12
chapter

Traditional Methods for Mineral Analysis

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12.1 INTRODUCTION

This chapter describes traditional methods for analysis of minerals involving titrimetric and colorimetric procedures, and the use of ion selective electrodes. Other traditional methods of mineral analysis include gravimetric titration (i.e., insoluble forms of minerals are precipitated, rinse, dried, and weighed) and redox reactions (i.e., mineral is part of an oxidation–reduction reaction, and product is quantitated). However, these latter two methods will not be covered because they currently are used little in the food industry. The traditional methods that will be described have maintained widespread usage in the food industry despite the development of more modern instrumentation such as atomic absorption spectroscopy and inductively coupled plasma-atomic emission spectroscopy (Chap. 24). Traditional methods generally require chemicals and equipment that are routinely available in an analytical laboratory and are within the experience of most laboratory technicians. Additionally, traditional methods often form the basis for rapid analysis kits (e.g., Quantab® for salt determination) that are increasingly in demand. Procedures for analysis of minerals of major nutritional or food processing concern are used for illustrative purposes. For additional examples of traditional methods refer to references (1–6). Slight modifications of these traditional methods are often needed for specific foodstuffs to minimize interferences or to be in the range of analytical performance. For analytical requirements for specific foods see the *Official Methods of Analysis* of AOAC International (5) and related official methods (6).

12.1.1 Importance of Minerals in the Diet

Calcium, phosphorus, sodium, potassium, magnesium, chlorine, and sulfur make up the dietary macro minerals, those minerals required at more than 100 mg/day by the adult (7–9). An additional ten minerals are required in milli- or microgram quantities per day and are referred to as **trace minerals**. These include iron, iodine, zinc, copper, chromium, manganese, molybdenum, fluoride, selenium, and silica. There is also a group of minerals called **ultra trace minerals**, including vanadium, tin, nickel, arsenic, and boron, that are being investigated for possible biological function, but that currently do not have clearly defined biochemical roles. Some mineral elements have been documented to be **toxic** to the body and should, therefore, be avoided in the diet. These include lead, mercury, cadmium, and aluminum. Essential minerals such as fluoride and selenium also are known to be harmful if consumed in excessive quantities, even though they do have beneficial biochemical functions at proper dietary levels.

The Nutrition Labeling and Education Act of 1990 (NLEA) mandated labeling of **sodium**, **iron**, and **calcium** contents largely because of their important roles in controlling hypertension, preventing anemia, and impeding the development of osteoporosis, respectively (see Fig. 3-1, Chap. 3). The content of these minerals in several foods is shown in Table 12-1. The content of other minerals may be included on the label at the producer's option, although this becomes mandatory if the mineral is the subject of a nutrient claim on the label. Implementation of the NLEA has led to an increased need for more rapid and accurate analysis of minerals and other food components.

12.1.2 Minerals in Food Processing

Minerals are of **nutritional** and **functional** importance, and for that reason their levels need to be known and/or controlled. Some minerals are contained at high levels in natural foodstuffs. For example, milk is a good source of calcium, containing about 300 mg of calcium per 8-ounce cup. However, direct acid cottage cheese is very low in calcium because of the action of the acid causing the calcium bound to the casein to be freed and consequently lost in the whey fraction. Similarly, a large portion of the phosphorus, zinc, manganese, chromium, and copper found in a grain kernel is lost when the bran layer is removed in processing. The enrichment law for flour requires that iron be replaced in white flour to the level at which it occurred naturally in the wheat kernel before removal of the bran.

Fortification of some foods has allowed addition of minerals above levels ever expected naturally. Prepared breakfast cereals often are fortified with minerals such as calcium, iron, and zinc, formerly thought to be limited in the diet. Fortification of salt with iodine has almost eliminated goiter in the USA. In other cases, minerals may be added for functionality. Salt is added for flavor, to modify ionic strength that effects solubilization of protein and other food components, and as a preservative. This increases significantly the sodium content of products such as processed meats, pickles, and processed cheese. Phosphorus may be added as phosphates to increase water-holding capacity. Calcium may be added to promote gelation of proteins and gums.

Water is an integral part of food processing, and **water quality** is a major factor to be considered in the food processing industry. Water is used for washing, rinsing, blanching, cooling, and as an ingredient in formulations. Microbiological safety of water used in food processing is very important. Also important, but generally not appreciated by the consuming public, is the mineral content of water used in food processing.

12-1 table **Mineral Content of Selected Foods**

Food Item	mg/g (Wet Weight Basis) Calcium	Iron	Sodium
Cereals, bread, and pasta			
Rice, brown, long-grain, raw	23	2	7
Corn flakes, plain	3	19	950
White rice, long-grain, regular, real, enriched	28	4	5
Wheat flour, whole-grain	34	4	5
Wheat flour, white, all-purpose, unenriched	15	1	2
Macaroni, dry, enriched	21	3	6
Rye bread	73	3	660
Dairy products			
Milk, whole, fluid, 3.3% fat	110	<1	40
Evaporated milk, whole	260	<1	110
Butter, with salt	24	<1	580
Cream, fluid, half and half	110	<1	41
Cheese, cottage, low fat, 2% milk fat	91	<1	330
Yogurt, plain, low fat	200	<1	77
Fruits and vegetables			
Apples, raw, with skin	6	<1	1
Bananas, raw	5	<1	1
Cherries, sweet, raw	13	<1	<1
Raisins, seedless	50	2	11
Potatoes, raw, skin	30	3	10
Tomatoes, red, ripe, raw	10	<1	5
Meats, poultry, and fish			
Eggs, whole, raw, fresh	53	2	40
Fish fillet, battered or breaded, and fried	18	2	530
Pork, fresh, leg (ham), whole, raw	5	<1	47
Bologna, chicken, pork, beef	92	1	1,100
Chicken, broilers or fryers, breast meat only, raw	11	<1	65
Beef, chuck, arm pot roast, raw	16	2	74

From US Department of Agriculture, Agricultural Research Service (2009) USDA National Nutrient Database for Standard Reference. Release 22. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl

Waters that contain excessive minerals can result in clouding of beverages. Textural properties of fruits and vegetables can be influenced by the "**hardness**" or "**softness**" of the water used during processing.

12.2 BASIC CONSIDERATIONS

12.2.1 Nature of Analyses

Mineral analysis is a valuable model for understanding the basic structure of analysis procedures to separate and measure. Separation of minerals from the food matrix is often specific, such as **complexometric titrations** (Sect. 12.3.1) or **precipitation titrations** (Sect. 12.3.2). In these cases of specific separation, nonspecific measurements such as volume of titrant are made and are later converted to mass of mineral based on fundamental stoichiometric relationships. In other cases, separation of mineral involves nonspecific procedures such as **ashing** or **acid extraction**. These nonspecific separations require that a specific measurement be made as provided by **colorimetry** (Sect. 12.3.3), **ion-selective electrodes** (ISE) (Sect. 12.3.4), **atomic absorption spectroscopy**, or **inductively coupled plasma-atomic emission spectroscopy** (Chap. 24).

Because determination of mass of mineral is the final objective of analysis, measures other than mass are considered to be surrogate, or stand-in, measures. **Surrogate measures** are converted into mass of mineral via fundamental stoichiometric and physiochemical relationships or by empirical relationships. Empirical relationships are those associations that need to be established by experimentation because they do not follow any well-established physiochemical relationship. An example of a surrogate measurement is the absorbance of a chromogen–mineral complex (Sect. 12.3.3). It may be possible to convert absorbance into mass of mineral using the fundamental relationships defined by the molar absorptivity and stoichiometry of the chromogen–mineral complex. However, it is more commonly required that the absorbance: concentration relationship be empirically developed using a series of standards (i.e., a standard curve).

12.2.2 Sample Preparation

Some sample preparation is generally required for traditional methods of mineral analysis to ensure a well-mixed and representative sample and to make the sample ready for the procedure to follow. A major concern in mineral analysis is **contamination** during sample preparation. **Comminution** (e.g., grinding or chopping) and mixing using metallic instruments can add significant mineral to samples and, whenever possible, should be performed using nonmetallic instruments or instruments not composed of the sample mineral. For example, it is standard practice in our laboratories to use an aluminum grinder for comminution of meat samples undergoing iron analysis. **Glassware** used in sample preparation and analysis should be scrupulously cleaned using acid washes and triple rinsed in the purest water. The latter may necessitate installation of an **ultrapure water system** in the laboratory to further purify the general supply of distilled water.

Solvents, including water, can contain significant quantities of minerals. Therefore, all procedures involving mineral analysis require the use of the

purest reagents available. In some cases, the cost of ultrapure reagents may be prohibitive. When this is the case, the alternative is to always work with a reagent blank. A **reagent blank** is a sample of reagents used in the sample analysis, quantitatively the same as that used in the sample but without any of the material being analyzed. This reagent blank, representing the sum of the mineral contamination in the reagents, is then subtracted from the sample values to more accurately quantify the mineral.

A method such as near-infrared spectroscopy (Chap. 23) allows for mineral estimation without destruction of the carbon matrix of carbohydrates, fats, protein, and vitamins that make up foods. However, traditional methods generally require that the minerals be freed from this organic matrix in some manner. Chapter 7 describes the various methods used to ash foods in preparation for determination of specific mineral components of the food. In water samples, minerals may be determined without further preparation.

12.2.3 Interferences

Factors such as **pH**, **sample matrix**, **temperature**, and other **analytical conditions** and **reagents** can interfere with the ability of an analytical method to quantify a mineral. Often there are specific interfering substances that must be removed or suppressed for accurate analysis. Two of the more common approaches are to isolate the sample mineral, or remove interfering minerals, using selective precipitations or ion exchange resins. Water may need to be boiled to remove carbonates that interfere with several traditional methods of mineral analysis.

If other interferences are suspected, it is a common practice to develop the standard curve using sample mineral dissolved in a background matrix containing interfering elements known to be in the food sample. For example, if a food sample is to be analyzed for calcium content, a **background matrix solution** of the known levels of sodium, potassium, magnesium, and phosphorus should be used to prepare the calcium standards for developing the standard curve. In this manner, the standard curve more closely represents the analysis response to the sample mineral when analyzing a food sample. Alternatively, the standard curve can be developed using a series of sample mineral spikes added to the food sample. A **spike** is a small volume of a concentrated standard that is added to the sample. The volume is small enough so as to not appreciably change the overall composition of the sample, except for the mineral of interest. Thus, measurements of both the standards and the sample are made in the presence of the same background. If the spikes are added before implementation of the analysis protocol, possible effects of incomplete extractions, sample mineral degradation, and other losses are integrated into the standard curve.

12.3 METHODS

12.3.1 EDTA Complexometric Titration

12.3.1.1 Principles

The hexadentate ligand **ethylenediaminetetraacetate** (EDTA) forms stable 1:1 complexes with numerous mineral ions. This gives complexometric titration using EDTA broad application in mineral analysis. Stability of mineral–EDTA complexes generally increases with valence of the ion, although there is significant variation among ions of similar valence due to their coordination chemistry. The complexation equilibrium is strongly pH dependent. With decreasing pH the chelating sites of EDTA become protonated, thereby decreasing its effective concentration. Endpoints are detected using mineral chelators that have coordination constants lower than EDTA (i.e., less affinity for mineral ions) and that produce different colors in each of their complexed and free states. **Calmagite** and **Eriochrome Black T** (EBT) are such indicators that change from blue to pink when they complex with calcium or magnesium. The endpoint of a complexometric EDTA titration using either Calmagite or EBT as the indicator is detected as the color changes from pink to blue.

The pH affects a complexometric EDTA titration in several ways and must be controlled for best performance. The pH must be 10 or more for calcium or magnesium to form stable complexes with EDTA. Also, the sharpness of the endpoint increases with increasing pH. However, magnesium and calcium precipitate as their hydroxides at pH 12, and titration pH should probably be no more than 11 to ensure their solubility. Considering all factors, EDTA complexometric titration of calcium and magnesium is specified at pH 10 ± 0.1 using an ammonia buffer (10).

12.3.1.2 Procedure: Hardness of Water Using EDTA Titration

Water hardness is determined by EDTA complexometric titration of the total of calcium and magnesium, in the presence of Calmagite, and expressed as the equivalents of calcium carbonate (mg/L) (*Standard Methods for the Examination of Water and Wastewater*, Method 2340, Hardness) (10) (Fig. 12-1). The calcium–Calmagite complex is not stable, and calcium alone cannot be titrated using the Calmagite indicator. However, Calmagite becomes an effective indicator for

WATER HARDNESS-EDTA TITRATION

Titration of Water Sample

Dilute 25 ml sample (or such volume to require <15 ml titrant) to 50 ml in a flask.

⇓

Bring pH to 10±0.1 by adding 1–2 ml buffer solution (NH_4 in NH_4OH, combined with Na_2EDTA and $MgSO_4$ or $MgCl_2$) and 1–2 drops Calmagite indicator solution.

⇓

Titrate with a standard solution of ca. 0.01 *M* EDTA to a blue endpoint.

Standardization of EDTA

Weigh 1.000 mg $CaCO_3$ into a 500-ml Erlenmeyer flask and add HCl (1 : 1 dilution with water) until dissolved. Add 200 ml H_2O and boil a few minutes to expel CO_2. Let cool.

⇓

Add a few drops of methyl red indicator and adjust to intermediate orange color with 3 *N* NH_4OH or HCl (1 : 1) as required. Transfer to 1 L flask and dilute to volume.

⇓

Titrate calcium standard solution with EDTA solution, to Calmagite endpoint.

⇓

Determine $CaCO_3$ equivalents as mg $CaCO_3$/ml EDTA solution.

Calculations

Hardness (EDTA) as mg $CaCO_3$/L = (mg $CaCO_3$/ml EDTA x ml EDTA)/L sample

12-1 figure

Procedure for determination of water hardness by EDTA titration. *Standard Methods for the Examination of Water and Wastewater*, Method 2340, Hardness. [Adapted from (10).]

calcium titration if we include in the buffer solution a small amount of neutral magnesium salt and enough EDTA to bind all magnesium. Upon mixing sample into the buffer solution, calcium in the sample replaces the magnesium bound to EDTA. The free magnesium binds to Calmagite, and the pink magnesium–Calmagite complex persists until all calcium in the sample has been titrated with EDTA. The first excess of EDTA removes magnesium from Calmagite and produces a blue endpoint.

12.3.1.3 Applications

The major application of EDTA complexometric titration is testing calcium plus magnesium as an indicator of water hardness (10). However, EDTA complexometric titration is suitable for determining calcium in the ash of fruits and vegetables (AOAC Method 968.31) (5) and other foods that have calcium without appreciable magnesium or phosphorus. The water hardness application of the EDTA complexometric titration is made easy using test strips impregnated with Calmagite and EDTA (e.g., **AquaChek**, Environmental Test Systems, Inc., a HACH Company, Elkhart, IN). The strips are dipped into the water to test for total hardness caused by calcium and magnesium. The calcium displaces the magnesium bound to EDTA, and the released magnesium binds to Calmagite, causing the test strip to change color.

12.3.2 Precipitation Titration

12.3.2.1 Principles

When at least one product of a titration reaction is an insoluble precipitate, it is referred to as **precipitation titrimetry**. Few of the many gravimetric methods, however, can be adapted to yield accurate volumetric methods. Some of the major factors blocking the adaptation are long times necessary for complete precipitation, failure of the reaction to yield a single product of definite composition, and lack of an endpoint indicator for the reaction.

Nonetheless, precipitation titration has resulted in at least two methods that are used widely in the food industry today. The **Mohr method** for chloride determination is a direct or **forward titration** method, based on the formation of an orange-colored solid, silver chromate, after silver from silver nitrate has complexed with all the available chloride.

$$Ag^+ + Cl^- \rightarrow AgCl\,(\text{until all } Cl^- \text{is complexed}) \quad [1]$$

$$2Ag^+ + CrO_4^{2-} \rightarrow Ag_2CrO_4\,(\text{orange only after } Cl^- \text{is all complexed}) \quad [2]$$

The **Volhard method** is an indirect or **back-titration** method in which an excess of a standard solution of silver nitrate is added to a chloride-containing sample solution. The excess silver is then back-titrated using

a standardized solution of potassium or ammonium thiocyanate with ferric ion as an indicator. The amount of silver that is precipitated with chloride in the sample solution is calculated by subtracting the excess silver from the original silver content.

$$Ag^+ + Cl^- \rightarrow AgCl \text{ (until all } Cl^- \text{is complexed)} \quad [3]$$

$$Ag^+ + SCN^- \rightarrow AgSCN \text{ (to quantitate silver not complexed with chloride)} \quad [4]$$

$$SCN^- + Fe^{3+} - [FeSCN]^{2+} \text{ (red when there is any } SCN^- \text{not complexed to } Ag^+) \quad [5]$$

12.3.2.2 Procedures

12.3.2.2.1 Mohr Titration of Salt in Butter (AOAC Method 960.29) Salt in foods may be estimated by titrating the chloride ion with silver (Fig. 12-2). The orange endpoint in this reaction occurs only when all chloride ion is complexed, resulting in an excess of silver to form the colored silver chromate. The endpoint of this reaction is therefore at the first hint of an orange color. When preparing reagents for this assay, use boiled water to avoid interferences from carbonates in the water.

12.3.2.2.2 Volhard Titration of Chloride in Plant Material (AOAC Method 915.01) In the Volhard method (Fig. 12-3), water must be boiled to minimize errors due to interfering carbonates, because the solubility product of silver carbonate is much less than the solubility product of silver chloride. Once chloride is determined by titration, the chloride weight is multiplied by 1.648 to obtain salt weight, if salt content is desired.

12.3.2.3 Applications

Precipitation titration methods are well suited for any foods that may be high in chlorides. Because of added salt in processed cheeses and meats, these products should certainly be considered for using this method to detect chloride; then salt content is estimated by calculation. Precipitation titrations are easily automated, thus ensuring that these traditional methods will see continued use in the analytical food laboratory. For example, the automatic titration system commonly used to rapidly measure the salt content of potato

SALT — MOHR TITRATION

Titration of Butter Sample

Weigh about 5 g of butter into 250-ml Erlenmeyer flask and add 100 ml of boiling H_2O.

⇓

Let stand 5–10 min with occasional swirling.

⇓

Add 2 ml of a 5% solution of K_2CrO_4 in d H_2O.

⇓

Titrate with 0.1 *N* $AgNO_3$ standardized as below until an orange-brown color persists for 30 sec.

Standardization of 0.1 *N* $AgNO_3$

Accurately weigh 300 mg of recrystallized dried KCl and transfer to a 250-ml Erlenmeyer flask with 40 ml of water.

⇓

Add 1 ml of K_2CrO_4 solution and titrate with $AgNO_3$ solution until first perceptible pale red-brown appears.

⇓

From the titration volume subtract the milliliters of the $AgNO_3$ solution required to produce the endpoint color in 75 ml of water containing 1 ml of K_2CaO_4.

⇓

From the next volume of $AgNO_3$ calculate normality of the $AgNO_3$ as:

$$\text{Normality } AgNO_3 = \frac{\text{mg KCl}}{\text{ml } AgNO_3 \times 74.555 \text{ g KCl/mole}}$$

Calculating Salt in Butter

$$\text{Percent salt} = \frac{\text{ml } 0.1\,N\ AgNO_3 \times 0.585}{\text{g of sample}}$$

$$[0.585 = (58.5 \text{ g NaCl/mol})/100]$$

12-2 figure Procedure of Mohr titration of salt in butter. AOAC Method 960.29 [Adapted from (5)].

SALT — VOLHARD TITRATION

Titration of Sample

Moisten 5 g of sample in crucible with 20 ml of 5% Na_2CO_3 in water.

⇓

Evaporate to dryness.

⇓

Char on a hot plate under a hood until smoking stops.

⇓

Combust at 500°C for 24 hr.

⇓

Dissolve residue in 10 ml of 5 *N* HNO_3.

⇓

Dilute to 25 ml with d H_2O.

⇓

Titrate with standardized $AgNO_3$ solution (from the Mohr method) until white AgCl stops precipitating and then add a slight excess.

⇓

Stir well, filter through a retentive filter paper, and wash AgCl thoroughly.

⇓

Add 5 ml of a saturated solution of $FeNH_4(SO_4)2 \bullet 12H_2O$ to the combined titrate and washings.

⇓

Add 3 ml of 12 *N* HNO_3 and titrate excess silver with 0.1 *N* potassium thiocyanate.

Standardization of Potassium Thiocyanate Standard Solution

Determine working titer of the 0.1 *N* potassium thiocyanate standard solution by accurately measuring 40–50 ml of the standard $AgNO_3$ and adding it to 2 ml of $FeNH_4(SO_4)2 \cdot 12H_2O$ indicator solution and 5 ml of 9 *N* HNO_3.

⇓

Titrate with thiocyanate solution until solution appears pale rose after vigorous shaking.

Calculating Cl Concentration

Net volume of the $AgNO_3$ = Total volume $AgNO_3$ added – Volume titrated with thiocyanate

1 ml of 0.1 *M* $AgNO_3$ = 3.506 mg chloride

12-3 figure Procedure for Volhard titration of chloride in plant material. AOAC Method 915.01. [Adapted from (5).]

chips is simply doing a Mohr titration. Also, the **Quantab® chloride titration** used in AOAC Method 971.19 is an adaptation of the principles involved in the Mohr titration method. This test strip adaptation allows for very rapid quantitation of salt in food products and is accurate to ±10% over a range of 0.3–10% NaCl in food products.

12.3.3 Colorimetric Methods

12.3.3.1 Principles

Chromogens are chemicals that, upon reaction with the compound of interest, form a colored product. Chromogens are available that selectively react with a wide variety of minerals. Each chromogen reacts with its corresponding mineral to produce a soluble colored product that can be quantified by absorption of light at a specified wavelength. The relationship between concentration and absorbance is given by **Beer's law** as detailed in Chap. 22. Generally, concentration of mineral in a sample is determined from a standard curve developed during the analysis, although in some cases it is possible to directly calculate concentration based on molar absorptivity of the chromogen–mineral complex.

Samples generally must be ashed or treated in some other manner to isolate and/or release the minerals from organic complexes that would otherwise inhibit their reactivity with the chromogen. The mineral of interest must be solubilized from a dry ash and subsequently handled in a manner that prevents its precipitation. The soluble mineral may need to be treated (e.g., reduced or oxidized) to ensure that all mineral is in a form that reacts with the chromogen (2). Ideally, the chromogen reacts rapidly to produce a stable product. This is not always the case in practice, and time constraints may be established for color development and reading of absorbance. As with all mineral analysis of food, special efforts must be put in place to avoid contamination during sampling and analysis.

12.3.3.2 Procedures: Colorimetric Determination of Iron in Meat

The total iron content of foods can be quantified spectrophotometrically as shown in Fig. 12-4. In this method, the absorption of light at 562 nm is converted to iron concentration in the sample via a regression equation generated from a standard curve developed during the analysis using a standard solution. In meat systems, this method has been coupled with a method specific for heme iron to determine the ratio of heme iron to total iron, which is important nutritionally as the former is more bioavailable (11). Another interesting aspect of this method of interest to the food scientist is the fact that the ferrozine reagent only reacts with ferrous iron, and not ferric. The addition of ascorbic acid in the second to last step is necessary to convert all ionic iron to the detectable ferrous form. Repeating the procedure with and without ascorbic acid allows determination of total and ferrous ionic iron, respectively. Ferric iron is calculated by difference.

12.3.3.3 Applications

Colorimetry is used for the detection and quantification of a wide variety of minerals in food, and it is often a viable alternative to atomic absorption spectroscopy and other mineral detection methods. Colorimetric methods generally are very specific and usually can be performed in the presence of other minerals, thereby avoiding extensive separation to isolate the mineral of interest. They are particularly robust and often immune to matrix effects that can limit the usefulness of other methods for mineral analysis. With minimal effort and expense, many colorimetric methods will perform with precision and accuracy similar to that obtained by experienced personnel using atomic absorption spectroscopy (11).

12.3.4 Ion-Selective Electrodes

12.3.4.1 Principles

Many electrodes have been developed for the selective measurement of various cations and anions, such as bromide, calcium, chloride, fluoride, potassium, sodium, and sulfide (12, 13). The pH electrode described in Chap. 13 is a specific example of an **ISE**. For any ISE, an **ion-selective sensor** is placed such that it acts as a "bridging electrode" between two reference electrodes carefully designed to produce a constant and reproducible **potential**. The sensor can take on many forms (e.g., glass, single crystal, precipitate based, solvent polymer), although each provides an ion-selective electronic coupling that allows a potential to develop across the sensor. The exact mechanism(s) of charge transport across the sensor is not completely understood, but it is brought about by ion-selective species incorporated within the sensor itself and has been described by analogy to the response of billiard balls to an impact. If the ion-selective species within the sensor are imagined to be a row of billiard balls, it can be envisioned how the impact of sample ions on one sensor surface is translocated to the other surface. In this manner, the potential within the

IRON DETERMINATION OF MEAT—COLORIMETRIC ASSAY

Preparation of Standards

Prepare solutions of 10, 8, 6, 4, 2 µg iron/ml from a stock solution of 10 µg iron/ml.
Make dilutions using 0.1 *N* HCl.

Analysis of Sample

Place ~5 g sample into crucible and accurately weigh.
⇓
Heat on hot plate until well charred and sample has stopped smoking.
⇓
Ash in furnace at ca 550°C until ash is white.
⇓
Dissolve ash in small amount 1 *N* HCl and dilute to 50 ml volume with 0.1 *N* HCl
⇓
Transfer 0.500 ml of diluted sample and standards into 10 ml test tubes.
⇓
Add 1.250 ml ascorbic acid (0.02% in 0.2 *N* HCl, made fresh daily). Vortex and let set 10 min.
⇓
Add 2.000 ml 30% ammonium acetate. Vortex. (pH needs to be >3 for color development)
⇓
Measure absorbance at 562 nm. Determine iron concentration in sample digest (µg iron/ml) from standard curve.

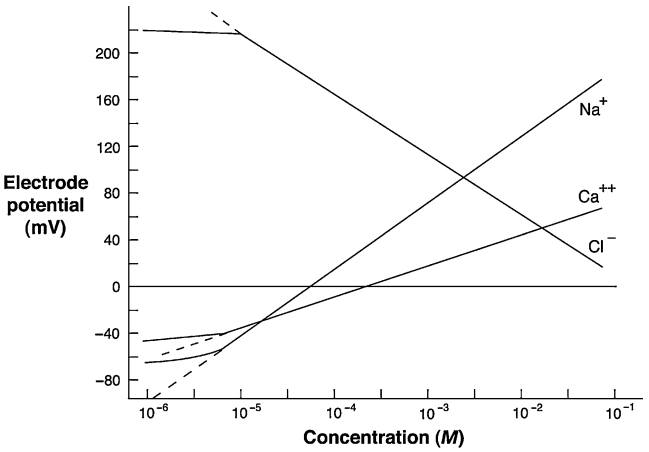
12-4 figure Procedure for determination of iron in meat by colorimetry. [Adapted from (11).]

sensor remains constant, while potentials develop at the sensor surfaces according to the **Nernst equation** (Sect. 13.3.2.2), dependent on sample **ion activity** in the solutions contacting each surface.

Typically, the inside surface of the ion-selective sensor is in contact with the **negative reference electrode** (anode) via a filling solution having a constant concentration of sample ion, while the outside surface of the ion-selective sensor is in contact with the **positive reference electrode** (cathode) via sample solutions having varying concentration of sample ion. Because sample ion activity of the internal solution is fixed, the potential varies across the sensor depending solely on the activity of sample ion in the sample solution. As described by the Nernst equation, the potential of the outside sensor surface increases by $0.059/n$V (where n is the number of electrons involved in the half reaction of the sample ion) for each tenfold increase in activity of a mineral cation. Conversely, the potential decreases by $0.059/n$V for each tenfold increase in activity of mineral anion. These changes have a direct effect on the overall ISE potential because the outside sensor surface is orientated toward the positive reference electrode. **Ion concentration** is generally substituted for ion activity, which is a reasonable approximation at low concentrations and controlled ionic strength environments. Indeed, this is observed within limitations set by electrode and instrumental capabilities (Fig. 12-5).

12.3.4.2 General Methodology

For ISE analysis, one simply attaches an ISE for the sample ion to a **pH meter** set on the **mV scale** and follows instructions for determination. However, the performance of an ISE must be considered when first selecting an electrode and later when designing sampling and analysis protocols. Detailed information regarding the performance of specific ISEs is available from vendor catalogs. Typical ISEs likely to be employed for analysis of foods operate in the range of 1–10^{-6} M, although the electrode response may be distinctly nonlinear at the lower concentrations.



12-5 figure Examples of ion-selective electrode calibration curves for ions important in foods. (Courtesy of Van London pHoenix Co., Houston, TX.)

Electrode performance is affected by the presence of **interfering ions**, often with the strongest interference from those ions having size and charge similar to the ion of interest. Relative response of ISE to interfering ions may be expressed as selectivity coefficients or as concentration of interfering ion that leads to 10% error. If the selectivity coefficient relative to an interfering ion is 1000 (i.e., the ISE is 1000-fold more responsive to the sample ion than the interfering ion), 10% or greater error can be expected when measuring μM levels of the sample ion with interfering ion present at mM levels. Most ISEs operate over a broad pH range, although pH may need to be controlled for best performance. Minimum response times for ISEs fall in the range of 20 s to 1 min.

Despite inherent limitations of electrode design and construction, the analyst can adjust the sample and control measurement conditions to minimize many practical problems that otherwise limit the specificity and precision of ISEs. Because the ISE responds to ionic activity, it is important that the **activity coefficient** be kept constant in samples and calibration standards. The activity coefficient (γ) is used to relate ion activity (A) to ion concentration (C) $(A = \gamma C)$. Activity coefficient is a function of ionic strength, so **ionic strength adjustment** (ISA) **buffers** are used to adjust the samples and standards to the same ionic strength. These ISA buffers are commercially available. The use of ISA buffers also adjusts the pH, which may be necessary if H^+ or OH^- activities affect the ion-specific sensor or if they interact with the analyte. In the case of metals having insoluble hydroxides, it is necessary to work at a pH that precludes their precipitation. Depending on the selectivity of the ISE, it may be necessary to remove interfering ions from the sample by selective precipitation or complexation.

In view of temperature effects on standard potentials and slopes of electrodes (see Nernst Equation [8] in Chap. 13), it is important to keep the electrode and solutions at a constant temperature. This may involve working in a room that is thermostatically controlled to 25 °C (one of the internationally accepted temperatures for electrochemical measurements), and allowing sufficient time for all samples and standards to equilibrate to this temperature. Solutions should be gently stirred during the measurement to attain rapid equilibrium and to minimize concentration gradients at the sensor surface. Finally, it is important to allow sufficient time for the electrode to stabilize

before taking a reading. ISEs may not completely stabilize within a practical timeframe, so a decision needs to be made of when to take the reading. The reading may be taken when the rate of change has fallen below some predetermined value or at a fixed time after the electrode was placed in solution. A problem with the latter is that many ISEs respond more rapidly, as samples are changed, to an increase in concentration of sample ion as compared to a decrease in concentration of sample ion.

12.3.4.3 Electrode Calibration and Determination of Concentration

In using an ISE, ion concentration can be determined using either a calibration curve, standard addition, or endpoint titration. It is common practice to develop a **calibration curve** when working with an ISE because it allows a large number of samples to be measured rapidly. The electrode potential (volts) is developed in a series of solutions of known concentration and plotted on **semilog paper** against the standard concentrations. Examples of calibration curves for various ions are given in Fig. 12-5. Upon analysis of a test sample, the observed electrode potential is used to determine ion concentration by referring to the calibration curve. Note the nonlinear region of the curve at the lowest concentrations. Total ionic strength and the concentration of interfering ions are especially important factors limiting selective detection of low levels of ions.

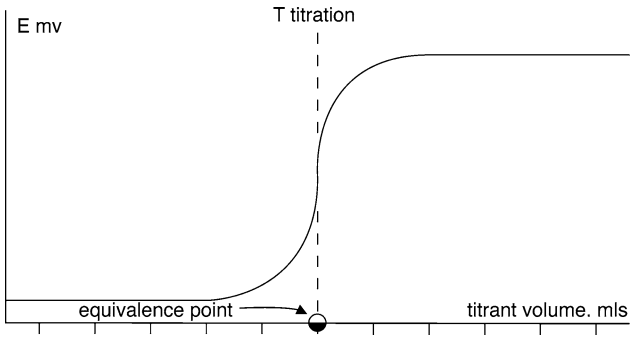
The **standard addition method** is of great value when only a few samples are to be measured and time does not permit the development of a calibration curve. This method also eliminates complex and unknown background effects that cannot be replicated when developing a calibration curve using standards. The ISE is immersed in the sample and the resulting voltage is recorded (E_{sample}). An aliquot, or **spike**, containing a known amount of the measured species is added to the sample, and a second measurement of electrode potential is determined (E_{spike}). Concentration of active species in the original sample is determined from the absolute difference in the voltage readings ($\Delta E = |E_{\text{spike}} - E_{\text{sample}}|$) according to the following relationship algebraically derived from the Nernst equations.

$$C_O = \frac{C_\Delta}{(10^{\Delta E/S} - 1)} \qquad [6]$$

where:

C_O = original concentration of sample ion (mol/L)

C_Δ = change in sample ion concentration when spike was added (mol/L)



12-6 figure A typical T-type titration. [From (2), used with permission.]

E = difference in potential between the two readings (V)

S = 0.059/number of electrons in the 1/2 reaction of the sample ion

Finally, ISEs can be used to detect the **endpoints of titrations** using species that form a precipitate or strong complex with the sample ion. If an ISE is selected that detects titrant species, a T-type titration curve results from the large increase in titrant activity detected at the equivalence point (see Fig. 12-6 for a cation titrant). If an ISE is selected that detects the sample ion, an S-type titration curve results from the removal of sample ion activity at the equivalence point. In either case, sample concentration is calculated from titrant volume to reach the equivalence point and the stoichiometric relationship between titrant species and sample ion.

12.3.4.4 Applications

Some examples of applications of ISEs are salt and nitrate in processed meats, salt content of butter and cheese, calcium in milk, sodium in low-sodium ice cream, carbon dioxide in soft drinks, potassium and sodium levels in wine, and nitrate in canned vegetables. An ISE method applicable to foods containing $<$100 mg sodium/100 g is an official method of AOAC International (Method 976.25). This method employs a sodium combination ISE, pH meter, magnetic stirrer, and a semilog graph paper for plotting a standard curve. Obviously, there are many other applications, but the above serve to demonstrate the versatility of this valuable measuring tool.

A major *advantage* of ISEs is their ability to measure many anions and cations directly. Such measurements are relatively simple compared to most other analytical techniques, particularly because a pH meter may be used as the voltmeter. Analyses are independent of sample volume when making direct

measurements, while turbidity, color, and viscosity are all of no concern.

A major *disadvantage* in the use of ISEs is their inability to measure below 2–3 ppm, although there are some electrodes that are sensitive down to 1 part per billion. At low levels of measurement (below 10^{-4} M), the electrode response time is slow. Finally, some electrodes have had a high rate of premature failure or a short operating life and possible excessive noise characteristics.

12.4 COMPARISON OF METHODS

For labeling, processing, and even practical nutrition, we are concerned only with a few minerals, which generally can be analyzed by traditional methods. The traditional methods available for mineral analysis are varied, and only a very limited number of examples have been given in this chapter. Choice of methods for mineral analysis must be made considering method performance regarding accuracy, sensitivity, detection limit, specificity, and interferences. Information on method performance is available from the collaborative studies referenced with official methods (Chap. 1). Other factors to be considered include cost per analysis completed, equipment availability, and analytical time compared to analytical volume.

Generally, for a small laboratory with skilled analytical personnel, the traditional methods can be carried out rapidly, with accuracy, and at minimal costs. If a large number of samples of a specific element are to be run, there is certainly a time factor in favor of using atomic absorption spectroscopy or emission spectroscopy, depending on the mineral being analyzed. The graphite furnace on the atomic absorption spectrophotometer is capable of sensitivity in the parts per billion range. This is beyond the limits of the traditional methods. However, for most minerals of practical concern in the food industry, this degree of sensitivity is not required.

Modern instrumentation has made it possible to quantify an entire spectrum of minerals in one process, some into the parts per billion range. Instrumentation capable of such analysis is expensive and beyond the financial resources of many quality assurance laboratories. Large numbers of samples to be analyzed may justify the automation of some routine analyses and perhaps the expense of some of the modern pieces of equipment. However, the requirements for only occasional samples to be analyzed for a specific mineral will not justify the initial costs of much instrumentation. This leaves the options of sending samples out to certified laboratories for analysis or utilizing one of the more traditional methods for analysis.

12.5 SUMMARY

The mineral content of water and foodstuffs is important because of their nutritional value, toxicological potential, and interactive effects with processing and texture of some foods. Traditional methods for mineral analysis include titrimetric and colorimetric procedures. The basic principles of these methods are described in this chapter, along with discussion of ISE methodology that has general application for mineral analysis.

Procedures are described in this chapter that illustrate use of these traditional methods to quantify minerals of concern in the food industry. These procedures generally require chemicals and equipment routinely available in an analytical laboratory and do not require expensive instrumentation. These methods may be suited to a small laboratory with skilled analytical personnel and a limited number of samples to be analyzed. The traditional procedures will often perform similarly to procedures requiring more instrumentation and may be more robust in actual practice.

Foods are typically ashed prior to traditional analyses because the methods generally require that the minerals be freed from the organic matrix of the foods. Sample preparation and analysis must include steps necessary to prevent contamination or loss of volatile elements and must deal with a variety of potential interferences. Various approaches are described to account for these possible errors including use of reagent blanks, addition of spikes, and development of standard curves using appropriate mineral matrix background.

Traditional methods for mineral analysis are often automated or adapted to test kits for rapid analysis. Tests for water hardness and the Quantab® for salt determination are examples currently being used. The basic principles involved in traditional methods will continue to be utilized to develop inexpensive rapid methods for screening mineral content of foods and beverages. Familiarity with the traditional principles will allow the food analyst to obtain the best possible performance with the kits and adapt to problems that may be encountered.

12.6 STUDY QUESTIONS

1. What is the major concern in sample preparation for specific mineral analysis? How can this concern be addressed?
2. If the ammonia buffer is pH 11.5 rather than pH 10 in the EDTA complexometric titration to determine the hardness of water, would you expect to overestimate or underestimate the hardness? Explain your answer.

3. This chapter includes descriptions of the EDTA complexometric titration method and ISE methodology for quantifying calcium. Differentiate these techniques with regard to the principles involved, and discuss primary advantages and disadvantages of these two techniques.
4. The Mohr and Volhard titration methods often are used to determine the NaCl content of foods. Compare and contrast these two methods, as you explain the principles involved.
5. In a back-titration procedure, would overshooting the endpoint in the titration cause an over- or underestimation of the compound being quantified? Explain your answer.
6. Describe how and why to employ standards in background matrix, spikes, and reagent blanks.
7. Explain the principles of using an ISE to measure the concentration of a particular inorganic element in food. List the factors to control, consider, or eliminate for an accurate measure of concentration by the ISE method.
8. You have decided to purchase an ISE to monitor the sodium content of foods produced by your plant. List the advantages this would have over the Mohr/Volhard titration method. List the problems and disadvantages of ISE that you should anticipate.
9. What factors should be considered in selecting a specific method for mineral analysis for a food product?

12.7 PRACTICE PROBLEMS

1. If a given sample of food yields 0.750 g of silver chloride in a gravimetric analysis, what weight of chloride is present?
2. A 10-g food sample was dried, then ashed, and analyzed for salt (NaCl) content by the Mohr titration method $(AgNO_3 + Cl \rightarrow AgCl)$. The weight of the dried sample was 2 g, and the ashed sample weight was 0.5 g. The entire ashed sample was titrated using a standardized $AgNO_3$ solution. It took 6.5 ml of the $AgNO_3$ solution to reach the endpoint, as indicated by the red color of Ag_2CO_4 when K_2CrO_4 was used as an indicator. The $AgNO_3$ solution was standardized using 300 mg of dried KCl as described in Fig. 12-2. The corrected volume of $AgNO_3$ solution used in the titration was 40.9 ml. Calculate the salt (NaCl) content of the original food sample as percent NaCl (wt/wt).
3. A 25-g food sample was dried, then ashed, and finally analyzed for salt (NaCl) content by the Volhard titration method. The weight of the dried sample was 5 g, and the ashed sample weighed 1 g. Then 30 ml of 0.1 *N* $AgNO_3$ was added to the ashed sample, the resultant precipitate was filtered out, and a small amount of ferric ammonium sulfate was added to the filtrate. The filtrate was then titrated with 3 ml of 0.1 *N* KSCN to a red endpoint.
   (a) What was the moisture content of the sample, expressed as percent H_2O (wt/wt)?
   (b) What was the ash content of the sample, expressed as percent ash (wt/wt) on a dry weight basis?
   (c) What was the salt content of the original sample in terms of percent (wt/wt) NaCl? (molecular weight Na = 23; molecular weight Cl = 35.5)
4. Compound X in a food sample was quantified by a colorimetric assay. Use the following information and Beer's law to calculate the content of Compound X in the food sample, in terms of mg Compound X/100 g sample:
   (a) A 4-g sample was ashed.
   (b) Ashed sample was dissolved with 1 ml of acid and the volume brought to 250 ml.
   (c) A 0.75-ml aliquot was used in a reaction in which the total volume of the sample to be read in the spectrophotometer was 50 ml.
   (d) Absorbance at 595 nm for the sample was 0.543.
   (e) The absorptivity constant for the reaction (i.e., extinction coefficient) was known to be 1574 L/M cm.
   (f) Inside diameter of cuvette for spectrophotometer was 1 cm.
5. Colorimetric analysis
   (a) You are using a colorimetric method to determine the concentration of Compound A in your liquid food sample. This method allows a sample volume of 5 ml. This volume must be held constant but can comprise diluted standard solution and water. For this standard curve, you need standards that contain 0, 0.25, 0.50, 0.75, and 1.0 mg of Compound A. Your stock standard solution contains 5 g/L of Compound A.

   Devise a dilution scheme(s) for preparing the samples for this standard curve that could be followed by a lab technician. Be specific. In preparing the dilution scheme, use no volumes less than 0.5 ml.
   (b) You obtain the following absorbance values for your standard curve:

Sample (mg)	*Absorbance (500 nm)*
0.00	0.00
0.25	0.20
0.50	0.40
0.75	0.60
1.00	0.80

   Construct a standard curve and determine the equation of the line.
   (c) A 5-ml sample is diluted to 500 ml, and 3 ml of this solution is analyzed as per the standard samples; the absorbance of 0.50 units at 500 nm. Use the equation of the line calculated in part (b) and information about the dilutions to calculate what the concentration is of Compound A in your original sample in terms of g/L.
6. What is the original concentration of copper in a 100-ml sample that shows a potential change of 6 mV after the addition of 1 ml of 0.1 *M* $Cu(NO_3)_2$?

Answers

1.

$$\frac{x \text{ g Cl}}{0.750 \text{ g AgCl}} = \frac{35.45 \text{ g/mol}}{143.3 \text{ g/mol}}$$

$$x = 0.186 \text{ g Cl}$$

2.

$$N\text{AgNO}_3 = \frac{0.300\text{ g KCl}}{\text{ml AgNO}_3 \times 74.555\text{ g KCl/mol}}$$

$$0.0984\,N = \frac{0.300\text{ g}}{40.9\text{ ml} \times 74.555}$$

Percent salt

$$= \left(\frac{0.0065\text{ L} \times 0.0984\ N\ \text{AgNO}_3 \times 58.5\text{ g/mol}}{10\text{ g}}\right) \times 100$$

Percent salt = 0.37%

3.

(a) $\dfrac{25\text{ g wet sample} - 5\text{ g dry sample}}{25\text{ g wet sample}} \times 100 = 80\%$

(b) $\dfrac{1\text{ g ash}}{5\text{ g dry sample}} \times 100 = 20\%$

(c) mol Ag added = mol Cl^- in sample + mol SCN^- added

$$\text{mol Ag} = (0.1\text{ mol/L}) \times (0.03\text{ L}) = 0.003\text{ mol}$$

$$\text{mol SCN}^- = (0.1\text{ mol/L}) \times (0.003\text{ L}) - 0.0003\text{ mol}$$

$$0.003\text{ mol Ag} = \text{mol Cl}^- + 0.0003\text{ mol SCN}^-$$

$$0.0027\text{ mol} = \text{mol Cl}^-$$

$$(0.0027\text{ mol Cl}^-) \times \frac{58.5\text{ g NaCl}}{\text{mol}} = 0.1580\text{ g NaCl}$$

$$\frac{0.1580\text{ g NaCl}}{25\text{ g wet sample}} = \frac{0.00632\text{ g NaCl}}{\text{g wet sample}} \times 100$$

$$= 0.63\%\text{ NaCl (w/w)}$$

4.

$$A = abc$$

$$0.543 = (1574\text{ L g}^{-1}\text{ cm}^{-1})(1\text{ cm})\,c$$

$$c = 3.4498 \times 10^{-4}\text{ g/L}$$

$$c = 3.4498 \times 10^{-4}\text{ mg/ml}$$

$$\frac{3.4498 \times 10^{-4}\text{ mg}}{\text{ml}} \times 50\text{ ml} = 1.725 \times 10^{-2}\text{ mg}$$

$$\frac{1.725 \times 10^{-2}\text{ mg}}{0.75\text{ ml}} \times \frac{250\text{ ml}}{4\text{ g}} = 1.437\text{ mg/g}$$

$$= 143.7\text{ mg/100 g}$$

5.

(a) Lowest dilution volume for 1 ml of stock:

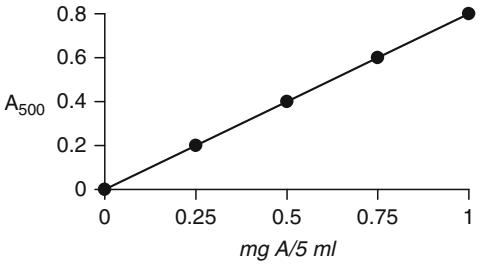
$$\frac{0.25\text{ mg}}{0.5\text{ ml}} = \frac{1\text{ ml}}{x\text{ ml}} \times \frac{5\text{ mg}}{\text{ml}}$$

$$x = 10\text{ ml}$$

Therefore, use a volumetric pipette to add 1 ml of stock to a 10-ml volumetric flask. Bring to volume with ddH_20 to give a diluted stock solution of 0.25 mg/0.5 ml. Use this to make up standards according to the following table.

mg A/5 ml	*ml Diluted stock solution*	*ml H_2O*
0	0	5.0
0.25	0.5	4.5
0.50	1.0	4.0
0.75	1.5	3.5
1.0	2.0	3.0

(b)



Equation of the line: $y = 0.8x + 0$

(c)

$$A_{500} = 0.50 = y$$

$$0.50 = 0.8x + 0$$

$$x = 0.625$$

$$\frac{0.625\text{ mg}}{5\text{ ml}} \times \frac{5\text{ ml}}{3\text{ ml}} \times \frac{500\text{ ml}}{5\text{ ml}} = 20.8\text{ mg/ml}$$

$$= 20.8\text{ g/L}$$

6.

$$\text{C}_\text{O} = \frac{0.001\text{ L} \times \dfrac{0.001\text{ moles}}{\text{L}} \times \dfrac{1}{0.100\text{ L}}}{10^{0.006/0.0285} - 1} = 1.6\text{ m}M$$

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