21 chapter

Traditional Methods for Mineral Analysis

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21.1 INTRODUCTION

This chapter describes traditional methods for analysis of minerals involving titrimetric and colorimetric procedures, ion-selective electrodes, and instruments to measure salt content. 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 not used much 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 ion chromatography (Chap. 13), atomic absorption spectroscopy, and inductively coupled plasma-optical emission spectroscopy (Chap. 9). 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., AquaChek® for calcium and Quantab® for salt determination) and for automated benchtop analyzers for salt content. In these cases, it is useful to understand the principles employed in the analysis. For additional examples of traditional methods, refer to references [1–6]. For analytical requirements for specific foods, see the *Official Methods of Analysis* of AOAC International [5] and related official methods [6].

21.1.1 Importance of Minerals in the Diet

Dietary **macrominerals** (calcium, phosphorus, sodium, potassium, magnesium, chlorine, and sulfur) are required at more than 100 mg per day by the adult [7–9]. Additional ten minerals (iron, iodine, zinc, copper, chromium, manganese, molybdenum, fluoride, selenium, and silica) are required in milli- or microgram quantities per day and are referred to as **trace minerals. Ultra trace minerals**, including vanadium, tin, nickel, arsenic, and boron, are being investigated for possible biological function, but they currently do not have clearly defined biochemical roles. **Heavy metals** (lead, mercury, cadmium, and arsenic) have been documented to be toxic to the body and should, therefore, be avoided in the diet. 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 Chap. 3, Fig. 3.1). The newest US food labeling regulations require the addition of **potassium** to the label, due to its numerous health benefits (see Chap. 3). The content of these minerals in several foods are shown in Table 21.1. The content of other minerals may be included on the nutrition label at the producer's option, although this becomes mandatory if the mineral is the subject of a nutrient content claim on the label (Chap. 3, Sect. 3.2.3).

21.1.2 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-oz 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 the 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 affects solubilization of protein and other food components, and as a preservative. This significantly increases the sodium content of products such as processed meats, pickles, and processed cheese. Phosphorus may be added as phosphates to increase water-holding capacity in meats and to change the texture of processed cheese. Calcium may be added to promote gelation of proteins and gums. Textural properties of fruits and vegetables can be influenced by the "hardness" or "softness" of the water used during processing.

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. Waters that contain excessive minerals can result in clouding of beverages.

21.1 table Mineral content of selected foods

Food item	mg/g (wet weight basis) Calcium	Iron	Sodium	Potassium
Cereals, bread, and pasta				
Rice, brown, long grain, raw	9	1	5	250
Rice, white, long grain, regular, raw, enriched	28	4	5	115
Wheat flour, whole grain	34	4	2	363
Wheat flour, white, all-purpose, unenriched	15	1	2	107
Pasta, dry, enriched	21	3	6	223
Dairy products				
Milk, whole, 3.25% milk fat with added vitamin D	113	<1	43	132
Butter, salted	24	<1	643	24
Cheese, cottage, low-fat, 2% milk fat	111	<1	308	125
Fruits and vegetables				
Apples, raw, with skin	8	<1	1	107
Bananas, raw	5	<1	1	358
Raisins, seedless	50	2	11	749
Potatoes, raw, skin	30	3	10	413
Tomatoes, red, ripe, raw	10	<1	5	237
Meats, poultry, and fish				
Eggs, whole, raw, fresh	56	2	142	138
Fish fillet, battered or breaded, and fried	14	<1	561	251
Pork, fresh, leg (ham), whole, raw	6	1	55	369
Beef, chuck, arm pot roast, raw	16	2	62	290
Bologna, chicken, pork, beef	92	1	1,120	313

From US Department of Agriculture, Agricultural Research Service (2016) USDA National Nutrient Database for Standard Reference. Release 28. Nutrient Data Laboratory Home Page, https://ndb.nal.usda.gov/

Water quality standards in the USA are established by the Environmental Protection Agency as a result of the Safe Drinking Water Act (Chap. 2, Sect. 2.2.5.2), and local water suppliers must ensure regulated substances, such as heavy metals, are below maximum contaminant levels. Tests ensure that levels of total microorganisms, coliforms, inorganic contaminants, disinfection by-products (such as chlorine), and radioactive contaminants are below maximum contaminant levels. In addition, food processors should measure other water quality parameters such as pH, turbidity, hardness, heavy metals, iron, nitrates, and volatiles.

Water hardness is a measure of the dissolved calcium and magnesium salts in water and is commonly expressed in parts per million (ppm). Hardness of water is determined using the following scale: 0–60 ppm is soft, 60–120 ppm is moderately hard, 120–180 ppm is hard, and >180 ppm is very hard. Use of hard water can affect processing in several ways. For example, over time, calcium and magnesium salts may precipitate out of hard water and form scale in pipes and on other surfaces. In addition, hard water reduces the effectiveness of soaps and sanitizers. If the water source at a food production facility contains excess minerals, it can be softened using ion exchange resins. Table 21.2 lists other treatments that may be used to improve water quality for use in food production.

21.2 BASIC CONSIDERATIONS

21.2.1 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. 21.3.1) or **precipitation titrations** (Sect. 21.3.2). In these cases of specific separation, nonspecific measurements such as volume of titrant are made and 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. 21.3.3), **ion-selective electrodes** (ISE) (Sect. 21.3.4), **atomic absorption spectroscopy** (AAS) (Chap. 9), or **inductively coupled plasma-optical emission spectroscopy** (ICP-OES) (Chap. 9).

Because determination mineral mass is the final objective of analysis, measures other than mass are considered to be surrogate, or stand-in, measures. **Surrogate measures** are converted into mineral mass via fundamental stoichiometric and physiochemical relationships or by empirical relationships. Empirical relationships are those associations that need be established by experimentation because they do not follow any well-established physiochemical relationship. An example of a surrogate measurement is the wavelength-specific absorbance of a chromogen-mineral complex (Sect. 21.3.3). It may be possible to convert absorbance into mass of mineral using the fundamental relationships defined by the molar absorptivity and stoichiom-

21.2 table Common water treatments and their rationales for use

	Treatments						
Quality parameter	*Filtration*	*Membrane filtration*	*Ion exchange*	*Chlorination/ ozonization*	*UV radiation*	*pH adjustment*	*Activated carbon*
Solids	X	X					
Salts, including hardness		X	X				
pH correction							
Other chemical contaminants, e.g., organic residues	X	X	X				X
Bacteria		X		X	X		
Viruses		X		X	X		
Protozoa		X		X	X		
Algal bloom (toxin)							X

Adapted from [14]

etry of the chromogen-mineral complex. However, it is more commonly required that the absorbance vs. concentration relationship be empirically developed using a series of standards (i.e., a standard curve).

21.2.2 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, using an aluminum grinder is standard practice 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 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. 8, Sect. 8.4) 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 16 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.

21.2.3 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.

21.3 METHODS

21.3.1 EDTA Complexometric Titration

21.3.1.1 *Background Information*

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. Endpoints are detected using mineral chelators that have coordination constants lower than EDTA (i.e., indicator has less affinity for mineral ions than does EDTA) and that produce different colors in each of their complexed and free states. **Calmagite** and **Eriochrome Black T** (EBT) are such indicators that are pink when complexed with calcium or magnesium, but blue when no metal ions are complexed. The endpoint of a complexometric EDTA titration using either Calmagite or EBT as the indicator is detected when 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 complexation equilibrium is strongly pH dependent. With decreasing pH the chelating sites of EDTA become protonated, thereby decreasing its effective concentration. 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, so 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].

21.3.1.2 *Principle*

Based on the background information above, a summary of the principle of the EDTA complexometric titration method follows (described for a sample containing just calcium): The EDTA in the titrant solution complexes with calcium, 1:1, at pH 10. The EDTA binds to calcium ions stronger than the indicator binds to the calcium. When all the calcium present in the sample has reacted with the EDTA titrant (i.e., there is no calcium left to react with the indicator), the indicator changes in color from pink to blue. The moles of calcium in the sample are equivalent to the moles of EDTA used to do the titration.

21.3.1.3 *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]. The calcium-Calmagite complex is not stable, and calcium alone cannot be titrated using the Calmagite indicator. However, Calmagite becomes an effective indicator for calcium titration if the buffer solution contains 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.

21.3.1.4 *Applications*

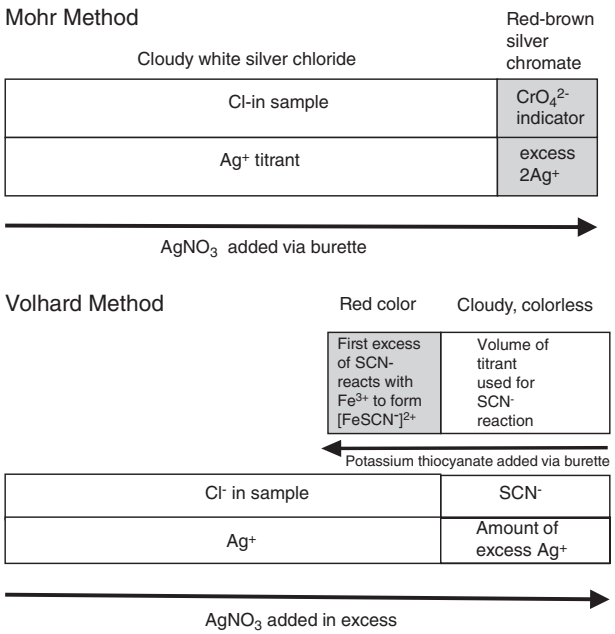
The major application of EDTA complexometric titration is testing calcium plus magnesium as an indicator of water hardness [10]. However, EDTA complexometric titration is suitable for determining calcium in the ash of fruits and vegetables (AOAC Method 968.31) [5] and other foods that have calcium without appreciable magnesium or phosphorus.

Several of the methods for mineral analysis described in this chapter have been incorporated into portable test strips that can be used to test samples in real time. For example, 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. The color is compared to a reference standard to estimate the calcium concentration.

21.3.2 Precipitation Titration

21.3.2.1 *Principles*

When at least one product of a titration reaction is an insoluble precipitate, it is referred to as **precipitation titrimetry**. Major factors that have limited the usefulness



21.1 figure Comparison of the Mohr (*forward*) and Volhard (*backward*) chloride titration methods

and accuracy of these methods have been the 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: Mohr titration and Volhard titration.

The **Mohr method** for chloride determination is a direct or **forward titration** method, in which the chloride (in NaCl) is titrated with silver nitrate in the presence of potassium chromate (Fig. 21.1). The arrow in the figure shows the titrant addition. Initially, the silver reacts with the chloride and the solution is cloudy white. When all the chloride is reacted, the excess silver reacts with chromate to form an orange-colored solid, silver chromate. The volume of silver nitrate titrant and the molarity of that solution are used to calculate the amount of chloride ions, which can be used to calculate the amount of NaCl:

$$Ag^+ + Cl^- \rightarrow AgCl \left(\text{until all } Cl^- \text{ is complexed}\right) \quad (21.1)$$

$$2Ag^+ + CrO_4^{2-} \rightarrow Ag_2CrO_4 \,(\text{orange only after } Cl^- \text{ is all complexed}) \quad (21.2)$$

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 which is shown by the bottom arrow in Fig. 21.1. The excess silver is then back-titrated (top arrow) using a standardized solution of potassium or ammonium thiocyanate, with ferric ion as an indicator. The volume of thiocyanate solution used in the titration is proportional to the amount of excess silver that did not react with the chloride in the sample. The moles of total silver are equal to the sum of the moles of chloride in the sample and moles of thiocyanate in the titrant:

$$Ag^+ + Cl^- \rightarrow AgCl \text{ (until all } Cl^- \text{ is complexed)} \quad (21.3)$$

$$Ag^+ + SCN^- \rightarrow AgSCN \text{ (to quantitate silver not complexed with chloride)} \quad (21.4)$$

$$SCN^- + Fe^3 \rightarrow [FeSCN]^{2+} \text{ (red when there is any } SCN^- \text{ not complexed to } Ag^+) \quad (21.5)$$

21.3.2.2 *Procedures*

21.3.2.2.1 Mohr Titration

Salt in foods may be estimated by titrating the chloride ion with silver. For example, to measure the salt content of butter (AOAC Method 960.29), a butter sample is solubilized in boiling water, potassium chromate is added as the indicator, and then the solution is titrated with a standardized solution of silver nitrate. 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, boiled water must be used to avoid interferences from carbonates in the water.

21.3.2.2.2 Volhard Titration

To measure the chloride content of cheese by the Volhard method (AOAC 935.43), excess silver nitrate is added to a cheese sample that is boiled in nitric acid solution. After cooling and filtering, the excess silver nitrate is titrated with potassium thiocyanate, using ferric aluminum as the indicator. Once chloride is determined by titration, the chloride weight is multiplied by 1.648 to obtain salt weight (based on molecular weight of Cl vs. NaCl). As in the Mohr titration method, water must be boiled to minimize errors due to interfering carbonates.

21.3.2.3 *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. A second example of a traditional mineral analysis method that has been adapted to a test strip format is the **Quantab® chloride titration** used in AOAC Method 971.19. This method 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.

21.3.3 Colorimetric Methods

21.3.3.1 *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. 7. Generally, the concentration of a specific 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 then 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, but if not, absorbance is read at a specific time. As with all mineral analysis of food, special efforts must be put in place to avoid contamination during sampling and analysis.

21.3.3.2 *Procedures: Determination of Phosphorus in Milk*

The total phosphorus content of foods can be quantified spectrophotometrically using modification of the Murphy-Riley method [11]. In this analysis, food-derived phosphorus reacts with ammonium molybdate at low pH to form phosphomolybdic acid. This product is subsequently reduced by ascorbic acid to a blue color, and antimony potassium tartrate is added to facilitate the reduction. The blue-colored phosphomolybdate complex formed has maximum absorptivity at 880 nm, a wavelength in the infrared range. However, this complex absorbs sufficient radiation at 700 nm, in the visible range, to be measured by most spectrophotometers. A requirement of the assay is that phosphorous must be soluble, and therefore solid food samples must first be ashed. To conduct the measurement, the ashed sample is solubilized in strong acid and then mixed with the Murphy-Riley reagent producing a colored product. A standard curve is prepared along with the samples with known amounts of phosphorus, and a regression equation is used to determine the concentration of phosphorus in the sample.

21.3.3.3 *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. The assays are particularly robust and often immune to matrix effects that can limit the usefulness of other methods for mineral analysis.

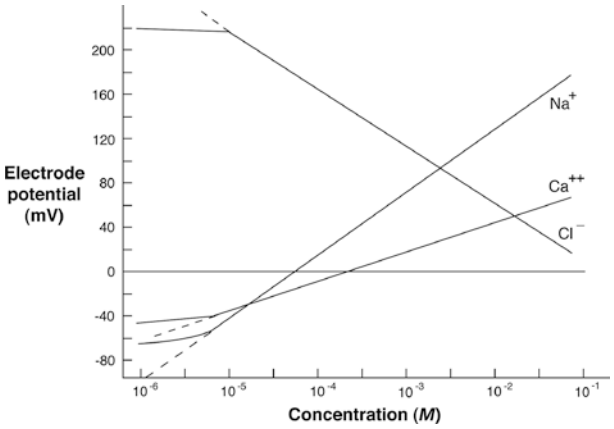
21.3.4 Ion-Selective Electrodes

21.3.4.1 *Background Information*

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. 22 is a specific example of an **ISE**. While the sensor of a pH electrode is specific to hydrogen ions, other sensors available are specific to individual mineral ions. 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**. In this manner, the potential within the sensor remains constant, while potentials develop at the sensor surfaces according to the **Nernst equation** (Chap. 22, Sect. 22.3.2.2), dependent on sample **ion activity** in the solutions contacting each surface. **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. 21.2).

21.3.4.2 *Principle*

The principle for using ISE to measure a specific mineral is the same as for measuring pH (i.e., uses Nernst equation), but the composition of glass in the sensing electrode is made to be specific for the element of interest. When sensing and reference electrodes (often as a combination electrode) are immersed in the sample solution,



21.2 figure

Examples of ion-selective electrode calibration curves for ions important in foods (Courtesy of Van London p Hoenix Company, Houston, TX)

the electrical potential that develops at the surface of the sensing electrode is measured by comparing it to the reference electrode. The voltage that develops between the two electrodes is related to the ion activity for the element of interest, measured as mV. Ion activity is related to ion concentration via the activity coefficient, which is controlled by ionic strength of the sample. The concentration of the element of interest is determined using a standard curve of mV vs. log concentration.

21.3.4.3 *General Methodology*

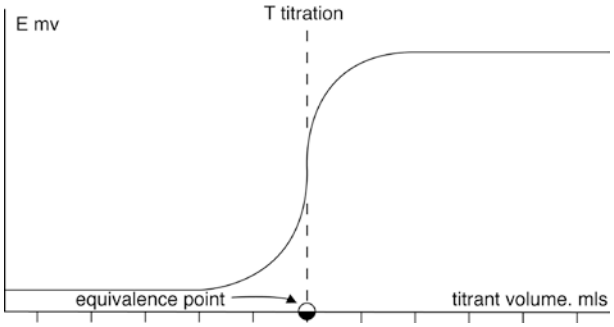
For ISE analysis, one simply attaches an ISE for the sample ion to a **pH meter** set on the **mV scale** and follows instructions for determination. Detailed information regarding the performance of specific ISEs is available from vendor catalogs. Typical ISEs likely to be employed for analysis of foods operate in the range of 1–10^{-6} M (Fig. 21.2), although the electrode response may be distinctly nonlinear at the lower concentrations. 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 prevents 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, 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 as samples are changed, many ISEs respond more rapidly to an increase in concentration of sample ion as compared to a decrease in concentration of sample ion.

21.3.4.4 *Electrode Calibration and Determination of Concentration*

In using an ISE, ion concentration can be determined using either a calibration curve 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 against the standard concentrations on a log scale. Examples of calibration curves for various ions are given in Fig. 21.2. 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.

ISEs can be used to detect the endpoints of potentiometric titrations by measuring the change in potential as the titration proceeds. One common use is for endpoint detection in the Mohr chloride assay previously described. In potentiometric silver chloride (Mohr) titrations, the ISE used for endpoint detection can either be specific for the titrant species (Ag^+) or the sample ion (Cl^-). If an ISE is used for Ag^+, a T-type titration curve results from the large increase in titrant activity detected at the equivalence point (see Fig. 21.3 for a cation titrant). Sample concentration is calculated from titrant volume to reach the equivalence point, taking into account the stoichiometric relationship between titrant species and sample ion.



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

21.3.4.5 *Applications*

Some examples of applications of ISEs are measuring 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, and magnetic stirrer. Several official methods exist for using ISEs to detect the endpoint in chloride and salt measurement, such as AOAC methods 971.27 (sodium chloride in canned vegetables), 976.18 (salt in seafood), and 986.26 (chloride in milk-based infant formula).

21.4 BENCHTOP RAPID ANALYZERS FOR SALT

There are several rapid methods of salt determination used in food analysis that are not necessarily based on the traditional methods described above in this chapter. Nonetheless, it is useful for food science students to be aware of the methods and to compare the principles to the other methods previously described.

Salt concentration can be measured by either hydrometry or refractometry (Chap. 15, Sects. 15.2.5.2 and 15.2.5.3). A **salometer** is a hydrometer that measures brine strength using a scale that indicates percent of salt saturation. A **salinity refractometer** can be either a manual or automatic device that gives a measurement analogous to that of sugar concentration, or brix, using a typical refractometer. An advantage of the automatic devices, over the less expensive manual models, is that they can correct for changes in the refractive index of salt solutions as a function of temperature.

Several instrument manufacturers sell devices that can be used to determine the salt content of a sample in under a minute using an automatic titration. In these instruments, both the titrant dosing and the endpoint detection are controlled by the instrument which increases the precision over manual titrations where there is subjective error in both determining the titrant dose as well as the endpoint color change. These instruments work on the basic principle of precipitation titrations, as silver ions are still used to precipitate chloride ions, but there are a few differentiating features. For example, some instruments couple automated silver nitrate addition with endpoint determination using an ion-sensitive electrode. Several manufacturers offer models using this technique including Mettler-Toledo (Columbus, OH), Hanna Instruments (Woonsocket, RI), and Metrohm (Herisau, Switzerland). A second type of automatic chloride titrator does not rely on using a silver nitrate titrant but rather generates silver ions in the sample using the principle of coulometry. In instruments manufactured by Nelson-Jameson (Marshfield, WI), Sherwood Scientific (Cambridge, United Kingdom), and DKK-TOA (Toyko, Japan), silver ions are generated from a silver wire in situ via an electric current, and they complex with chloride ions in solution. The potential of the solution is monitored via an ISE, and the reaction is complete when the potential shifts dramatically as in Fig. 21.3. The electric current used to generate silver ions then may be converted to chloride and subsequently salt concentration. One advantage of this method is that no silver nitrate titrant is needed, and thus a standardization step is not necessary.

Another common method of salt determination in commercial laboratories is via conductance (e.g., DiCromat Salt Analyzer, Arrow Scientific, Gladesville, Australia). Salts in solution dissociate into negative and positive ions and thus increase conductance in proportion to their concentration. Conductance-based salt analyzers are rapid and also correct for differences in conductivity as a function of temperature.

21.5 COMPARISON OF METHODS

For labeling, processing, and even practical nutrition, the focus is on only a few minerals that 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 perfor-

mance regarding accuracy, sensitivity, detection limit, specificity, and interferences. Information on method performance is available from the collaborative studies referenced with AOAC official methods. 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. Atomic absorption spectrophotometers with a graphite furnace or inductively coupled plasma-mass spectrometers are capable of sensitivity in the parts per billion range, which is much lower than the limits of traditional methods. Yet, instrumentation capable of such analysis is expensive and beyond the financial resources of many quality assurance laboratories, and often this degree of sensitivity is not required. This leaves the options of sending samples out to certified laboratories for analysis or utilizing one of the more traditional methods for analysis.

21.6 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, and some benchtop analyzers for salt content. Table 21.3 gives a summary of many methods covered in this chapter, with comparison to AAS and ICP-OES (Chap. 9).

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.

21.7 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. 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.
4. Describe how and why there is a need to employ standards in background matrix, spikes, and reagent blanks.
5. 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.
6. 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.
7. What factors should be considered in selecting a specific method for mineral analysis for a food product?

21.3 table Summary of mineral analysis methods

Method	*Principle*	*Advantages*	*Disadvantages*	*Applications*	*AOAC method number*
EDTA titration method	Description for sample containing just calcium: EDTA of titrant solution complexes with calcium, 1:1, at pH 10; EDTA binds to calcium ions stronger than the indicator binds to calcium. When all the calcium present in the sample has reacted the EDTA titrant, and no calcium is bound to the indicator, the indicator changes from pink to blue. Moles of calcium in the sample are equivalent to moles of EDTA used in titration	Rapid, especially when done with test strip. Inexpensive	Can be interfering compounds. Endpoint is subjectively determined	Testing water hardness. Calcium in ash of fruits and vegetables	AOAC Method 968.31, calcium in canned vegetables
Mohr titration	A forward titration. Chloride (in NaCl) is titrated with silver nitrate in the presence of potassium chromate. Silver reacts with the chloride; when all chloride is reacted, the excess silver reacts with chromate to form an orange-colored solid, silver chromate. Volume and molarity of silver nitrate are used to calculate amount of chloride, which relates to amount of NaCl	Does not require expensive equipment, highly trained personnel, or ashing of sample. Fewer reagents and less time-consuming than Volhard titration. Inexpensive (unless automated equipment)	Subjectivity of determining endpoint of titration (if manual titration)	Salt content of variety of foods. Test strip version and automated instruments available	AOAC Method 960.29, salt in dairy products and butter
Volhard titration	A backward titration. An excess of a standard solution of silver nitrate is added to a chloride-containing solution. Excess silver nitrates is back-titrated with a standardized solution of potassium or ammonium thiocyanate, with ferric ion as an indicator. Volume of thiocyanate solution used is proportional to the excess silver. Moles of total silver is equal to the sum of the moles of chloride in the sample and moles of thiocyanate in the titrant	Does not require expensive equipment, highly trained personnel, or ashing of sample. Rapid. Inexpensive	Subjectivity of determining endpoint of titration. Requires more reagents and time than Mohr titration	Salt content of variety of foods	AOAC Method 935.43 chloride (Total) in cheese; AOAC Method 915.01, chloride in plants
Colorimetric methods	Description for just mineral analysis. Chromogen in reagent reacts with mineral of interest to form a soluble colored compound that can be quantitated by absorption of light at a specific wavelength. Concentration of mineral of interest is determined from standard curve of absorbance vs. concentration, based on Beer's law	Applicable to wide variety of minerals. Very specific. Other minerals usually don't interfere. Less expensive than AAS or ICP-OES, but similar accuracy and precision	Requires significant technician time	Low-cost method for analyzing single element	AOAC Method 944.02, iron in wheat flour

(continued)

Ion-selective electrode	Principle is the same as for measuring pH (i.e., uses Nernst equation), but by varying the composition of the glass in the sensing electrode, the electrode can be sensitive to a specific mineral. Sensing and reference electrodes (often as combination electrode) are immersed in solution with element of interest; electrical potential that develops at surface of sensing electrode is measured by comparing to reference electrode with fixed potential. Voltage between sensing and reference electrode is related to ion activity, measured in mV. Ion activity is related to ion concentration via the activity coefficient, which is controlled by ionic strength. Concentration of element is determined using standard curve of mV vs. log concentration	Can measure many anions and cations directly. Does not require expensive equipment (only pH meter), highly trained personnel, or ashing of sample. Analysis is independent of turbidity, color, or viscosity	Cannot measure at low concentration. Electrode response can be slow. Sensing and reference electrode must be specific to element being measured. High rate of premature failure for some electrodes	Quality control, especially for Na or K	AOAC Method 976.25, sodium in foods for special dietary use
Atomic absorbance spectroscopy (AAS)	Heat energy from a flame converts molecules to atoms and then energy (at specific wavelength, from a hollow cathode lamp (HCL)) raises atoms of a specific element from ground to excited state. Measure absorption of energy, which is linearly related to concentration of element	Can measure many elements (i.e., not just sodium). More sensitive than Mohr or Volhard titration for sodium	Expensive instrument. Requires highly trained personnel. Requires ashing of most types of samples. Requires a different HCL for each element. Use potentially explosive fuel gas. More interferences than ICP-OES. Response is not linear over a broad concentration range	Single-element analysis in a given food or biological sample	AOAC Method 975.03, metals in plants and pet foods; AOAC Method 985.35, minerals in infant formula, enteral products, and pet foods
Inductively coupled plasma-optical emission spectrometry (ICP-OES)	Energy from a plasma converts molecules to atoms and ions and then plasma energy raises atoms from ground to excited state. Measure emission of energy (at specific wavelength) as excited atoms returns to a lower energy state; amount of radiant energy emitted is proportional to concentration of specific element	Can measure many elements (i.e., not just sodium) simultaneously. More sensitive than Mohr or Volhard titration for sodium. Few interferences. Response linear over a large concentration range	Expensive instrument. Requires highly trained personnel. Requires ashing of most types of samples	Multiple elements in a large number of samples. Nutrition labeling	AOAC Method 984.27, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc in infant formula; AOAC Method 985.01, metals and other elements in plants and pet foods

21.8 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→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. 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 be comprised of 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 is 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.

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

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

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

$$\text{Percent salt} = 0.37\%$$

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) $\text{mol Ag added} = \text{mol Cl}^-\text{ in sample} + \text{mol SCN}^-\text{ added}$

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

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

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

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

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

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

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

4. $A = abc$

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

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

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

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

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

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

5. (a) Lowest dilution volume for 1 mL of stock:

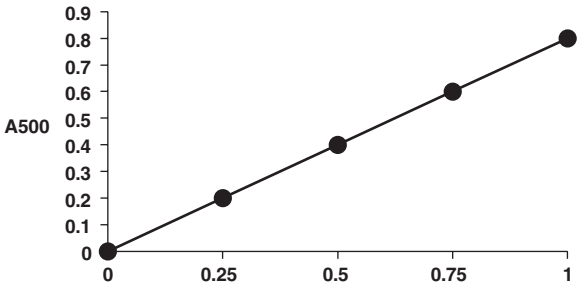
$$\frac{0.25\,\text{mg}}{0.5\,\text{mL}} = \frac{1\,\text{mL}}{x\,\text{mL}} \times \frac{5\,\text{mg}}{\text{mL}}$$

$$x = 10\,\text{mL}$$

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. $$C_O = \frac{0.001\text{L} \times \dfrac{0.001\text{moles}}{\text{L}} \times \dfrac{1}{0.100\,\text{L}}}{\left(10^{0.006/0.285} - 1\right)} = 1.6\,\text{m}M$$

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