Ligand-Exchange Chromatography of Aziridines and Ethanolamines

Kazuko Shimomura,[1] Tong-Jung Hsu,[2] and Harold F. Walton
University of Colorado, Boulder, Colo. 80302

Chromatographic separations on metal-loaded ion-exchange resins are described. Ethylenimine, propylenimine, *N*-ethylaziridine and *N*-(2-hydroxyethyl)-aziridine were separated from each other and from mono- and diethanolamine; mono-, di-, and triethanolamine were separated from one another, as well as from glycerol and *N*-dimethylethanolamine. Refractometric monitoring was used; 0.1-mg quantities gave easily measurable peaks. Best results were obtained with nickel-loaded sulfonic and carboxylic resins. Eluents were aqueous ammonia solutions.

LITTLE HAS BEEN PUBLISHED on chromatographic separations of ethylenimine and its derivatives, the aziridines. *Cis*- and *trans*-*N*-substituted aziridines were separated by gas chromatography (*1*) and it was shown that ethylenimine could combine with alkyl and aryl isocyanates, forming derivatives that were susceptible to separation by gas chromatography (*2*). Aziridines are very reactive. They hydrolyze rapidly in acidic solutions to give alkanolamines. They cannot, therefore, be analyzed by conventional cation-exchange chromatography. They are, however, stable in alkaline solutions, and it seemed reasonable to hope that they could be separated by ligand-exchange chromatography, in which the mobile phase is aqueous ammonia and the stationary phase is an ion-exchange resin loaded with metal ions (*3*, *4*).

Ethanolamine is of biological interest. It occurs in combined form in phospholipids, along with its *N*-methyl derivatives. Mono-, di-, and triethanolamines are used in industry. Ethanolamine and its *N*-methyl derivatives have been separated by gas chromatography (*5*) and by thin-layer chromatography on magnesium silicate (*6*). Several reports mention the detection of monoethanolamine during ion-exchange chromatography of amino acids (*7*, *8*). Mono-, di-, and triethanolamine were separated by cation-exchange chromatography, using hydrochloric acid eluent (*9*), and more effectively by using 0.05*M* borax (*10*). In both cases triethanolamine was eluted first, then di-, then monoethanolamine.

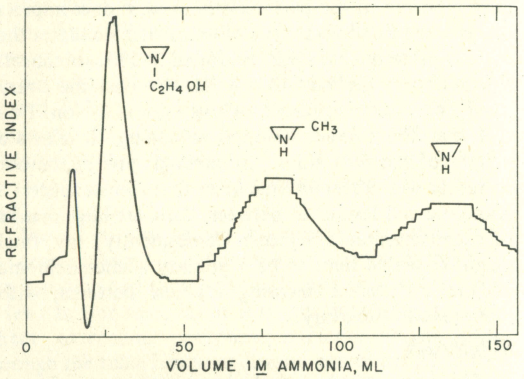

Figure 1. Elution of aziridines

Dowex 50-Ni, 33 cm × 1.1 cm; 10 mg each of ethyleneimine and propylenimine, 5 mg *N*-(2-hydroxethyl)aziridine; flow rate 38 ml/hr. Maximum change of refractive index is about 10^{-4} unit

The order of elution was reversed with zirconium phosphate cation exchanger, and the separation was much poorer (*11*).

In the work to be described, we have separated mono-, di-, and triethanolamine from each other, ethanolamines from aziridines, and three substituted ethylenimines (aziridines) from each other and from ethylenimine itself. The stationary phases were ion-exchange resins of various kinds carrying nickel(II) and copper(II) ions, and the mobile phase was aqueous ammonia.

EXPERIMENTAL

Materials. The following ion-exchange resins were used: AG50W × 8, 200–400 mesh, which is the polystyrene–sulfonic acid resin Dowex-50W, refined and sold by the Bio-Rad Corp., Richmond, Calif.; Chelex-100, 200–400 mesh, a polystyrene–iminodiacetate chelating resin made by the Dow Chemical Company and refined and sold by the Bio-Rad Corp.; Amberlite CG-50, 100–200 mesh, a polymethacrylate resin made by the Rohm and Haas Co., Philadelphia, Pa., and refined and sold by the Mallinckrodt Chemical Works, St. Louis, Mo.

Resins were converted to their nickel or copper forms by stirring with solutions of the metal–ammonia complexes, then transferring to wide columns and washing with more metal–ammonia solutions, then water. The carboxylic resin CG-50 was mixed with about 10% its weight of cellulose powder (Cellex-N, Bio-Rad Corp.) before packing into the chromatographic column (*4*).

Aziridines were provided by the Dow Chemical Co. They were: ethylenimine, C_2H_4NH; 1-methylaziridine or propylenimine, $CH_3 \cdot C_2H_3NH$; *N*-ethylaziridine, $C_2H_4NC_2H_5$; *N*-(2-hydroxyethyl)aziridine, $C_2H_4NCH_2CH_2OH$. They were used as received with no further purification. The compounds mono-, di-, and triethanolamine, 2-dimethylamino-

[1] Present address, Kazuko Shimomura Aramaki, Tokyo Woman's Christian College, Tokyo, Japan.
[2] Present address, Taipei Medical College, Taipei, Taiwan.

(1) R. L. Van Etten and A. T. Bottini, *J. Chromatogr.*, **21**, 408 (1966).
(2) W. L. Zielinski, L. Fishbein, R. O. Thomas, and T. E. Welsko, *ibid.*, **29**, 58 (1967).
(3) K. Shimomura, L. Dickson, and H. F. Walton, *Anal. Chim. Acta*, **37**, 102 (1967).
(4) C. M. de Hernandez and H. F. Walton, ANAL. CHEM., **44**, 890 (1972).
(5) R. L. Lester and D. C. White, *J. Lipid Res.*, **8**, 565 (1967).
(6) H. Grasshof, *J. Chromatogr.*, **20**, 165 (1965).
(7) R. S. Steele, K. Brendel, E. Scheer, and R. W. Wheat, *Anal. Biochem.*, **34**, 206 (1970).
(8) A. Saifer, E. A'Zary, C. Valenti, and L. Schneck, *Clin. Chem.*, **16**, 891 (1970).
(9) L. O. Pilgeram, E. M. Gal, E. N. Sassenrath, and D. M. Greenberg, *J. Biol. Chem.*, **204**, 367 (1953).
(10) Y. Yoshino, H. Kinoshita, and H. Sugiyama, *Nippon Kagaku Zasshi*, **86**, 405 (1965).
(11) R. L. Rebertus, ANAL. CHEM., **38**, 1089 (1966).

Reprinted from **ANALYTICAL CHEMISTRY**, Vol. 45, Page 501, March 1973

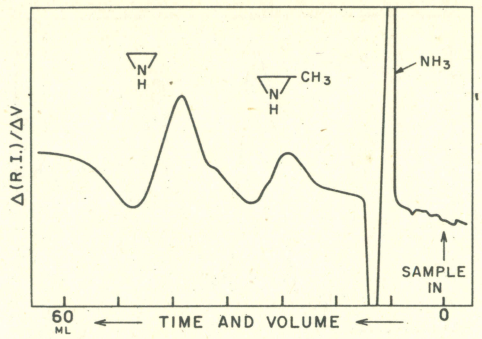

Figure 2. Derivative curve

Dowex 50-Ni, 23 cm × 1.1 cm; 10 mg each of ethylenimine and propylenimine

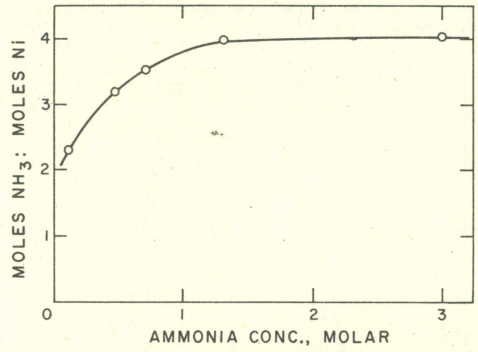

Figure 3. Absorption isotherm, nickel–carboxylic resin

On nickel-loaded Amberlite CG-50 resin. Ordinates are ratios of NH_3:Ni absorbed on the resin; abscissae, concentrations in solution

ethanol, and glycerol were obtained in reagent grades from commercial sources; monoethanolamine was redistilled before use. Carbon-14-labeled ethanolamines were obtained from the New England Nuclear Co.

Equipment. Columns and fittings were obtained from Chromatronix, Inc., Berkeley, Calif. In the earlier experiments, gravity flow was used with columns 11-mm and 7.5-mm diameter. Later, columns 6 mm in diameter were used, and the eluent solutions were contained in a stainless steel cylinder and driven under moderate pressures (15–50 pounds) of helium gas. Samples were introduced by syringe and septum. A fraction collector was used in some cases, but more often the effluents were monitored by a differential refractometer. In the experiments with aziridines (Figures 1 and 2) we used an early model refractometer with mechanical balancing. In the experiments with ethanolamines, we used a Model R-4 Laboratory Differential Refractometer (Waters Associates, Inc., Framingham, Mass.). The eluents passed first through the reference cell of the refractometer, then through the sample injection port, into the column, and out through the sample cell of the refractometer.

The differential refractometer was calibrated by experiments with aqueous ethanol solutions of known concentrations.

In a few tests, the refractometer was used in a "derivative mode." The column effluent passed through the reference cell, then through a short loop of narrow Teflon (Du Pont) tubing, and finally through the sample cell. A graph of the first derivative of refractive index was obtained. The idea

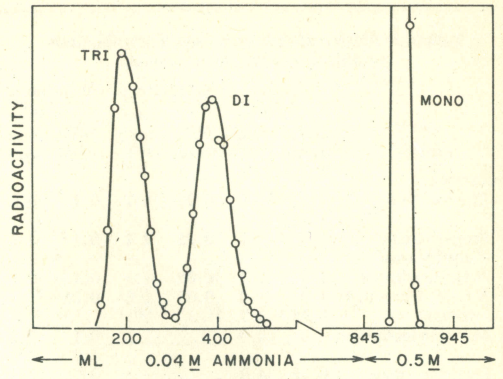

Figure 4. Chromatography of ethanolamines

Dowex 50-Ni, 30 cm × 0.75 cm; 5 mg ethanolamine, 2.5 mg diethanolamine, 1.5 mg triethanolamine plus ^{14}C-14 tracers. Flow rate; 40 ml/hr

was to minimize the effects of base-line drift and, eventually, to make it feasible to use the refractometer for gradient elution. Figure 2 shows one of the curves thus obtained.

Analytical Methods. Carbon-14 compounds were measured with a Packard Tri-Carb liquid scintillation spectrometer. Ethylenimine was determined in some experiments by a spectrophotometric method (*12*). It reacts with a freshly-prepared solution of 1,2-naphthoquinone-4-sulfonic acid sodium salt at pH 11.7 to form a red product that is extracted by chloroform. Ammonia and ethanolamine give red compounds that are not so extracted. The absorbance was measured at 420 nm.

Copper and nickel concentrations of effluents were measured by atomic absorption.

Ammonia Binding by Carboxylic Resin. Isotherms of the ammonia absorption by nickel- and copper-loaded chelating resin and by nickel-loaded polystyrene sulfonic resin were already available (*3*) but the isotherm for nickel-loaded carboxylic resin was not. We prepared some air-dried nickel-saturated Amberlite CG-50 resin and determined its nickel content by extracting a weighed portion with hydrochloric acid and titrating with EDTA. Then we shook weighed portions of this resin, each about 0.5 gram, with 10-ml quantities of aqueous ammonia solutions of known concentrations. Equilibrium was reached in 1–2 hours. (The reaction is slowest at the start, since the dry nickel-loaded resin is almost anhydrous and unswollen.) After 2–3 hours, the ammonia concentration in the solution is measured by titration, and the ammonia in the resin calculated by difference. The absorption curve is shown in Figure 3.

RESULTS AND DISCUSSION

Aziridines. Figures 1 and 2 show elution curves; Table I summarizes the data obtained with three metal–resin combinations. The best discrimination is obtained with nickel-loaded resins.

Substitution on the nitrogen atom has a marked effect. It reduces the strength of binding, and the effect is completely consistent with the effects we have noted earlier (*3*, *4*) and with the chromatography of substituted ethanolamines (see below). Any obstruction to the basic nitrogen atom weakens the coordination to the metal ion.

Comparing *N*-ethylaziridine with *N*-(2-hydroxyethyl)-aziridine, we note that the hydroxy group strengthens the

(12) D. Rosenblatt, P. Hlinka, and J. Epstein, ANAL. CHEM., **27**, 1290 (1955).

Table I. Elution of Aziridines and Ethanolamines
(Multiples of bulk column volume)

Resin:	Ni-sulfonic NH_3 concn	Ni-sulfonic Elution vol.	Ni-chelating NH_3 concn	Ni-chelating Elution vol.	Cu-chelating NH_3 concn	Cu-chelating Elution vol.
Substance:						
Ethylenimine	1.0	4.6	0.25	6.1	0.1	5.8
	1.4	2.7	...	...	...	...
Propylenimine	1.0	2.8	0.25	2.2	0.1	5.1
(1-methylaziridine)	1.4	1.8	...	...	...	...
N-Ethyl-aziridine	1.0	0.7	0.05	1.3	0.1	1.1
N-(2-Hydroxyethyl)	1.0	1.0	0.05	2.6	0.25	1.4
aziridine	0.25	2.7	...	...	...	...
Monoethanolamine	1.0	4.0	...	...	0.1	7.2
					1.0	2.4
Diethanolamine	0.25	4.0	...	...	0.25	2.3

binding to nickel. It may strengthen the binding to copper also, but the attractions are so weak in this case that the effect is hard to notice.

We had already observed (*3*) that ethylenimine is retained on nickel–ammonia-loaded resins for 24 hours without appreciable decomposition. The same is true of copper-loaded resins.

Aziridines Distinguished from Ethanolamines. The product of hydrolysis of ethylenimine is monoethanolamine; that of *N*-(2-hydroxyethyl)aziridine is diethanolamine. It is therefore important to know whether the aziridines can be distinguished from their hydrolysis products by ligand-exchange chromatography.

Mixtures of ethylenimine with ethanolamine-^{14}C were passed through columns of Ni-AG50 resin and Cu–Chelex resin, each 1.1 cm in diameter and 120 cm long. In both cases the eluent was 1.0*M* ammonia. Fractions were collected; ethanolamine was determined by counting, while ethylenimine was determined photometrically (*12*). The nickel-loaded resin retained ethylenimine slightly more strongly than ethanolamine but gave no significant separation. The copper-loaded resin retained ethanolamine much more strongly than ethylenimine, the peak retention volumes being 80 ml and 48 ml, respectively. Separation was excellent.

Similar tests were made with *N*-(2-hydroxyethyl)aziridine and diethanolamine, using refractometric monitoring. Both copper-loaded and nickel-loaded columns gave adequate separation, but the elution order was different. The nickel resin retained the aziridine more strongly than diethanolamine; copper-loaded chelating resin held diethanolamine more strongly and gave better separation, the ratio of corrected elution volumes being 2:1. Diethanolamine did remove a little copper from the chelating resin, but the peak copper concentration was below 5 mg/ml. The eluent was 0.25*M* ammonia.

Ethanolamines. Figure 4 shows the elution of a mixture of mono-, di-, and triethanolamine to which tracer amounts of the respective ^{14}C-labeled compounds had been added. The separation is excellent, but the elution volumes are inconveniently large. Monoethanolamine is held much more strongly than di- or triethanolamine, and the ammonia concentration had to be increased to get it off the column, which was nickel-loaded polystyrene sulfonic resin.

Tests were next made with refractometric monitoring and columns of Amberlite CG-50 carboxylic resin loaded with copper and nickel ions. Not surprisingly, copper was stripped off the resin, giving blue effluents. Copper(II) forms

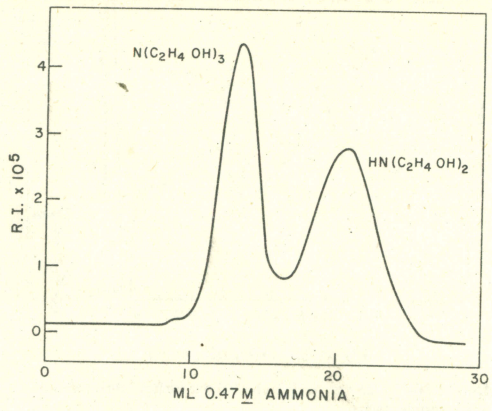

Figure 5. Di- and triethanolamines

Amberlite CG-50-Ni, 100-200 mesh, 44 cm × 0.6 cm; 1.25 mg of each; flow rate 19 ml/hr

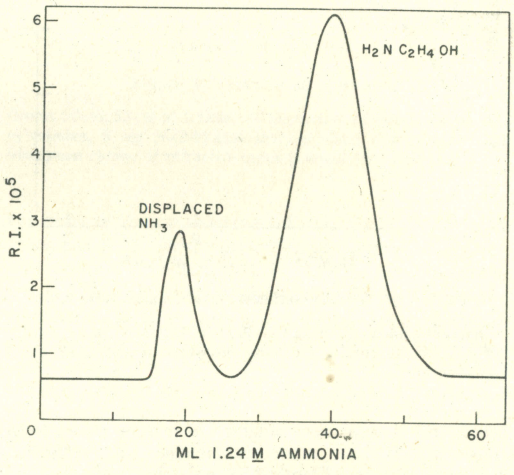

Figure 6. Monoethanolamine elution

Same column as Figure 5; 5.7 mg ethanolamine; flow rate 37 ml/hr

an uncharged complex with diethanolamine, and this fact can be used in chromatographic separations of metals (*13*). Stripping of nickel ions was negligible, so a nickel-loaded carboxylic resin column was selected for further study.

With this column, we again found that monoethanolamine was retained much more strongly than di- or triethanolamine. Di- and triethanolamine were separated by 0.47*M* ammonia; see Figure 5. Monoethanolamine needed more concentrated ammonia for effective elution.

Figure 6 shows a typical refractometer curve for monoethanolamine along. There are two peaks. The first, smaller peak is caused by a pulse of ammonia released when the ethanolamine sample enters the column and displaces ammonia from coordination with the nickel resin. We have commented on this phenomenon before (*3*). It is an unfortunate consequence of using refractive index for detec-

(13) F. Hilgeman, K. Shimomura, and H. F. Walton, *Separ. Sci.*, **4**, 111 (1969).

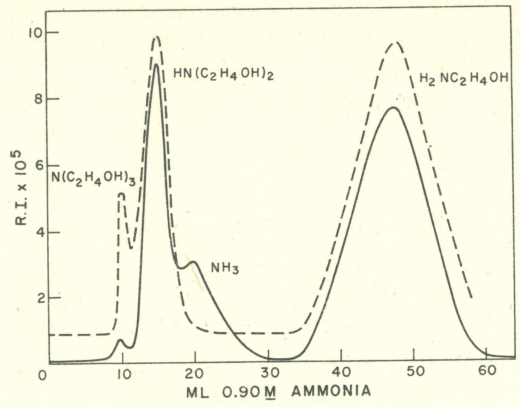

Figure 7. Mono- and diethanolamines

Same column as Figure 5; 7.3 mg mono-, 1.9 mg diethanolamine plus tracers; flow rate 21 ml/hr. Dashed curves show radioactivity

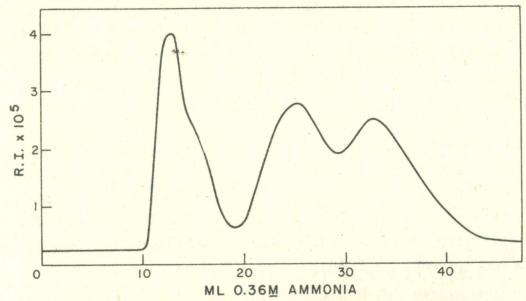

Figure 8. Glycerol, mono-, di-, triethanolamines

Same column as Figure 5; 0.65 mg glycerol, 0.37 mg triethanolamine, 0.90 mg diethanolamine, 10.5 mg monoethanolamine; flow rate 22 ml/hr. The monoethanolamine was eluted later with 1*M* ammonia

tion. Any change in solution composition, from whatever cause, affects the refractive index.

Evidence that the first peak of Figure 6 is due to ammonia is as follows. Its area did not diminish when the ethanolamine was redistilled; the ratios of the areas of the first and second peaks, 1:6.8, is the same as the (experimentally determined) ratio of the partial molal refractive indices of ammonia and ethanolamine in dilute aqueous solutions; where ^{14}C-labeled ethanolamine was used, the first peak had no radioactivity (see Figure 7); injection of a small amount of concentrated ammonia solution produced a peak at the same elution volume and injection of water produced a corresponding negative peak.

The elution volume of the ammonia peak may be predicted from the theory of the "concentration pulse" (*14*):

$$v = v_i(1 + \Delta C_s/\Delta C_m) = v_i + mx$$

Here, v is the peak elution volume in ml, v_i the effective interstitial volume of the column (which includes all void space from the point of sample introduction to the detector), C_s and C_m are the quantities of ammonia in the stationary and moving phases per unit column segment, m is the number of millimoles of nickel ions in the entire column, and x is the slope of the isotherm in Figure 3, expressed as the increase in the NH_3:Ni mole ratio for unit increase in the molar concentration of ammonia in solution.

(14) F. Helfferich, *Science*, **142**, 661 (1963).

Table II. Elution of Ethanolamines from Ni-Carboxylic Resin
(Multiples of bulk column volume)

Ammonia concn, *M*	Glycerol	Dimethylethanolamine	Triethanolamine	Diethanolamine	Monoethanolamine	Ammonia pulse
2.9	...	...	0.90	0.9	1.6	...
1.5	...	...	0.90	0.95	2.6	...
1.14	...	...	0.90	1.07	3.3	1.48
0.90	...	...	0.90	1.20	3.75	1.55
0.47	...	1.07	1.07	1.7	6.8	2.25
0.34	1.05	...	1.40	2.3	10.3	2.8
0.23	1.00	1.32	1.50	2.65	...	...

The concentration of nickel ions in the packed column was found to be 0.66 mole/liter; the bulk column volume was 12.5 ml. The slope of the isotherm is hard to establish accurately, but the calculated values of v agree reasonably well with the observed values. The ammonia peak travels more slowly, the more dilute the solution.

No ammonia peak was observed when diethanolamine and triethanolamine were present alone. These substances are much more weakly absorbed than monoethanolamine, and if they displace anything from the column they probably displace water as well as ammonia. Any refractive index pulse that they might produce would be less noticeable than with monoethanolamine because their own refractive indices are greater.

Figure 7 shows the elution curve for a mixture of mono- and diethanolamine to which radioactive tracers were added. Both refractive index and radioactivity are shown. The ammonia peak follows that for diethanolamine. Just before the diethanolamine peak is a small peak that seems to be due to triethanolamine present as an impurity. In the radioactivity curve this peak is prominent, and it was enhanced when tracer triethanolamine was added.

The question then arose whether the ammonia peak produced by a large amount of monoethanolamine would mask the presence of a small amount of diethanolamine. To answer this question a solution was prepared containing mono- and diethanolamine in the ratio 12:1 by weight. It was injected and eluted with 0.36*M* ammonia. The diethanolamine peak emerged at 25.5 ml, the ammonia peak at 33 ml; see Figure 8. We estimate that diethanolamine could be detected in the presence of at least 50 times its weight of monoethanolamine. The ammonia peak could serve as a rough measure of the amount of monoethanolamine, but if monoethanolamine itself were to be determined with maximum accuracy, it would be best to perform another elution with 1*M* ammonia.

The mixture whose chromatogram is shown in Figure 8 also contained glycerol. Glycerol is not retained by the column, and the peaks due to glycerol and triethanolamine are partially resolved. With 0.25*M* ammonia, the resolution of glycerol and triethanolamine is better.

Table II presents elution volumes for various concentrations of ammonia. Dimethylethanolamine is more weakly retained than triethanolamine, but more strongly than glycerol. It should be possible to analyze a mixture of triethanolamine, dimethylethanolamine, and glycerol by using

a column of finer-mesh resin and eluting with a low ammonia concentration.

Quantitative Measurements. The peak areas on the recorder chart were proportional to the quantities of solutes added, within the errors of measurement, which were about ±3%. Peak areas must be calibrated for each solute individually. At first sight, one would expect a given mass of triethanolamine to give a bigger peak than the same mass of di- or monoethanolamine, for the refractive indices of the three amines are, respectively, 1.4852, 1.4776, and 1.4541, compared to 1.3325 for water. However, the refractive index of a mixture depends on mole fraction rather than weight fraction, and calculation shows that on a weight basis the three amines affect the refractive index almost equally. Calibration is essential, because peak areas may be influenced by displacement of ammonia from the resin.

We did not try to see how small a quantity of amine could be measured, but one can form an impression of the sensitivity from the first small peak in Figure 7. This peak corresponds to 0.1 mg of triethanolamine in the injected sample. The refractometer attenuation was 4×, and it should be possible to detect one-tenth of this amount, namely, 0.01 mg, under favorable conditions.

CONCLUSIONS

These chromatographic separations of ethanolamines and aziridines illustrate generalizations we have made before (*4*). The strongest binding to the metal-loaded resins occurs with primary amino groups; carbon atoms attached to the amino nitrogen or to the adjacent carbon atom obstruct the binding and cause the compounds to be eluted earlier. Thus diethanolamine is eluted before monoethanolamine, and *N*-ethyl and 1-methylaziridines are eluted before ethylenimine. We must consider the coordination of the metal ions with hydroxyl groups, however, as well as their coordination with nitrogen atoms. Triethanolamine is more strongly bound by the nickel resin than is dimethylethanolamine, and the sugar amines glucosamine and galactosamine, which we have tested, are held almost as strongly as monoethanolamine in spite of their "obstructed" amino groups. Copper ions seem to coordinate more strongly with hydroxyl groups than nickel ions, to judge by the elution orders of aziridine–ethanolamine mixtures (see Table I).

Chromatographic partition ratios depend on various factors, of which the metal–ligand interaction is only one, but this is probably the main effect in the systems studied here. We have shown that metal–ethanolamine complexes are only slightly less stable in an ion-exchange resin than in aqueous solution (*15*). We could use metal–ligand association constants in solution as guides to ligand-exchange chromatography if we had the data. For Ni(II) and monoethanolamine, $K_1 = 950$ (*15*). We do not have the constants for di- and triethanolamine, and they may be too small to measure by the usual techniques.

Whatever the reason, monoethanolamine is held much more strongly than di- and triethanolamine, and our method of analysis is very effective for measuring small amounts of monoethanolamine in the presence of much di- and triethanolamine. It is almost as effective for measuring small amounts of di- and triethanolamine in the presence of much monoethanolamine. Our method is the only one we have seen reported for chromatographic analysis of simple aziridines.

RECEIVED for review July 7, 1972. Accepted October 2, 1972. Work was supported by the U.S. Atomic Energy Commission, Contract AT-(11-1)-499, by the National Science Foundation, Grant No. GP-25727, and by a fellowship awarded to one of us (T-J.H.) by the National Science Council of the Republic of China.

(15) L. Cockerell and H. F. Walton, *J. Phys. Chem.*, **66**, 75 (1962).