Introduction to Analytical Separations

In the vast majority of real analytical problems, we must identify and quantitate one or more components from a complex mixture. Isolating the desired unknown is the challenging first step. This chapter discusses the fundamentals of analytical separations, and the next three chapters describe specific methods.

23-1 Solvent Extraction

Extraction is the transfer of a solute from one phase to another. Common reasons to carry out an extraction in analytical chemistry are to isolate or concentrate the desired analyte or to separate it from species that would interfere in the analysis. The most common case is the extraction of an aqueous solution with an organic solvent. Diethyl ether, toluene, and hexane are common solvents that are immiscible with and less dense than water. They form a separate phase that floats on top of the aqueous phase, as shown in Color Plate 21. Chloroform, dichloromethane, and carbon tetrachloride are common solvents that are denser than water.\(^1\) In the two-phase mixture, one phase is predominantly water and the other phase is predominantly organic.

Suppose that solute S is partitioned between phases 1 and 2, as depicted in Figure 23-1. The partition coefficient, \(K\), is the equilibrium constant for the reaction

\[
S\text{ (in phase 1)} \Leftrightarrow S\text{ (in phase 2)}
\]

Partition coefficient:

\[
K = \frac{A_{S_2}}{A_{S_1}} = \frac{[S]_2}{[S]_1}
\]  
(23-1)

where \(A_{S_1}\) refers to the activity of solute in phase 1. Lacking knowledge of the activity coefficients, we will write the partition coefficient in terms of concentrations.

\(^1\)Whenever a choice exists between CHCl\(_3\) and CCl\(_4\), the less toxic CHCl\(_3\) should be chosen. Hexane and toluene are greatly preferred over benzene, which is a carcinogen.

Figure 23-1 Partitioning of a solute between two liquid phases.
Suppose that solute S in V₁ mL of solvent 1 (water) is extracted with V₂ mL of solvent 2 (toluene). Let m be the moles of S in the system and let q be the fraction of S remaining in phase 1 at equilibrium. The molarity in phase 1 is therefore \( qm/V₁ \). The fraction of total solute transferred to phase 2 is \((1 - q)\), and the molarity in phase 2 is \((1 - q)m/V₂\). Therefore,

\[
K = \frac{[S]₂}{[S]₁} = \frac{(1 - q)m/V₂}{qm/V₁}
\]

from which we can solve for q:

\[
\text{fraction remaining in phase 1 after 1 extraction} = q = \frac{V₁}{V₁ + KV₂}
\] (23-2)

Equation 23-2 says that the fraction of solute remaining in the water (phase 1) depends on the partition coefficient and the volumes. If the phases are separated and fresh toluene (solvent 2) is added, the fraction of solute remaining in the water at equilibrium will be

\[
\text{fraction remaining in phase 1 after 2 extractions} = q^2 = \left(\frac{V₁}{V₁ + KV₂}\right)^2
\]

After n extractions with volume V₂, the fraction remaining in the water is

\[
\text{fraction remaining in phase 1 after n extractions} = q^n = \left(\frac{V₁}{V₁ + KV₂}\right)^n
\] (23-3)

**EXAMPLE Extraction Efficiency**

Solute A has a partition coefficient of 3 between toluene and water (with 3 times as much in the toluene phase.) Suppose that 100 mL of a 0.010 M aqueous solution of A is extracted with toluene. What fraction of A remains in the aqueous phase (a) if one extraction with 500 mL is performed and (b) if five extractions with 100 mL are performed?

**SOLUTION** (a) Taking water as phase 1 and toluene as phase 2, Equation 23-2 says that after a 500-mL extraction, the fraction remaining in the aqueous phase is

\[
q = \frac{100}{100 + (3)(500)} = 0.062 = 6\%
\]

(b) With five 100-mL extractions, the fraction remaining is given by Equation 23-3:

\[
\text{fraction remaining} = \left(\frac{100}{100 + (3)(100)}\right)^5 = 0.00098 = 0.1\%
\]

It is more efficient to do several small extractions than one big extraction.

**pH Effects**

If a solute is an acid or base, its charge changes as the pH is changed. Usually, a neutral species is more soluble in an organic solvent and a charged species is more soluble in aqueous solution.

**Distribution Coefficient**

which becomes

\[
\alpha_B = \frac{K}{\alpha_A}
\]

Substituting K

**Distribution of between two phases**

where \( \alpha_B \) is the distribution coefficient of solute B between two phases such as B and A.

To extract BH⁺ (Figure 2) enough to com

**Challenge**

**Distribution between two phases**

where \( \alpha_{HA} \) is

**EXAMPLE**

Suppose that t of the dissociation of amine is extra remaining in t

**SOLUTION**

10⁻⁹ + 1.0 \times fraction remaini

The concentr
soluble in aqueous solution. Consider a basic amine whose neutral form, B, has partition coefficient \( K \) between aqueous phase 1 and organic phase 2. Suppose that the conjugate acid, BH\(^+\), is soluble only in aqueous phase 1. Let's denote its acid dissociation constant as \( K_a \). The distribution coefficient, \( D \), is defined as

\[
D = \frac{\text{total concentration in phase 2}}{\text{total concentration in phase 1}}
\]  

which becomes

\[
D = \frac{[B]_{2}}{[B]_{1} + [BH^+]}  
\]  

(23-5)

Substituting \( K = [B]_{2}/[B]_{1} \) and \( K_a = [H^+][B]/[BH^+] \) into Equation 23-5 leads to

\[
\text{Distribution of base between two phases: } D = \frac{K \cdot K_a}{K_a + [H^+]} = K \alpha_B
\]  

(23-6)

where \( \alpha_B \) is the fraction of weak base in the neutral form, B, in the aqueous phase. The distribution coefficient \( D \) is used in place of the partition coefficient \( K \) in Equation 23-2 when dealing with a species that has more than one chemical form, such as B and BH\(^+\).

To extract a base into water, you should use a pH low enough to convert it to BH\(^+\) (Figure 23-2). To extract the acid HA into water, you should use a pH high enough to convert the acid to A\(^-\).

**Challenge** Suppose that the acid HA (with dissociation constant \( K_a \)) is partitioned between aqueous phase 1 and organic phase 2. Calling the partition coefficient \( K \) for HA and assuming that A\(^-\) is not soluble in the organic phase, show that the distribution coefficient is given by

\[
\text{Distribution of acid between two phases: } D = \frac{K \cdot [H^+]}{[H^+] + K_a} = K \alpha_{HA}
\]  

(23-7)

where \( \alpha_{HA} \) is the fraction of weak acid in the form HA in the aqueous phase.

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**Example** Effect of pH on Extraction

Suppose that the partition coefficient for an amine, B, is \( K = 3.0 \) and the acid dissociation constant of BH\(^+\) is \( K_a = 1.0 \times 10^{-9} \). If 50 mL of 0.010 M aqueous amine is extracted with 100 mL of solvent, what will be the formal concentration remaining in the aqueous phase (a) at pH 10.00? (b) at pH 8.00?

**Solution** (a) At pH 10.00, \( D = K K_a / ([H^+] + [H^+]) = (3.0)(1.0 \times 10^{-9}) / (1.0 \times 10^{-9} + 1.0 \times 10^{-10}) = 2.73 \). Using \( D \) in place of \( K \), Equation 23-2 says that the fraction remaining in the aqueous phase is

\[
q = \frac{50}{50 + (2.73)(100)} = 0.15 \quad \Rightarrow \quad 15\% \text{ left in water}
\]

The concentration of amine in the aqueous phase is 15% of 0.010 M = 0.0015 M.
(b) At pH 8.00, $D = (3.0)(1.0 \times 10^{-9})/(1.0 \times 10^{-9} + 1.0 \times 10^{-8}) = 0.273$. Therefore, 

$$q = \frac{50}{50 + (0.273)(100)} = 0.65 \Rightarrow \text{65\% left in water}$$

The concentration in the aqueous phase is 0.006 5 M. At pH 10, the base is predominantly in the form B and is extracted into the organic solvent. At pH 8, it is in the form BH$^+$ and remains in the water.

### Extraction with a Metal Chelator

Most complexes that can be extracted into organic solvents must be neutral. Charged complexes, such as Fe(EDTA)$^{-}$ or Fe(1,10-phenanthroline)$^{2+}$, are not very soluble in organic solvents. One scheme for separating metal ions from one another is to selectively complex one ion with an organic ligand and extract it into an organic solvent. Three ligands commonly employed for this purpose are shown here.

![Figure 23-3](image)

Figure 23-3 Extraction of a metal ion with a chelator. The predominant form of metal in the aqueous phase is M$^{n+}$, and the predominant form in the organic phase is ML$_n$.

Each ligand can be represented as a weak acid, HL, which loses one proton when it binds to a metal ion through the atoms shown in **bold** type.

$$\text{HL}(aq) \rightleftharpoons \text{H}^+(aq) + \text{L}^-(aq) \quad \quad K_a = \frac{[\text{H}^+]_{aq}[\text{L}^-]_{aq}}{[\text{HL}]_{aq}} \quad (23-8)$$

$$n\text{L}^-(aq) + \text{M}^{n+}(aq) \rightleftharpoons \text{ML}_n(aq) \quad \quad \beta = \frac{[\text{ML}_n]_{aq}}{[\text{M}^{n+}]_{aq}[\text{L}^-]_aq^{n}} \quad (23-9)$$

Each of these ligands can react with many different metal ions, but some selectivity is achieved by controlling the pH.

Let's derive an equation for the distribution coefficient of a metal between two phases when essentially all the metal in the aqueous phase (aq) is in the form M$^{n+}$ and all the metal in the organic phase (org) is in the form ML$_n$ (Figure 23-3). We define the partition coefficients for ligand and complex as follows:

$$\text{HL}(aq) \rightleftharpoons \text{HL}(org) \quad K_L = \frac{[\text{HL}]_{org}}{[\text{HL}]_{aq}} \quad (23-10)$$

$$\text{ML}_n(aq) \rightleftharpoons \text{ML}_n(organic) \quad K_M = \frac{[\text{ML}_n]_{org}}{[\text{ML}_n]_{aq}} \quad (23-11)$$
Sample Preparation

Sample preparation is the process of transforming a sample into a form that is suitable for analysis. This process might involve extracting analyte from a complex matrix, preconcentrating very dilute analytes to get a concentration high enough to measure, removing or masking interfering species, or chemically transforming the analyte into a more convenient or more easily detected form. Chemical transformation of analyte, called derivatization, was shown in Box 24-1, in which nonvolatile amino acids were converted into volatile derivatives for gas chromatography. Chapter 28 is devoted to sample preparation, so here we describe only two techniques that are especially applicable to gas chromatography.

Solid-phase microextraction is a simple method to extract compounds from liquids, air, or even sludge without using any solvent. The key component is a fused silica fiber coated with a 10- to 100-μm-thick film of nonvolatile liquid stationary phase similar to those used in gas chromatography. Figure 24-17 shows the fiber attached to the base of a syringe with a fixed metal needle. The fiber can be extended from the needle or retracted inside the needle. Figure 24-18 demonstrates the procedure of exposing the fiber to a sample solution (or the gaseous headspace above the liquid) for a fixed length of time while stirring and, perhaps, heating. Only a fraction of the analyte in the sample is extracted into the fiber.

After sampling, the fiber is retracted and the syringe is injected into the inlet of a gas chromatograph. The fiber is extended inside the hot injection liner, where analyte is thermally desorbed from the fiber in the splitless mode for a fixed time. Cold trapping (Section 24-2) focuses the desorbed analyte at the head of the column prior to chromatography. If there will be a long time between sampling and injection, the atmosphere.

onde

Mass of ana

$V_f$ is extracted, as being extrac solution: $K$ the concentration such that mass extrac quantitative solutions. A for solid-ph solids (such them into a only removing 100 lytes from). Figure carbonated bled through of analysis contai

![Figure 24-17](image-url) Syringe for solid-phase microextraction. The fused silica fiber is withdrawn inside the steel needle after sample collection and when the syringe is used to pierce a septum.

![Figure 24-18](image-url) Sampling by solid-phase microextraction and desorption of analyte from the coated fiber into a gas chromatograph. [Adapted from Supelco Chromatography Products catalog, Bellefonte, PA.]
form that is from a concentration high clinically transform. Chemistry in Box derivatives for so here we nomatography, sounds from species on is a liquid state-17 shows the fiber can -18 demonstrate the gaseous and, perhaps, the fiber into the inlet liner, where it fixed time, of the col-umpling and injection, the needle should be inserted into a septum to seal the fiber from the atmosphere. Figure 24-19 shows a chromatogram of chemical warfare nerve agents isolated from seawater by solid-phase microextraction and detected with a nitrogen-phosphorus detector. The chromatogram is deceptively simple because the detector only responds to compounds containing N and P.

In solid-phase microextraction, the mass of analyte \( m, \mu g \) absorbed in the coated fiber is

\[
Mass \, of \, analyte \, extracted: \quad m = \frac{KV_mC_oV_s}{KV_f + V_s} \quad (24-3)
\]

where \( V_f \) is the volume of film on the fiber, \( V_s \) is the volume of solution being extracted, and \( C_o \) is the initial concentration (\( \mu g/mL \)) of analyte in the solution being extracted. \( K \) is the partition coefficient for solute between the film and the solution: \( K = C_f/C_s \), where \( C_f \) is the concentration of analyte in the film and \( C_s \) is the concentration of analyte in the solution. If you extract a large volume of solution such that \( V_s >> KV_f \), then Equation 24-3 reduces to \( m = KV_mC_o \). That is, the mass extracted is proportional to the concentration of analyte in solution. For quantitative analysis, you can construct a calibration curve by extracting known solutions. Alternatively, internal standards and standard additions are both useful for solid-phase microextraction.

**Purge and trap** is a method for removing volatile analytes from liquids or solids (such as groundwater or soil), concentrating the analytes, and introducing them into a gas chromatograph. In contrast to solid-phase microextraction, which only removes a portion of analyte from the sample, the goal in purge and trap is to remove 100% of the analyte from the sample. Quantitative removal of polar analytes from polar matrices can be difficult.

Figure 24-20 shows an apparatus for measuring volatile flavor components in carbonated cola beverages. Helium purge gas from a stainless steel needle is bubbled through the cola in the sample vial, which is heated to 50°C to aid evaporation of analytes. Purge gas exiting the sample vial passes through an adsorption tube containing three layers of adsorbent compounds with increasing adsorbent

![Figure 24-19 Gas chromatogram of nerve agents sampled by solid-phase microextraction for 30 min from seawater spiked with 60 nL of each agent per liter (60 ppb by volume). The fiber had a 65-\( \mu \)m-thick coating of copoly(dimethylsiloxane/divinylbenzene). The nitrogen-phosphorus detector had a detection limit of 0.05 ppb. Analytes were desorbed from the fiber for 2 min at 250°C in splitless mode in the injection port. The column temperature was 30°C during desorption and then it was ramped up at 10°C/min during chromatography. The column was 0.32 mm × 30 m with a 1-\( \mu \)m coating of (phenyl)\(_{0.06}\)(methyl)\(_{0.95}\)polysiloxane. Soman appears as a split peak because it has two isomers. [From H.-Å. Lakso and W. F. Ng, *Anal. Chem.* 1997, 69, 1866.]

![Figure 24-20 Purge and trap apparatus for extracting volatile substances from a liquid or solid by flowing gas.](image-url)
24 Gas Chromatography

You need to establish the time and temperature required to purge 100% of the analyte from the sample in separate control experiments.

Order of decisions:

1. goal of analysis
2. sample preparation
3. detector
4. column
5. injection

Garbage in—garbage out!

The method responds to electron cap flame ionization. Each analyte is usually resolved into individual peaks. Sensitivity of the detector is determined by the detector's ability to detect small concentrations of the analyte.

Selecting

The basic considerations of extraction, sample preparation, and instrumental analysis are important. The choice of column, stationary phase, and detector are critical. The selectivity of the detector is determined by the detector's ability to detect small concentrations of the analyte.

Choosing the Detector

The next step is to choose a detector for chromatography. Do you need information about everything in the sample or do you want a detector that is specific for a particular element or a particular class of compounds?
This technique fills a gap between gas and liquid chromatography, because the solvent properties are between those of gas and liquid. Consider the following phase diagram of carbon dioxide. At a temperature of $-78^\circ$C, solid CO$_2$ (Dry Ice) is in equilibrium with gaseous CO$_2$ at 1 atm. The solid sublimes without turning to liquid. At any temperature above the **triple point** at $-56.6^\circ$C, there is a pressure at which liquid and vapor coexist as separate phases. For example, at 0°C, liquid is in equilibrium with gas at 34.4 atm. Moving up the liquid-gas boundary, two phases always exist until the **critical point** is reached at 31.3°C and 72.9 atm. Above this temperature, only one phase exists, no matter what the pressure. We call this phase a **supercritical fluid** (Color Plate 23). Its density and viscosity are between those of the gas and liquid, as is its ability to act as a solvent.

![Supercritical fluid diagram](image)

**Critical constants**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Critical temperature (°C)</th>
<th>Critical pressure (atm)</th>
<th>Critical density (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>31.3</td>
<td>72.9</td>
<td>0.448</td>
</tr>
<tr>
<td>Ammonia</td>
<td>132.3</td>
<td>111.3</td>
<td>0.24</td>
</tr>
<tr>
<td>Water</td>
<td>374.4</td>
<td>226.8</td>
<td>0.344</td>
</tr>
<tr>
<td>Methanol</td>
<td>240.5</td>
<td>78.9</td>
<td>0.272</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>193.6</td>
<td>36.3</td>
<td>0.267</td>
</tr>
</tbody>
</table>

Superization increases separation efficiency of traditional liquid and gas chromatography. Supercritical fluid chromatography can achieve higher separation speed and resolution than traditional liquid and gas chromatography, leaving carbon dioxide as the most popular supercritical fluid.
Supercritical fluid chromatography provides increased speed and resolution, relative to liquid chromatography, because of the increased diffusion coefficients of solutes in supercritical fluids. (However, speed and resolution are slower than those of gas chromatography.) Unlike gases, supercritical fluids can dissolve nonvolatile solutes. When the pressure on the supercritical solution is released, the solvent turns to gas, leaving the solute in the gas phase for easy detection. Carbon dioxide has been the supercritical fluid of choice for chromatography because it is compatible with the versatile flame ionization detector of gas chromatography, it has a low critical temperature, and it is nontoxic. Unfortunately, it is not a particularly good solvent for highly polar or high molecular weight solutes.

Equipment for supercritical fluid chromatography is similar to that for HPLC, with open tubular columns similar to those of gas chromatography. Flame ionization and ultraviolet detectors are most common. Eluent strength is increased in HPLC by gradient elution and in gas chromatography by raising the temperature. In supercritical fluid chromatography, eluent strength is increased by making the solvent denser (by increasing the pressure). The following chromatogram illustrates density gradient elution.

Capillary supercritical chromatogram of aromatic compounds with CO₂ using density gradient elution at 140°C. [From R. D. Smith, B. W. Wright, and C. R. Yonker, Anal. Chem. 1988, 60, 1323A.]
this book once wrote, “I have seen someone substitute perchloric acid for sulfuric acid in a Jones reductor experiment with spectacular results—no explosion but the tube melted!”

The combination of Fe$^{2+}$ and H$_2$O$_2$, called Fenton’s reagent, oxidizes organic material in dilute aqueous solutions. For example, organic components of urine were destroyed in 30 min at 50°C to release traces of mercury for analysis.$^{12}$ A 50-mL sample was adjusted to pH 3–4 with 0.5 M H$_2$SO$_4$. Then 50 $\mu$L of saturated aqueous ferrous ammonium sulfate, Fe(NH$_4$)$_2$(SO$_4$)$_2$, were added, followed by 100 $\mu$L of 30% H$_2$O$_2$.

### 28-3 Sample Preparation Techniques

**Sample preparation** is the series of steps required to transform a sample so that it is suitable for analysis. Sample preparation could include dissolving the sample, extracting analyte from a complex matrix, concentrating a dilute analyte to a level that can be measured, chemically converting analyte to a detectable form, and removing or masking interfering species.

### Liquid Extraction Techniques

In *extraction*, analyte is dissolved in a solvent that does not necessarily dissolve the entire sample and does not decompose the analyte. In a typical *microwave-assisted extraction* of pesticides from soil, a mixture containing soil and the solvents acetone and hexane is placed in a Teflon-lined bomb (Figures 28-6 and 28-10) and heated by microwaves to 150°C.$^{13}$ This temperature is 50°C to 100°C higher than the boiling points of the individual solvents in an open vessel at atmospheric pressure. Soluble pesticides dissolve, but most of the soil remains behind. The liquid is then analyzed by chromatography.

**Figure 28-10** Extraction vessels in a microwave oven that processes up to 12 samples in under 30 min. Each 100-mL vessel has a vent tube that releases vapor if the pressure exceeds 14 atmospheres. Vapors from the chamber are ultimately vented to a fume hood. The temperature inside each vessel can be monitored and used to control the microwave power. [Courtesy CEM Corp., Matthews, NC.]
Some chelators can extract metal ions into supercritical CO₂ (containing small quantities of methanol or water). The ligand below dissolves lanthanides and actinides:\(^\text{15}\)

![Chemical Structure](image)

**Figure 28-11** (a) Apparatus for supercritical fluid extraction. (b) Vessel for extracting house dust at 50° C with 20 mol % methanol/80 mol % CO₂ at 24.0 MPa (238 atm). (c) Gas chromatogram of CH₂Cl₂ solution of extract using a 30 m × 0.25 mm diphenyl/0.25%phenyl-1.0%siloxane column (1 μm film thickness) with a temperature ranging from 40° to 280° C and flame ionization detection. [From T. S. Reighard and S. V. Olesik, *Anal. Chem.* 1996, 68, 3612.]

**Supercritical fluid extraction** uses a supercritical fluid (Box 25-2) as the extraction solvent.\(^\text{14}\) Carbon dioxide is the most common supercritical fluid because it is relatively inexpensive and because it eliminates the need for costly disposal of waste organic solvents. Addition of a second solvent such as methanol increases the solubility of polar analytes.

Figure 28-11a shows how a supercritical fluid extraction can be carried out. Pressurized fluid is pumped through a heated extraction vessel. Fluid can be left in contact with the sample for some time or it can be pumped through continuously. At the outlet of the extraction vessel, the fluid flows through a capillary tube to release pressure. Exiting CO₂ evaporates, leaving extracted analyte in the collection vessel. Alternatively, the CO₂ could be bubbled through a solvent in the collection vessel to leave a solution of analyte.

Figures 28-11b shows the extraction of organic compounds from dust collected with a vacuum cleaner from door mats at the chemistry building of Ohio State University. The chromatogram of the extract in Figure 28-11c exhibits a myriad of organic compounds that you and I inhale in every breath.

Figure 28-12 shows glassware for continuous liquid-liquid extraction of a nonvolatile analyte. In Figure 28-12a, the extracting solvent is denser than the liquid being extracted. Solvent boils from the flask and condenses into the extraction vessel. Dense droplets of solvent falling through the liquid column extract the analyte. When the liquid level is high enough, extraction solvent is pushed through the return tube to the solvent reservoir. By this means, analyte is slowly transferred from the light liquid at the left into the dense liquid in the reservoir. Figure 28-12b shows the procedure when the extraction solvent is less dense than the liquid being extracted.

**Solid-Phase Extraction\(^\text{16}\)**

**Solid-phase extraction** uses a small volume of a chromatographic stationary phase to isolate desired analytes from a sample. The extraction removes much of
Figure 28-12  Continuous liquid-liquid extraction apparatus used when extraction solvent is (a) denser than the liquid being extracted or (b) lighter than the liquid being extracted.

Figure 28-13  Steps in solid-phase extraction.
Then rinses with 4 mL of 40 vol % methanol/60% water and 4 mL of 20% acetone/80% water remove less polar substances. Finally, elution with two 0.5-mL aliquots of 73% methanol/27% water washes the steroids from the column. Figure 28-14 shows the chromatogram of the extracted steroids.

Solid-phase extractions can reduce solvent consumption in analytical chemistry. For example, a standard procedure approved by the U.S. Environmental Protection Agency for the analysis of pesticides in wastewater requires 200 mL of dichloromethane for the liquid-liquid extraction of 1 L of water. The same analytes can be isolated by solid-phase extraction on C$_{18}$-silica disks. The pesticides are recovered from the disks by supercritical fluid extraction with CO$_2$ that is finally vented into a small volume of hexane. This one kind of analysis can save 10$^5$ kg of CH$_2$Cl$_2$ per year.$^{17}$

**Preconcentration**

Trace analysis often requires **preconcentration** of analyte to bring it to a higher concentration prior to analysis. Metal ions in natural waters can be preconcentrated with cation-exchange resin. For example, a 500-mL volume of seawater adjusted to pH 6.5 with ammonium acetate and ammonia was passed through 2 g of Chelex-100 in the Mg$^{2+}$ form to trap all the trace metal ions. Washing with 2 M HNO$_3$ eluted the metals in a total volume of 10 mL, thereby giving a concentration increase of 500/10 = 50. Metals in the HNO$_3$ solution were then analyzed by graphite furnace atomic absorption, with a typical detection limit for Pb being 15 pg/mL. The detection limit for Pb in the seawater is therefore 50 times lower, or 0.3 pg/mL. Figure 28-15 shows the effect of pH on the recovery of metals from seawater. At low pH, H$^+$ competes with the metal ions for ion-exchange sites and prevents complete recovery.

![Figure 28-14](image)

**Figure 28-14** High-performance liquid chromatography of anabolic steroids preconcentrated from urine by solid-phase extraction with 1 mL of C$_{18}$-silica. [Courtesy Supelco, Bellefonte, PA.]

![Figure 28-15](image)

**Figure 28-15** The pH dependence of the recovery of trace metals from seawater by Chelex-100. The graph shows the pH of the seawater when it was passed through the column. [From S.-C. P'ai, *Anal. Chim. Acta* **1988**, *211*, 271.]