

Figure 3. Titration of barium with 0.001610M EDTA

Preliminary work in the application of the colored complex to a spectrophotometric determination of barium indicated that color fading caused serious errors. The fading was apparently due to the conversion of the phenolphthalein part of the indicator molecule to a colorless form in alkaline solution. However, fading was no problem in the titration itself, which could be carried out in a reasonable length of time. The titration can be easily completed within 30 minutes.

The photometric method is recommended for the determination of 0.05 to 12 mg. of barium. Larger quantities can be determined readily by the visual method (1).

ACKNOWLEDGMENT

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Table III. Photometric Titration of Barium

Molarity of EDTA	Barium Taken, Mg.	Barium Found (Difference), Mg.
0.01340	2.48	+0.03, +0.06, +0.03
	6.20	-0.02
	9.92	+0.02, +0.01, +0.01
	12.40	+0.01, ±0.00, -0.04
0.001610	0.050	+0.007, ±0.000, -0.009
	0.099	+0.002, +0.001, +0.003
	0.149	-0.001, -0.004, -0.004
	0.248	+0.002, +0.002, +0.002
	0.496	±0.000, +0.003, +0.034
	0.99	-0.01, -0.01, ±0.00
	1.24	±0.00, ±0.00, -0.01
	1.98	-0.01, -0.01, -0.01

LITERATURE CITED

- (1) Anderegg, G., Flaschka, H., Sallman, R., Schwarzenbach, G., *Helv. Chim. Acta* **37**, 113 (1954).
- (2) Goddu, R. F., Hume, D. N., *ANAL. CHEM.* **22**, 1314 (1950).
- (3) Manns, T. J., Reschovsky, M. Y., Certa, A. J., *Ibid.*, **24**, 909 (1952).
- (4) Rowley, K., Stoenner, R. W., Gordon, L., *Ibid.*, **28**, 136 (1956).
- (5) Schwarzenbach, G., "Die Komplexometrische Titration," Ferdinand Enke Verlag, Stuttgart, 1955.

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Effect of Iron on Determination of Tin in Brass and Bronze Application of Radioisotope Techniques

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Radioisotope tracer techniques have been utilized for quantitative evaluation of the effect of iron on the nitric acid precipitation of tin in brass and bronze. Two digestion temperatures were studied, with iron-59 and tin-113 used as tracers. The degree of accuracy of the gravimetric procedure, the amount of iron coprecipitated, and the effect of the presence of iron on the solubility of the metastannic acid precipitate were determined. Coprecipitation of iron is shown to follow Freundlich's adsorption equation.

TIN in brass and bronze can be determined (7, 11) by digesting with nitric acid, filtering, igniting the resultant metastannic acid, and weighing as stannic oxide. Two significant sources of error are inherent in this method: Iron, copper, and zinc coprecipitate, and iron increases the solubility of the metastannic acid (7). Detailed quantitative studies of these interferences have not been reported. The purpose of the present study is to provide more extensive data by utilizing the improved sensitivity of radioisotope tracer techniques.

Earlier investigators recognized the existence of these errors in the determination of tin. In 1922 Meyer (8) experienced difficulty in the quantitative recovery of tin in alloys containing iron in excess of 0.5%. Lundell and Hoffman (7) state, "The precipitation of tin as metastannic acid

is not complete if the alloy contains much iron, say above 0.25%." They further maintain that the metastannic acid that separates from solution is always contaminated by compounds of iron, copper, and zinc. If the alloy contains antimony, phosphorus, silicon, or arsenic, these are also found in the precipitate, often quantitatively (2). Willard and Furman (13) state that the volumetric determination of tin is capable of greater accuracy than the gravimetric determination because the coprecipitation error is eliminated. The present paper applies radioisotope tracer techniques to investigate the gravimetric procedure, to evaluate quantitatively the effect of iron on the precipitation of tin as metastannic acid in brass and bronze, and to elucidate its mechanism.

Various investigators differ regarding the digestion temperature of the metastannic acid precipitate. Hillebrand and Lundell (4) recommend digestion at boiling temperatures to help minimize the effects of the colloidal character and solubility of the precipitate. Kolthoff and Sandell (6) state that the digestion should be carried out at 90° to 100° C. to ensure a more quantitative separation of tin. Norwitz, Boyd, and Bachtiger (9) suggest that the acid digestion of the alloy be maintained at a boil for low-tin samples and at 95° C. for high-tin samples. Goldberg (3) advocates a nitric acid digestion at a boiling temperature for manganese bronzes.

It was desirable to resolve this problem of digestion temperature with respect to the two sources of error by investigating two digestion temperatures with samples of varied iron contents. This approach is in line with the research of Kolthoff and Moltzan

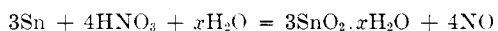


Table I. NBS Standards and Composites

Sample	Wt., Gram	Tin, %
Bronze 52c	1.0	7.85 ± 0.01
Bronze 52c plus brass 37d	0.5 each	4.410 ± 0.007
Brass 37d	1.0	0.970 ± 0.004

Composition of Standard Alloys, %

Sample	Cu	Zn	Sn	Pb	Ni	Fe
Bronze 52c	82.25	2.12	7.85	0.011	0.76	0.004
Brass 37d	70.78	26.65	0.97	0.94	0.58	0.076

Table II. Data for Below-Boiling Digestion

Fe, %	Sn ^a , Uncorr., %	Cocprecipitants, %			Sn, Corr., %	Recovery, Corr.
		Fe ^b	Cu ^c	Zn ^c		
Sn = 7.85 ± 0.01%						
0.10	7.81	0.068	0.06	0.006	7.68	97.8
0.30	7.92	0.163	0.05	0.004	7.70	98.1
0.55	7.93	0.229	0.04	0.006	7.65	97.5
1.05	7.96	0.346	0.03	0.001	7.58	96.6
2.05	7.90	0.442	0.03	0.001	7.43	94.6
4.05	7.33	0.506	0.01	0.004	6.81	86.8
Sn = 4.41 ± 0.01%						
0.10	4.52	0.057	0.03	0.012	4.42	100.2
0.30	4.62	0.144	0.02	0.005	4.45	100.9
0.55	4.65	0.192	0.02	0.009	4.43	100.4
1.05	4.60	0.198	0.03	0.014	4.36	98.9
2.05	4.46	0.288	0.02	0.010	4.14	93.9
4.05	3.82	0.323	0.01	0.003	3.48	78.9
Sn = 0.970 ± 0.004%						
0.09	1.036	0.018	0.008	0.006	1.014	104.5
0.39	1.039	0.046	0.010	0.007	0.976	100.6
0.59	1.038	0.055	0.006	0.006	0.971	100.0
1.09	0.987	0.055	0.010	0.006	0.916	94.4
2.09	0.830	0.075	0.010	0.005	0.740	76.3
4.09	0.462	0.100	0.009	0.006	0.347	35.8

^a Gravimetric assay.^b Radiometric assay.^c Polarographic assay.

(5), who have demonstrated that adsorption is not a simple process, but often the result of several variables, among which are temperature and concentration of the ions involved.

APPARATUS

The radioactive counting equipment consisted of a shielded scintillation counter (Model DS-3, Nuclear Instrument & Chemical Corp., Chicago, Ill.) with a Harshaw well-type, sodium iodide, thallium-activated crystal. The crystal was coupled to a DuMont 6292 (K1186) phototube. This is a ten-stage multiplier phototube of the end-window type with a spectral response predominantly in the visible region. Dow Corning DC 200 silicone fluid was used for optical contact between crystal and phototube. The pulses were fed through a preamplifier to a Model 182 Ampli-count scaler (Nuclear Instrument & Chemical Corp.) operating at 1900 volts at a 100-mv. threshold. The samples were contained in 16 × 150 mm. glass test tubes and inserted in the circular well of the sodium iodide crystal.

REAGENTS AND RADIOISOTOPE STANDARDS

Standard inert ferric nitrate solution containing 5 mg. of iron per ml. was prepared by dissolving 1 gram of pure iron wire (99.85% iron) in 20 ml. of 70% nitric acid and 5 ml. of distilled water. The solution was boiled gently for 20 minutes, cooled, and diluted to exactly 200 ml. with 70% nitric acid.

Standard radioactive ferric nitrate solution was prepared by dissolving 1 gram of pure iron wire in 20 ml. of 1 to 1 nitric acid, boiling gently for 10 minutes, and cooling. To the resultant solution was added 0.2 ml. of radioactive ferric chloride solution containing an initial activity of 1 mc. per ml., with a specific activity 3.39 mc. per mg. of iron. The solution was made slightly alkaline with ammonium hydroxide, boiled gently, filtered while hot through a No. 40 Whatman filter paper, and washed with hot ammonium hydroxide (1%). The precipitate was dissolved with a minimum of 1 to 1 nitric acid. The solution was evaporated to approximately 10 ml., cooled, and adjusted to exactly 200 ml. with 70% nitric acid. The resultant solution contained 5 mg. of iron and 1 μ c., initially, of radioactivity per ml.

Standard radioactive tin chloride solution was prepared in a 100-ml. quantity to contain 0.2 ml. of the high specific activity Atomic Energy Commission processed isotope, Sn-113P, as tin chloride in 6N hydrochloric acid. The resultant solution contained 0.39 mg. of tin and 2 μ c. of initial radioactivity per ml.

In order to obviate the necessary corrections in radiation measurements caused by the decay of the radioisotopes, aliquots of standard solutions were measured concurrently with the analytical samples.

PROCEDURE

To each of seven 1-gram samples in a set (NBS standards and composites, Table I), contained in 300-ml. beakers, was added 0.10 ml. of standard radioactive ferric nitrate solution. Increments of inert ferric nitrate standard solution were added corresponding to a range from 0.05 to 4.05% iron in the sample. The acid content of the ferric nitrate solution was supplemented with 70% nitric acid so that each beaker contained a total of 12 ml. of the acid. Approximately 25 ml. of hot distilled water was added and the solutions were boiled gently for 10 minutes to effect complete solution and to expel nitric oxide fumes. This was followed by the addition of approximately 60 ml. of hot distilled water to each beaker, with subsequent digestion for 30 minutes at 80° ± 5° C. This is referred to as the below-boiling procedure. A similar series of seven samples was treated as described above, except that the solutions were diluted to approximately 150 ml. and boiled gently for 30 minutes at 102° C. This is referred to as the boiling procedure. The solutions were filtered while hot through a No. 40 Whatman filter paper containing a small quantity of paper pulp. The precipitates were washed thoroughly with hot 1% nitric acid and transferred to tared porcelain crucibles. The filter papers were carefully charred and the precipitates were ignited at 810° C. for 1 hour under oxidizing conditions. The resulting oxides were cooled and weighed.

The following procedures were used to determine the amount of oxide impurities of iron, copper, and zinc coprecipitated with the metastannic acid.

Iron. The ignited residues were quantitatively transferred

Table III. Data for Boiling Digestion

(102° C.)

Fe, %	Sn ^a , Uncorr., %	Cociprecipitants, %			Sn, Corr., %	Recovery, Corr.
		Fe ^b	Cu ^c	Zn ^c		
Sn = 7.85 ± 0.01%						
0.10	7.81	0.082	0.044	0.004	7.68	97.8
0.30	8.02	0.201	0.040	0.004	7.77	99.0
0.55	8.10	0.284	0.016	0.001	7.80	99.4
1.05	8.17	0.401	0.028	0.001	7.74	98.6
2.05	8.25	0.476	0.024	0.006	7.74	98.6
4.05	7.74	0.580	0.012	0.004	7.14	91.0
Sn = 4.410 ± 0.007%						
0.10	4.51	0.071	0.026	0.012	4.40	99.8
0.30	4.59	0.154	0.018	0.009	4.41	100.0
0.55	4.56	0.196	0.018	0.007	4.34	98.4
1.05	4.51	0.255	0.020	0.012	4.22	95.7
2.05	4.25	0.307	0.018	0.006	3.92	94.9
4.05	3.69	0.379	0.015	0.008	3.29	74.6
Sn = 0.970 ± 0.004%						
0.09	1.013	0.018	0.008	0.006	0.981	101.1
0.39	1.042	0.044	0.006	0.009	0.983	101.3
0.59	1.001	0.048	0.006	0.006	0.941	97.0
1.09	0.973	0.059	0.010	0.007	0.897	92.2
2.09	0.822	0.069	0.005	0.006	0.742	76.5
4.09	0.581	0.095	0.008	0.007	0.471	48.5

^a Gravimetric assay.^b Radiometric assay.^c Polarographic assay.**Table IV. Data for Boiling Digestion Using Tin-113 as Tracer with Brass 37d**

Fe, %	Sn, C.P.M.			Sn Recovery, %	Solubility Loss, %
	Ppt.	Filtrate	Total		
0.09	27,550	310	27,860	95.9	1.1
0.39	26,890	360	27,250	95.7	1.3
0.59	26,480	980	27,460	93.0	3.6
1.09	24,410	3,200	27,610	85.8	11.5
2.32	21,830	5,880	27,710	76.4	21.1
4.09	14,770	12,440	27,210	52.7	45.7

to 16 × 150 mm. glass test tubes which were placed in a scintillation well counter for measurement of the gamma radiation. Radiation emitted by radioactive iron-59 consists of two beta rays of moderate energy and two gamma rays of high energy (1.3 and 1.1 m.e.v.) decaying with a half life of 46.3 days. In order to obviate the need for self-absorption and Geiger dead time corrections associated with moderate beta counting, the iron content of the samples was determined solely by the gamma emission with the beta rays effectively screened out. The per cent radioactive iron present was determined by comparing the radiation count of the oxide residue to the count obtained with 0.10 ml. of standard radioactive ferric nitrate. This eliminated corrections for decay.

Copper and Zinc. In another series of experiments the metastannic acid precipitates obtained were not ignited but were redissolved by fuming with 96% sulfuric acid, adding 70% nitric acid as necessary to destroy the filter paper. Tin was volatilized by repeated fumings with hydrobromic acid (48.5%), and the copper and zinc were determined by polarographic methods.

Corrections for the coprecipitated impurities in the ignited residue were made by applying the following formula:

$$\% \text{ Sn (corr.)} = \left[A - \left(\frac{B \times C \times 1.42}{D} + E + F \right) \right] 0.7877 \times 100$$

where

- A = SnO₂ residue in grams
- B = activity of residue (c.p.m.)
- C = iron (iron content of standard bronze + added iron)
- D = activity of iron-59 standard (c.p.m.)
- E = CuO in residue
- F = ZnO in residue
- 1.42 = Fe₂O₃/Fe ratio
- 0.7877 = Sn/SnO₂ ratio

In an analogous procedure employing boiling digestion, aliquots of a standard solution of radioactive tin chloride, equivalent to 0.08 mg. of radioactive tin, were added to each 1-gram sample of brass 37d. Radioactive tin containing 0.4 μc. gave a total activity of 27,000 counts per minute ±3%. The iron concentrations in this procedure were the same as above but were not radioactive.

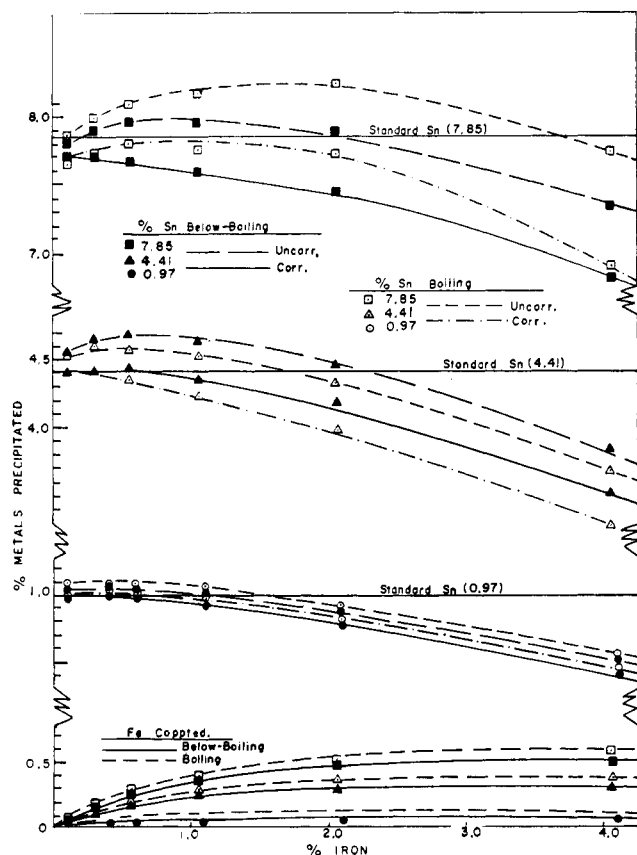


Figure 1. Effect of iron on precipitation of metastannic acid

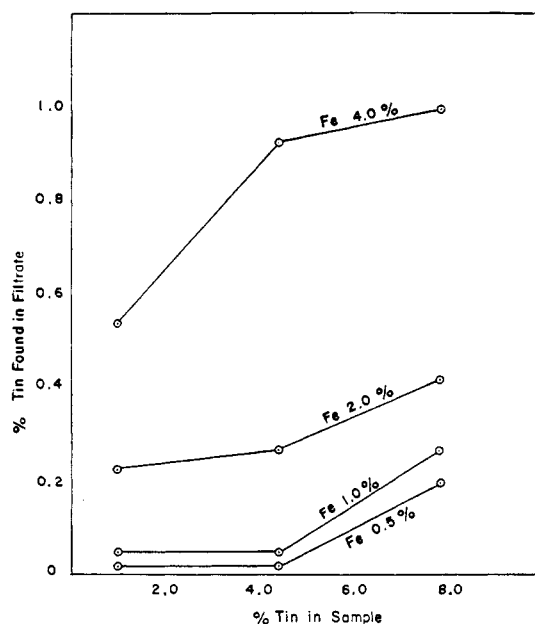


Figure 2. Relationship between tin present and tin found in filtrate for different iron contents

Below-boiling digestion

DISCUSSION

The quantitative data obtained confirmed earlier reports that the precipitation of tin as metastannic acid is subject to the iron-induced, partially compensating errors of coprecipitation and solubility. The detailed data are presented in Tables II and III, and Figure 1.

For purposes of routine gravimetric analysis with no corrections applied for adsorbed elements, the data reveal that the below-boiling digestion is superior to the boiling digestion for samples containing 7.85% tin and equivalent for those containing 4.41 and 0.97% tin.

Further confirmatory evidence of the solubility effect due to iron are the results obtained with the use of tin-113 as tracer. Data are presented in Table IV showing the amounts of metastannic acid contained in the filtrate when NBS standard brass 37d was used at boiling digestion.

The relationship between the tin present and tin found in the filtrate for different iron contents at below-boiling temperatures is illustrated in Figure 2. It is evident that the amount of tin dissolved increases or remains constant with the tin content of the sample for all iron and tin ranges investigated.

Figure 3 is an enlargement of the lower part of Figure 1, showing a sharp increase in adsorption up to 1% iron and a gradual increase from 1 to 4% iron.

The below-boiling digestion curves of Figure 1 are linear in the range from 0.5 to 2.0% iron. The deviation from 100% recovery is due to the solubility of the metastannic acid precipitate. It is, therefore, possible to derive a solubility correction equation of the form

$$y = ax + b \quad (1)$$

where y is per cent tin (corrected for adsorbates), x is per cent iron in the alloy, and a and b are constants. Equations for the tin ranges studied, derived empirically, are listed as follows:

$$0.97\% \text{ Sn: } y = -0.15x + 1.07 \quad (2)$$

$$4.41\% \text{ Sn: } y = -0.15x + 4.47 \quad (3)$$

$$7.85\% \text{ Sn: } y = -0.15x + 7.75 \quad (4)$$

If K is assumed to represent the NBS value of tin in the bronze, then the tin correction may be expressed as:

$$\text{Sn (corr.)} = K - (ax + b) \quad (5)$$

At each tin level the following equations are derived by substituting the respective constants for a and b of Equations 2 to 4 in Equation 5:

$$0.99\% \text{ Sn (corr.)} = 0.15x - 0.10 \quad (6)$$

$$4.41\% \text{ Sn (corr.)} = 0.15x - 0.06 \quad (7)$$

$$7.85\% \text{ Sn (corr.)} = 0.15x + 1.00 \quad (8)$$

Equations 6 and 7 show similar values for a and b within experimental error and are averaged to produce a general equation useful in the analysis of tin with iron contents up to 4.4% as follows:

$$\text{Sn (corr.)} = 0.15x - 0.08 \quad (9)$$

Hence

$$K' = y + 0.15x - 0.08 \quad (10)$$

where K' is the calculated per cent tin in the sample.

In Table V NBS values for tin are compared with values obtained when applying Equation 10 to the experimental results.

Table V. Comparison of Calculated Tin with NBS Values

Tin, NBS Value, %	Iron Present (x)	Tin, %	
		Corrected for adsorbates (y)	Calcd. ^a (K')
4.41	0.10	4.42	4.36
	0.30	4.45	4.42
	0.55	4.43	4.43
	1.05	4.36	4.44
	2.05	4.14	4.38
0.970	0.09	1.014	0.94
	0.39	0.976	0.96
	0.59	0.971	0.98
	1.09	0.916	1.01
	2.09	0.740	0.98

^a $K' = y + 0.15x - 0.08$.

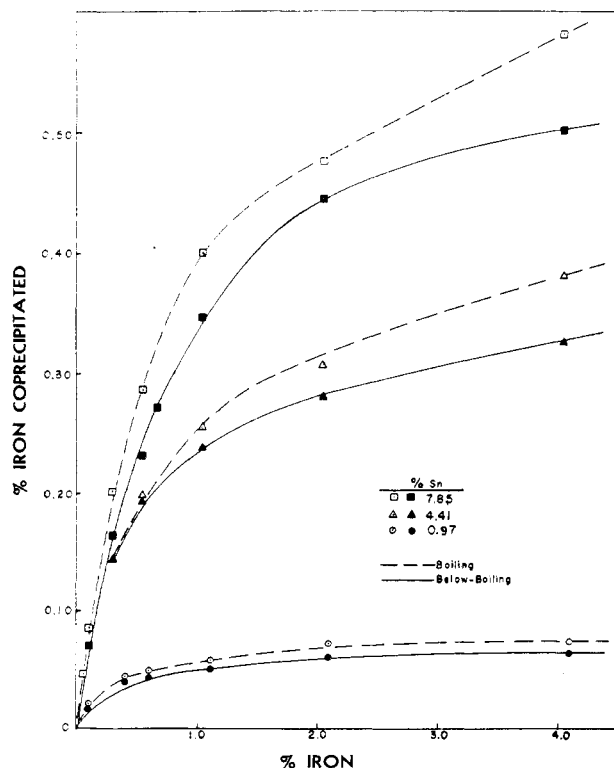


Figure 3. Relationship between iron content and amount of iron coprecipitated with metastannic acid

The average deviation is 0.025%, which is satisfactory for routine analysis.

Weiser (12) states that hydrous stannic oxide does not precipitate in the usual way from a nitric acid solution of tin containing iron. Hydrous stannic oxide is peptized by ferric nitrate or a mixture of ferric nitrate and nitric acid and coagulation does not take place at below-boiling or boiling temperatures. The colloid stabilizing action of the strongly adsorbed ferric ion contributes to the solubility of the metastannic acid precipitate. The present investigation has shown quantitatively that there is a definite solubility of the hydrous stannic oxide due to the presence of iron above 0.5%, both at boiling and below-boiling temperatures. Zsigmondy (14) has suggested that stannic oxide reacts with hydrogen ions to give positively charged stannic ions. In the analytical procedure for tin, stannic oxide peptized in the nitric acid medium may give the positively charged stannic ion, $(\text{SnO}_2 \cdot \text{H}_2\text{O}) \text{Sn}^{++}$. Ferric oxide sols have been obtained by Powis (10), and in the nitric acid medium ferric oxide behaves similarly. The structure of the colloidal unit may be written as $(\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}) \text{Fe}^{+++}$. The similarity of these two structures makes more likely the entrainment of the ferric ion into the structure of the colloidal positively charged stannic ion, and contributes to the solubility of metastannic acid.

The present work shows that the adsorption of interfering ions is greater at boiling temperatures for the 8% tin and practically equivalent or slightly greater at the 4 and 1% tin levels. This phenomenon apparently does not conform to theoretical considerations, as indicated by the Gibbs equation:

$$a = - \frac{c}{RT} \frac{d}{dc}$$

in which a is moles absorbed per sq. cm. of interface, dc is change in concentration of solute, and d is change in interfacial tension. This equation indicates that the increase in temperature should result in decreased adsorption, due in part to the fact that the solubility of most impurities is increased by rise of temperature. Actually an increase in digestion temperature may permit an exchange and penetration of the interfering ions into the sub-microscopic capillary structure of the colloidal metastannic acid, with development of an effective interface resulting in a greater degree of physical and chemical adsorption. The exchange of ions may be possible because of the similarity of the colloidal stannic oxide ion $(\text{SnO}_2 \cdot \text{H}_2\text{O}) \text{Sn}^{++}$ and ferric oxide ion $(\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}) \text{Fe}^{+++}$.

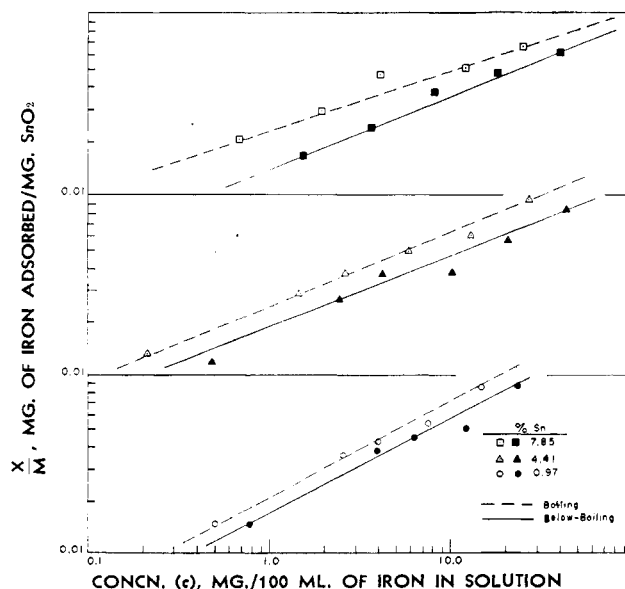


Figure 4. Freundlich's adsorption isotherm of effect of iron on metastannic acid

The data presented point to the possibility that the coprecipitation of iron by metastannic acid is governed by the Freundlich equation, $\frac{x}{m} = Kc^{1/n}$ (1), where x is milligrams of adsorbate (iron), m is milligrams of stannic oxide, and c is concentration of iron (mg. per ml.) remaining unadsorbed at equilibrium. The constants K and $1/n$ are particular to the system. A plot of $\frac{x}{m}$ against c on logarithmic paper resulted in a straight line, with a slope equal to $1/n$ as illustrated in Figure 4 for all tin levels studied. This indicated that the mechanism of coprecipitation of iron can be interpreted as a true Freundlich adsorption process.

Radioisotope techniques, utilizing iron-59 and tin-113 as tracer elements, provide a rapid, sensitive, and quantitative method for evaluating the interference of iron in the gravimetric determination of tin in brass and bronze alloys. The quantitative data obtained by this means confirm earlier reports of the dual phenomenon of solubility and coprecipitation exerted by iron on metastannic acid.

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LITERATURE CITED

- (1) Freundlich, H., Schucht, H., *Z. physik. Chem.* **85**, 660 (1913).
- (2) Gates, O., Silverman, L., *IND. ENG. CHEM., ANAL. ED.* **11**, 370 (1939).
- (3) Goldberg, C., *Iron Age* **166**, 84 (1950).
- (4) Hillebrand, W. F., Lundell, G. E. F., "Applied Inorganic Analysis," p. 236, Wiley, New York, 1944.
- (5) Kolthoff, I. M., Moltzan, D. R., *Chem. Revs.* **17**, 293 (1935).
- (6) Kolthoff, I. M., Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," p. 698, Macmillan, New York, 1948.
- (7) Lundell, G. E. F., Hoffman, J. I., "Outlines of Methods of Chemical Analysis," pp. 47, 210, Wiley, New York, 1945.
- (8) Meyer, A., *Chem.-Ztg.* **46**, 209 (1922).
- (9) Norwitz, G., Boyd, T. F., Bachtiger, F., *ANAL. CHEM.* **21**, 1291 (1949).
- (10) Powis, F., *Z. physik. Chem.* **89**, 91-110 (1914).
- (11) Rodden, C. J., "Analytical Chemistry of the Manhattan Project," vol. VIII-1, p. 375, McGraw-Hill, New York, 1950.
- (12) Weiser, H., *J. Phys. Chem.* **26**, 681 (1922).
- (13) Willard, H. H., Furman, N. H., "Elementary Quantitative Analysis," p. 485, Van Nostrand, New York, 1940.
- (14) Zsigmondy, R., *Z. anorg. Chem.* **49**, 697 (1901).

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Some Theoretical and Experimental Considerations of pH Gradient Elution Analysis

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In order to aid in the selection of optimum conditions for buffered pH gradient elution of chromatographic columns, some general equations relating pH change to effluent volume were derived. The equations are applicable to pH changes produced by the addition of one buffer to another or by the addition of acid (or base) to a buffer under a variety of different experimental conditions. With the proper choice of conditions nearly any gradient can be obtained. Superimposed concentration gradients were also considered.

MAINTENANCE of a sorption gradient on a chromatographic column by means of a continuous change in the composition of the eluting solution is referred to as gradient elution. Sorption gradients were apparently first considered by Tiselius as reported by Synge (17) and later by Strain (16). A theoretical treatment of concentration gradient elution was first developed by Alm, Williams, and Tiselius (2). The method was first used by Donaldson, Tulane, and Marshall (8), Alm (1), and Busch, Hurlbert, and Potter (5). A thorough theoretical discussion of sorption gradients and concentration gradient elution with particular reference to the effect on chromatographic behavior is the subject of a recent paper by Drake (9).

The experimental arrangement for gradient elution consists of a mixing chamber containing a solution of low eluting power and a reservoir of a solution of high eluting power. Solution is delivered continuously from the reservoir to the mixing chamber as the mixture passes to the column. The types of apparatus which have the greatest utility have been discussed by Bock and Ling (3). It is simplest and usually sufficient to employ either a constant volume or an equal level arrangement. In

the former case the mixing chamber is a closed system, with the result that the flow rates into and out of the mixing chamber are equal. In the latter case the mixing chamber and reservoir are set at the same level and connected with a siphon or similar arrangement (14). During the progress of elution, the levels fall together. For vessels of identical size the rate of flow into the mixing chamber will be one half the flow out. For straight-sided but different sized vessels the ratio of the flow rates (f_1/f_2) is simply related to the cross sections (A) by the expression $A_1/(A_1 + A_2)$ where subscript 1 refers to the reservoir and subscript 2 to the mixing chamber. More complicated arrangements are possible (3, 9) but these are sufficiently versatile for the great majority of applications.

The concentration gradients obtained from these simple devices are a function of the composition of the solutions, the volumes, and the relation between the flow rates. Equations relating these variables to concentration changes in the eluent were first derived for constant volume mixers (2, 6, 8) and later extended to systems with variable flow rates (11, 12). Equations have also appeared describing multiple mixers (9) and arrangements where the ratio of the flow rates is continuously variable (3).

These equations are not directly applicable to chromatography employing buffered solutions where the sorption gradient is maintained by a continuous change in pH with, if desired, a constant eluent concentration. Because pH-gradient elution has been shown to have considerable utility (4, 7, 10, 13, 15, 18), it is of interest to discuss some of the general considerations.

THEORY

Considering the volume relationships of an experimental arrangement consisting of a reservoir and mixing chamber where