basis, so that 1 mole of theophylline is 1 equivalent weight. An alternative method would be to use a measured excess of standard solution of silver nitrate to react with the sample. Bring the mixture up to a specified volume, filter, and collect an aliquot of the filtrate to determine the amount of unreacted silver nitrate by titration with ammonium thiocyanate, as is done in the assay of aminophylline for the theophylline content. Another alternative would be to titrate the hydrogen ion displaced by the silver ion with standard base. These methods however are being replaced with the newer nonaqueous titrimetric method.

Table 18. Substances Assayed by Direct Titration with Ammonium Thiocyanate

Substance	mEq. Gm	Official requirement, percent*
U.S.P.		
Aminophylline suppositories	0.22823	$C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$ = 90 to 110 l.a.
Meralluride	0.18017	$C_7H_8N_4O_2$ = 94 to 106 l.a.
Meralluride injection	0.19817	$C_7H_8N_4O_2 \cdot H_2O$ = 95 to 105 l.a.
Mercuric acetate (R)	0.15934	$Hg(C_2H_3O_2)_2$ = 98
Mercuric nitrate (R)*	0.16230	$Hg(NO_3)_2$ = 99
Mercuric sulfate (R)*	0.10030	Hg = 67 to 67.5
Mercury, ammoniated	0.12604	$HgNH_2Cl$ = 98
Silver nitrate	0.16987	$AgNO_3$ = 99.8
Silver nitrate ophthalmic solution	0.16987	$AgNO_3$ = 0.95 to 1.05 *W/W*
Silver nitrate, toughened	0.16987	$AgNO_3$ = 94.5
Sodium mercaptomerin	0.30300	$C_{16}H_{25}HgNNa_2O_6S$ = 95 to 105
Sodium mercaptomerin, sterile	0.30300	$C_{16}H_{25}HgNNa_2O_6S$ = 95 to 105
N.F.		
Mercuric oxide, yellow	0.10830	HgO = 99.5
Mercuric oxide ointment, yellow	0.10830	HgO = 0.9 to 1.1
Mercurophylline	0.25497	$C_{14}H_{24}HgNNaO_5$ = 94 to 106
Mercurophylline	0.18017	$C_7H_8N_4O_2$ = 94 to 106
Mercurophylline injection	0.25497	$C_{14}H_{24}HgNNaO_5$ = 94 to 106 l.a.
Mercurophylline injection	0.18017	$C_7H_8N_4O_2$ = 94 to 106 l.a.
Mercurophylline tablets	0.25497	$C_{14}H_{24}HgNNaO_5$ = 90 to 110 l.a.
Mercurophylline tablets	0.18017	$C_7H_8N_4O_2$ = 90 to 110 l.a.
Nitromersol	0.17585	$C_7H_5HgNO_3$ = 98
Nitromersol solution	0.17585	$C_7H_6HgNO_3$ = 0.18 to 0.22 *W/V*
Nitromersol tincture	0.17585	$C_7H_5HgNO_3$ = 0.45 to 0.55 *W/V*
Phenylmercuric nitrate	0.10032	Hg = 62.75 to 63.50
Silver nitrate solution, ammoniacal	0.10788	Ag = 28.5 to 30.5 *W/W*
Silver protein, mild	0.10788	Ag = 19 to 23
Thimerosal	0.20240	$C_9H_9HgNaO_2S$ = 97 to 101
Thimerosal solution	0.20240	$C_9H_9HgNaO_2S$ = 0.095 to 0.105 *W/V*
Thimerosal tincture	0.20240	$C_9H_9HgNaO_2S$ = 0.095 to 0.105 *W/V*

* = ferric nitrate indicator.
l.a. = labeled amount; (R) = reagent.

The use of dichlorofluorescein in direct titrations with standard silver nitrate is exemplified in the following exercise.

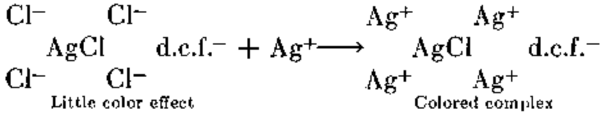
Exercise 36

Object. Assay of Azacyclonol Hydrochloride.

Materials Required. 500 mg of Azacyclonol Hydrochloride
Dichlorofluorescein T.S.
0.1 *N* silver nitrate

Procedure. "Dissolve about 500 mg. of Azacyclonol Hydrochloride, previously dried at 105° for 2 hours and accurately weighed, in a mixture of 40 ml. of alcohol and 20 ml. of water, add dichlorofluorescein T.S., and titrate with 0.1 *N* silver nitrate. Each milliliter of 0.1 *N* silver nitrate is equivalent of 30.38 mg. of $C_{18}H_{21}NO \cdot HCl$."

Dichlorofluorescein, like the other adsorptive indicators, is a weak organic acid, and the extent of its ionization is dependent on the pH of the titrated solution. In highly acidic solutions where the pH is lower than 4, the anions present are so few as to make the end point very faint. The pH of a 1 in 200 solution of azacyclonol is between 5 and 7. Alcohol is used in the assay to help keep the precipitated silver chloride in a colloidal state and thus give sharpness to the color change which occurs on the precipitate surface.



$$\begin{array}{ccc} \text{Cl}^- & & \text{Cl}^- \\ & \text{AgCl} & \\ \text{Cl}^- & & \text{Cl}^- \\ \multicolumn{3}{c}{\text{Little color effect}} \end{array} \quad \text{d.c.f.}^- + \text{Ag}^+ \longrightarrow \begin{array}{ccc} \text{Ag}^+ & & \text{Ag}^+ \\ & \text{AgCl} & \\ \text{Ag}^+ & & \text{Ag}^+ \\ \multicolumn{3}{c}{\text{Colored complex}} \end{array} \quad \text{d.c.f.}^-$$

A color change does not take place during the titration until the Ag^+ is in excess, at which time the colloidal silver chloride particles bear a positive charge because of a primary adsorption layer of silver cations. A secondary adsorption of the indicator, dichlorofluorescein, anions results in a change in the electron distribution of the anions and a concomitant change in color. Before the end point is reached, halide ions are primarily adsorbed, and the resulting negatively charged particles repel the adsorption of the indicator anions.

Exercise 37

Object. Assay of Sodium Cyanide reagent.

Materials Required. 400 mg of Sodium Cyanide
Potassium iodide solution (1 in 10)
1 ml of stronger ammonia water
0.1 *N* silver nitrate

Procedure. "Weigh accurately about 400 mg., and dissolve in 30 ml. of water. Add 4 drops of potassium iodide solution (1 in 10) and 1 ml. of stronger ammonia water, and titrate with 0.1 *N* silver nitrate to a slight, permanent turbidity: each milliliter

TABLE 20. SUBSTANCES ASSAYED BY RESIDUAL TITRATION OF SILVER NITRATE WITH AMMONIUM THIOCYANATE

Substances	mEq, Gm	Official requirement, percent
U.S.P.		
Acetylcholine chloride (R)	0.03545	Cl = 19.3 to 19.8
Aminophylline	0.18018	$C_7H_8N_4O_2$ = 84 to 86
Aminophylline injection	0.22823	$C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$ = 93 to 107 l.a.
Aminophylline tablets	0.22823	$C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$ = 93 to 107 l.a.
Ammonium chloride	0.05349	NH_4Cl = 99.5
Ammonium sulfide solution (R)	0.03408	$(NH_4)_2S$ = 16 to 20
Benzene hexachloride, gamma	0.09694	$C_6H_6Cl_6$ = 99
Bethanechol chloride	0.03545	Cl = 17.7 to 18.3
Calcium chloride (R)	0.05549	$CaCl_2$ = 74 to 78
Calcium chloride anhydrous (R)	0.05549	$CaCl_2$ = 96
Chlorobutanol	0.05916	$C_4H_7Cl_3O$ = 98
Chlorophenothane	0.07089	$C_{14}H_9Cl_5$ = 96 to 102
Dextrose and sodium chloride injection	0.05844	NaCl = 95 to 105 l.a.
Dibucaine hydrochloride	0.03545	Cl = 9.1 to 9.5
Dimenhydrinate	0.21462	$C_7H_7ClN_4O_2$ = 44 to 47
Dimenhydrinate tablets	0.46998	$C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$ = 95 to 105 l.a.
Hydriodic acid (R)	0.12791	HI = 47
Methyl iodide (R)	0.14194	CH_3I = 98.5
Potassium chloride	0.07456	KCl = 99
Potassium chloride injection	0.07456	KCl = 95 to 105 l.a.
Potassium chloride tablets	0.07456	KCl = 95 to 105 l.a.
Pyridoxal hydrochloride (R)	0.03545	Cl = 17.2 to 17.7
Pyridoxamine dihydrochloride (R)	0.03545	Cl = 29.1 to 29.6
Ringer's injection	0.05844	NaCl = 0.82 to 0.90 *W/V*
Ringer's injection, lactated	0.05844	NaCl = 0.57 to 0.63 *W/V*
Sodium chloride	0.05844	NaCl = 99.5
Sodium chloride injection	0.05844	NaCl = 0.85 to 0.95 *W/V*
Sodium chloride solution	0.05844	NaCl = 0.85 to 0.95 *W/V*
Sodium chloride tablets	0.05844	NaCl = 95 to 105 l.a.
Sodium sulfobromophthalein	0.07991	Br = 36 to 39
Tetramethylammonium bromide (R)	0.15406	$(CH_3)_4NBr$ = 98
Tetramethylammonium chloride (R)	0.10960	$(CH_3)_4NCl$ = 98
Thiourea (R)	0.03806	$(NH_2)_2CS$ = 99
Trihexyphenidyl hydrochloride	0.03545	Cl = 10 to 10.7
Zinc chloride (R)	0.06814	$ZnCl_2$ = 95
N.F.		
Ammonium bromide	0.09795	NH_4BR = 99 to 101
Bromides elixir, three	0.10590	Total bromides = 23 to 25 *W/V*
Bromides tablets, three	0.07991	Br = 70 to 81 total bromides
Chlorotrianisene	0.38088	$C_{23}H_{21}ClO_3$ = 95 to 105
Fructose and sodium chloride injection	0.05844	NaCl = 95 to 105 l.a.

0.05 *M* disodium ethylenediaminetetraacetate, 20 ml. of ammonium acetate buffer T.S., 50 ml. of alcohol, and 2 ml. of dithizone T.S. Titrate the solution with 0.05 *M* zinc sulfate until the color changes from green-violet to rose-pink. Record the volume of 0.05 *M* disodium ethylenediaminetetraacetate consumed."

Aluminum and zinc have about the same stability constants and also behave similarly at about pH 4. The faint turbidity produced by the ammonia water is insoluble aluminum hydroxide. It is much more insoluble than zinc hydroxide. The acid redissolves it; then the pH of the titration mixture is controlled by the ammonium acetate buffer. Aluminum hydroxide would precipitate at a pH more alkaline than 4.5. Alcohol is used to solubilize the metal-indicator complex, which is insoluble in water. Alcohol will also increase the stability of the metal-EDTA complex. The pH for the transition stage of the dithizone indicator is 4.6.

3. "*Determination of zinc*—Pipet 25 ml. of *Assay Preparation* into a 250-ml. beaker and add 10 ml. of stronger ammonia water, 5 ml. of triethanolamine solution (3 in 10), and 5 ml. of ammonia–ammonium chloride buffer T.S. Cool the mixture to below 5° in a cooling bath, add 0.1 ml. eriochrome black T.S., and titrate with 0.05 *M* disodium ethylenediaminetetraacetate to a blue color, maintaining the temperature of the solution below 5° during the titration. Record the volume of 0.05 *M* disodium ethylenediaminetetraacetate consumed by the zinc present."

The titration of the zinc alone in the presence of the aluminum is accomplished using the masking technique. When the sample is treated with the stronger ammonia water, triethanolamine, and the ammonia-ammonium chloride buffer, the resulting pH is about 9 to 11, and the temperature at below 5° highly favors the formation of triethanolamine-aluminum complex which will not compete with the zinc for the EDTA nor for the eriochrome indicator. EDTA reacts with all metals in a 1:1 ratio. The volume of titrant used in the zinc determination subtracted from the volume of EDTA consumed by the aluminum and zinc titration is the volume equivalent to just the aluminum present in the 25-ml aliquot or one-tenth of the original sample weight. The atomic weight of Al is 26.9815, and 1 ml of a $\frac{1}{20}$ *M* solution would be equal to $26.9815/20 \times 1{,}000$ or 1.349 mg of Al.

Questions and Problems

1. Define complexation and chelation with simple schematic equations.

2. What volume of 0.05 *M* EDTA solution would be equivalent to 100 ml of a 0.025 *M* solution of titratable divalent metal cations? Trivalent? Tetravalent?

3. How could EDTA be used to determine quantitatively such anions as sulfate and arsenate?

4. How many grams of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2HO$) is required to prepare 250 ml of a 0.025 *M* solution? What would the titer of this solution be in terms of $CaCl_2$; $2H_2O$; $MgSO_4$; and $ZnCl_2$?

5. A 200-mg sample of pure calcium carbonate was acidified and dissolved in 500 ml of solution. A 50-ml sample required 25.0 ml of an EDTA solution for titration. Find the molarity of the EDTA solution.