Table I. Experiments Illustrating Enrichment and Permeability of Ion Exchange Membranes to Counter Ions and Co-Ions

Experiment	Wrapped electrode combination[a]	Sensed ion	Membrane type and form	Solution compositions		Time (min) required for inside solution to reach $N \times$ outside concentration of sensed ion		
				Inside	Outside	$N = 0.1$	$N = 1$	$N = 10$
1	A–D	H^+	Cation–K^+	0.1M KCl, pH 6	10^{-1} to $10^{-5}M$ HCl	0.03–0.05	0.05–1	2.5–10
2	B	H^+	Anion–Cl^-	0.1M KCl, pH 6	10^{-2} to $10^{-4}M$ HCl	*b*	*b*	*b*
				0.1M KCl, pH 6	$10^{-1}M$ HCl	60	*b*	*b*
3	A–D	OH^-	Anion–acetate$^-$	0.1M KOAc, pH 8	10^{-2} to $10^{-4}M$ KOH	0.8–2.8	1.8–5.5	>11, $N = 5$
4	A–D	OH^-	Cation–K^+	0.1M KOAc, pH 8	10^{-3} & $10^{-4}M$ KOH	*b*	*b*	*b*
				0.1M KOAc, pH 8	$10^{-2}M$ KOH	60	*b*	*b*
				0.1M KOAc, pH 8	$10^{-1}M$ KOH	5	60	*b*
5	C–D	NH_4^+	Cation–$Tris^+$	0.1M Tris–sulfate buffer, pH 8	10^{-2} to $10^{-4}M$ $(NH_4)_2SO_4$	0.2–1	2.5–9	10–20
6	C–D	NH_4^+	Anion–SO_4^{2-}	0.1M Tris–sulfate buffer, pH 8	10^{-3} & $10^{-4}M$ $(NH_4)_2SO_4$	*b*	*b*	*b*
				0.1M Tris–sulfate buffer, pH 8	$10^{-2}M$ $(NH_4)_2SO_4$	15	*b*	*b*
				0.1M Tris–sulfate buffer, pH 8	$10^{-1}M$ $(NH_4)_2SO_4$	2	30	*b*

[a] A = Sargent S-30050-19 flat glass electrode. B = Sargent S-30072-15 combination glass electrode. C = Beckman #39137 monovalent cation electrode. D = Sargent S-30080-15A S.C.E. reference electrode. [b] Denotes that concentration of ion in the inside solution had not reached this level after 60 minutes elapsed time.

Figure 3 is a log–log plot of the rate of buildup of counter ion in the inside solution against its concentration in the outside solution. There is fair linearity of the plot over about 2 decades of concentration, and the average slope is 1.1, indicating that the rate of buildup inside is proportional to the outside concentration. Such proportionality is also shown by the transport of a trace counter ion from one solution to another through an ion exchange membrane (*3*). With suitable control over rate determining parameters, and with more uniform wrapping of the membrane around the electrode, the buildup rate might be of analytical use in measuring outside concentrations that are too low to be directly measurable with the sensor electrode.

CONCLUSIONS

The preceding experiments show that thin modern ion exchange membranes exhibit high permeability to counter ions, and virtually prohibit diffusion of co-ions. Further, ion exchange membranes may give concentration enrichment of counter ion species by factors as high as 100, which may be of analytical use in sampling and in increasing the sensitivity of ion selective electrodes. Work is presently under way in our laboratories on coupled ion exchange–immobilized enzyme membrane systems which would increase sensitivity and reduce interferences.

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Quantitative Conversion of Cyclamate to *N,N*-Dichlorocyclohexylamine, and Ultraviolet Spectrophotometric Assay of Cyclamate in Food

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THE OLDEST and most widely known method for determining cyclamate is using nitrous acid to convert cyclamate to sulfuric acid and cyclohexene, and subsequent formation of barium sulfate precipitate (*1*). The other product, cyclohexene, is, however, of little use in evaluating cyclamate, since the gas chromatographic method for cyclohexene determination is lengthy and laborious (*1*). Johnson *et al.* (*2*) found that quantitative hydrolysis of cyclamate to cyclohexylamine can be achieved by heating cyclamate in aqueous 1.3N HCl at 125 °C (15 psi) for at least 7 hours. This procedure for determining cyclamate is time-consuming.

While searching for a faster and better approach to split cyclamate, we found that an equivalent amount of *N,N*-

(1) M. L. Richardson, *Talanta*, **14**, 385 (1967).

(2) Darryl E. Johnson, Helmut B. Nunn, and Stanley Bruckenstein, ANAL. CHEM., **40**, 368 (1968).

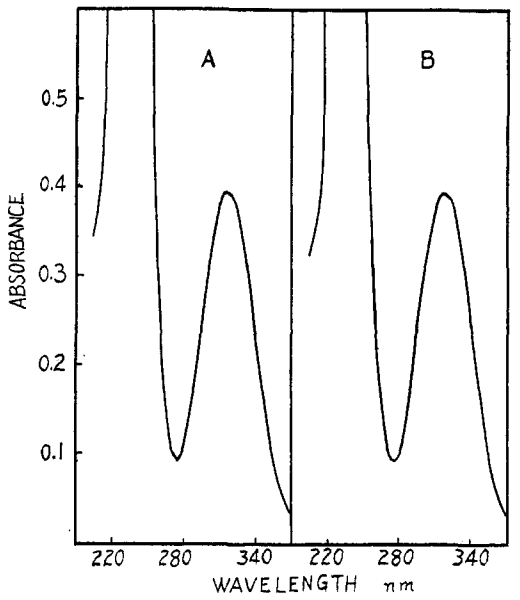


Figure 1. Ultraviolet spectra

A. N,N-dichlorocyclohexylamine, made from 0.99 mg of cyclohexylamine, in 10 ml of cyclohexane
B. N,N-dichlorocyclohexylamine, made from 2.01 mg of sodium cyclamate, in 10 ml of cyclohexane

dichlorocyclohexylamine formed upon adding excess chlorine water or hypochlorite to an acidic, aqueous solution of cyclamate. This was verified by analyzing the electropositive chlorine content of the product and by comparing with the *N,N*-dichlorocyclohexylamine prepared from cyclohexylamine. By applying the UV-absorption property of *N,N*-dichlorocyclohexylamine, it was possible to determine cyclamate content as low as 0.001% in liquid foods.

EXPERIMENTAL

Apparatus. Spectra and absorbance measurements were taken with a Hitachi 124 spectrophotometer connected to a Perkin-Elmer 165 recorder

Reagents. Sodium cyclamate, Kjeldahl method for nitrogen yielded a purity of 99.3%.

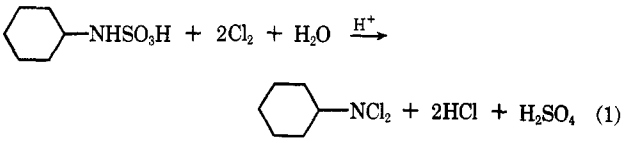
Cyclohexylamine, titration with HCl in water yielded a purity of 99.6%.

Quantitative Dehydrochlorination of Cyclohexylamine to *N,N*-Dichlorocyclohexylamine. Small modifications were made in applying the methods of Jackson *et al.* (*3*) and Baumgarten *et al.* (*4*) in synthesizing *N,N*-dichlorocyclohexylamine. About 50 mg of cyclohexylamine in 30 ml of water in a separator was acidified with 5% acetic acid to slightly acidic, and buffered by adding 0.2 gram of sodium bicarbonate. Then 40 ml of cyclohexane and 20 ml of sodium hypochlorite (1% available chlorine) which was previously acidified by acetic acid to pH 7–8.0 and buffered by 0.2 gram of sodium bicarbonate was added to the separator. After the separator was shaken for 1 minute, the water layer was drained into a flask. The cyclohexane layer was shaken with 50 ml of 0.1*N* NaOH to remove any free chlorine in the organic layer, and the water layer was drained into the flask.

To the NaOH-washed cyclohexane layer (ready for spectrum taking) was added 4 ml of glacial acetic acid, a pinch of potassium iodide (about 100 mg), and 1 ml of water. After shaking for 1 minute, the inner wall and lid of the separator were flushed with water, and the resultant triiodide solution was drained into another flask. The shaking with acetic KI–water, and flushing were repeated until the upper layer was colorless. The combined triiodide solution, after being titrated with sodium thiosulfate, was found to have two equivalent amounts of electropositive chlorine. When the combined water layer was analyzed by the A.O.A.C. method (*5*), it was found that four equivalent amounts of available chlorine were consumed and two equivalent amounts of chlorine in the form of chloride were produced.

A spectrum of *N,N*-dichlorocyclohexylamine in 10 ml of cyclohexane was made from 0.99 mg of cyclohexylamine. See Figure 1.

Quantitative Conversion of Cyclamate to *N,N*-Dichlorocyclohexylamine. About 100 mg of sodium cyclamate in 30 ml of water in a separator was acidified with 2 ml of sulfuric acid (H_2SO_4:H_2O = 3:7 by volume). Then 40 ml of cyclohexane and 20 ml of sodium hypochlorite (1% available chlorine) were added successively. After shaking for 1 minute, the water layer was drained into a flask containing enough NaOH to neutralize the sulfuric acid used. The cyclohexane layer was shaken with 50 ml of 0.1*N* NaOH to remove free chlorine; and the alkaline water layer was drained into the flask. The NaOH-washed cyclohexane layer, when analyzed in the same manner as before for electropositive chlorine, was found to have two equivalent amounts of chlorine. By analyzing the combined water layer (*5*), it was found that four equivalent amounts of available chlorine were consumed and two equivalent amounts of chlorine in the form of chloride were produced. Furthermore, when HCl, instead of sulfuric acid, was used as the acidifying agent, and an excess amount of barium chloride was added to the water layer, one equivalent amount of barium sulfate was obtained. Therefore, an overall equation was proposed:



A spectrum of *N,N*-dichlorocyclohexylamine in 10 ml of cyclohexane was made from 2.01 mg of sodium cyclamate. It coincides perfectly with that from 0.99 mg of cyclohexylamine. See Figure 1.

Assay of Cyclamate in Food. PROCEDURE. In a separator, 100 ml of sample solution containing 1–20 mg of sodium cyclamate was acidified with two 5 ml of concentrated sulfuric acid while swirling the separator, cooled twice under tap water, and shaken gently for 2 minutes with 100 ml of ethyl acetate. Then 80 ml of clear ethyl acetate layer (obtained by filtering through cotton or by centrifuging if emulsion occurred) was measured into another separator, and was shaken with 3 × 30 ml of water. The combined water layer, in a third separator, after being made alkaline with 2 ml of 10*N* NaOH, was shaken with 10 ml of cyclohexane for 1 minute. The alkaline water layer was transferred to a fourth separator. The inner wall of the third separator was washed with 5–10 ml of water, and the washing was drained into the fourth separator. To the fourth separator was added 5 ml of sulfuric acid (H_2SO_4:H_2O = 3:7 by volume), 10 ml of cyclohexane, and 10 ml of sodium hypochlorite (1% available chlorine) successively, and the separator was shaken for 2 minutes (when the two layers separate, the cyclohexane layer should be greenish yellow; otherwise, addition of more sodium hypochlorite is necessary). After draining off the lower layer, the cyclohexane layer was shaken with 50 ml of 0.5*N* NaOH for 1 minute. Then the alkaline water layer was drained off,

(3) L. K. Jackson, G. N. R. Smart, and George F. Wright, *J. Amer. Chem. Soc.*, **69**, 1539 (1947).
(4) Henry E. Baumgarten and Frank A. Bower, *ibid.*, **76**, 4561 (1954).
(5) William Horwitz, "Methods of Analysis A.O.A.C.," 10th ed., 1965, p 58.

and the inner wall of the separator was flushed with about 50 ml of water, which was again drained off. The cyclohexane layer was then run through a plug of cotton for spectrum taking in the range of 260–370 nm, using cyclohexane as reference. The sodium cyclamate content was determined by comparing absorbance at 314 nm with the standard curve.

PREPARATION OF STANDARD CURVE. Volumes of 100 ml of water containing 2.00, 4.00, 8.00, 12.00, 16.00, and 20.00 mg of sodium cyclamate were carried through the above procedure for the assay of cyclamate. The amount (mg) of sodium cyclamate used was plotted against absorbance at 314 nm. A straight line obeying Beer's law was obtained.

RESULTS AND DISCUSSION

One-hundred milliliter solutions of various foods with 5 and 10 mg of sodium cyclamate added, were tested. Results are in Table I. Slight interference was found with some foods. This is why the recovery of added cyclamate is sometimes a little higher than 100%.

Amino acids, urea, ammonia, various aliphatic amines, and food additives such as salicylic acid, benzoic acid, *p*-hydroxybenzoates, potassium sorbate, saccharin, and dulcin were tested for interference. Only dulcin was found to interfere slightly, but its presence was revealed by a brown coloration of the water layer in the step when cyclohexane layer was shaken with 50 ml of 0.5*N* NaOH. As is shown in Figure 1, *N*,*N*-dichlorocyclohexylamine has two absorption peaks. Although the absorptivity at 222 nm is much larger than at 314 nm, wavelength 314 nm was adopted because interference was found negligibly slight at 314 nm for the various foods we had tested. A spectrum with a peak at 314 nm and a valley at 274 nm definitely gives a qualitative picture, disregarding the slight interference.

When 100 ml of ethyl acetate was used to extract 100 ml of water containing a certain amount of sodium cyclamate (1–20 mg), it was found 7.5 ml of concentrated sulfuric acid was the critical amount needed to acidify the aqueous solution so that the maximum proportion of cyclamic acid was extracted (about 46.5%). Beyond this critical amount, when 10, 15, and 20 ml were tested, the maximum proportion of cyclamic acid being extracted remained the same. The reason that 10 ml of sulfuric acid was used in our procedure is that after possible consumption of part of the sulfuric acid by the sample solution, the remaining sulfuric acid would acidify the sample solution strongly enough to ensure maximum-proportion extraction.

Table I. Average Recovery of Sodium Cyclamate, Obtained from Three Analyses for Each Cyclamate-Added Sample

Sample (100 ml)[a]	Average recovery, % 5 mg added	10 mg added
Guava juice	105.0 ± 0.2	103.5 ± 0.4
Orange juice	104.5 ± 0.5	103.0 ± 0.2
Tomato juice	100.2 ± 0.1	100.0 ± 0.2
Mango juice	100.5 ± 0.2	100.9 ± 0.3
Cola A	104.8 ± 0.3	104.3 ± 0.1
Cola B	104.4 ± 0.3	103.7 ± 0.2
Soy sauce A	104.2 ± 0.6	104.0 ± 0.5
Soy sauce B	103.5 ± 0.4	102.4 ± 0.3
Pork lean extract	99.7 ± 0.2	99.2 ± 0.4
Fish extract	100.3 ± 0.4	100.1 ± 0.3
5% Milk powder	102.2 ± 0.5	101.7 ± 0.5
30% Vanilla ice cream	100.6 ± 0.3	100.2 ± 0.2
	Total av 102.2 ± 1.8%	

[a] Fruit juices and soy sauces were diluted by a ratio of 1:1.

Determination of cyclamate without previous extraction with ethyl acetate can be achieved only with some drinks. With others, as in the case of soy sauce, consumption of hypochlorite was too big to render the determination possible. Emulsion problems did usually occur with some food products. In extraction with ethyl acetate, suspensions of dairy products of considerably high concentration, led to serious emulsion. But clear ethyl acetate, although less in volume than originally used, was easily obtained by centrifuging followed by filtering the upper supernatant through cotton. For fruit juices and soy sauces, slight emulsion occurred in between the two layers, After draining off the water layer and the emulsion part, clear ethyl acetate was obtained by filtering through cotton. In case the clear ethyl acetate layer obtained is less than 80 ml, the last result of assay could still be obtained by correction to 80 ml. In the subsequent extractions with cyclohexane, emulsion did not occur.

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CORRESPONDENCE

A Simplified Separation of Strontium, Radium, and Lead from Environmental Media by Precipitation Followed by Fractional Elution

SIR: Procedures for strontium-89 and -90 in media such as water, soil, food ash, and bone ash commonly take advantage of the insolubility of $Sr(NO_3)_2$ in strong nitric acid to effect a separation of strontium from calcium. Sensitivity requirements in environmental analysis dictate amounts of sample often containing up to 3 grams of calcium. In addition, there is a restriction on the amount of strontium used as carrier, typically 20 mg, to minimize self-absorption of beta particles during counting. Thus the calcium:strontium ratio is often greater than 100:1. A suitable choice of nitric acid concentration and repeated treatments are needed to effect a satisfactory separation with minimum loss of strontium.

An early study (*1*) established the optimum nitric acid concentration at about 80% w/w. This concentration is obtained by dilution of unpleasant, hazardous, fuming nitric acid (nominally 95% w/w). Since then, however, successful separations have been achieved using nitric acid at about the

(1) H. H. Willard and E. W. Goodspeed, IND. ENG. CHEM., ANAL. ED., **8**, 414 (1936).