1
Preparation of Liquid and Solid Samples

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1.1
Introduction

Sampling and sample preparation of liquids and solids often present significant challenges for real-world quantitative analyses using spectrometric techniques (e.g., UV–vis and infrared absorption, luminescence and Raman spectroscopies). Very often, the native form of a sample is unsuitable for analysis. This could be due to (i) the complex nature of the object, which could provide false measurements due to interferences or masking agents; (ii) the size of the object being too large to analyze in its entirety (e.g., laboratory sample of contaminated soil); or (iii) the awkward shape of the object, preventing it from fitting within the instrument in which the measurement is to be made. To overcome these problems, some sort of sample preparation must be performed. In many cases, sample preparation is required before any quantitative analysis, and both can have dramatic impacts on the measured results and their accuracy.

The previous chapter presented the main criteria to be followed for solid and liquid sample collection. This chapter presents a general overview of various methods for sample preparation. This general topic has been described extensively in a variety of research papers and review chapters in the literature [1–6], with specific variations for particular applications often being necessary. This chapter deals with the sample preparation required to provide a material suitable for spectrometric analysis.

1.2
Preparation of Samples for Analysis

1.2.1
Measurement Process

Samples collected for spectral analysis can generally be classified into three categories based on their state: (i) solids, (ii) liquids, and (iii) gases. Samples

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are typically classified on the basis of their state as a method of providing an initial means of handling/treating them. A general flow diagram depicting the measurement process for various samples is shown in Figure 1.1.

1.2.2 Preparation of Samples for Analysis

Once a representative sample has been obtained from the object of interest, the next step is to prepare the sample for analysis. Since sample preparation depends upon both the analyte (e.g., iron in water, polycyclic aromatic compounds in benzene, etc.) and the instrumentation used to perform the spectroscopic measurement (e.g., UV–vis or IR absorption, luminescence, Raman, HPLC-fluorescence, GC–MS, etc.), details of the preparation process will vary from analysis to analysis. Many general procedures have been developed over the years for the preparation of various types of samples prior to analysis. Most of these procedures can be classified depending upon the type of samples that are to be analyzed, either solid or liquid. Within each of these categories exist several subcategories based upon the type of analyte to be measured.

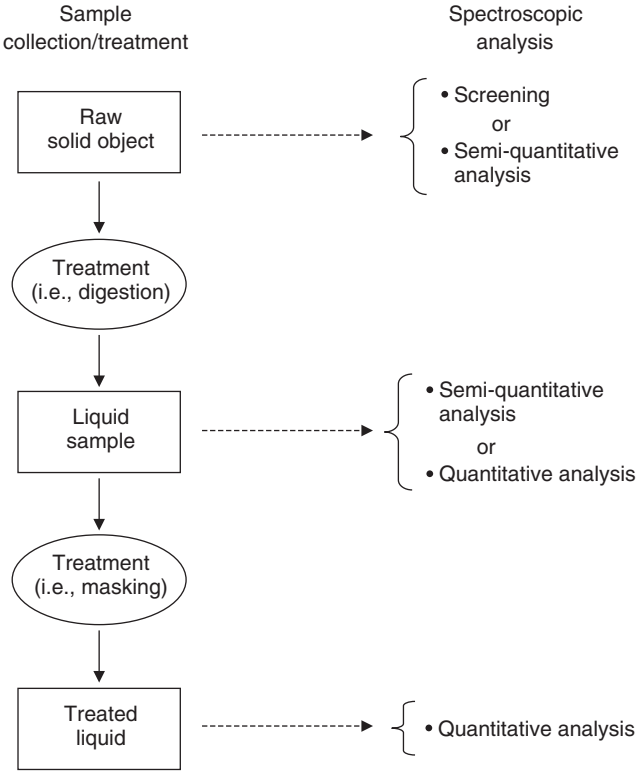

Figure 1.1 Schematic diagram depicting generalized sample preparation and analysis steps.

1.2.3 Solid Samples

The first of the two categories that we will discuss is solid samples. The various types of solid samples that are most often encountered have been discussed elsewhere in the sampling section of this chapter (i.e., powders, chunks, or cores). In the case of the latter two sample types (chunks and cores), the first preparation step involves grinding the larger pieces into a powder, which is much easier to deal with, and introduce it into the measuring instrument. The most common method for obtaining powders from these samples involves grinding a solid sample into a powder using either a mortar and pestle or a ball mill. Mortars typically come in two different types: the agate version (or ceramic) for relatively soft solid materials (e.g., large crystalline substances) that must be ground into a fine powder; or steel mortars, which are used for crushing much harder materials. In agate mortars, the material to be ground is placed in the depression of the mortar and then the sample is pressed down with the pestle in a rotating movement. When using agate or ceramic mortars and pestles, it is important to clean them thoroughly to avoid sample contamination. Less expensive mortars are typically softer and hence can be scratched more easily than more expensive ones. This is especially the case for ceramic mortars. Once scratched, they are much more difficult to clean, and may require the use of an abrasive or even a strong HCl solution. Steel mortars, also known as *percussion mortars*, have a hardened steel sleeve and pestle that fit snuggly into the mortar, and a hammer is then used to strike the pestle and subsequently crush the sample.

Another grinding tool that is often used to grind solid samples is the ball mill. A ball mill is a ceramic drum within which are placed the sample and many small balls made of hard ceramic. To grind the sample, the drum is rotated, producing a very fine powder. Ball mills are often used on softer solids, as the time taken for grinding is directly proportional to the hardness of the material. To ensure that none of the material that is being ground sticks to the walls of the mill during the grinding process, thereby producing larger pieces, the samples are typically dried to 100–110 °C prior to grinding to expel any water.

1.2.3.1 Sample Preparation for Inorganic Analysis

Most commercially available instruments for quantitative analyses in chemical and biological sciences are designed for the analysis of liquid samples. Because of this, solid samples that are to be analyzed are typically dissolved in a suitable solvent, usually following conversion to a powder by one of the previously described methods. The solvent chosen for this dissolution process may be either polar (e.g., water) or nonpolar (e.g., benzene) depending on the polarity and reactivity of the sample. In order to ensure that the entire analyte has been dissolved in the solution of interest, a solvent is chosen that can dissolve the entire solid sample (analyte as well as other materials). If the sample cannot be readily dissolved in these mild conditions, many other techniques are available for dissolution. As inorganic

materials often present the greatest difficulty in dissolution, this section will deal primarily with these materials.

1.2.3.1.1 **Acid Digestion** Acid digestion of inorganic materials is a common alternative to the mild solvents used for dissolution, as described above. When using acids to digest metallic materials, great care should be taken not to change the speciation of the metal or metallic species to be analyzed. When analyzing a reduced state of a metal or metallic species, several nonoxidizing acids can be used. These include HF, HCl, HBr, H_3PO_4, dilute H_2SO_4, and dilute $HClO_4$. These acids dissolve most metals with negative reduction potentials. However, in some cases (i.e., aluminum), a protective oxide layer is formed that prevents the metal from being dissolved, which must be removed prior to dissolution. Substances that cannot be dissolved in the nonoxidizing acids described above are often soluble in the oxidizing acids: HNO_3, hot and concentrated H_2SO_4, and hot and concentrated $HClO_4$.

In most cases, the solubility of a metal dramatically increases by heating the acid. To improve the dissolution of samples in hot acids, a device often referred to as a *digestion bomb* has been developed. This device is comprised of a Teflon™-lined sample container that can be sealed and placed in a microwave oven for heating. An alternative to using the digestion bomb is to heat the acids in an open container, thereby allowing volatile species created during the reaction (e.g., H_2S, H_3BO_3, etc.) to escape. However, in rare cases, some metal halides (e.g., $SnCl_4$, $HgCl_2$, OsO_4, and RuO_4) are volatile and can escape as gases.

1.2.3.1.2 **Nonoxidizing Acids** HCl and HBr are typically used for the dissolution of most metals, oxides, sulfides, phosphates, and carbonates. HCl and HBr digestions are typically performed with a concentration of 37% and 48–65%, respectively. When using hot acids, HCl has a constant boiling composition of 20% at 109 °C, and HBr has a constant boiling composition of 48% at 124 °C. H_2SO_4 is an excellent solvent for most materials when used at its boiling point, which is 338 °C. The composition of H_2SO_4 for digestion purposes is typically 95–98%. Heating H_2SO_4 causes the sample to become dehydrated while dissolving the metals and, in addition, causes any organic material to become oxidized. To dissolve refractory oxides that are insoluble in other acids, hot H_3PO_4 can be used at a concentration of 85%. As the temperature of the acid is increased, it dehydrates. At temperatures above 150 °C, it becomes anhydrous; at temperatures greater than 200 °C, it dehydrates to pyrophosphoric acid; and, finally, at temperatures greater than 300 °C, it is converted to meta-phosphoric acid. A 50% HF solution is often used for the dissolution of silicates. Since glass is comprised primarily of silica, HF must be used in Teflon, silver, or platinum containers. At 112 °C, HF has a constant boiling composition of 38%.

1.2.3.1.3 **Oxidizing Acids** HNO_3 is capable of dissolving most metals, with the exception of gold and platinum. To dissolve these two metals, a 3 : 1 volumetric mixture of HCl and HNO_3 (also known as *aqua regia*) can be used. As described

above, H_2SO_4 is typically considered a nonoxidizing acid with respect to metals; however, it provides a useful means of oxidizing organic material in the sample. When the organic material in the sample cannot be oxidized by either HNO_3 or H_2SO_4, a 60–72% solution of hot $HClO_4$ can be used. In either cold or dilute conditions, $HClO_4$ is not oxidizing. However, at high temperatures, $HClO_4$ becomes an explosive oxidizer. Because of this extreme oxidizing potential, it is important to evaporate and destroy as much organic material as possible with hot HNO_3 prior to using $HClO_4$.

It should be noted that mineral acids used to digest solid samples may contain a large number of metals in different concentration ranges (usually parts per million or subparts per million levels) themselves. This could provide a source of contamination, especially significant for trace analysis work. One way to account for this contamination source is to include a blank preparation with the digestion procedure. This involves exposing an extra beaker or flask, identical to the one containing the sample, to the same digestion treatment (added acids, thermal treatment, dilutions, etc.) to which the sample was exposed. The blank solution prepared this way will contain an approximately equal amount of contaminants introduced to the sample by the acid digestion.

1.2.3.1.4 **Fusion Reactions** Fusion is a process by which a finely powdered sample is mixed with 5–10 times its mass of inorganic material (flux) and heated in a platinum crucible to temperatures of 300–1200 °C, thereby melting the flux and the sample. While in the molten state, chemical reactions between the flux and the sample produce new species which are more soluble. After the sample has been thoroughly melted, the molten solution is allowed to cool slowly. During this cooling process, the crucible is swirled to create a thin layer of solidified material on the walls of the container. The newly solidified material is then dissolved in a dilute acid. Many different flux materials have been used over the years, with Na_2CO_3, $Li_2B_4O_7$, $LiBO_2$, $Na_2B_4O_7$, NaOH, KOH, Na_2O_2, $K_2S_2O_7$, B_2O_3, and a 2 : 1 mixture (w/w) of $Li_2B_4O_7$ and Li_2SO_4 being the most common. Fluxes are typically classified as acidic, basic, or amphoteric, with basic fluxes being best suited to dissolve acidic oxides of silicon and phosphorus and acidic fluxes being best suited to the dissolution of basic oxides, alkali metals, alkaline earths, lanthanides, and aluminum. The basic fluxes listed above include Na_2CO_3, $LiBO_2$, NaOH, KOH, and Na_2O_2. The acidic fluxes include $Li_2B_4O_7$, $K_2S_2O_7$, B_2O_3, and $Na_2B_4O_7$.

Na_2CO_3 is one of the most common fluxes, and is typically used for dissolving silicates (e.g., clays, rocks, minerals, glasses, etc.) as well as refractory oxides and insoluble sulfates and phosphates. To dissolve aluminosilicates, carbonates, and samples with high concentrations of basic oxides, $Li_2B_4O_7$, $LiBO_2$, or $Na_2B_4O_7$ is typically used. Analysis of both silicates and SiC-based materials can be performed using a flux of either NaOH or KOH. When using these two fluxes, however, frothing may occur in the absence of water. Therefore, best results are often achieved by first melting the flux and then adding the sample. It is also important to note that, when using NaOH and KOH as fluxes, either a gold or silver crucible should be used for the reaction. For silicates that cannot be dissolved using

Na_2CO_3, a more powerful oxidant such as Na_2O_2 can be used. This flux is good for dissolving iron and chromium alloys and should be used in a nickel crucible. Owing to the strong oxidizing and basic properties of Na_2O_2, the crucible used for this reaction should be coated with a thin layer of Na_2CO_3, which melts at a higher temperature than the peroxide and therefore protects the crucible. To dissolve refractory oxides and not silicates, $K_2S_2O_7$ is the flux of choice. $K_2S_2O_7$ is prepared by either heating $KHSO_4$ until all the water is driven off and all the foaming has stopped or decomposing $K_2S_2O_8$ with heat. B_2O_3 is a very useful flux for the dissolution of oxides and silicates. Its main advantage over the other fluxes listed previously is that the flux can be removed from the crucible completely, following reaction with the sample, as a volatile methyl borate, by simply washing several times with HCl in methanol. For relatively fast dissolution of refractory silicates and oxides (10–20 min at 1000 °C), a 2 : 1 mixture (w/w) of $Li_2B_4O_7$ and Li_2SO_4 works well. One gram of this flux can dissolve 0.1 g of sample, and the resulting material can be easily dissolved in hot HCl. While fusion has proven to be a necessary method for the dissolution of many compounds, it should be used only as a last resort, because of the possibility of introducing impurities into the sample as well as being a very time-consuming process.

1.2.3.2 Decomposition of Organics

1.2.3.2.1 Ashing When elemental analysis of an organic sample or quantitative analysis of inorganic species complexed with organic species is desired, the first step of the process is to decompose the organic material. This process of decomposition of organic matter is often termed *ashing*. Ashing is typically subdivided into two different categories: those processes that do not require the use of a liquid, that is, dry ashing; and those processes that rely on liquids for the decomposition, that is, wet ashing. Fusion can be used as a type of ashing, with the most common fluxes used in these processes being Na_2O_2 and alkali metals, but the simplest and most common form of dry ashing is via combustion. In this procedure, the organic material is burned in a stream of oxygen gas, with catalysts added for more complete combustion. The released CO_2 and H_2O are then trapped and analyzed quantitatively. Variations of this procedure are also used to perform quantitative analyses of nitrogen, sulfur, and halogens in organic matter.

Wet ashing methods have existed for over several hundred years. One such method, which has been used since 1883, is known as the *Kjeldahl procedure*. This procedure is one of the most accurate and widely applicable methods for determining the nitrogen composition of organic matter. The first step in this procedure is to digest the organic matter in boiling H_2SO_4, which converts the nitrogen atoms in the sample to NH_4^+ while oxidizing other elements such as carbon and hydrogen. To speed up the process, K_2SO_4 can be added, which increases the boiling point of the H_2SO_4 to 338 °C.

Another common procedure that has been developed is known as the *Carius method*. This procedure, which involves the digestion of organics in fuming HNO_3, is carried out in a heavy-walled, sealed glass container that is heated to 200–300 °C.

A very powerful technique that can be widely applied to the decomposition of organic matter is refluxing the sample in a mixture of HNO_3 and $HClO_4$. However, perchloric acid is a strong explosive, and great care should be taken by the experimentalist to shield himself/herself from the digestion process. In this procedure, the sample is first heated in boiling HNO_3, and the solution is then evaporated until almost dry. This process is repeated several times to remove any easily oxidized material that might explode in the presence of $HClO_4$. The sample is then collected and the process repeated with $HClO_4$.

One of the fastest and easiest methods of wet ashing organic matter involves the use of a Teflon-lined digestion bomb (described earlier in the section on dissolution) and a microwave oven for heating. While many different procedures have been developed for various analyses, they all generally involve the addition of the sample and a liquid into the digestion bomb, which is then placed in the microwave oven and heated. An example of such a procedure is the decomposition of animal tissue using a 1 : 1 mixture of HNO_3 and H_2SO_4 and heating it in a microwave oven for 1 min. Another example is a modified version of the Kjeldahl reaction in which H_2SO_4 and H_2O_2 are mixed in a Teflon-lined bomb and heated, thereby reducing the digestion time to approximately 15 min.

In contrast to these wet ashing procedures that rely on concentrated acids, a mild form of wet ashing has also been developed. This procedure uses hydroxyl radicals that are produced using Fenton's reagent, a combination of H_2O_2 and $Fe(NH_4)_2(SO_4)_2$, to oxidize the organic materials. The mixture is then heated to 50 °C with the organic material present, allowing the radicals to oxidize the sample (Table 1.1).

Table 1.1 Various sample preparation methods for solid samples.

Dissolution of solids
Acid digestion
Nonoxidizing acids
HF, HCl, HBr, H_3PO_4, dilute H_2SO_4, dilute $HClO_4$
Oxidizing acids
HNO_3, hot and concentrated H_2SO_4, hot and concentrated $HClO_4$
Fusion reactions
Basic fluxes
Na_2CO_3, $LiBO_2$, NaOH, KOH, Na_2O_2
Acidic fluxes
$Li_2B_4O_7$, $K_2S_2O_7$, B_2O_3, $Na_2B_4O_7$
Decomposition of organics
Ashing methods
Dry ashing
Combustion in O_2, and fusion with Na_2O_2 or alkali metals
Wet ashing reagents
Hot H_2SO_4, fuming HNO_3, $HClO_4$, hydroxyl radicals (H_2O_2 and $Fe(NH_4)_2(SO_4)_2$)

1.2.4 Liquid Samples

1.2.4.1 Extraction/Separation and Preconcentration

Once a liquid sample has been obtained, either from an original liquid object or from the dissolution of a solid object, the various species of interest must be isolated for analysis. In the case of a liquid suspension, filtration or centrifugation is often performed prior to analysis to remove any solid particles. In the case of a solution, there are many methods available for isolating analytes, including complexation, separation, or extraction. These procedures are performed prior to analysis, for many reasons. Most often, these procedures are performed either to remove any species that may cause interferences in the particular analysis or to provide a means of concentrating the analyte prior to analysis.

1.2.4.1.1 **Extraction** Extraction has historically been one of the most common means of isolating a particular species from a solution. Several different types of extraction are commonly used for analyte isolation, including liquid/liquid extraction and solid-phase extraction. In any extraction procedure, the isolation of particular components is based upon the affinity of the particular species for two different phases. In liquid/liquid extractions, the two phases are both liquid and are immiscible in each other (e.g., an aqueous phase and an organic phase), creating two layers with a distinct boundary. The affinity of the various components within the sample for each of the two layers is used to separate them. The distribution of the analyte or solute between the two different phases is described as the partition coefficient (the ratio of the solute's concentration in one solvent to its concentration in the second). Therefore, the ideal extraction would happen with either a very large or a very small partition coefficient. When this is not the case, and the solute is only slightly more soluble in one solvent than the other, multiple extractions may have to be performed to remove most of the solute. In addition, as this extraction is based on fractional partitioning of the solute, it is impossible to extract 100% into any one phase. Therefore, to determine the amount of analyte that has been extracted, one needs to keep track of the number of extractions that were performed and the partition coefficient of the process.

Another type of extraction commonly used on liquid samples is based upon the partitioning of an analyte between the liquid in which it is dissolved and a solid support. Such extractions are typically based upon adsorption of the solute onto the solid. An example of such an extraction is in the adsorption of hydrocarbons in an aqueous solution onto activated charcoal. This process has long been used in such areas as pollution control (e.g., oil spills in water) and much more recently is beginning to be implemented more in trace analysis procedures as a technique called *solid-phase microextraction*. The main disadvantage of extraction techniques is typically the time that is required to recover most of the solute. Because of this problem, extraction in quantitative analyses is typically performed as a last choice.

1.2.4.1.2 **Complexation** To increase the specificity of a particular analysis, it is often necessary to remove components from the solution that could produce

erroneous results. One means of performing this task is through complexation reactions. One such procedure, known as *masking*, involves the complexation of an interfering species with a chelating agent. The reaction between the two species forms a stable complex which cannot undergo certain chemical reactions that are essential for quantification of the analyte. Therefore, by complexing possible interferents, a more selective measurement can be obtained. Another form of complexation that is often employed for the removal of interferences is precipitation. In precipitation reactions, an insoluble complex is selectively formed with either the interfering species or the analyte itself. Once the precipitate is formed, it can be removed and discarded in the case of an interfering species or analyzed in the case of the analyte. Complexation reactions typically involve elaborate procedures, and depend upon many parameters such as the chemical composition of the solution, its pH, and the temperature. When these factors are considered, complexation procedures can provide excellent results. For example, uranium can be isolated from associated metals in solution with the addition of carbonates. Carbonates form a soluble complex with uranium, while most other metals form insoluble carbonate of hydroxide precipitates. The complexation of a particular species is dependent on the chemical equilibria of the various species involved. Table 1.2 provides a general list of the most common complexing agents and the species with which they react.

Using Table 1.2 and the particular formation constants and solubility constants of the involved species, at the correct pHs and temperatures, determination of the best complexing agents for a liquid sample should be possible. In the case where two agents form complexes with the same elements, the particular solution parameters (e.g., pH) should be used to determine which is the most suitable. For

Table 1.2 Common masking agents.

Masking agent	Ions of elements complexed
1. Carbonate	Be, Th, U
2. Citrates and tartrates	Be, Mg, Ca, Sc, Ti, Cr, Mn, Fe, Ga, Sr, Y, Zr, Nb, Mo, In, Sb, Ba, La, Hf, Ta, W, Re, Tl, Pb, Bi, Ce, Th, U
3. Cyanide and amine	Co, Ni, Cu, Zn, Ru, Rh, Pd, Ag, Cd, Os, Ir, Pt, Au, Hg
4. EDTA	Mg, Al, Ca, Sc, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Cd, In, acetic Ba, La, Pb, Bi, Ce, Th
5. Fluoro (F^-) agents	B, Al, Si, Ti, Zr, Nb, Mo, Hf, Ta, W
6. Halides (Cl^-, Br^-, I^-)	Ge, As, Ru, Rh, Pd, Cd, Sn, Os, Ir, Pt, Au, Hg, Tl
7. Oxalate	V, Cr, Fe, Co, Ni, Ge, In, Re, Tl, Bi
8. Oxo and hydride	Be, B, Al, P, V, Cr, Mn, Zn, Ga, Ge, As, Se, Mo, Ru, Sn, Sb, Te, W, Re, Os, Pb
9. Peroxo agents	Ti, V, Zr, Nb, Hf, Ta, U
10. Sulfide	As, Sn, Sb

EDTA, ethylenediaminetetraacetic acid.

instance, citrates usually form more stable complexes in acidic solutions, whereas tartrates are typically more stable in alkaline solutions.

1.2.4.2 **Chromatographic Separation**

With continued developments in chromatographic columns and equipment, separation of various analytes from a sample via chromatography has become the most common pretreatment to complex liquid samples [7]. The combination of chromatography and spectroscopy is described in detail later in the chapter on Hyphenated Techniques in this Handbook. This section only provides a brief discussion of the separation methods used in sample treatment prior to spectrochemical analysis. As with solid extraction procedures, chromatographic separation is based upon the partitioning of the various solutes between two different phases, namely a liquid phase and a solid phase. However, unlike extractions, the two different phases are not separated to allow removal of the component of interest. Instead, the liquid containing the solute is flowed across the solid phase, and the partitioning of the various components in the liquid between the two phases causes them to be retained temporarily and eluted from the solid matrix at different times. The time of elution from the solid matrix, or the retention time, is determined by the partitioning coefficient of the particular component between the solid and the liquid. Many chromatographic techniques exist for separating various solutes in liquids. These techniques are generally classified by the types of interactions that occur between the analytes and the solid phase, or matrix. These categories include (i) adsorption, (ii) ion exchange, (iii) partition, (iv) thin layer, and (v) size exclusion. In adsorption chromatography, the separation is based upon the polarity of the solid matrix and the solutes. Solid matrices for adsorption chromatography can include alumina, charcoal, clay, diatomaceous earth, silica, silica gels, cellulose, or starch, which are packed into a glass column. In the case of alumina, which is a polar matrix, the sample would be flowed down the column, with the nonpolar solutes eluting first and the polar solutes eluting later because of their stronger interactions with the matrix.

Ion-exchange chromatography is similar to adsorption chromatography, with the exception that elution of the various components is based upon the affinity of ions for the solid matrix. The solid support matrix for such separations is some form of ion-exchange resin, depending upon the materials to be separated. The mobile, or liquid phase, in ion-exchange chromatography is generally an aqueous solution. Ion-exchange chromatography is used to separate solute molecules based upon their charge. Under optimum conditions, ions of equal charge, such as the alkali metals, can even be separated in an ion-exchange column. In particular separations, the effectiveness of ion-exchange chromatography can be enhanced by the addition of chelating agents to the mobile phase, thus reducing the ionic interactions of particular species and making them elute earlier in time.

The third type of liquid chromatography, partition chromatography, is performed by placing the sample on a column of solid support that has been impregnated with a liquid. The sample is then flowed down the column, with a second liquid as the mobile phase and immiscible in the liquid used to moisten the column. Therefore,

as the sample flows down the column, the various components are partitioned between the solid and liquid phases based upon their solubility in the two solvents, and thus elute at different times.

Thin-layer chromatography is performed using a glass plate that has been evenly coated with an absorbent such as alumina or silica gel. To ensure binding of the adsorbent to the glass, starch, plaster of paris, collodion, or a plastic dispersion is often added. The coated plates are then dried in an oven prior to use. Once dried, the sample is spotted on one end of the plate, which is then placed in a dish containing a solvent. The solvent then travels up the plate via capillary action, and the various components in the sample travel at a different rates and therefore different distances during a specific time depending upon their solubility in the solvent. Therefore, by changing the solvent used, the separation of the components can be varied until the particular analyte of interest is separated out from other components.

Another form of liquid chromatography that can be used for separation of components in a solution is known as *size-exclusion chromatography*. In this technique, the solid matrix, which has well defined pore sizes, is placed in a column through which the liquid sample is flowed. The size of the pores varies from matrix to matrix, and it is these pore sizes that are used to separate the compounds. As the components travel down the column, their elution times are based upon their size, with the larger unretained species eluting first and the smaller species being held up in the pores and eluting later. This technique typically works best for larger molecules such as biomolecules or polymers.

Another more recently developed means of separating components in a solution is known as *electrophoresis*. This technique is used for the separation of components based upon their ability to travel in an electric field. Many different matrices have been used for electrophoretic separations, including buffered solutions and gels (e.g., agarose gel). Gel electrophoresis has been used extensively for the separation of biomolecules; however, it is often slow and irreproducible. A faster and more reliable form of electrophoretic separation is known as *capillary electrophoresis*. In this technique, a buffer-filled capillary is used to span the distance between two containers of the same buffer solution. A potential of 20–30 kV is typically applied between the two containers, and a small amount of sample is injected into the capillary. The individual components of the sample are then separated, which is based on the combination of their overall charge and their friction within the solvent. The individual components can then either be collected or detected upon elution from the column.

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