with its steady-state concentration.

Indirect absorption can apply not only to inorganic ions but also to low molecular weight organics, for example, morpholine (M), *N*-methylmorpholine (NMM) and *N*-methylmorpholine-*N*-oxide (NMMO). Among these, NMM and M are major degradation products formed from NMMO which is used for the production of cellulose fibers. It is known that those products either

can deteriorate the technical properties of the spinning baths with respect to the quality of the fibers produced or can affect the purity of NMMO. The separation and detection of NMM and M in the presence of an excess of NMMO has been described with high-performance liquid chromatography (HPLC)⁸ and capillary isotachopheresis (ITP) coupled with a conductivity detector.⁹ Both methods, however, require relatively complicated procedures including the application of two chromatography systems or two electrolyte systems.

In this work, a UV-VIS absorption bipolar capillary electrophoresis system was built on the basis of on-column detection. For the application study of CZE, analysis of inorganic anions, such as Cl^- , SO_4^{2-} , and NO_3^- are demonstrated. The separation of M, NMM and NMMO using this indirect UV absorption technique is discussed as well.

Experimental

Apparatus

The laboratory-made capillary electrophoresis system with on-column UV-VIS detector was constructed as shown in Fig. 1.

A high voltage power supply (0 – 30 kV DC, Spellman High Voltage Electronics Corp., NY, USA) was used to drive the electrophoresis process across the capillary. A fused silica capillary (60 cm×75 μm i.d., Polymicro Technologies, Inc., USA) was used as the separation column. This separation system, except the detector, was constructed in a similar way to that described previously.¹⁰

For the UV detector, light from D₂ lamp (Hamamatsu, Model L613) of 30 W passed through a 255 nm inter-

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Fig. 1 Block diagram of experimental arrangement for UV-VIS capillary electrophoresis. 1, deuterium lamp; 2, interference filter; 3, detection cell; 4, optical fiber; 5, PMT; 6, pre-amplifier; 7, computer; 8, HV power supply.

ference filter (Optical Corp. of America, Model 4TFN, 255 nm center, 12 nm bandpath) and was focused into the capillary column through a 100 μ m diameter pinhole by a ball lens (Yunam Hitech Inc., Korea). The absorption light was collected with a single UV transparent optical fiber (Oriel, Model 77573, 0.8 mm diameter). A home-made PMT power supply adjustable up to -2.0 kV operated a photomultiplier tube (Hamamatsu, Model IP8) with a locally-constructed housing. The output of the photomultiplier tube was fed to the current pre-amplifier (Oriel, Model 70710) and transferred to a data acquisition board. The data acquisition system consisted of an I/O interface with A/D converter and a computer with locally-made graphic software.

Chemicals

Chromate buffers in the range of pH 5 to pH 7 were prepared by titration of potassium chromate (K_2CrO_4 , Aldrich, A.C.S reagent) solution with chromic acid

(CrO_3 , Aldrich, 99.9%) solution. Morpholine (tetrahydro-1,4-oxazine, M) was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). *N*-Methylmorpholine (NMM) and *N*-methylmorpholine-*N*-oxide (NMMO) were obtained from Aldrich (Milwaukee, WI, USA). Stock solutions of Cl^- , SO_4^{2-} , NO_3^- were prepared using sodium chloride, sodium sulfate and sodium nitrate (Duksan Pharmaceutical, Korea).

Results and Discussion

Optimization and analysis of inorganic anions

In order to optimize the voltage and the temperature in the system, current is measured as the voltage is varied as shown in Fig. 2. The figure shows that the chromate buffers higher than 10 mM generated linear Ohm's law curves with the current more than 100 μ A at 20 kV. Such high current results in the increase of the rate of Joule heat production. Because the heat removal capacity of the system is being exceeded whenever the graph shows a positive deviation from linearity, operating on the linear portion of the curve will generally yield the highest number of theoretical plates.

The peaks of Cl^- , SO_4^{2-} , NO_3^- of 100 ppm were separated at pH 8.0 and 20 kV with 5 mM chromate buffer that served as the chromophore to make indirect UV detection possible, as shown in Fig. 3. The linear calibration curves for three anions with the linear regression coefficients of larger than 0.994 were reasonably obtained when the sample was manually injected by hydrodynamic technique. The detection limits at signal-to-noise ratio of 2 for those anions were in the range of about 5 ppm at 25 nl injection.

Fig. 2 Ohm's law plot for chromate buffer of various concentrations at pH 8.0.

Fig. 3 Electropherogram of a model mixture of Cl^- , NO_3^- and SO_4^{2-} , using 5 mM chromate buffer (pH 8.0). Separation voltage 20 kV.

Separation of M, NMM and NMMO

Indirect UV absorption using chromate buffer of 10 mM is used for the separation of M, NMM and NMMO because the absorption coefficients of them are small at 254 nm. Adjustment of the buffer pH is the most important procedure in order to optimize the separation conditions due to the acid and base properties of the analytes as well as those of buffer constituents.

The effect of buffer pH on the separation of 500 ppm M, 500 ppm NMM and 1000 ppm NMMO is illustrated in Fig. 4. The figure shows that the migration span is getting shorter as the pH increases. The system peak is observed at about 182 for pH 7.0, and at about 270 for pH 5.0. The electroosmotic flow (EOF) will be fast if the buffer pH is high, which will lead to the short migration time span. Consequently, the greater separation is observed for the higher-pH buffer in the figure. Changing the pH will impact on the mobility of the analyte which is governed by the charge to size ratio. The net charge on an analyte can be calculated using the Henderson-Hasselbalch equation¹¹ and values are listed in Table 1. Since the pK_a values of M, NMM and NMMO are known to be 8.50, 7.38 and <5, respectively⁹, the pH of the buffer solution was varied between 5.0 and 7.0 by 0.5. At pH 7.0, the calculated charges for M and NMM are 0.969 and 0.706, respectively. At pH 5.0 the calculated charges become 1.000 and 0.996. The greater resolution is therefore expected and observed at the higher-pH buffer since the mobilities are better dis-

Fig. 4 Electropherograms of a model mixture of morpholine (M), *N*-morpholine (NMM) and *N*-morpholine-*N*-oxide (NMMO) using 10 mM chromate buffer at separation voltage of 20 kV. (a) pH 5.0; (b) pH 6.5; (c) pH 7.0.

Table 1 Calculation of net charge for morpholine (M), *N*-methylmorpholine (NMM) and *N*-methylmorpholine-*N*-oxide (NMMO)

	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0
M ^a	1.000	0.999	0.997	0.990	0.969
NMM ^b	0.996	0.987	0.960	0.884	0.706
NMMO ^c	0.500	0.240	0.091	0.031	0.010

a. pK_a of M is 8.50.

b. pK_a of NMM is 7.38.

c. pK_a of NMMO is assumed to be 5.0.

tinguished. The actual separation of M and NMM occurs at pH 6.5, which is shown in Fig. 4(b).

It should also be mentioned that NMMO is not observed when the buffer pH is higher than 5.0. The

peak of NMMO however becomes separated from the system peak at pH 5.0 near pK_a because NMMO is protonated and is migrating electrophoretically. The detection limits for M, NMM and NMMO estimated from the peak height are in the range of 70 ppm. As a result, M, NMM and NMMO can be separated using indirect absorption CZE by adjusting the buffer pH.

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