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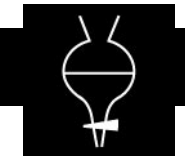
EXTRACTION



Analytical Extractions

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Introduction

The process of generating analytical data involves some combination of planning, sampling, sample preparation, quantification, data review and reporting. Initially, each step in the method required comparable effort. Sample preparation, generally involving some form of extraction followed by analyte enrichment, has in the past been a laborious process, with only a few tools available. Likewise, quantification usually consisted of a 'wet' chemistry process such as titration or precipitation. Before the development of personal computers, the planning, sampling, data review and reporting steps also required considerable effort. Since each step presented formidable challenges to the analytical scientist, the relative importance of each step remained about the same.

Modern techniques, particularly chromatography, have changed the situation. The rapid and successful development of gas and liquid chromatography dramatically reduced quantification steps from hours or days to a matter of minutes, often with better accuracy and precision. The other steps in the method, especially sample preparation, were regarded as of secondary importance, serving only to support the ultimate (i.e. chromatographic) step in the method. Since most of the creative – and financial – resources flowed into chromatography development, research in the other areas slowed and sample preparation came to be viewed as the 'low tech' part of the method.

Chromatography is now considered a mature science, being an integral part of nearly every analytical laboratory. The slower pace of chromatographic research, coupled with outside pressures to improve the efficiency of the entire analytical method, has finally resulted in an increased interest in sample preparation. These efforts have produced a number of advances that improve efficiency, selectivity and time required. The discussion will provide an overview of some of the many sample preparation principles and techniques available, focusing on the analytical extraction part of the process. The goal is to provide the reader with a more balanced view of this important part of analytical methodology.

Principles of Extraction

Developing a successful extraction as part of an analytical method requires an understanding of the chemical and physical principles involved. Thus, we will begin this discussion of analytical extraction by focusing on the underlying principles that make the techniques work. Only with understanding and

appreciation of these principles can full advantage be taken of them.

Definition of Analytical Extraction

Extraction is the process of moving one or more compounds of interest (analytes) from their original location (usually referred to as the sample or matrix) to a physically separate location where further processing and analysis occur. The sample may be a solid, liquid or gas. The separate location is usually a fluid (an extracting solvent), but extractions into the gas phase and on to solid sorbents are also common. Finally, the word analytical implies that this process involves small amounts of analyte (as opposed to preparative extraction). Most analytical methods aim at complete extraction although situations frequently exist where good analytical results are possible with only partial extraction.

Thermodynamics and Kinetics

These two terms are often interchanged, when in fact they have very different chemical meanings. Thermodynamics is the study of energy, in this case the energy associated with the chemical process of extraction. Through this study of energy, we can determine if the process is favourable or unfavourable. That is, will this extraction give a good result or a bad one? Even if the process is favourable, it may not happen quickly because of kinetic factors. Kinetics is the study of the rate at which these processes occur.

It is important to realize that these two principles are completely independent of each other. Complete extraction is not necessarily a fast process and development of a successful extraction method requires that consideration to be given to both aspects.

Like Dissolves Like

A compound will be soluble in, or mix with, another compound that is chemically like it. That is, the two compounds must be from the same, or similar, chemical families. This simple principle is an implied requirement in every analytical extraction. The concept of moving analytes from the matrix to some other location requires them to be transported using some medium in which they are soluble. Therefore, we must carefully consider how the like-dissolves-like concept can help to achieve the desired result: extracting the desired compounds and not extracting the undesired ones.

Figure 1 illustrates how simple changes in molecular structure can have a profound influence on solubility behaviour. This plot shows the solubility of three related amino acids in water. Amino acids are generally considered to be polar, so their solubility in the polar solvent, water, is generally high. As nonpolar functionality is added to the molecule, in the sequence from glycine to phenylalanine, the nonpolar character of the entire molecule increases (i.e. it becomes less like water). The result is a significant reduction in water solubility.

The same situation exists when considering the relative solubility of any compound in a series of solvents. A higher solubility will be observed when the solvent is most like the compound in question. The reader is referred to the Further Reading section for additional examples.

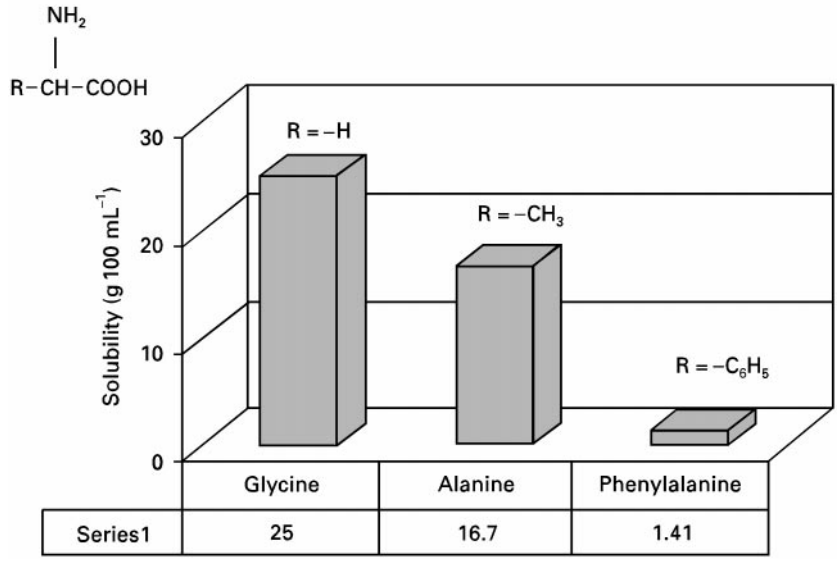


Figure 1 Solubility of amino acids in water as a function of structure.

Temperature Effects

Temperature has an effect on three important phenomena: solubility, vapour pressure and kinetics. While increasing temperature generally increases the magnitude of each effect, there are some aspects of this principle that are particularly relevant to analytical extractions.

Effect of temperature on solubility In most cases involving organic analytes, increasing the temperature of a liquid results in increased analyte solubility. **Figure 2** illustrates that for the same three amino acids as shown in Figure 1 a temperature increase from 0 to 75°C results in a three- to fourfold increase in solubility. Even marginally soluble compounds show a dramatic improvement from this simple change in conditions. Indeed, as will be seen later, this principle is used in most extraction procedures.

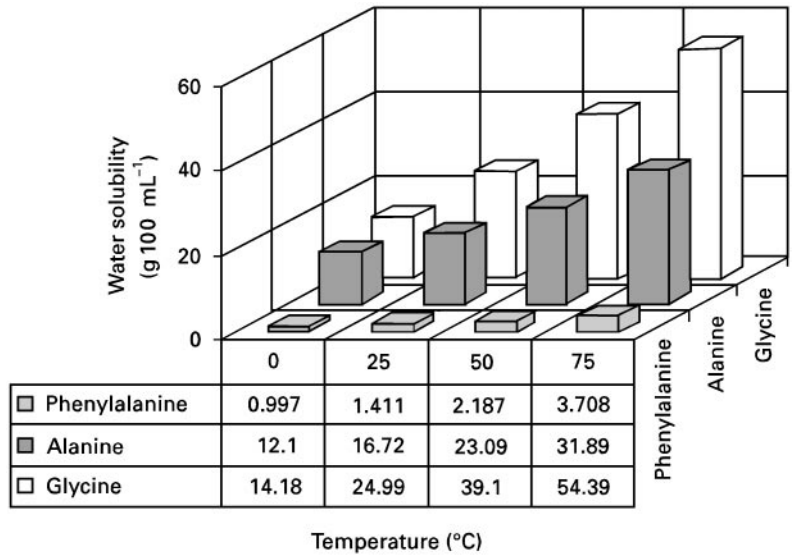
Effect of temperature on vapour pressure Increasing the temperature of a liquid will result in an increase in vapour pressure. Boiling occurs when the vapour pressure above the liquid equals the applied (usually atmospheric) pressure.

Figure 3 shows calculated vapour pressures for several common solvents. Note that the vapour pressure is relatively large at temperatures as much as 20°C below the boiling point of the solvent. Simple evaporation in a stream of nitrogen at room temperature uses this fact to evaporate a solvent rapidly without boiling. If the applied pressure is raised, the boiling point is also raised, so that the solvent can be maintained in its liquid state at higher temperatures. Pressurized fluid extraction uses this phenomenon to advantage. Similarly, lowering the applied pressure, as in a rotary evaporator, reduces the boiling point, allowing faster evaporation at lower temperatures. Finally, at any given temperature, the relative vapour pressure of each compound above the liquid phase provides an estimate of the relative evaporation rates of the liquids. Such knowledge is essential when performing critical steps such as solvent evaporation or solvent exchange.

Effect of temperature on kinetics All chemical processes are affected by the temperature at which the process is occurring, although the exact change in reaction rate with temperature is unique for any process. However, many reaction rates will approximately double for each 10°C increase in temperature, and this rule of thumb can be a helpful guide in understanding the effects of temperature changes. These changes can be either positive or negative, depending on whether the temperature change is increasing or decreasing. For example, storing samples and solutions at low temperatures slows down evaporation and degradation. These processes are about four times slower if the solution is stored at 4°C compared to room temperature.

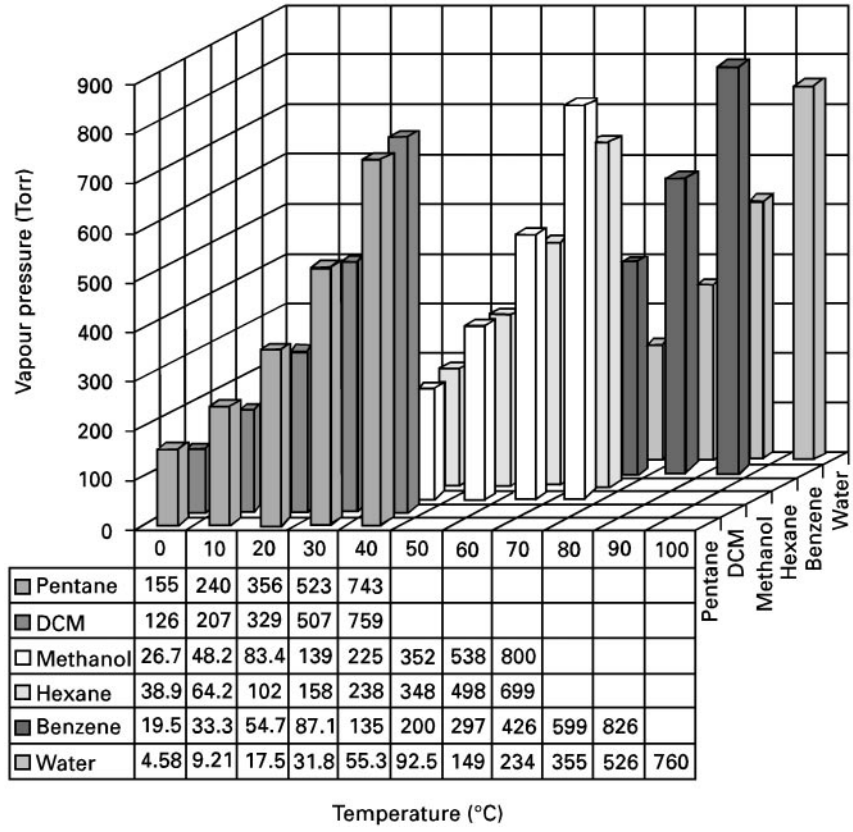
Effect of pH

The pH of an aqueous sample will influence the success or failure of an extraction for acidic and basis analytes. Acids and bases involve an equilibrium between two forms, one neutral and one ionic. Each form has significantly different chemical and physical properties, as noted in **Table 1**.



	0	25	50	75
Phenylalanine	0.997	1.411	2.187	3.708
Alanine	12.1	16.72	23.09	31.89
Glycine	14.18	24.99	39.1	54.39

Figure 2 Solubility of amino acids in water as a function of temperature.



	0	10	20	30	40	50	60	70	80	90	100
Pentane	155	240	356	523	743						
DCM	126	207	329	507	759						
Methanol	26.7	48.2	83.4	139	225	352	538	800			
Hexane	38.9	64.2	102	158	238	348	498	699			
Benzene	19.5	33.3	54.7	87.1	135	200	297	426	599	826	
Water	4.58	9.21	17.5	31.8	55.3	92.5	149	234	355	526	760

Figure 3 Vapour pressaure of common solvents as a function of temperature. (Calculated from data in *Handbook of Chemistry and Physics* (1971).)

Extraction of organic acids from water is only practical at pH values more than two units below the pK_a of the acid. Only at this pH is most of the compound in the neutral form and amenable to extraction with an organic solvent. Similarly, to keep a base, such as an aromatic amine, in the neutral (extractable) form, the pH of the solution must be adjusted to at least two units above the pK_b of the base. Readings with a pH meter are likely to be unstable and/or unreliable in the presence of organic solvents, and the equilibrium constant, K_a, is also likely to change, often in an unpredictable way. Any required pH adjustments and measurements must, therefore, be made before the organic component is added.

Table 1 Properties of individual forms in acid–base equilibria

Neutral form		*Ionic form*
$HA + H_2O$	$\rightleftarrows$	$A^- + H_3O^+$
Acid		Conjugate base
$B + H_2O$	$\rightleftarrows$	$BH^+ + OH^-$
Base		Conjugate acid
More soluble in organic solvents		Less soluble in organic solvents
Insoluble in water		Soluble in water
More volatile		Nonvolatile
Sour/bitter taste, bad odour		Little odour

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Two-phase Distribution Equilibria

Definitions The process of extraction, by definition, requires that the analyte be transferred from the matrix to a different phase. When the extracting medium first contacts the matrix, the analytes will become distributed between the two phases in a well-defined ratio. Since the matrix is usually a liquid or solid, and the extracting medium can be a solid, liquid or fluid, this usually refers to liquid–liquid and liquid–solid distribution equilibria.

These distribution equilibria can be described by several important equations. First, the distribution ratio, D, for extracting from phase 1 into phase 2 is defined as:

$$D = \frac{C_2}{C_1}$$

where C is the stoichiometric concentration of the analyte in each of the phases. (Actually, D is related

to the ratio of activities rather than concentration, but in dilute solution the difference is negligible.)

This ratio is a constant that depends on the analyte, the two phases, the composition of the phases (pH, ionic strength, etc.) and the temperature.

The fraction extracted, θ, in any one equilibration is defined as:

$$\theta = \frac{D\beta}{1 + D\beta}$$

where β is the phase ratio, the ratio of the volumes of the two phases ($= V_2/V_1$). The fraction remaining in the initial phase (V_1) is, of course $1 - \theta$.

The amount extracted depends on the physicochemical interactions between the two phases and the analyte, and the volume of each phase. A change in these variables will cause a change in the extraction result.

Effect of analyte structure on D Actual values of D in **Table 2** show how simple changes in molecular structure have a profound influence on the success of an extraction.

The addition of nonpolar functional groups (methyl- and chloro-) to benzene make the molecule more nonpolar, so that the new molecule favours the hexane phase (larger value for D). Conversely, adding polar groups (amine, hydroxy, carboxylic acid) makes the molecule more like the water phase (smaller D). It is important to keep these general principles in mind when developing an extraction method and understanding the results.

Multiple extractions When multiple extractions are performed on the same sample, the amount extracted into phase 2 and the amount remaining in phase 1 are calculated using the equations shown in **Table 3**.

In general, several extractions with the same total volume of extracting solvent will always produce better recovery than a single extraction with the same volume of solvent, although it is seldom worth carrying out more than three extractions.

Table 2 Distribution ratios for extraction from water into hexane

Analyte	*Added functional group*	*Functional group category*	*D (25°C)*
Benzene			275
Toluene	$-CH_3$	Nonplar	970
Chlorobenzene	–Cl	Nonpolar	950
Nitrobenzene	$-NO_2$	Moderately polar	31.2
Aniline	$-NH_2$	Polar	0.90
Phenol	–OH	Polar	0.13
Benzoic acid	–COOH	Very polar	0.051

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Table 3 Equations used for multiple extractions

Extraction number	*Fraction extracted into phase 2*	*Fraction remaining in phase 1*
1	θ	$1 - \theta$
2	$\theta(1 - \theta)$	$(1 - \theta)^2$
3	$\theta(1 - \theta)^2$	$(1 - \theta)^3$
n	$\theta(1 - \theta)^{n-1}$	$(1 - \theta)^n$

Effect of variations in D and β The effects of variations in D and β on extraction results are shown in **Table 4**. The total recovery after multiple extractions is calculated for various combinations of D and β. These calculations show the importance of all three variables: phase ratio, distribution ratio and number of extractions.

In summary, two-phase distribution equilibria are an important part of every analytical extraction, and the laboratory scientist must ensure that all critical variables are controlled in order to generate reliable results.

Other Principles

The preceding principles do not represent an exhaustive list. Certainly, there are other chemical principles that contribute to the extraction process, but play a more minor role. Some of these are discussed briefly below.

Time A longer extraction time will usually produce better recoveries, but this effect assumes that the

Table 4 Total per cent recovery as a function of β, D and number of extractions

Phase ratio $\beta = V_2/V_1$	*D*	*After 1st extraction*	*After 2nd extraction*	*After 3rd extraction*	*After 4th extraction*
1/1	1	50	75	88	94
	2	67	89	96	
	10	91	99		
	100	99			
1/4	1	20	36	49	59
	2	33	56	70	80
	10	71	92	98	99
	100	96	99		
1/10	1	9	17	25	32
	2	17	31	42	52
	10	50	75		
	100	91	99		

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analytes, reagents and solvents are nonvolatile, stable and do not react with each other. If these assumptions are not valid, longer extraction times may actually produce poorer recoveries.

Ionic strength The addition of ionic species to an aqueous solution results in a 'salting-out' effect. This procedure often enhances extraction of neutral organic analytes from water by increasing D.

Surface area Reducing the particle size of a solid matrix, thereby increasing contact areas between phases, can cause a dramatic increase in extraction rates.

Stirring/mixing Adequate stirring enhances the rate of procedures that are otherwise limited by diffusion processes.

Analytical Extraction Techniques

This discussion will focus on the most popular traditional techniques, and provide an introduction to some of the newer extraction technologies. In each case, the principles involved will be considered together with some practical operating tips.

Liquid–Liquid Extraction Techniques

Separatory funnel techniques There are few limitations on what size or type of liquid samples can be extracted, except that the two liquid phases must be immiscible and unreactive with each other. Separatory funnels are available to handle samples from as small as a few millilitres to several litres. Extraction times vary from 1 to 15 min, depending on the specific requirements of the method, but equilibration is usually fast in all but the most viscous liquids. As noted in the section on principles of extraction, multiple extractions with a smaller volume of extracting solvent are preferred over a single extraction with a larger volume. The primary disadvantages of separatory funnels are the labour necessary, the need to evaporate an often large volume of solvent and the formation of emulsions.

The following practical points should be considered:

- Funnel size: to allow adequate mixing, the flask size should be chosen so that at least 25% of the funnel volume is free space.
- Venting: regular venting is a required safety procedure, especially at the start of the extraction process.
- Draining layers: too much time should not be wasted draining off one layer, except after the final equilibration.
- Shaking: the most important variable is the time spent shaking the two layers, not the intensity of the shaking. Because of this, automated shakers provide adequate extraction, even though the intensity of mixing may be quite low.

Continuous liquid–liquid extractors These systems are usually reserved for larger water samples and/or situations where a long extraction time is required. There are two basic design types, depending on whether the extracting solvent is more dense (**Figure 4**) or less dense (**Figure 5**) than water. In each design, the solvent in the flask is heated to boiling, causing solvent vapours to collect in the condenser. The condensed solvent then passes through the sample in the main chamber.

The principles are the same as for separatory funnel extractions. However, each drop of solvent represents a separate two-phase distribution system with a small phase ratio but high surface area and fast extraction kinetics. Since each drop represents an equilibration step, the extraction consists of thousands of multiple extractions. The result is generally a high analyte

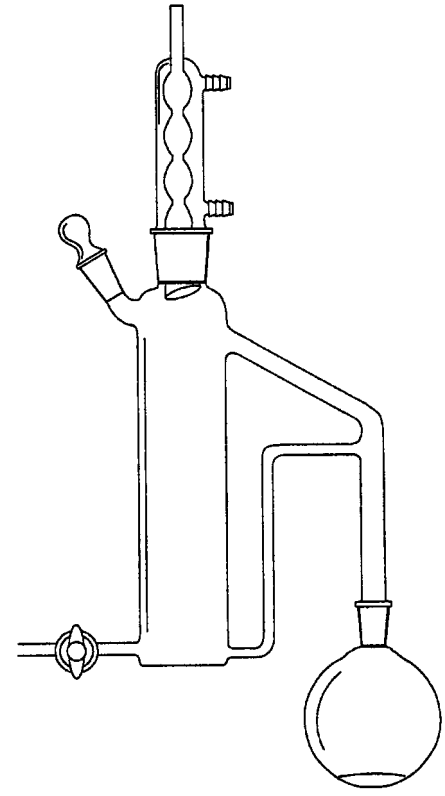

Figure 4 Continuous liquid–liquid extractor for use with extracting solvents that are denser than water. (Reproduced from Burford and Hawthorne (1994) *Journal of Chromatography A* 65: 75–94, with permission of ACCTA, Inc.)

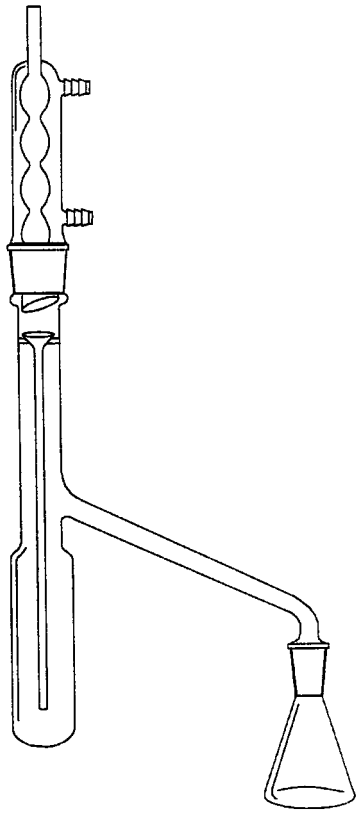

Figure 5 Continuous liquid–liquid extractor for use with extracting solvents that are less dense than water. (Reproduced with permission of ACCTA, Inc.)

recovery. The primary disadvantages are the set-up time, lengthy extraction time (6–24 h), and large quantities of solvent. The latter problem has been solved somewhat by integrated extraction systems that allow extraction, evaporation and concentration of solvent in one apparatus.

For continuous liquid–liquid extractors the following considerations are important:

- The extractor only works properly if the condensed solvent passes through the bulk of the sample, rather than along the sides of the flask.
- The reflux (boiling) rate determines the overall extraction rate, and some minimum rate must be maintained to ensure complete extraction.
- Emulsions can be a problem. See below for ways to deal with them.

Other liquid–liquid extraction devices While the chemistry and mechanics of liquid–liquid extraction have not changed, many practical variations have improved the speed and convenience of the technique. Two examples of these improvements are the Mixxor™ extractor (New Biology Systems Ltd, Haifa, Israel) and the VectaSep CLE® system (Whatman, Inc., Clifton, NJ, USA).

The Mixxor™ system (**Figure 6**) consists of a receiver and piston assembly. The aqueous sample is placed in the receiver (B) with a small quantity of immiscible organic solvent (D). The sample is extracted by moving piston (A) up and down a number of times. After extraction, the plunger is moved to the bottom and the separated organic solvent is forced into the axial chamber (C), where it is easily removed. The entire extraction and separation process is completed in less than 5 min and can provide a concentration factor of 30 or more. Extractors are available for samples with volumes ranging from 2 to 50 mL. This system is more convenient than separatory funnels, although at the expense of some flexibility in sample and extraction solvent volumes. Also, the design precludes the use of heavier-than-water solvents.

The VectaSep CLE® system (**Figure 7**) is particularly useful for smaller samples. The extraction solvent is placed in the larger tube. The sample (1.5 mL) is added to the sample dispenser, which is then placed in the extraction tube and centrifuged, typically for 10 min at 3500 rpm. Centrifugal force pushes the sample through a dispersion membrane in the bottom of the sample dispenser, causing the sample to emerge as small droplets which travel along

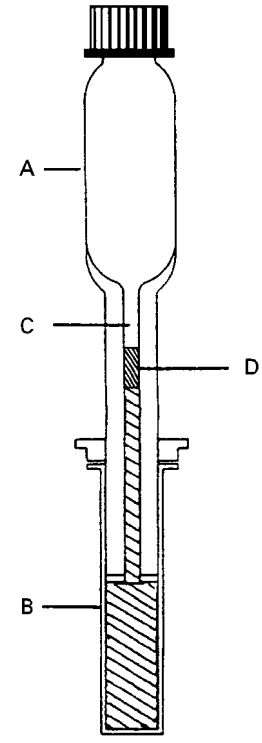

Figure 6 Mixxor™ extraction device. (A) Upper chamber; (B) sample reservoir; (C) axial chamber; (D) organic solvent. (Reproduced with permission of New Biology Systems Ltd.)

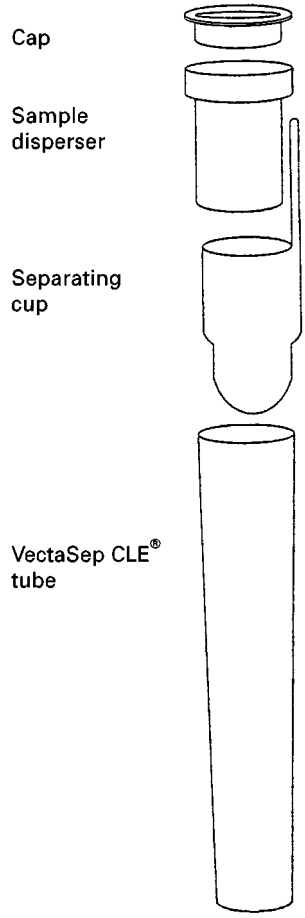

Figure 7 VectaSep CLE extraction system. (Reproduced with permission of Whatman, Inc.)

the inside of the tube to the bottom. After centrifugation, the sample dispenser is removed and the separating cup is added to locate the phase separation boundary. Evaporation of the upper organic layer then deposits extracted analytes in the separating cup, where they can be readily redissolved in an appropriate solvent. Although sample size and extraction solvent choices are somewhat limited, this system makes clever use of the extraction principles discussed earlier. In this case, the sample is passed through the extraction solvent (a reverse of the other methods) in small droplets, increasing surface area (kinetics) and offering a very favourable phase ratio.

These systems offer advantages in terms of sample handling, safety and efficiency considerations. So, despite their limitations, both of these systems, and others like them, merit consideration as a replacement for the more traditional techniques.

Emulsions No discussion of liquid–liquid extraction would be complete without mention of the emulsion problem. Emulsions are a mixture of two normally immiscible phases that won't separate in practice. This problem is often caused by the presence of surfactants or solids at the phase interface, high viscosity of one of the phases, or a small phase ratio (not enough organic phase). Although each emulsion is unique, one of the following remedies will often result in separation into two distinct layers:

- Wait: many emulsions will disappear with sufficient time.
- Gentle mechanical agitation/stirring with a glass rod or spatula.
- Immersion in an ultrasonic cleaning bath.
- Add 'a salt': this makes the aqueous phase less like the organic phase.
- Increase the phase ratio: add more organic phase.
- Pass through a bed packed with glass wool or diatomaceous earth.
- Centrifuge.
- Freeze the aqueous layer with dry ice/acetone or liquid nitrogen, then simply pour off the organic layer.

Liquid–Solid Extraction Techniques

Soxhlet techniques The Soxhlet extractor (**Figure 8**) is one of the oldest extraction systems available but is still very common. A solid sample is placed in an extraction thimble inside the middle chamber. Upon boiling, the solvent vapours from the bottom flask travel up to the condenser and then drip through the sample. The sample is soaked in solvent (a two-phase distribution equilibrium), which then returns to the flask when the liquid reaches the top of the siphon. The sample is exposed to fresh solvent after every siphon cycle, usually at a rate of about six cycles per hour. Typical extraction times are 6–24 h. Once assembled and operating, there is little that can go wrong with this system. However, operators must be aware of the following general hints:

- Proper cycling is required: the rate (cycles per hour) is usually specified in the method, and the operator must ensure that the unit siphons in distinct events rather than continuously draining.
- Solvent level in the thimble: if too high, sample may be lost from the thimble, contaminating the extract.
- Moisture content: dry samples work best; add a drying agent to remove free moisture.

The system requires a large volume of organic solvent, and extraction time is long. Despite these limitations, the Soxhlet extractor is still in widespread

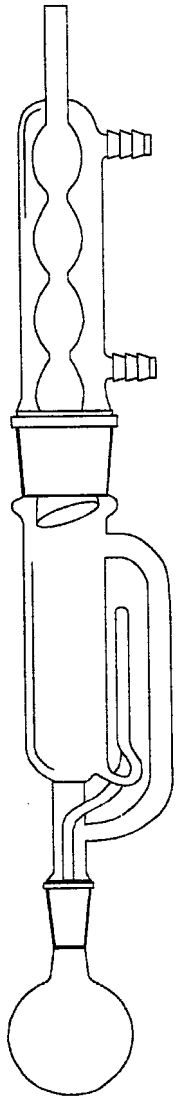

Figure 8 Soxhlet extractor. (Reproduced with permission of ACCTA, Inc.)

use, primarily because of its excellent reputation for providing complete extraction. Indeed, Soxhlet values are often used as the standard against which other extraction methodologies are compared.

Modified Soxhlet extractors The lengthy Soxhlet extraction times have prompted the development of modified extractors, such as the Soxtec® system (Foss Tecator AB, Höganäs, Sweden). The sample is placed in an extraction thimble, but the thimble is then directly immersed in boiling solvent, rather than bathed in cooler condensed solvent. The increased temperature means faster extraction kinetics. After about 1 h equilibration, the sample is removed from the solvent and flushed with fresh condensed solvent for an additional hour. The apparatus even allows the evaporation and collection of solvent, further improving efficiency. These alternatives offer considerable advantages in terms of time and solvent use, and results are generally comparable to the traditional method.

Solid-phase extraction Solid-phase extraction (SPE) is an alternative to liquid–liquid extraction where the extraction solvent is replaced with a solid sorbent. The sorbent is usually packed into a cartridge (**Figure 9**) that can vary in size from about 1 mL to more than 50 mL. The quantity of sorbent can range from about 500 mg to 10 g. Extraction is accomplished by forcing the aqueous sample past the sorbent (via vacuum or pressure), causing analytes in the sample to be sorbed. This two-phase distribution is similar to the partitioning that occurs in chromatography. After the sample has passed through the sorbent bed, the sorbed analytes are eluted with a strong solvent, such as methanol, acetonitrile or carbon disulfide.

The SPE process involves the following sequential steps:

- Conditioning/cleaning of the sorbent with an organic solvent such as methanol
- Extraction of the sample.
- Air drying or rinsing to remove any remaining sample.
- Elution of analytes using a strong organic solvent.

SPE offers three primary advantages over conventional liquid–liquid extraction: reduced solvent usage, extraction speed and selective chemistry. In an ideal method, only a few millilitres of organic solvent may be necessary for an extraction and it is possible to extract and elute 10 100-mL samples or more in as little as 15–20 min. Finally, by varying the nature of the sorbent, it is possible to achieve selective extraction and/or selective elution. For example, a minor change in bonded phase from a C_{18} phase to a C_8 phase can actually result in a significant change in selectivity. The shorter chain C_8 phase is less retentive towards more hydrophobic molecules and exposes somewhat more of the polar character from the underlying silica. This trend can be extended using even shorter aliphatic bonded phases or by adding a polar functional group to the chain (e.g. cyano- or phenyl-). There are no analogous series in liquid–liquid systems.

There are a host of sorbents available, including more polar functional groups, polymer-based, ion exchange, affinity and chelating materials. Nearly every liquid–liquid extraction method has an SPE counterpart, and almost all provide equal if not better

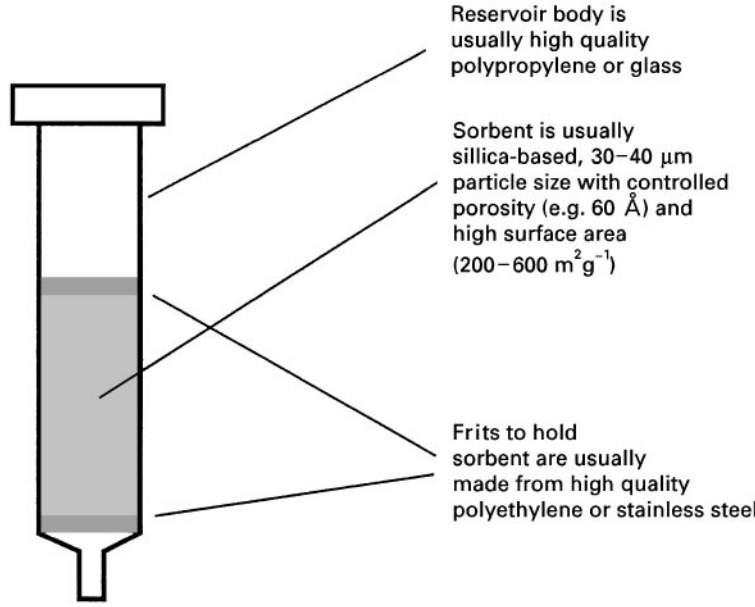

Figure 9 Solid-phase extraction (SPE) cartridge design. (Reproduced with permission of ACCTA, Inc.)

results, with considerably less effort. It should also be noted that SPE can be performed on nonaqueous samples using a polar sorbent, but this application is usually used for sample clean-up rather than extraction.

Finally, it is important to note SPE's limitations:

- High particulate samples will often plug the frits.
- Extracting capacity (total extractable mass) is more limited than with conventional solvent extraction.
- Reproducibility (batch-to-batch) can be a problem, although this is less of a concern now than during early development of the technique.

Membrane disc extraction Membrane extraction discs, first sold under the brand name Empore® (3M, St Paul, MN, USA), are an alternative SPE system. In the membrane discs the sorbent is enclosed in a support network rather than simply being packed into a cartridge. The unique Empore® design consists of 90% (w/w) sorbent particles (8–10 μm diameter), in a network of polytetrafluoroethylene fibrils, in a disc format that resembles a thicker version of conventional synthetic membrane filters. A typical disc is about 0.5 mm thick with diameters ranging from 1 to 90 cm.

A membrane disc extraction method would typically consist of the following steps:

- Pre-washing the disc with the final eluting solvent.
- Pre-wetting the disc with methanol or some other solvent that is miscible with the sample (which is usually aqueous).
- Extraction, i.e. drawing the sample through the disc.
- Elution of analytes, which involves a soak with the elution solvent for a period of time, followed by elution with the aid of a vacuum. This elution step may be repeated with different solvents if necessary.

Membrane discs have the same advantages over liquid–liquid extraction as SPE but are superior to conventional SPE because the extraction rate is faster; flow rates of 100–200 mL min^{-1} are typical. The small particles also provide greater capacity and uniformity of packing. Unfortunately, the discs are more sensitive to the presence of particulates, so a pre-filter is often necessary.

Early applications of membrane discs focused on environmental analysis, where large sample sizes made the fast extraction rates attractive. Membrane discs can also be formulated into SPE-like cartridges, allowing the efficient processing of small clinical samples (e.g. serum, urine, etc.). Use of membrane disc applications continues to grow, although the number of reported applications is not as high as SPE, due to the relative age of the two techniques.

Solid-phase microextraction Solid-phase microextraction (SPME) is another version of liquid–solid extraction techniques. In this system, the extraction

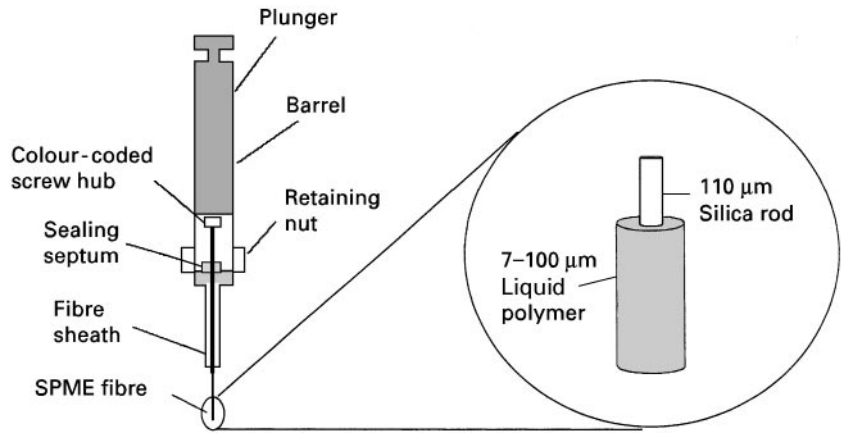

Figure 10 Solid-phase microextraction system. (Reproduced with permission of ACCTA, Inc.)

phase consists of a fused silica fibre coated with a sorbent (e.g. dimethylsilicone or other immobilized polymer) with thicknesses ranging from about 10 to 100 μm (**Figure 10**). The fibre is placed in contact with the liquid or gas sample and analytes are sorbed on to the phase, from which they are directly desorbed into a chromatograph.

Unlike the other techniques discussed here, SPME is a completely solvent-free extraction method. The extraction step tends to be rapid, usually requiring 10–20 min, and desorption can take only a few seconds. Thus, a fast analysis with a good lower limit of detection is possible, since the entire extract is analysed. Furthermore, the selectivity and extractability can be affected by changes in fibre chemistry as well as solution pH, ionic strength, etc. Sampling by immersion in the sample or extraction from the headspace above the liquid (often a faster extraction) provides additional flexibility. However, SPME, by its nature, is not applicable to as wide a range of samples as other techniques, and there are currently a more limited number of sorbents available compared to SPE. However, SPME offers some unique advantages that make it an attractive alternative for many applications.

Pressurized Fluid Extraction

So far, each extraction medium discussed has been either a conventional liquid or solid, each performing its function at or near room pressure and at or below the boiling point of the liquid phase. Experimentally, these conditions are easiest to attain in the laboratory and require relatively unsophisticated equipment. Unfortunately, in some cases these conditions can also result in slow extraction kinetics and/or incomplete extraction because of the (relatively) mild conditions employed. Such problems can often be solved simply by maintaining the extraction fluid at a higher pressure so that higher temperatures can be used. This can result in a dramatic improvement in extraction efficiency. In addition to the pressurization of conventional solvents in a closed vessel, supercritical fluids may also be used at high temperature and pressure.

Accelerated liquid extraction As noted earlier, increased temperature improves solubility and extraction kinetics, and increases the vapour pressure. In addition, an increase in applied pressure causes an increase in the boiling point of a liquid. Logically, then, one would expect improvement in extraction results at higher applied pressure, where the increased boiling point would then allow liquid extractions above the normal boiling point of the solvent. This approach has been successfully applied in two different ways.

In the first method, called microwave-assisted solvent extraction (MASE), the sample and extraction solvent are placed in a sealed vessel, usually constructed of polytetrafluoroethylene or other inert polymer. When placed in a microwave field, polar materials (e.g. water) absorb energy and the sample heats up. Since the vessel is closed, the pressure also increases, resulting in a significantly elevated boiling point. For example, hexane–acetone mixtures can be used at 115°C, which is more than 40°C above the boiling point of either solvent. The increased temperature has several beneficial effects on the extraction, such as increased solubility, faster diffusion, reduced viscosity and reduced surface tension (increased wettability).

This approach is also used for sample digestion in inorganic analysis, and succeeds for the same reasons – temperature-related improvements in reaction rates.

The second approach uses conventional electrical conduction heating in a sealed stainless steel vessel (Dionex Corporation, Salt Lake City, UT, USA) to accomplish the same effect. With this equipment, the pressure and temperature can be set independently, whereas in the MASE process the pressure increase results from the temperature increase.

Both methods allow Soxhlet-type extractions to be completed in 30 min or less and require small solvent volumes. This approach has received widespread acceptance because it draws from existing experience with organic extraction solvents. The basic chemistry of the extraction does not change significantly, only the rate. In theory, then, any liquid solvent-based extraction method could be adapted for accelerated liquid extraction.

The primary disadvantages involve the safety aspects associated with the use of organic solvents at high temperatures and pressures. In addition, the same process that enhances analyte extraction may also cause extraction of other unwanted components from the matrix. However, the reductions in solvent use and extraction time make accelerated liquid extraction an attractive alternative to unpressurized techniques.

Supercritical fluid extraction As the temperature and pressure on a compound are raised, a point is reached, called the critical point, where the substance is no longer a gas or liquid, but has properties intermediate between these two states. Supercritical fluids are good solvents with gas-like viscosities and diffusivities and no surface tension.

Carbon dioxide is the most popular choice for a supercritical fluid, because of its relatively low critical point (31°C, 73 atm). Supercritical fluid extraction (SFE) then involves placing the sample in a high pressure vessel and contacting the sample with the supercritical fluid. Extraction temperature can be varied from about 40°C to more than 150°C while pressures may be adjusted between 100 and as high as 680 atm or more. Since carbon dioxide is actually a nonpolar fluid, 10–20% of polar modifiers such as methanol can be added to improve the range of solubilities. A typical extraction will be complete in less than 20 min.

SFE receives much attention because the extractions are fast and, with carbon dioxide as the extraction fluid, evaporation of the extracting medium is spontaneous upon decompression to atmospheric conditions. The ability to control extracting power, through changes in temperature, pressure and modifier, offers more selectivity and flexibility than with liquid solvents. While SFE is not a universal replacement for liquid solvent-based methods, it is clearly the best choice for many specific applications, especially foods, natural products, polymers and environmental samples.

Final Comments

Analytical extraction (and sample preparation in general) has returned to its rightful place as an equally important part of the analytical method. There are many options available to achieve extraction, depending on the type and size of sample as well as other more practical considerations. The laboratory worker can choose from 100-year-old techniques that still provide excellent results, or instrumental-based technologies that offer faster extractions on smaller samples.

The extraction step, as a distinct part of the analytical method, will retain its importance as long as chromatographic procedures are used for quantification. The objective of moving the analytes from the sample to the point of quantification will still be required. Future research is likely to focus on better ways of accomplishing this movement, resulting in reduced solvent usage and sample size, automation and online transfer of extracts to subsequent processing and quantification steps. But throughout these changes, it will be important to remember that, although the names may change, the chemistry will remain the same.

Further Reading

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