A Polarographic Cell for the Continuous Monitoring of Column Effluents and Its Application to the Determination of Nitropyridine Derivatives

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(Received August 30, 1974)

The construction of a microdetector, based on the principle of polarography, for liquid chromatography and its application are described. The characteristics of the flow cells with a horizontal dropping mercury electrode (HDME), a vertical dropping mercury electrode (DME), and a mercury plated platinum electrode (Hg–Pt) are compared with each other. The flow cell has been applied to the analysis of a mixture of nitropyridine derivatives, such as 2-amino-3-nitropyridine (A3N), 2-amino-5-nitropyridine (A5N), 2-hydroxy-3-nitropyridine (O3N), 2-hydroxy-5-nitropyridine (O5N), and 2-nitroaminopyridine (NA), by means of a reversed-phase partition column or an ion-exchange column. In quantitative analysis, a standard deviation of better than 3% can be obtained.

Various physicochemical detectors, *e.g.*, refractometric,[1] colorimetric,[2] thermometric,[3] and ionization detector,[4] for the continuous analysis of column effluents are well established, and measurements have been made successfully in recent years. Some of these detectors are excellent in sensitivity and stability, but all add to the complexity and expense of the liquid chromatographic equipment.

On the other hand, the polarographic method was first used for the continuous monitoring of the column effluents by Drake;[5] the technique was later extensively developed with the powerful studies by Kemula.[6,7] Thereafter, various investigations of the apparatus[8–15] and its application[16–23] have been reported by several authors.

The present paper will describe a polarographic cell for the continuous measurement of a flowing solution, the characteristics of the flow cell, and its application to the determination of nitropyridine derivatives separated by either a reversed-phase partition column or an ion-exchange column.

Experimental

Apparatus. *Polarographic Flow Cell*: Three cells for a horizontal dropping mercury electrode (HDME),[24] a vertical dropping mercury electrode (DME), and a mercury plated platinum electrode (Hg–Pt) were designed and compared with each other. As is shown in Fig. 1, the cell for HDME was constructed of a Teflon plate (5) on which an effluent canal (2×2 mm) (1) and depressions for a mercury pool electrode (2), a detector electrode (3), and a thermostated water passageway (4) were caved. The Teflon plate was sandwiched between two plates of transparent acrylics (6). Polyethylene film (7) was used to prevent the swelling of acrylics with an organic solvent contained in the effluent. Though the cell for DME was constructed in the same way as for HDME, the cell was modified for DME. The capillary had the following characteristics in Britton-Robinson's buffer (pH 9.0) containing 0.2 M sodium sulfite at −0.9 V *vs.* SCE, at h=35 cm: m=1.64 mg/s for both electrodes, t=1.51 for HDME and 5.54 s/drop for DME. The cell for Hg–Pt was the same as for HDME. The Hg–Pt was a mercury plated platinum wire (0.3 mm in diameter×0.5 mm in length) which was plated electrolytically in a plating mixture containing 0.05 M mercuric chloride, 0.1 M EDTA, and 0.5 M sodium carbonate.

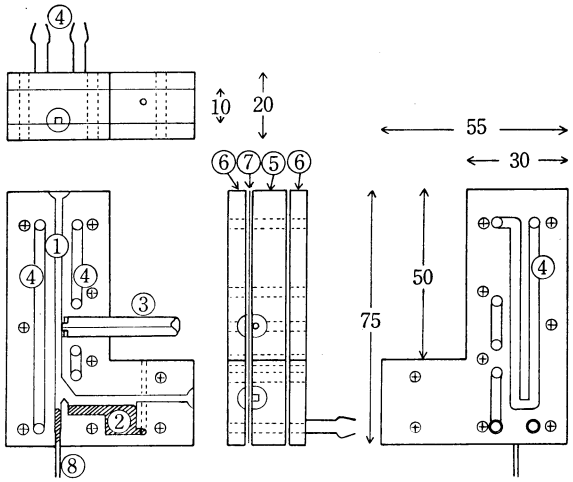

Fig. 1. Polarographic flow cell.
1) Effluent canal, 2) Hg pool anode, 3) HDME, 4) Thermostated water passageway, 5) Teflon, 6) Acrylics, 7) Polyethylene film, 8) Teflon tubing to Hg reservoir; ⊕: Setscrew; Scale in mm.

Voltage-applying and Current-measuring Equipment: The fundamental polarographic and chromato-polarographic measurements were carried out by using a Yanagimoto Automatic Recording Polarograph (PA-101) with a Toa Polyrecorder (EPR-2T). Simple equipment, as is shown in Fig. 2 (G and H), was also used for the practical chromato-polarography.

Chromatographic System: A schematic diagram of the apparatus used for liquid chromatography is shown in Fig. 2. The carrier liquid from an eluate reservoir (A) was conveyed into a saturator (C) by a Shibata micropump (SP-100; 0—120 ml/hr) (B). The saturator, made of glass (1 cm i.d. ×15 cm long) and filled with an organic solvent such as is used for the stationary phase in the column up to 10 cm from the bottom, ensures the proper equilibration of the moving phase with the stationary liquid. The carrier from the saturator passes through a sample injection device (D)[4] and is then delivered into the separation column (E) made of glass (0.5—0.9 cm i.d. × 5—30 cm long). The sample is introduced onto the top of the column by means of either a pipette or a calibrated syringe. Chromatographic peaks, leaving the column, pass through the flow cell (F) and are then presented on the recorder (H). The pulsing movement of the effluent due to the pump was damped by placing a nitrogen bubble (1—5 cm^3) on the top of the saturator and the column. The

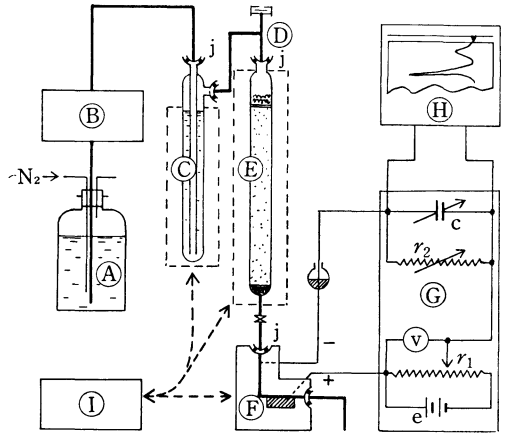

Fig. 2. Schematic diagram of chromato-polarograph. A) Eluate reservoir, B) Elution pump, C) Saturator, D) Sample injection device, E) Separation column, F) Flow cell, G) Polarograph, H) Recorder, I) Thermostat; e: battery (3 V), r_1: resistor (100 Ω), v: voltmeter (0—3 V), r_2: standard resistor (0.1 Ω—100 kΩ), c: damping capacitor (0—200 μF), j: O-ring joint.

Teflon tubing (0.9 mm i. d. and 1.7 mm o. d.) and various fittings were obtained from the Shibata Chemical Apparatus Mfg. Co., Ltd., Tokyo.

Preparation of the Column: The column packing was prepared as follows: after spherical polystyrene grains had been sufficiently swollen with an organic solvent (TCE or DCB), the excess solvent was removed by filtration with a small glass filter. The swollen polystyrene grains, which had been effectively washed with 10% ethanol, were suspended in 10% ethanol, and then the air bubbles cohesively attached to the grains were removed under reduced pressure. The suspension of the filler was quietly poured into the glass column plugged with a small wad of cotton and filled with 10% ethanol, while the filler liquid was quietly drained at the same rate. The column was then pressed under nitrogen pressure (about 2 atm) until the height of the carrier liquid above the packing was about 1 cm. These processes were repeated until the desired bed height was obtained. After filling the column, the top was then plugged with a small wad of cotton. The ion-exchange column was 0.5 cm i. d. × 5.6 cm long, and it was packed with Dowex 1×8 (100—200 mesh, nitrate form).

Materials and Reagents. The spherical polystyrene grains (7.1% cross-linked with divinylbenzene) employed as the support of the stationary phase were prepared by suspension polymerization according to the method of Winslow and Matreyek.[25] The polymer grains were sieved to five fractions of 100—115, 115—150, 150—200, 200—250, and above 250 mesh. The ion-exchange resin was Dowex 1×8 (100—200 mech), and the resin, supplied in the chloride form, was converted into the nitrate form by treating it with 1 M sodium hydroxyde and 1 M nitric acid. After having been washed with distilled water, the resin was stored in 1 M potassium nitrate. As for the stationary phase of the partition column, 1,1,2,2-tetrachloroethane (TCE) and *o*-dichlorobenzene (DCB) from Wako Pure Chemical Ind., Ltd., were used without further purification. The 2-amino-3-nitropyridine (A3N), 2-amino-5-nitropyridine (A5N), 2-hydroxy-3-nitropyridine (O3N), and 2-hydroxy-5-nitropyridine (O5N) were kindly donated by Professor Eiji Imoto of the University of Osaka Prefecture; their mp's were 164, 165—166, 226—228, and 192.5—193.5 °C respectively. The stock solutions (about 10^{-2} M) were prepared by dissolving the specimen in 10% ethanol. All the other chemicals were of a reagent grade or an equivalent. Britton-Robinson's buffer (BR) and 1 M potassium nitrate were used as the carrier liquid of the reversed partition and the ion-exchange chromatography respectively.

Procedures. After pre-conditioning the separation column under a fixed flow rate for 30—60 min at a constant temperature, an aliquot of the sample solution was introduced onto the top of the column and the current through the polarographic-flow cell at the constant applied voltage was recorded. The deaeration of the carrier liquid was carried out by means of either the addition of sodium sulfite (0.2 M) for neutral and alkaline solutions or a stream of nitrogen gas for an acidic solution.

Results and Discussion

Characteristics of the Flow Cell. To obtain some information concerning the characteristics of the three different flow cells described above, 2×10^{-4} M A3N in a BR buffer (pH 9.1) containing 0.2 M sodium sulfite was passed through the cell at a given flow rate and the polarographic current was recorded at an applied voltage of −0.9 V *vs.* Hg pool, which was sufficient for the reduction of A3N. The results obtained are presented in Fig. 3, where the current is plotted

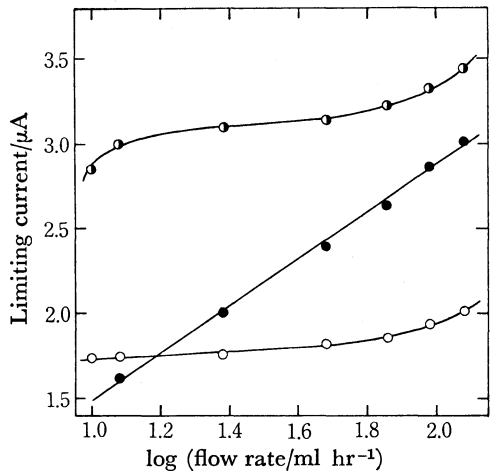

Fig. 3. Effect of flow rate on the polarographic current. ●: Hg–Pt, ◑: DME, ○: HDME.

against the logarithm of the flow rate. The effect of the flow rate on the current obtained with the HDME and DME is less pronounced than that on Hg–Pt. However, the slopes of the curves for both the dropping mercury electrodes exhibit a tendency to increase with the flow rate and to become similar to that for the stationary electrode[26] at a flow rate above 100 ml/hr. In the following experiments, the cell with HDME was used because the current oscillation, accompanied by the dropping of the mercury, can be easily reduced by the connection of a parallel capacitor (Fig. 2,c) and a good stability and reproducibility can be obtained.

The relationship between the limiting current in the flowing electrolyte and the height of the mercury reservoir is presented in Fig. 4; the change of the current with the mercury head was greater than that for the normal diffusion-controlled current, even in the

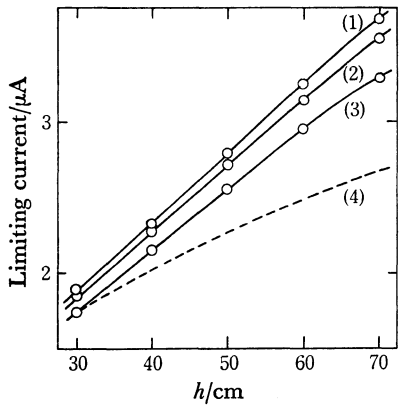

Fig. 4. Relationship between the limiting current (i) and the height (h) of Hg reservoir.
Flow rate of effluent: 1) 120, 2) 24, 3) 0 ml/hr, 4) diffusion current.

stationary solution. The steep increase in the current may be due to the stirring effect[27] of the rapidly dropping electrode in a narrow cavity. Takemori and Honda[15] found that the current through the particular cell designed as the detector of the liquid chromatography showed a constant value irrespective of the height of the mercury reservoir over a limited range. The discrepancy from the present observation seems to be caused by the difference in the flow rate in the electrode cavity and by the wall effect related to the electrode position.

Polarographic Behavior and Chromato-polarographic Separation of Nitropyridine Derivatives. Polarography: The course of the polarographic reduction was observed to be very similar in all the nitropyridines used (A3N, A5N, O3N, and O5N); they showed a diffusion-controlled and well-defined reduction wave (1st wave), followed by an ill-defined wave (2nd wave), at a potential, 0.8 V, more negative than that of first wave in an acidic buffered solution. The second wave became more ill-defined with an increase in the pH. The height of the first wave, except in the case of O5N, was observed to remain constant over the whole pH range studied and to be proportional to the concentration of the compounds in the range of $(0.5—20)\times10^{-4}$ M. On the other hand, at pH values heigher than 7 the splitting of the first wave for O5N into two waves was observed. The ratio of the two heights changed with the pH, the sum remaining constant.

As may be seen in Fig. 5, which presents the changes in the half-wave potentials of the nitropyridines with the pH, the polarographic separation of the mixtures in the normal manner seems to be difficult because the half-wave potentials are so close to each other.

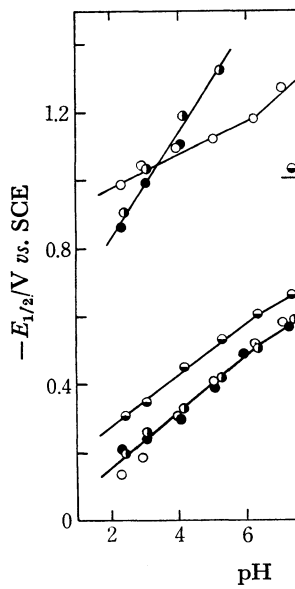

Fig. 5. pH-Dependence of the half-wave potentials of nitropyridines in BR buffer.
○: A3N, ●: A5N, ◑: O3N, ◒: O5N; Concn of nitropyridine: 2×10^{-4} M.

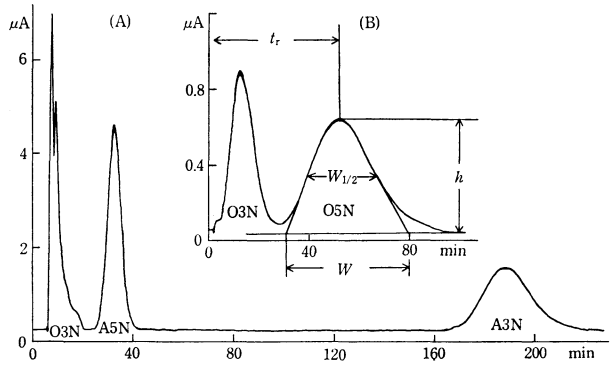

Fig. 6. Chromato-polarograms of nitropyridine mixtures at 30 °C.
A) Reversed-phase partition column (TCE), B) Ion-exchange column (Dowex 1×8, R-NO_3).

Chromato-polarography: Figure 6 (A) shows a typical chromato-polarogram obtained for a mixture of O3N, A5N, and A3N using a 0.9×10 cm column packed with spherical polystyrene grains (150—200 mesh) swollen by TCE, with a BR buffer (pH 9.1) containing 0.2 M sodium sulfite as a mobile phase, at a flow rate of 24 ml/hr. The current passing through the flow cell was recorded at an applied voltage of −0.8 V *vs.* Hg pool, which was sufficient for the reduction of all three compounds.

To examine the effect of the pH on the retention times of O3N, A5N, and A3N, such operating conditions as the sample size (1 μmol), the flow rate (24 ml/hr), and the column temperature (30 °C) were kept constant;

TABLE 1. THE EFFECT OF pH ON THE RETENTION TIMES OF NITEOPYRIDINES
Column: 0.9×11.5 cm, polystyrene grains (150—200 mesh) swollen by TCE

pH	Retention time/min		
	O3N	A5N	A3N
1.82	8.3	8.3	23.5
3.02	8.3	14.1	79.0
5.02	6.0	19.5	110.0
7.02	7.1	21.3	117.2
9.16	7.0	22.7	111.7
11.0	5.0	22.7	110.0

only the pH of the mobile phase (BR buffer) was changed. The results obtained are listed in Table 1. From the table it can be seen that, whereas the retention times of A5N and A3N are practically unaffected by the pH in the pH range above 5, the retention time decreases steeply with the change in pH in the acidic range below 5 because of the protonation of pyridine derivatives, and that the retention time of O3N is smaller than that of the former in the whole pH range studied. In the following experiments, the BR buffer with a pH of 9 was mainly used as the mobile phase, since a good separation can be obtained and since the dissolving oxygen can be removed easily by adding sodium sulfite.

The effects of the size of the packing polystyrene particle, the column temperature, the flow rates of the mobile phase, and the concentration of methanol in the mobile phase on the height equivalent to a theoretical plate (HETP) and the resolving power (R) of the column were also investigated; the results obtained are summarized in Fig. 7.

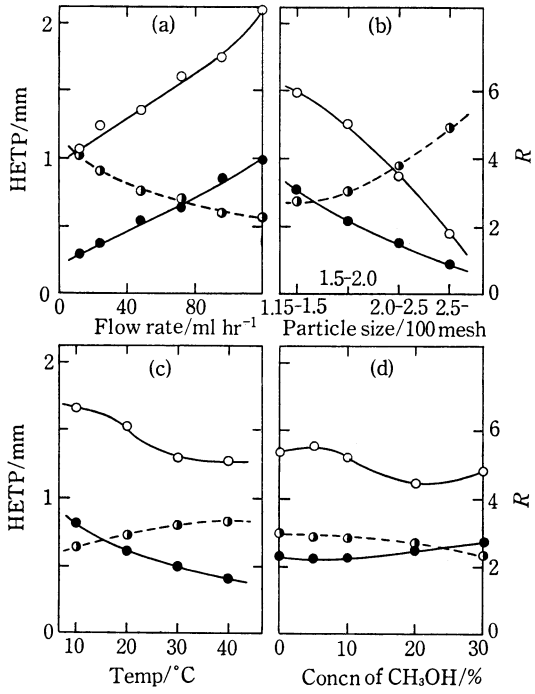

Fig. 7. Effect of flow rate (a), particle size (b), temperature (c), and methanol concentration (d) on the the HETP (——) and the resolving power (R,-----) of the column.
Concn of nitropyridine: 1 μmol; ●: A3N, ○: A5N.

The efficiency of the column was calculated by means of Eq. (1) of the plate theory:[4,28]

$$n = 16(t_r/W)^2 \quad (1)$$

where n is the number of the theoretical plate in the column, t_r is the retention time (min), and W is the peak width in min at the base. HETP was calculated from:

$$\text{HETP} = \text{column length in mm}/n \quad (2)$$

The resolving power (R) for the separation of A5N and A3N was calculated from:

$$R = 2\Delta t_r/\sum W \quad (3)$$

where Δt_r is the difference in the retention times of the two peaks and $\sum W$ is the sum of the peak widths at the base.

From Figs. 7a, b, and c, it can be seen that both the efficiency and the resolving power of the column used under the controlled experimental conditions increase with a decrease in the flow rate and the particle sizes and with an increase in the column temperature. With an increase in the methanol concentration in the mobile phase, no significant changes in the efficiency or the resolving power could be observed (Fig. 7 d), but the operating time for the separation was shortened.

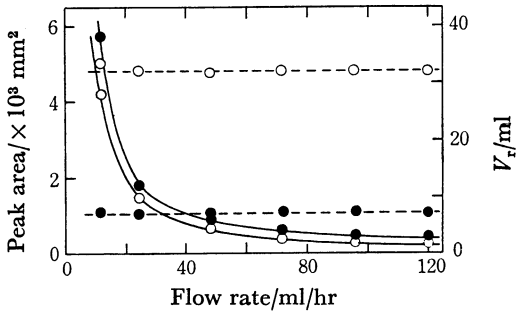

Fig. 8. Effect of flow rate on the peak area ($W_{1/2}\times h$, ——) and the retention volume (V_r,-----) at 30 °C.
Column: 0.9×5.7 cm, polystyrene grains (150—200 mesh) swollen by TCE; Elution: BR buffer (pH 9.2) contg. 0.2 M Na_2SO_3; Applied potential: −0.9 V *vs.* Hg pool; Sample: A5N (●, 1.29 μmol)+A3N (○, 1.26 μmol).

The changes in the peak areas corresponding to A5N and A3N with the flow rate are presented in Fig. 8, together with their retention volume, with the sample size and the experimental conditions being kept constant. With an increase in the flow rate, the peak areas for both A5N and A3N decreased hyperbolically, while their retention volumes showed fixed values independent of the flow rate. In determining components by means of chromato-polarography, therefore, the flow rate should be kept constant.

In order to obtain information concerning the reproducibility of the chromato-polarograms, the separation of a mixture of A5N (1.29 μmol) and A3N (1.26 μmol) was repeated five times under the same conditions; the results obtained are summarized in Table 2. The standard deviations of the peak heights and the peak areas (peak height×half-peak width) corresponding to each component were within 3.5 and 1.5% respectively; no appreciable change in the retention time was observed, even with ten runs of the separation.

The relationships between the chromato-polarographic responses obtained and the sample amounts added to the top of the column under the same operating conditions are presented in Table 3; linear relations were observed between the responses, the peak height, the peak areas (peak height×half-peak width and cutting-and-weighing method), and the amounts of A5N and A3N. In the analysis of the components in the sample by using the peak height, however, attention must be paid to keep the conditions strictly

Table 2. The reproducibility of chromato-polarograms for nitropyridines
Column: 0.9×5.3 cm, polystyrene grains (150—200 mesh) swollen by TCE, 30 °C; Elution; BR buffer (pH 9.15) contg. 0.2 M Na_2SO_3, 48 ml/hr; Applied potential: −0.9 V *vs.* Hg pool

Exp. No.	Peak height/mm		Peak area/mm²		Retention time/min	
	A5N	A3N	A5N	A3N	A5N	A3N
1	81.3	37.8	667	937	7.9	36.3
2	80.0	38.4	672	937	7.9	36.2
3	77.4	38.5	658	933	7.9	35.9
4	84.2	39.8	672	945	7.9	35.4
5	78.3	36.4	658	928	7.8	35.6
ave.	80.2_4	38.1_8	$665._4$	$936._0$	7.8_8	35.8_8

Table 3. The relationship between the chromato-polarographic responses and the sample size of nitropyridines
Column: 0.9×7.5 cm, polystyrene grains (150—200 mesh) swollen by TCE, 30 °C; Elution: BR buffer (pH 9.15) contg. 0.2 M Na_2SO_3, 60 ml/hr; Applied potential: −0.9 V *vs.* Hg pool; Recorder sens.: 0.0266 μA/mm; Chart speed: 180 mm/hr

Sample added /μmol		Peak height /mm		Peak area[a] /mm²		Peak area[b] /mg		Retention time /min		*R*
A5N	A3N	A5N	A3N	A5N	A3N	A5N	A3N	A5N	A3N	
0.2	1.0	19.5	42.7	156	1132	7.2	47.8	8.53	41.4	2.0
0.4	0.8	41.8	33.4	334	878	14.8	38.0	8.70	40.8	2.1
0.6	0.6	61.2	25.3	490	665	21.8	29.0	8.67	40.3	2.2
0.8	0.4	78.8	17.0	643	450	27.8	20.4	8.64	40.2	2.3
1.0	0.2	99.5	8.2	796	221	34.9	10.0	8.64	40.3	2.5

a) Peak height (h) × half-peak width ($W_{1/2}$). b) Cutting-and-weighing method.

controlled, since the height is markedly affected by even a small change in the operating conditions. The retention times for A5N and A3N were not pronouncedly affected by changes in the sample size, but the resolving power was observed to decrease slightly with an increase in the amounts.

Though all of the 2-hydroxy isomer could be easily separated from the mixture of 2-hydroxy and 2-amino nitropyridines by means of the reversed-phase partition column described above, as is shown in Fig. 6 (A), the separation of the mixture of 2-hydroxy isomers was not successful since they are sparingly soluble to such organic solvents as TCE and DCB. Thereupon, an ion-exchange column (5×56 mm) packed with Dowex 1×8 of 100—200 mesh was also used to separate the mixture of 2-hydroxy isomers. After adding the sample to the top of the column, the elution was carried out with 1 M potassium nitrate containing 0.2 M sodium sulfite at the flow rate of 24 ml/hr at 30 °C; a typical chromato-polarogram thus obtained is shown in Fig. 6 (B).

Application to the Determination of 2-Amino Nitropyridines in Nitrating Mixtures. The nitration was carried out by adding 5 ml of a nitrating reagent containing 1 ml of fuming nitric acid (d 1.52) in 20 ml of concentrated sulfuric acid to 100 ml of a concentrated sulfuric acid solution containing 0.5 g of 2-aminopyridine under continuous stirring at a temperature lower than 5 °C. One ml of the reaction mixture was pipetted out at a given time interval and transferred into a 25-ml volumetric flask. After having been neutralized with 5 M sodium hydroxyde under cooling by ice-water, the mixture was diluted to the desired volume with distilled water.

In a run, after a further tenfold dilution of the neutralized sample with a BR buffer (pH 7.1), the solution was polarographed in the usual way at 25 °C.

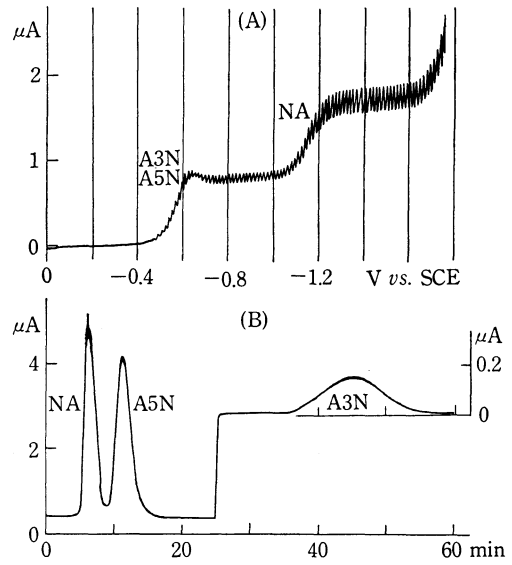

Fig. 9. Polarogram (A) and chromato-polarogram (B) of nitropyridines formed by nitration of 2-amino-pyridine.
Nitration: 5 °C, 30 min; Column: 0.5×10 cm, polystyrene grains swollen by DCB; Elution: 24 ml/hr; Applied potential: −1.5 V *vs.* Hg pool.

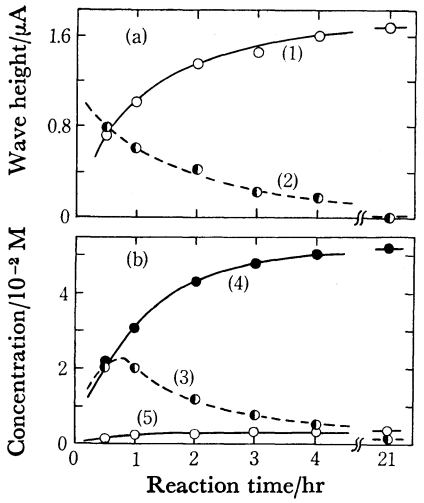

Fig. 10. Change of polarogram (a) and chromato-polarogram (b) during nitration.
1) 1st wave (A3N, A5N), 2) 2nd wave (NA), 3) 1st peak (NA), 4) 2nd peak (A5N), and 5) 3rd peak (A3N)

The polarogram thus obtained and the changes in the wave heights with the reaction times are shown in Figs. 9 (A) and 10 (a) respectively. From the latter figure, it can be seen that the second wave attains its maximum value rapidly after the addition of the nitrating reagent, while the first wave increases slowly at the expense of the second wave with the reaction time; the half-wave potential of the first wave coincides with that of A5N or A3N under the same conditions.

In another run, 0.5 ml of the neutralized sample was added to the top of the column described above and the column was eluted with BR buffer (pH 9) containing 0.2 M sodium sulfite at 30 °C. A typical chromato-polarogram is shown in Fig. 9 (B). The retention times of the second and the third peaks agreed with those for A5N and A3N respectively. The product corresponding to the second wave in Fig. 9 (A) and the first peak in Fig. 9 (B) may be concluded to be 2-nitroaminopyridine (NA).[29] Figure 10 (b) shows the changes in the concentration of the nitro compound formed in the reaction mixture with the reaction time, where the concentration of each substance was calculated by comparing the peak area obtained with that of a pure substance. From the findings described above, it may be deduced that 2-nitroaminopyridine forms rapidly at the first stage of the nitration and is then converted to A5N and A3N in the ratio of 14.5 to 1.

The authors wish to thank Professor Eiji Imoto for the gift of nitropyridine derivatives. They are also indebted to Mr. Hideo Oshima for his assistance in the experimental work.

References

1) R. D. Conlon, *Anal. Chem.*, **41**, 107A (1969).
2) J. J. Kirkland, *ibid.*, **40**, 391 (1968).
3) K. P. Hupe and E. Bayer, *J. Gas. Chromatog.*, **5**, 197 (1967).
4) T. E. Young and R. J. Maggs, *Anal. Chim. Acta*, **38**, 105 (1967).
5) B. Drake, *Acta Chem. Scand.*, **4**, 554 (1950); *Chem. Abstr.*, **44**, 8408g (1950).
6) W. Kemula, *Roczniki Chem.*, **26**, 281 (1952); *Chem. Abstr.*, **47**, 7344d (1953).
7) W. Kemula, "Proceeding of the International Symposium on Microchemistry 1958," Pergamon, London (1959), p. 248; "Progress in Polarography," Interscience, New York (1962), p. 397.
8) C. K. Mann, *Anal. Chem.*, **29**, 1385 (1957).
9) W. J. Blaedel and J. W. Todd, *ibid.*, **30**, 1821 (1958).
10) R. L. Robertus, R. J. Cappell, and G. W. Bond, *ibid.*, **30**, 1825 (1958).
11) W. J. Blaedel and J. H. Strohl, *ibid.*, **33**, 1631 (1961); **36**, 445 (1964).
12) E. B. Buchanan, Jr. and J. R. Bacon, *ibid.*, **39**, 615 (1967).
13) J. Janata and H. B. Mark, Jr., *ibid.*, **39**, 1896 (1967).
14) J. G. Koen, J. F. K. Huber, H. Poppe, and G. den Boef, *J. Chromatog. Sci.*, **8**, 192 (1970).
15) Y. Takemori and M. Honda, *Rev. Polarog.*, **16**, 96 (1970).
16) E. Sándi, *Z. Anal. Chem.*, **167**, 241 (1959).
17) W. Blaedel and J. W. Todd, *Anal. Chem.*, **33**, 205 (1961).
18) M. C. Corfield and A. Robson, *Biochem. J.*, **84**, 146 (1962).
19) M. Mohnke, R. Schmunk, and H. Schütze, *Z. Anal. Chem.*, **219**, 137 (1966).
20) W. Kemula, K. Butkiewicz, and D. Sybilska, "Modern Aspects of Polarography," Plenum, New York (1966), p. 36.
21) S. Tustanowski, *J. Chromatog.*, **31**, 266 (1967).
22) W. Kemula and D. Sybilska, *Anal. Chim. Acta*, **38**, 97 (1967).
23) P. Lanza, V. Concialini, and A. Benati, *J. Electroanal. Chem.*, **17**, 395 (1967).
24) I. Smoleń, *Collection Czech. Chem. Commun.*, **19**, 238 (1954).
25) F. H. Winslow and M. Matreyek, *Ind. Eng. Chem.*, **43**, 1108 (1951).
26) O. H. Müller, *J. Amer. Chem. Soc.*, **69**, 2992 (1947).
27) J. J. Lingane and B. A. Loveridge, *ibid.*, **72**, 438 (1950).
28) I. Rosenthal, A. R. Weiss, and V. R. Usdin, "Treatise on Analytical Chemistry," Part I, Vol. 3, ed. by I. M. Kolthoff and P. J. Elving, Interscience, New York (1961), p. 1411.
29) H. Meyer and E. R. von Beck, *Monatsh.*, **36**, 731 (1915).