Generalities of the Analytical Process

4

Abstract

This chapter, which starts Part II, explains how to extract (bio)chemical information from objects and systems. It describes the generalities of the analytical process, which are dealt with in greater detail in Chaps. 5 and 6, devoted to quantitative and qualitative analytical processes, respectively. Following a brief placement in context of the topic, the concept "analytical problem" is defined and the three general steps of the analytical process are described in separate sections on preliminary operations (sample collection and treatment), measurement and transducing of the analytical signal, and signal acquisition and processing to produce results in the required format.

Teaching Objectives

- To define the general features of analytical processes.
- To describe the preliminary operations (first step) of the analytical process.
- To describe sampling operations.
- To introduce students to analytical separations systems.
- To provide an overall description of measurement and transducing of the analytical signal.
- To describe manual and automatic systems for signal acquisition and data processing.

Electronic Supplementary Material The online version of this chapter (doi:10.1007/978-3-319-62872-1_4) contains supplementary material, which is available to authorized users.

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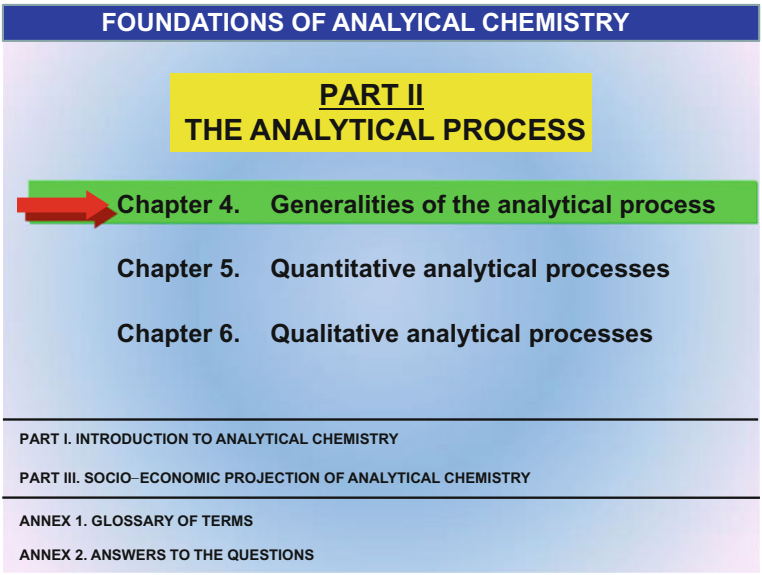
4.1 Explanation of the Slides

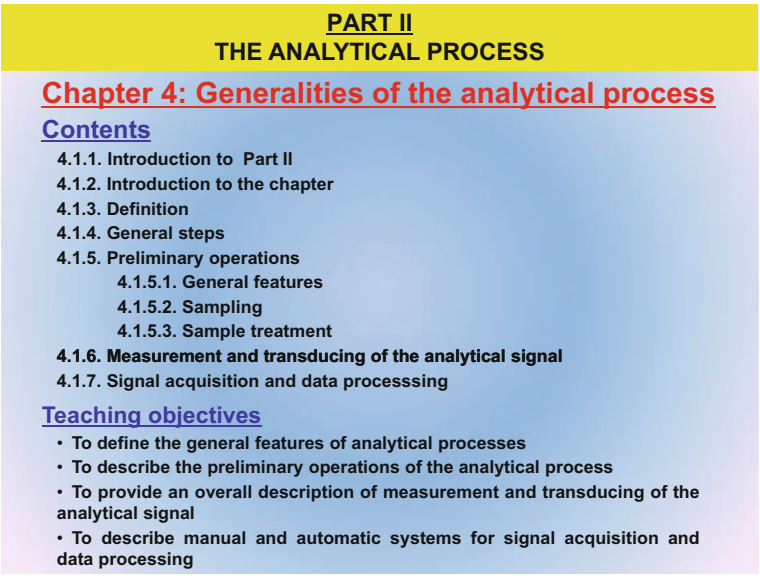
Slide 4.1



This slide places Part II (The Analytical Process) and shows the other two parts.

This is an introductory chapter approaching the generalities of the analytical measurement processes used to obtain (bio)chemical information (see Slide 1.4). Such processes can be quantitative (Chap. 5) or qualitative (Chap. 6) in nature.

Slide 4.2

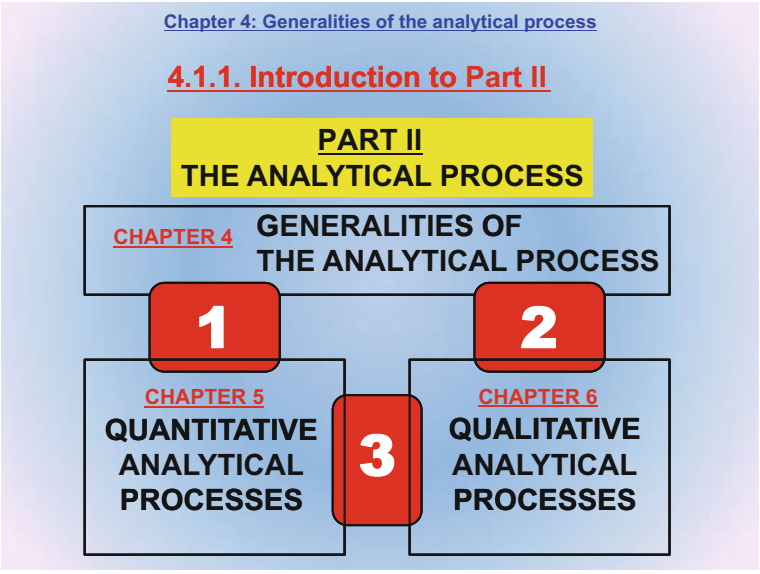


4.2.1. This slide shows the contents of this chapter, which span seven sections. The first section places the chapter in context within Part II and the next three provide an overview of analytical processes. The last three sections deal with the three main steps of the analytical process, namely: preliminary operations (4.5), measurement and transducing of the analytical signal (4.6), and data processing (4.7).

4.2.2. The slide also shows the main teaching objectives of the chapter, which can be summarized as follows: to provide an overview of analytical processes by describing their three general steps.

4.1.1 Introduction to Part II (1 Slide)

Slide 4.3



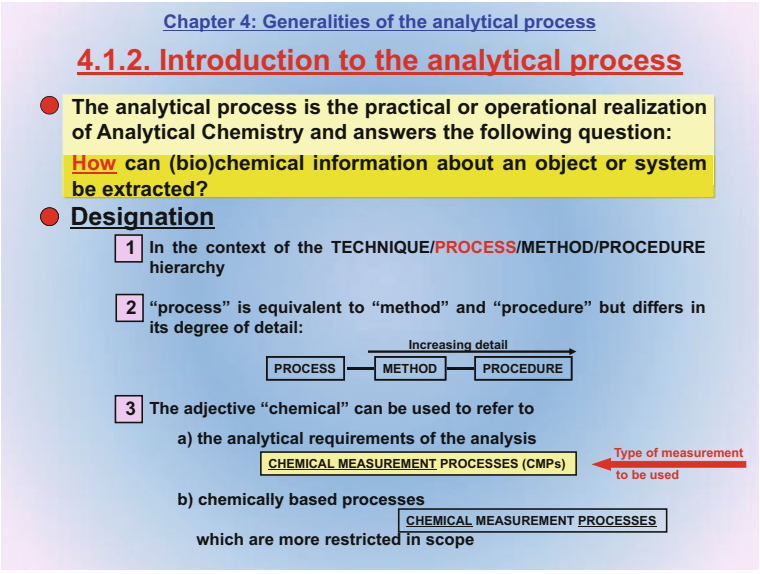
This slide depicts the relationships (interfaces 1–3) among the contents of this chapter and those of Chaps. 5, 6, which provide a general, harmonic answer to the following question: How can (bio)chemical information about an object or sample be extracted?

This chapter provides a framework for the generalities of the analytical process. The three chapters of Part II are related through the interfaces shown as follows:

- *Interfaces 1 and 2*. This chapter is general in scope and its contents pertain to both Quantitative Analysis (Chap. 5) and Qualitative Analysis (Chap. 6).
- *Interface 3*. The apparent difference between Quantitative Analysis (Chap. 5) and Qualitative Analysis is unrealistic. In fact, Qualitative Analysis frequently has quantitative connotations.

4.1.2 Introduction to the Analytical Process (1 Slide)

Slide 4.4



4.4.1. The analytical process is a set of operations separating a sample from its result (see Slide 1.22). It is the operational answer to the question "How can (bio)chemical information about an object (e.g., a lunar rock) or system (e.g., a river throughout the year) be obtained?" The object or system may be natural or artificial.

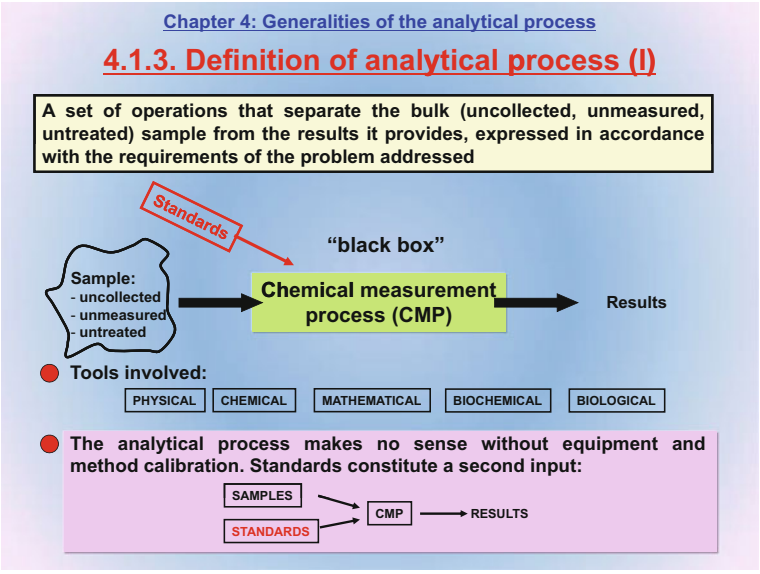
4.4.2. The designation "process" is intended to place Analytical Chemistry in the realm of Science and Technology. This designation is present in the hierarchy of Slide 1.22. The word "process" here represents how (bio)chemical information is obtained and materializes in increasingly detailed descriptions based on the words "method" and "procedure".

As can be seen, the adjective "chemical" is also troublesome because it can be applied indifferently to the information required and the tools needed to obtain it.

In this chapter, we use the designation *Chemical Measurements Processes* (CMPs), which are concerned with (bio)chemical information requirements even though they use chemical tools (reagents, solvents) to fulfil them.

4.1.3 Definition of Analytical Process (2 Slides)

Slide 4.5



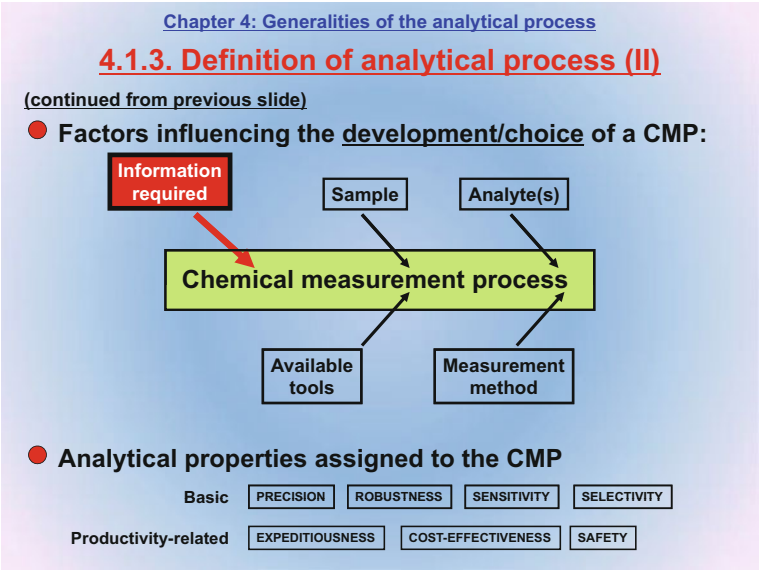
4.5.1. This slide provides a formal definition of "analytical process", which, as shown in the previous one, materializes in the acronym CMP.

As can be seen, a CMP is a sort of "black box" that receives samples as input and delivers results as output.

Depending on the characteristics of the analytical process, the "black box" can use *tools* of various types, namely: *physical* (e.g., apparatuses, instruments), *chemical* (e.g., reagents, solvents), *mathematical* [e.g., algorithms for converting raw data (signals) into results], *biochemical* (e.g., immobilized enzymes) and *biological* (e.g., tissue homogenate of banana peel to immobilize natural enzymes onto an electrode surface).

4.5.2. Since Analytical Chemistry is a metrological discipline (that is, one based on measurements), it requires using standards for comparison (see Slide 1.12). Measurement standards therefore constitute another input to the analytical process in addition to samples.

Slide 4.6



4.6.1. This slide completes the definition of "analytical process" by describing the factors governing its development or selection, and also the associated analytical properties (see Chap. 2).

An existing analytical process for determining a given analyte in a specific type of sample may be usable as such or require minimal adjustment. Such is the case with standard and official methods of analysis (see Slide 1.13). However, obtaining special information may require developing a new CMP from scratch. In any case, the development or selection of an analytical process should be guided by the following factors:

1. The specific *(bio)chemical information required* for well-grounded, timely decision-making, which, as shown in the slide, is crucial with a view to using the most suitable analytical process in each situation.

   The analytical process to be used for a given analyte will differ depending on whether the results are to be delivered expeditiously at the expense of accuracy or as accurately as possible at the expense of expeditiousness.

Two cases in point are the fast determination of the fat content of freshly harvested olives and that of moisture in an organic solvent. In the former case, the result should be delivered promptly because it will dictate the value of the olives. This can be accomplished by using a nuclear magnetic resonance (NMR) probe to measure the fat content with acceptable error (5–10%) virtually immediately. By contrast, moisture in an organic solvent must be determined with greater accuracy, which entails using a slower process such as Karl Fisher titrimetry with amperometric monitoring (a sluggish, expensive, labour-intensive process). These two examples illustrate how the type of information required and the expeditiousness with which it is to be delivered are two key factors in choosing or developing an analytical process for a given analytical purpose.

2. *Properties of the sample* such as state of aggregation (solid, liquid or gaseous), size (macroanalysis, microanalysis, etc., as shown in Slides 1.37 and 1.38) and availability (Slide 1.39), among others.
3. *Characteristics of the analyte(s)* such as nature (organic, inorganic, biochemical) (see Slide 1.34), number and concentration (macrocomponents to traces) (see Slide 1.37), among others.
4. *Available tools (apparatuses, instruments, reagents)*. Obviously, pesticides in soils can be more accurately and expeditiously determined with a gas chromatograph coupled to a mass spectrometer than with one equipped with a conventional detector. Thus, carcinogenic aflatoxins in milk can be more conveniently determined by direct immunoassay than with a liquid chromatograph coupled to a mass spectrometer—which requires labour-intensive sample treatment.
5. The *method of measurement*, which differs depending on whether qualitative or quantitative information is needed. For example, calculable methods (e.g., absolute methods) differ from relative methods in this respect (see Slides 5.11–5.14 and the sections that describe them).

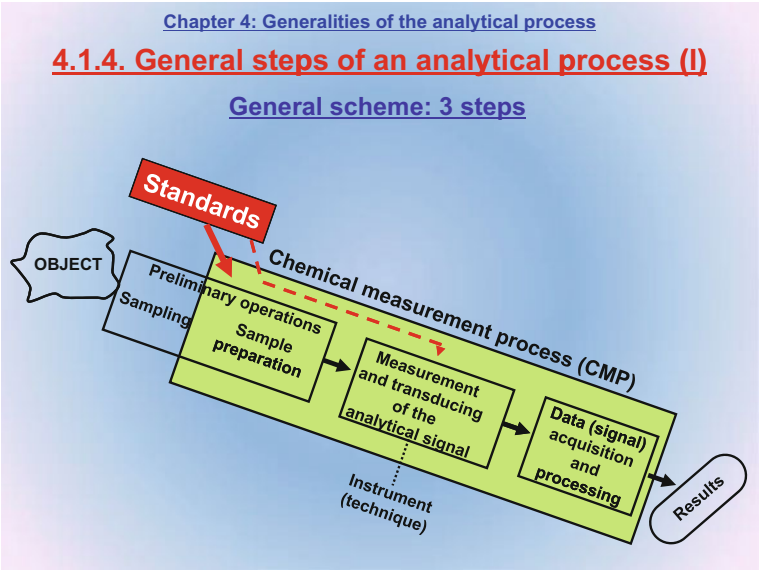
4.6.2. The analytical properties that dictate the quality of an analytical process depend on whether the process is of quantitative or qualitative kind.

Thus, Quantitative Analysis is linked to basic and productivity-related properties (Slide 2.4), and basic properties provide support for capital properties.

Because accuracy (a capital property) and precision do not apply to Qualitative Analysis, a new property called “reliability” is needed here (see Slides 6.14–6.16 and 6.21).

4.1.4 General Steps of an Analytical Process (2 Slides)

Slide 4.7

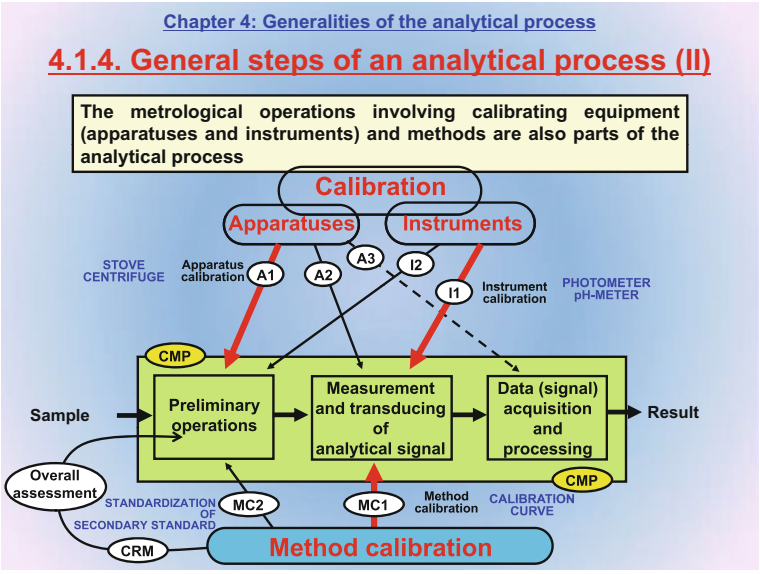


4.7.1. The definition of "analytical process" is completed in this section with a description of its main steps.

As shown in this slide, the analytical process comprises three steps separating the bulk sample from its results, namely: preliminary operations (sample collection and treatment), measurement of the analytical signal with an instrument, and acquisition and processing of raw signals to produce the results.

4.7.2. This slide emphasizes the crucial role of tangible measurement standards in the preliminary operations of the analytical process and in measurement of the analytical signal (see Chap. 3).

Slide 4.8



4.8.1. As noted in Chap. 3, a distinction should be made between equipment calibration, whose targets are apparatuses and instruments, and method calibration, where the target is the analytical process (see the example in Slide 3.19). Calibration is an essential part of the analytical process.

Equipment calibration can be aimed at two different types of targets, namely:

- Apparatuses (A.1) such as samplers, centrifuges, extractors, stoves or furnaces, which are typically used in the preliminary operations of the analytical process.
- Instruments (I.1) such as spectrophotometers, ammeters or chromatographs, which are normally used in the second step of the analytical process but may also be needed in the first (I.2) for purposes such as measuring volumes and weighing untreated or treated samples with labware (flasks, pipettes, burettes, balances) that requires calibration if accurate results are to be delivered.

Method calibration can be done for three main purposes (see Slides 3.19 and 3.20), namely:

- Establishing the signal–concentration relationship by constructing a calibration curve (see Slide 2.36) in the second step, where the instrument comes into play (CM1);
- Calibrating secondary standards by titration (CM2) with primary standards (see Slide 3.23).

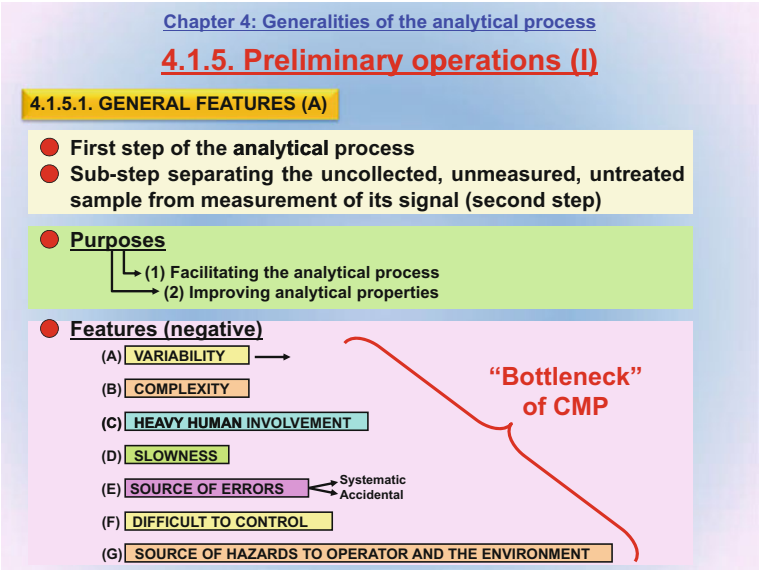
- Globally assessing analytical processes (see Slides 3.21 and 3.22) by application to a certified reference material (CRM) and statistical comparison of the results for the CRM and the samples.

4.8.2. These are selected examples of the two types of calibration in the analytical process.

4.1.5 Preliminary Operations of the Analytical Process (23 Slides)

4.1.5.1 General Features (4 Slides)

Slide 4.9



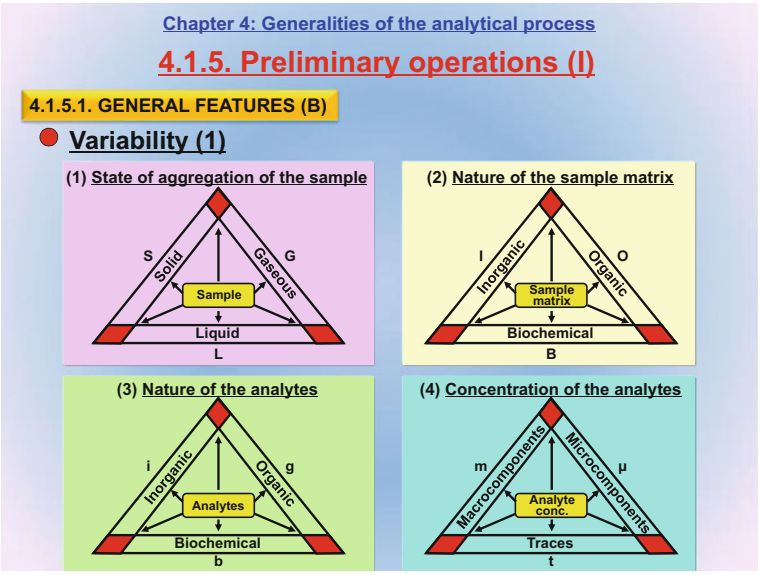
4.9.1. Slides 4.9–4.31 are concerned with the preliminary operations of the analytical process. The first four (4.9–4.12) explain its general features, the next eleven (4.13–4.23) sample collection and the last eight (4.24–4.31) sample treatment (with special emphasis on separation systems).

This slide defines "preliminary operations", which comprise sample collection and preparation (see Slide 4.7). Also, its describes their *general purposes* (namely, facilitating the analytical process and improving analytical properties) and their seven most salient features, which are as follows:

(A) Variability in the operations, which is a major hindrance. In fact, virtually each sample–analyte pair requires its own specific operation (see Slide 4.10), which precludes designing all-purpose equipment for this purpose.
(B) Preliminary operations are operationally complex as they typically involve transferring liquids, filtering, using analytical separation systems, measuring volumes, weighing, etc.
(C) As a result, they are labour-intensive and difficult to automate.
(D) Some operations (e.g., passing an eluent through a solid-phase extraction column, dissolving soil) are especially sluggish and take up 60–80% of the overall time spent in conducting an analytical process. This has made "direct methods of analysis" a priority goal to by-pass preliminary operations in the analytical process.
(E) One of the most negative features of preliminary operations is that they are the source of systematic and accidental errors. The former can arise from volume measurements with a poorly calibrated pipette, using inappropriate sampling equipment or not adhering to the recommended timing, for example. On the other hand, the latter are typically the result of human mistakes (e.g., distraction, poor readings, failing to distinguish colours). Properly performing preliminary operations is therefore very important because any errors made will propagate through the analytical process and have an adverse impact on the quality of the results.
(F) Preliminary operations are difficult to control because monitoring every single sub-step is nearly impossible. Calibrating apparatuses (e.g., extractors, stoves, centrifuges, thermometers) rarely suffices for this purpose. In fact, the best way to check that an analytical process is operating as expected is by assessing it—preliminary operations included—with a certified reference material (CRM). If the certified value and the result of the process are consistent, then the process can be validated and its preliminary operations assumed to be under control.
(G) Preliminary operations are the source of hazards for operators and the environment since they often use pressurized gas cylinders, toxic reagents and solvents, and high pressures and/or temperatures. Also, toxic waste from the operations can obviously affect analysts and the environment. So-called "green methods" are intended to minimize personal and environmental risks.

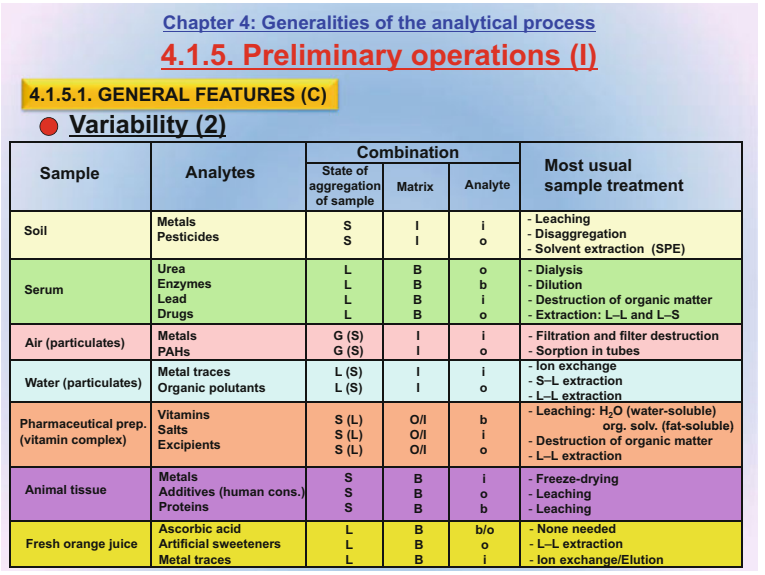
4.9.2. Based on the foregoing, the preliminary operations of the analytical process possess negative connotations although they are indispensable with a view to assuring integral quality in the analytical results.

Slide 4.10



This slide and the next illustrate the high variability of the preliminary operations of the analytical process (see also Slide 4.11) as regards state of aggregation of the sample (solid, liquid or gaseous), nature or the sample and analyte (organic, inorganic or biochemical), and concentration of the analyte (macrocomponents, microcomponents, traces). Also, the situation differs depending on whether one or more analytes are to be detected or determined in the same sample.

Slide 4.11



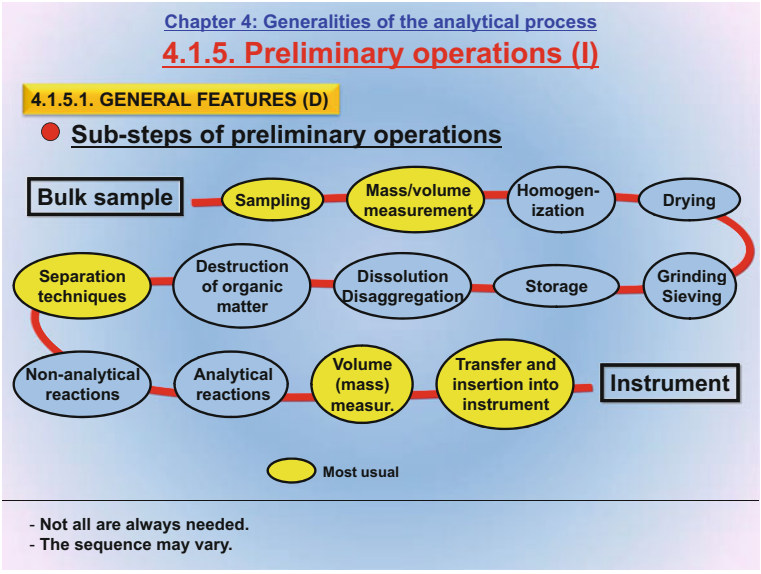
Sample	Analytes	Combination			Most usual sample treatment
		State of aggregation of sample	Matrix	Analyte	
Soil	Metals Pesticides	S S	I I	i o	- Leaching - Disaggregation - Solvent extraction (SPE)
Serum	Urea Enzymes Lead Drugs	L L L L	B B B B	o b i o	- Dialysis - Dilution - Destruction of organic matter - Extraction: L–L and L–S
Air (particulates)	Metals PAHs	G (S) G (S)	I I	i o	- Filtration and filter destruction - Sorption in tubes
Water (particulates)	Metal traces Organic polutants	L (S) L (S)	I I	i o	- Ion exchange - S–L extraction - L–L extraction
Pharmaceutical prep. (vitamin complex)	Vitamins Salts Excipients	S (L) S (L) S (L)	O/I O/I O/I	b i o	- Leaching: H_2O (water-soluble) org. solv. (fat-soluble) - Destruction of organic matter - L–L extraction
Animal tissue	Metals Additives (human cons.) Proteins	S S S	B B B	i o b	- Freeze-drying - Leaching - Leaching
Fresh orange juice	Ascorbic acid Artificial sweeteners Metal traces	L L L	B B B	b/o o i	- None needed - L–L extraction - Ion exchange/Elution

Each sample–analyte pair requires using a preliminary operation suited to the particular analytical purpose. This increases variability in the preliminary operations even further.

The examples in this slide show that the operations to be performed depend on the specific analyte to be detected or determined.

One example is the determination in animal or human serum of urea, enzymes, lead or drugs, which involves different types of preliminary operations such as dialysis, dilution, destruction of organic matter and solid–liquid extraction, respectively.

Slide 4.12



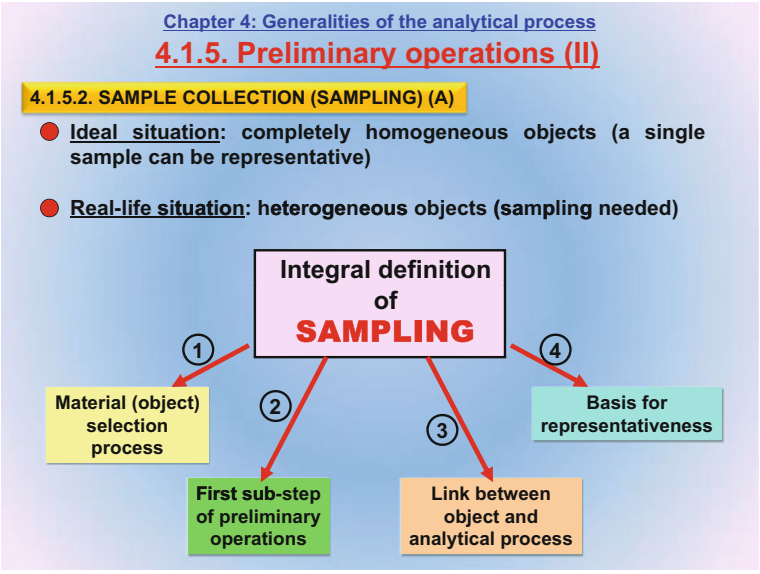
By definition, the preliminary operations of the analytical process separate the object (or bulk sample) from measurement with an instrument (second step of the process). This slide shows various types of operations for solid, liquid and gaseous samples. Those highlighted in yellow (namely, sampling, mass or volume measurement of the aliquot subjected to the analytical process, analytical separation, mass or volume measurement of the treated sample and insertion into the instrument) are the most usual.

In order to avoid misconceptions one should bear in mind that

(a) not every preliminary operation shown is always needed (e.g., no grinding or sieving is necessary with liquid samples); and
(b) the sequence of operations is not always as shown (e.g., non-analytical reactions may come before analytical separation in order to facilitate it).

4.1.5.2 Sample Collection (11 Slides)

Slide 4.13

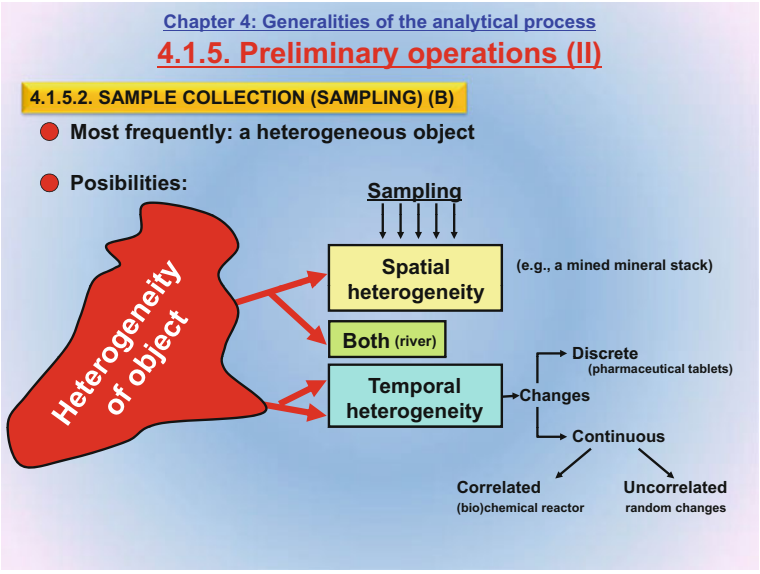


This slide starts the description of sample collection (sampling), which spans the next ten.

Sampling would be unimportant to the analytical process if the object were completely homogeneous since any sample withdrawn from it would be identical to and provide the same results as any other. This is an unrealistic scenario, however, because objects are nearly always heterogeneous, so any samples extracted from them will differ and lead to different results if subjected separately to the analytical process. As a consequence, the quality of the results depends critically on the quality of sampling, which is one of the most crucial preliminary operations.

This slide shows four complementary approaches to sample collection (sampling). The first defines sampling in technical terms, the second and third place it in context within the analytical process, and the last relate it to the capital property "representativeness",—which, together with accuracy, is an attribute of the results.

Slide 4.14

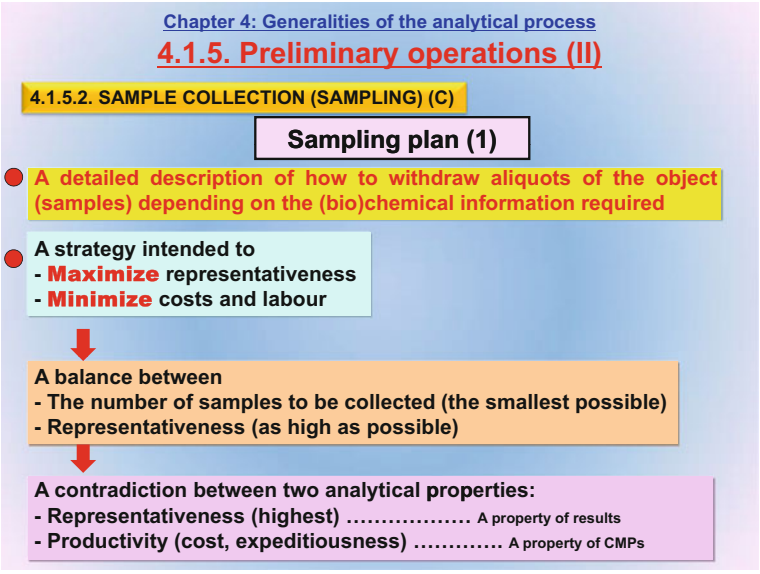


The degree of heterogeneity of the object (that is, its variability in space, time or both) dictates the sampling strategy to be used. There are three main types of object heterogeneity, namely:

(A) *Spatial heterogeneity* (e.g., differences in pesticide contents in the 10 cm deep layer of agricultural soil across a field of 1 ha).
(B) *Temporal heterogeneity* (that is, differences in object composition with time). The differences can be of two types:

- Discrete (e.g., those in the sweetener content of a non-alcoholic beverage among bottles).
- Continuous, whether predictable because changes in the object are mutually correlated (e.g., differences in the amount of glucose present in an enzymatic reactor used to produce it) or random in nature (i.e., following no well-defined pattern).
- *Spatial and temporal heterogeneity*, which is the most complex of the three (e.g., differences between heavy metal levels at different depths and in different seasons in a lake).

Slide 4.15



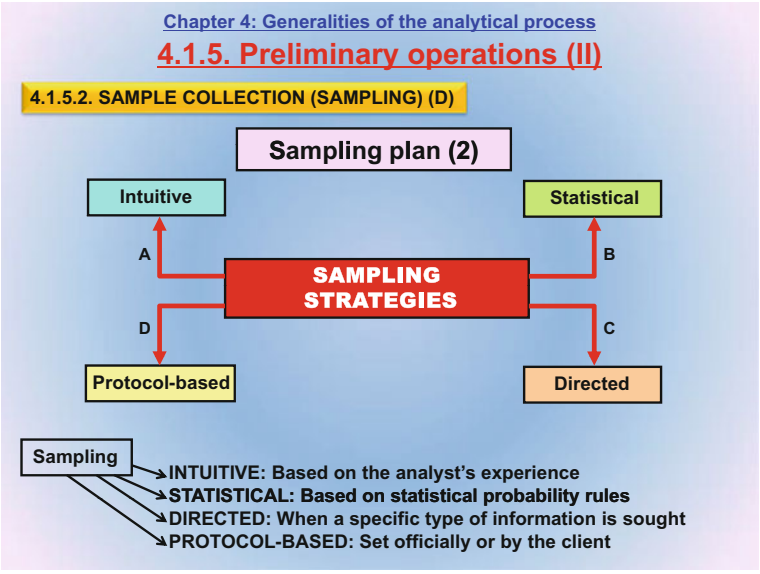
The so-called "sampling plan" or "sampling strategy" is a detailed description of the experimental procedure to be followed in order to collect samples, which differs depending on the particular information required. Thus, if contamination with organic matter by effect of vessel cleaning in a 1 ha beach strip is to be assessed, the sampling plan will differ depending on whether the target information is the average contaminant content of the whole beach, the shore or only tainted zones.

The sampling plan or strategy to be used in order to fulfil the information demand should afford *a balance* between

- representativeness (a capital analytical property as shown in Slide 2.4), which should be maximized, and
- productivity-related properties (namely, cost-effectiveness, and personnel and environmental safety).

The contradictory nature of these two aims is clearly apparent from Slide 2.60, which exposes the contradictions between analytical properties.

Slide 4.16



This slide defines the four most common types of sampling plans, which share some common traits despite their differences, and can be illustrated with the following examples:

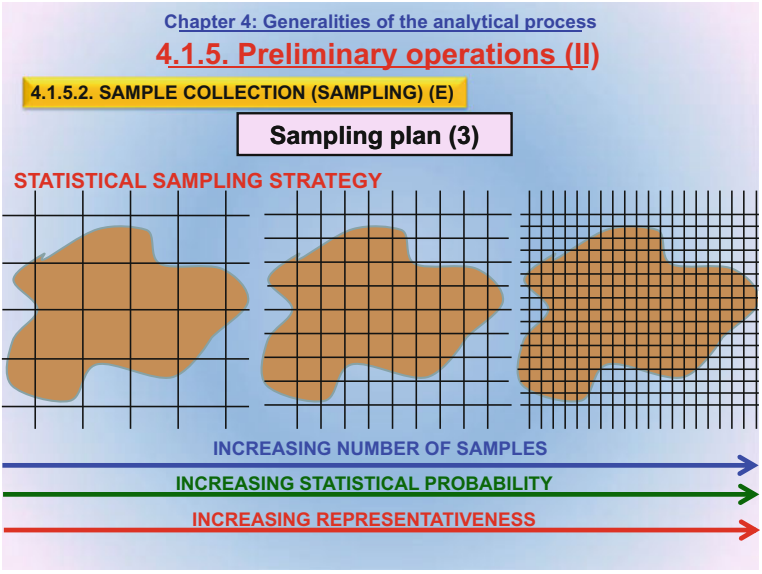
(A) *Intuitive sampling*. An expert collecting samples of mineral water from a spring will expand the collected set if any colour or odour change, or the presence of suspended matter, is observed.

(B) *Statistical sampling*. Representativeness of the samples is maximized according to a preset probability level. Thus, a field will be split into 200 squares for sampling if a high representativeness is sought but only 20 if moderate representativeness suffices.

(C) *Directed sampling*. This sampling strategy is used when very specific information such as the organic matter content of suspended particles in a water stream is needed—in which case samples will be collected by filtration.

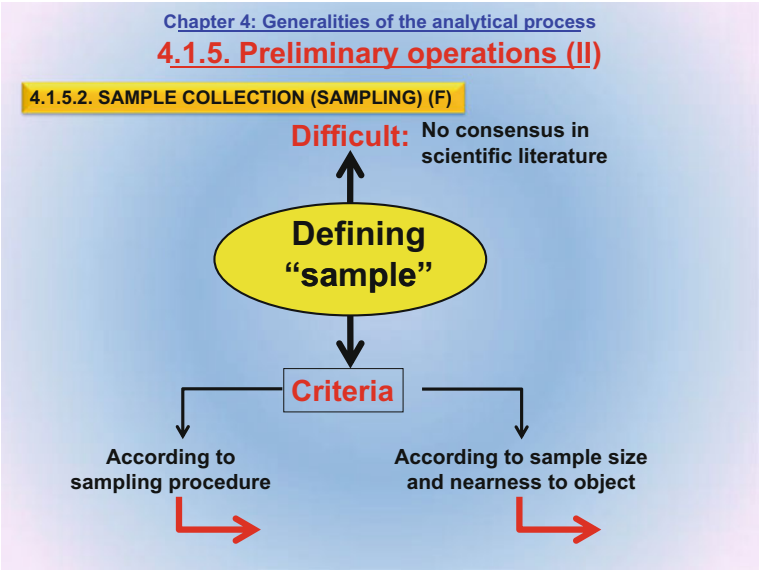
(D) *Protocol-based sampling*. This is the only choice when applicable regulations of the client require samples to be collected in a specific, carefully described manner. Such is the case, for example, with the determination of anabolic steroids in meat for human consumption, the sampling protocol for which is specified in an European Union directive.

Slide 4.17



This is an example of a statistical sampling plan. The aim is to determine available nitrogen in an agricultural field (the object). To this end, the field will be split into a variable number of imaginary squares depending on the desired level of representativeness. The probability of a sample being collected, and its representativeness, will increase with increasing number of squares. Samples will be collected from all squares or only from those previously selected in statistical terms.

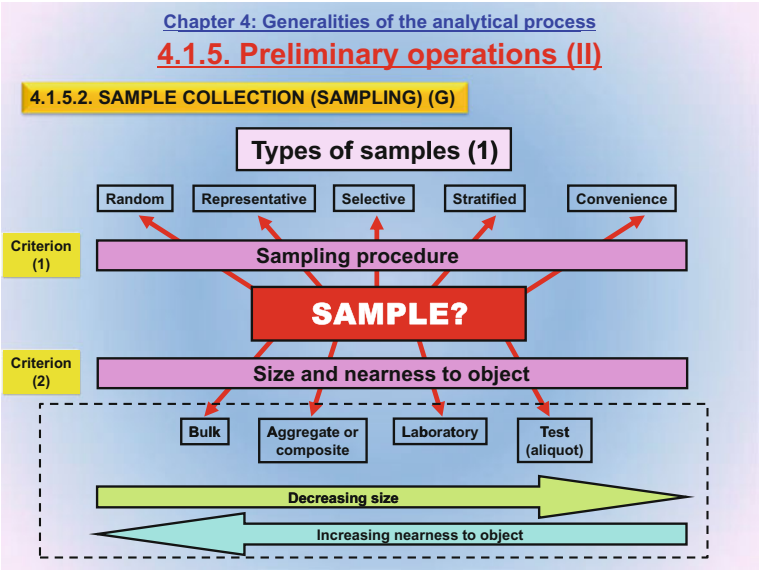
Slide 4.18



The word "sample" has been defined in a number of ways in the scientific and technical literature. This slide classifies sample designations according to two complementary criteria, namely: (1) sampling procedure, and (2) sample size and nearness to the object.

The two ensuing types of sample are shown in Slide 4.19 and defined in Slide 4.20.

Slide 4.19

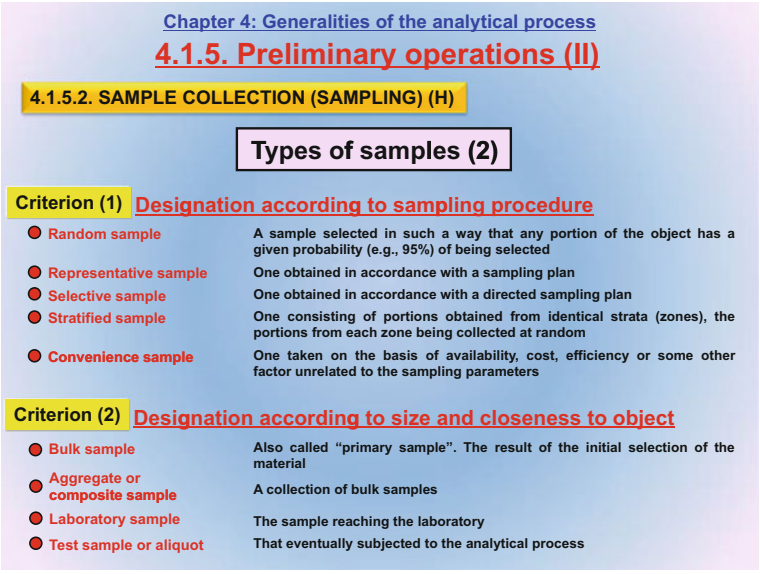


These are the different types of sample arising from the classification in the previous slide, based on the way samples are collected (Criterion 1), and their size and nearness to the object (Criterion 2).

These types of sample, designated by their qualifiers, are defined in the next slide.

Size decreases and nearness to the object increases from “bulk sample” to “test sample” among the sample types established according to Criterion 2.

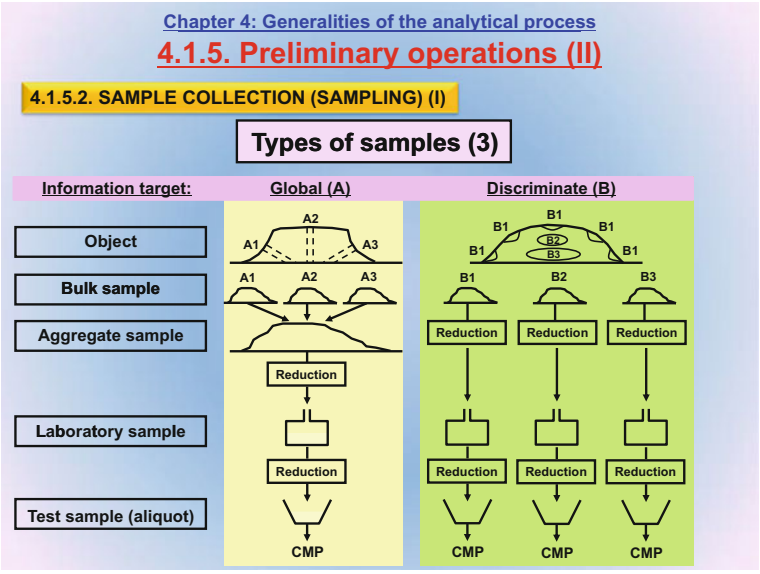
Slide 4.20



4.19.1. This slide defines the five types of samples established according to collection procedure (Criterion 1). The definitions are consistent with the sampling strategies in Slide 4.16.

4.19.2. The slide also defines the four types of samples according to size and nearness to the object (Criterion 2), which are illustrated in the next slide.

Slide 4.21



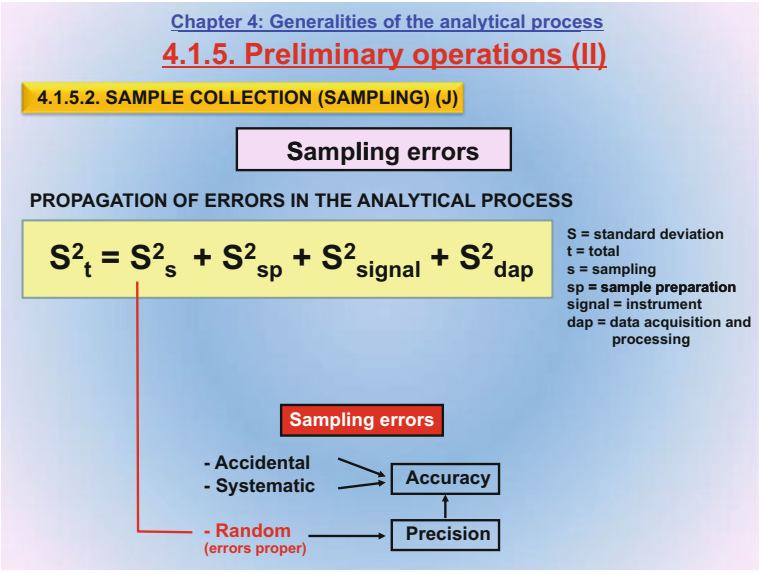
This slide illustrates the five types of samples established according to Criterion 2 in Slides 4.19 and 4.20 in relation to two different sampling strategies dictated by the type of information required, namely: (A) the mean content of the object (overall information) and (B) the contents of different parts of the object (discriminate information).

The example is the determination of the gold content in several tons of pyrite mining waste. The central and right-most columns show the object and the different types of samples that can be withdrawn from it.

If the aim is to know the concentration of gold (mg/kg) in the waste stack, and hence the total amount of gold that can be extracted from it, then the global sampling strategy should be used. For this purpose, a mechanically operated screw drill will be used to collect at least three samples (see Slide 4.23, solid sample) for mixing and homogenizing in order to obtain an aggregate or composite sample. The composite sample will be reduced in size for transfer to the laboratory, where it will be further reduced to a representative aliquot for direct application of the analytical process (CMP).

If the aim is to locate where the gold in the stack is, then bulk samples will be collected from the surface (B1), middle (B2) and bottom (B3) of the stock. Rather than being mixed, the three bulk samples will be reduced separately for delivery to the laboratory and independent processing with the CMP in order to obtain three different results that will reveal where gold in the stack is concentrated.

Slide 4.22



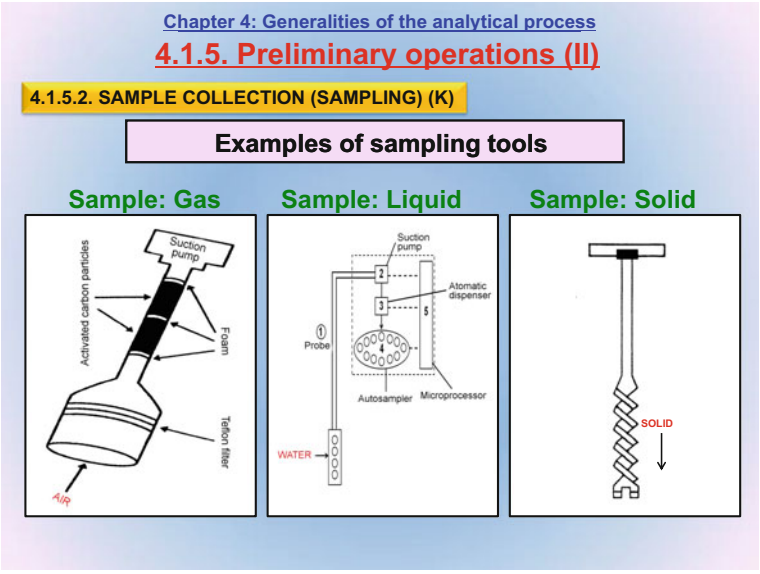
4.22.1. Based on the statistical theory of error propagation, the total error made in an analytical process is the combination of all errors made in its steps. Based on the principle of additivity of variances, the equation shows the approximate contribution of each step to the overall variance. As can clearly be seen, the preliminary operations of the analytical process (sampling and sample treatment) contribute especially markedly to its overall variance. Hence their strategic significance.

4.22.2. Similarly to the errors defined in Slides 2.9 and 2.10, sampling errors can be of the following types:

(1) *Accidental errors*, which occur in exceptional situations (e.g., operator distractions).
(2) *Systematic errors*, which will inevitable be present unless their source (e.g., poorly calibrated volume or mass measuring equipment) is eliminated.
(3) *Random errors*, which are due to chance in sampling and constitute "sampling errors" proper in statistical terms. They are denoted by S^2_S in the equation.

Accidental and systematic sampling errors have a direct impact on the accuracy of the results, whereas random errors affect precision—and hence accuracy (see Slide 2.10).

Slide 4.23



This slide exemplifies sampling systems for gaseous, liquid and solid samples. As can be seen, the sampling procedure of choice is again dictated by the type of information sought.

A gaseous object (e.g., air)

If the aim is to determine organic contaminants in the atmosphere near a chemical solvent factory, samples are obtained by using a portable suction pump with adjustable aspiration rate (e.g., in L/min) fitted with a nozzle. The pump includes a Teflon filter to retain air particulates and a sorption tube filled with foam containing activated carbon particles or carbon nanotubes. Organic molecules are retained on the filter as air circulates through the pump. When sampling is finished, the tube is transferred to the laboratory and the Teflon filter, which was previously tared, is weighed to determine the amount of particles present in the air. Then, retained contaminants are easily eluted with a volume of 2–5 mL of methanol.

A liquid object (e.g., lake water)

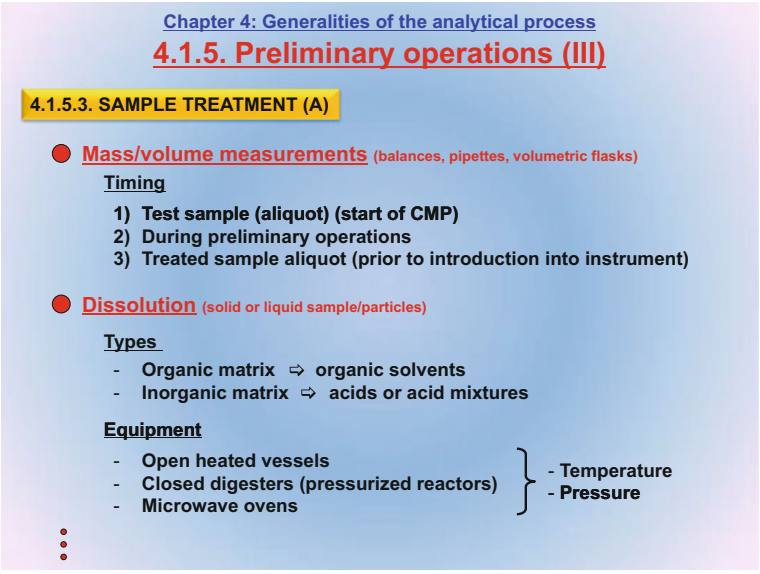
If the aim is to determine the concentration in mg/L of suspended particles at different depths in a mountain lake, then samples are obtained by using appropriate sampling equipment on board of a ship (see central image in the slide). The equipment comprises a sampling probe (1) that can be immersed to a variable depth; a suction pump (2) to aspirate water at a given flow-rate for a fixed time in order to collect samples at different depths; an automatic dispenser (3) to sequentially fill autosampler vials (4); and a microprocessor (5) to control the whole process. In this way, *n* discriminate samples are collected at different depths from the lake (the object).

A solid object (e.g., beach sand)

If the aim is to determine the concentration of organic contaminants at different depths in a beach sand under the potential influence of ship cleaning wastewater from a nearby port, then a screw drill such as that shown on the right picture is used. The operator will insert the drill in the ground with mechanical assistance and then draw it vertically in order to collect sand withdrawn at different depths from its thread.

4.1.5.3 Sample Treatment (8 Slides)

Slide 4.24



"Sample treatment" designates the body of operations performed to condition the bulk sample for insertion into the measuring instrument as depicted in Slide 4.12.

This slide and the next few provide a brief description of the most salient preliminary operations of the analytical process.

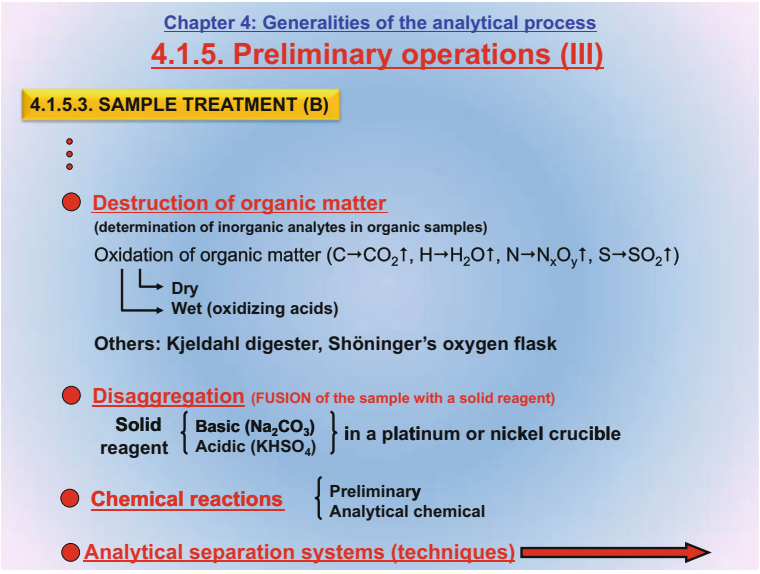
Mass and volume measurements, which are made with balances and volumetric ware (calibrated flasks, pipettes), respectively, constitute typical preliminary operations of the analytical process intended to establish the mass, in grams, or the volume, in millilitres, of test sample (aliquot) that is to be subjected to the CMP. Also, however, they are often made during the process or prior to inserting the sample portion to be measured into the instrument.

The preliminary operation *dissolution* (or *solubilization*) is only performed on samples containing solids. The "end-product" here is a transparent (colourless or otherwise) solution containing no suspended solids. The solvent to be used will depend on the nature of the sample matrix ("like dissolves like" is the rule of thumb here). Operationally, the solubilization procedure can vary widely. Thus, it may use

energy in the form of pressure or temperature, heated open tubes with or without a coolant, pressurized steel digesters, or microwave or ultrasound energy, for example. A detailed description of all possibilities is beyond the scope of this book, however.

The next slide describes additional types of sample preparation procedures.

Slide 4.25



This slide continues the description of sample treatment operations started in the previous one.

Destruction of organic matter is only used to determine inorganic analytes in organic samples (e.g., the total metal content of petroleum crude). Organic matter is "destroyed" by oxidizing carbon to carbon dioxide, hydrogen to water vapour, nitrogen to volatile oxides and sulphur to also volatile sulphur dioxide. These transformations can be accomplished simply by heating the sample until no further vapour is released—there may be losses through abrupt spillage—or by attack with an oxidant such as nitric acid. As a result, the sample first becomes brown, then black and eventually clear, leaving a residue of metal oxides.

Disaggregation can be considered an especially aggressive form of dissolution and is used when traditional solubilization procedures with acids or acid mixtures (e.g. *aqua regia*) fail to completely dissolve a sample—usually an inorganic sample. The procedure is usually as follows:

The solid sample to be dissolved is mixed with a fusion flux (e.g., a basic substance such as sodium carbonate) in a 1:10 ratio;

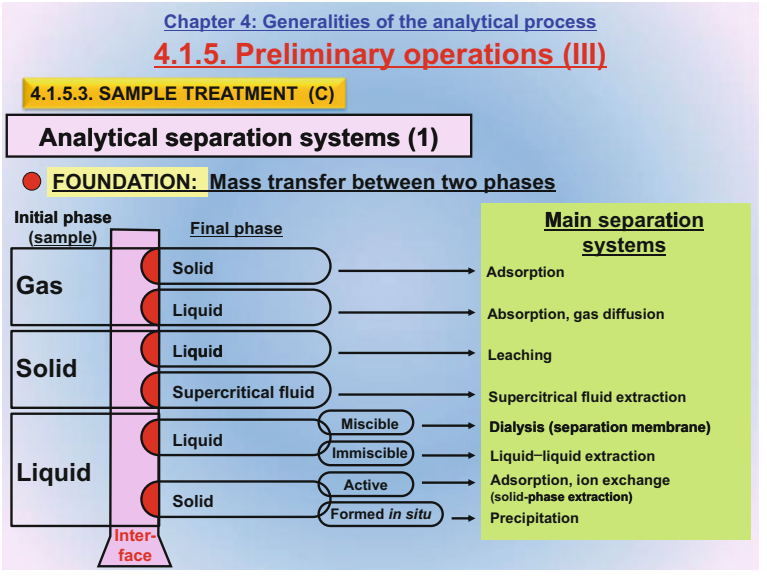
(1) the mixture is placed in a nickel or platinum crucible;
(2) the crucible is heated in a furnace at a high temperature until the mixture is completely melted;
(3) the crucible is allowed to cool down and
(4) the molten mass is dissolved at room temperature by immersion in the solvent to obtain a clear solution containing no suspended matter.

Preliminary chemical reactions are used to make the sample suitable for the intended purpose. One common preliminary reaction is that between a selective masking agent and the sample to form chelates with potentially interfering species present and improve the selectivity as a result (see Slide 6.29). Another common practice is to add a buffer in order to adjust the pH of the sample as required for its subsequent treatment. As a rule, preliminary chemical reactions involve the sample matrix rather than the analyte.

Unlike preliminary reactions, *analytical chemical reactions* involve the analyte to be detected or determined, which is "derivatized" (that is, converted into a suitable derivative for measurement in the second step of the analytical process). Thus, highly polar compounds must be subjected to a prior silylation reaction for determination by gas chromatography. Also, an analyte with inadequate photometric or fluorimetric properties must be derivatized for spectrophotometric or spectrofluorimetric determination, respectively, with adequate selectivity.

Analytical separation systems, which are the most relevant to sample preparation, are briefly described in Slides 4.26–4.31.

Slide 4.26



Analytical separation systems, often referred to as "analytical separation techniques" (ASTs) (see Slide 1.32), are extremely important in Analytical Chemistry because they help improve two essential properties: sensitivity and selectivity. Some authors have claimed that the history of Analytical Chemistry can be traced through progress in the development of separation systems.

Separation systems are operationally simple: a single or several analytes are partitioned between two phases on the assumption that they possess an increased affinity for either. Mass transfer between the two phases may also affect other components of the sample matrix—which should be removed in order to avoid interferences.

On the left of the slide are shown the phase types most usually involved in a separation process. The initial phase is that containing the sample and the final phase that added to the previous one or formed during the process. Mass transfer, which is the key to success in the separation, occurs at the interface between the two phases (red half-circles in the slide). Obviously, the interface should be as large as possible for easier transfer.

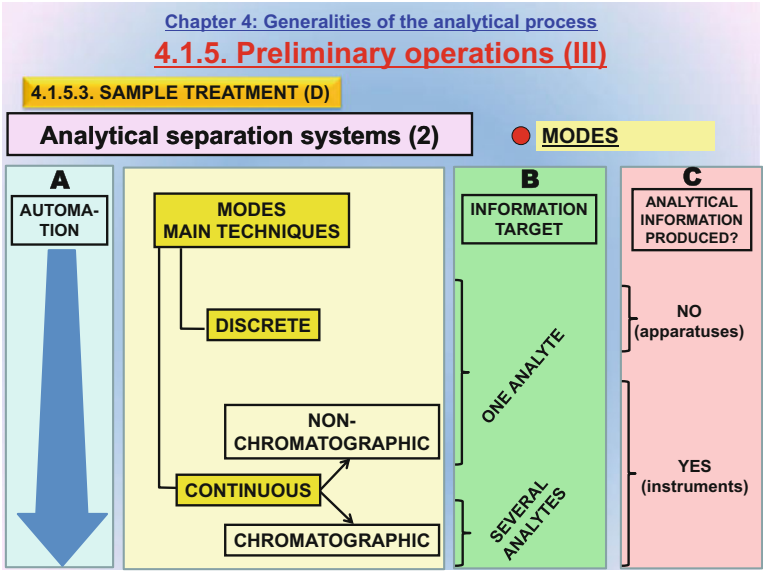
The phases involved are usually solids, liquids or gases, but can also be supercritical fluids. Combinations of the different types of phases have led to the development of a wide array of analytical separation systems for sample preparation the most salient of which are shown on the right of the slide.

If the sample (initial phase) is a *gas*, the phase to be added can be a solid for adsorption or a bubbling liquid for absorption (dissolution) or diffusion. Gas diffusion is a process by which the concentration gradient of a gas such as ammonia in a liquid causes dissolved volatile molecules to migrate to a porous membrane and pass through it into an acceptor stream (an acid stream for ammonia).

If the sample is a *solid*, the target component can be selectively dissolved with a suitable solvent (the final, liquid phase) for leaching or liquid–solid extraction (or supercritical fluid extraction if the final phase is supercritical CO_2, for example).

If the sample is a *liquid*, the final phase can be another liquid or a solid. The most common example of separation as a liquid phase—one that is formed in situ—is distillation, which is scarcely used for analytical purposes and not shown in the slide. If the liquid used as final phase is miscible with the liquid sample, the process is called "dialysis" and involves using a porous membrane for mass transfer (for example, a dialysis membrane to facilitate the passage of urea and uric acid from blood serum into an acceptor solution). On the other hand, if the final phase is not miscible with the sample, the process constitutes a typical liquid–liquid extraction. When the final phase is a surface-active solid, the transfer process involves absorption (as in solid-phase extraction, for example). Finally, a solid final phase can be formed in situ by precipitation from a liquid initial phase.

Slide 4.27



4.27.1. How are analytical separation systems implemented in the analytical process? This question is answered here by showing the most usual technical modes for this purpose.

- In *discrete separation systems*, contact between the two phases occurs in a single, unique manner, so only one separation equilibrium is established. Such is the case, for example, with liquid–liquid extraction in a separation funnel.
- In *continuous separation systems*, the so-called "mobile phase" is passed uninterruptedly through the so-called "stationary phase". In *non-chromatographic continuous separation systems*, the analyte partitions in a single thermodynamic equilibrium; in *chromatographic continuous separation systems*, however, it partitions in many different zones to give multiple analytes.

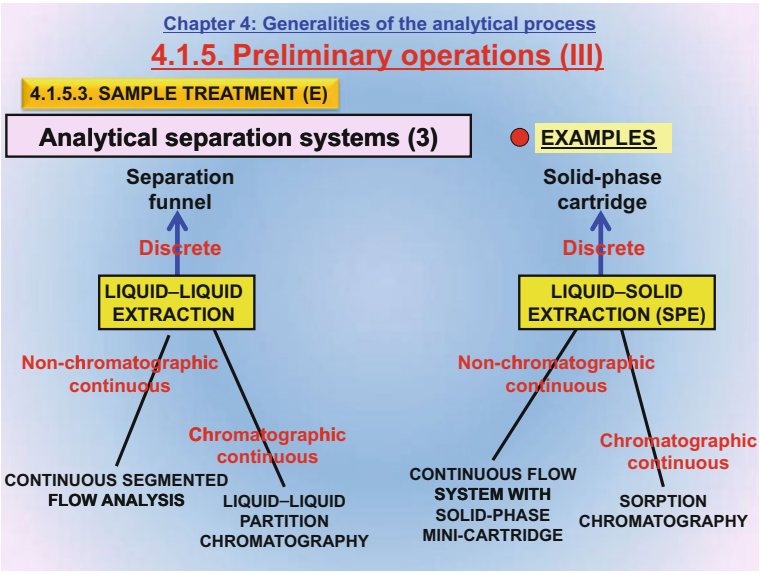
4.27.2. The ease of automation of analytical separation systems increases from discrete to continuous chromatographic systems by effect of commercial availability of separation equipment also increasing in that direction.

4.27.3. The information target(s) of analytical processes using separation systems can be (1) the presence or concentration of an analyte or analyte family with discrete and non-chromatographic continuous systems; and (2) the presence or concentration of individual analytes in a mixture with chromatographic continuous systems.

4.27.4. Discrete systems operate independently of analytical measuring equipment and are thus apparatuses because they produce no analytical information. On

the other hand, continuous separation systems, both chromatographic and non-chromatographic, invariably use a continuous detection system, whether destructive or non-destructive, and are thus instruments.

Slide 4.28

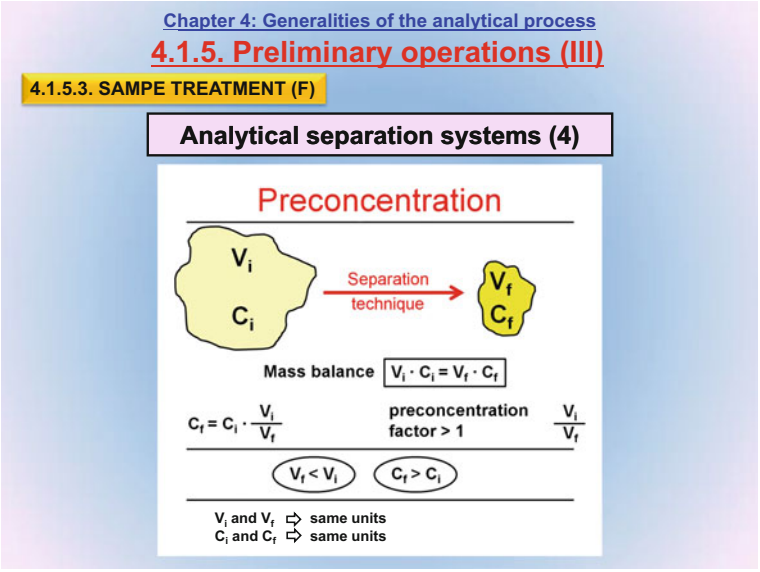


This slide exemplifies the separation modes explained in the previous one.

Liquid–liquid separation can be implemented in a discrete manner by using a separation funnel to mix appropriate volumes of the two phases. Also, it can be implemented by bringing the two phases into contact in a continuous segmented flow system for non-chromatographic continuous separation. Finally, it can be accomplished by having the analyte partition between a mobile liquid phase and a stationary solid phase (e.g., a chromatographic column) for chromatographic continuous separation.

Liquid–solid separation can be accomplished by inserting a solid-phase cartridge in a continuous-flow system (solid-phase extraction, SPE) or by having a liquid phase containing the analyte pass through a solid phase held in a column (sorption chromatography).

Slide 4.29



This slide illustrates one of the main purposes of separation systems, namely: preconcentrating analytes. If the analytes are highly diluted in the sample (that is, at very low concentrations, C_i, in a sample volume V_i), subjecting an aliquot to the analytical process will provide no measurable signal. However, reducing the sample volume (to V_f) can artificially increase the analyte concentrations to a high enough level C_f for a new sample aliquot to provide a measurable signal. Obviously, this procedure has a direct impact on the basic analytical property "sensitivity" (see Slide 2.33).

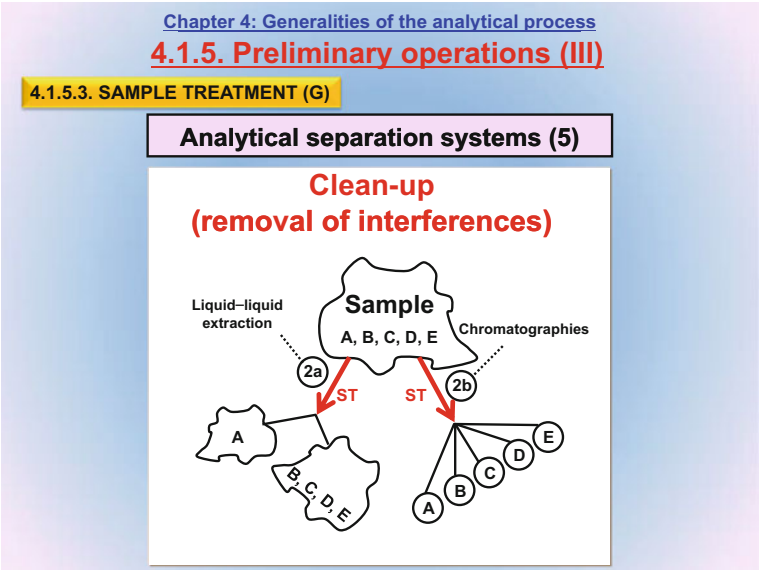
The final concentration of analyte, C_f, can be calculated from a mass balance (that is, from the amount of analyte present before and after reducing the sample volume, which will be identical).

The degree of preconcentration will increase with increasing preconcentration factor, which is the ratio of the initial to final sample volume: V_i/V_f.

For effective preconcentration, the final volume should obviously be smaller than the initial volume—and the final concentration higher than the initial concentration as a result.

The mass balance should be established by using volumes and concentrations in identical units.

Slide 4.30

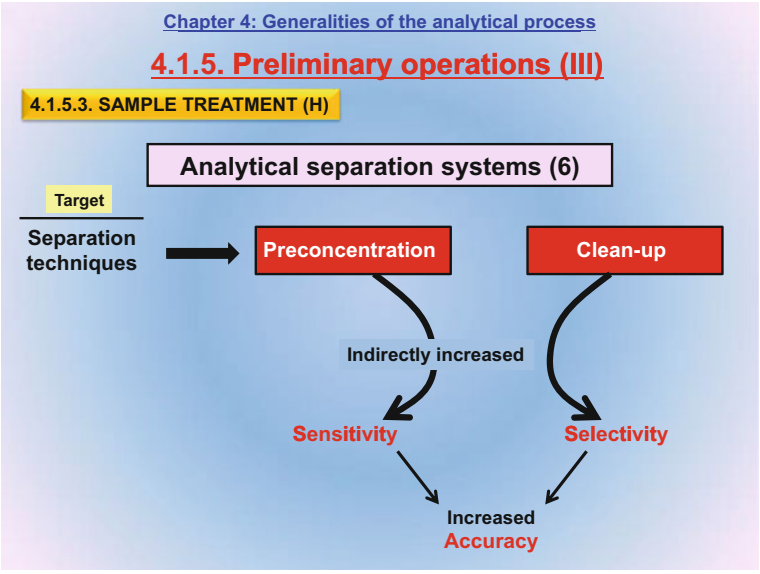


One other crucial purpose of analytical separation systems is the removal of interferences (so called "clean-up") to improve the basic analytical property "selectivity".

A sample can be cleaned up in two different ways with an analytical separation system depending on the nature of the information sought.

If the target information concerns a single analyte in the sample, the system to be used should allow the analyte to be physically isolated from all others in a separate phase. If a more ambitious goal such as determining the presence or concentration of more than one analyte is pursued, then each analyte should be isolated in its own, distinct zone by using a chromatographic separation system (see Slide 4.27).

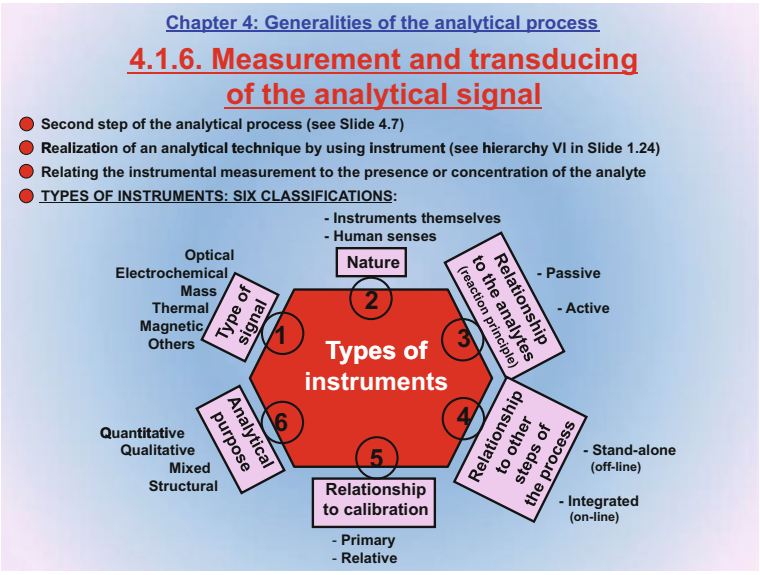
Slide 4.31



In summary, the primary purposes of using analytical separation systems for sample preparation in the second sub-step of the preliminary operations of the analytical process are preconcentration (Slide 4.29) and sample clean-up (Slide 4.30), which provide an indirect means for increasing sensitivity and selectivity, respectively, in addition to accuracy (see Slide 2.4).

4.1.6 Measurement and Transducing of the Analytical Signal (1 Slide)

Slide 4.32



The second step of the analytical process (see Slide 4.7) involves measuring and transducing[1] the analytical signal (primary data), which is processed to obtain the analytical results in the third. This operation requires using a measuring instrument (see the hierarchy in Slide 1.24).

A number of instruments for signal measurement and transducing based on a variety of principles have been made commercially available in the past fifty years that can be classified in a non-mutually exclusive manner as follows:

(1) According to the nature of the signals they measure, instruments can be of the *optical, electroanalytical, mass, thermal* or *magnetic* type, among others.
(2) According to their own nature, instruments can be the *human senses*, which are typically used in Classical Qualitative Analysis, or instruments proper (e.g., pH-meters, photometers, fluorimeters) (see Slide 6.27).
(3) According to their relationship to the analytes to be detected or quantified (or their reaction products with derivatizing agents), instruments can be *passive* (that is, simply receiving the analytical signal, as in mass weighed with a

[1]In this context, the word "transducing" designates the transformation of the signal originally produced by the instrument (usually in volts or millivolts) into the typical unit for the measured quantity (e.g., absorbance in UV-Visible absorption spectroscopy), which is that used in the third step of the analytical process.

balance), or *active* (that is, causing the analyte to emit a signal by applying it some form of energy such as light in photometry or fluorimetry).

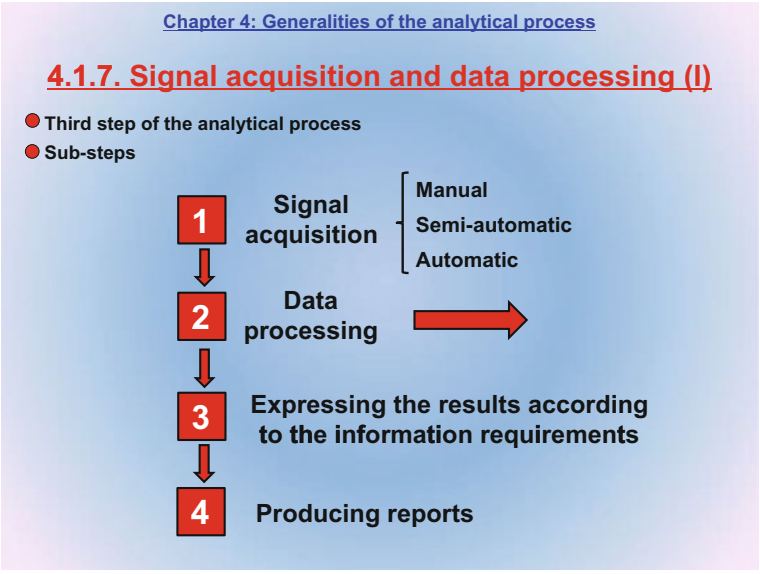
(4) According to their relationship to the first step of the analytical process, the instruments used in its second step can be of the *stand-alone* (*off-line*) type, to which samples are usually delivered by hand (e.g., by filling a cuvette for placement in a photometer); or of the *integrated* (*on-line*) type, which come with their own, automatic sample delivery system (e.g., the flame ionization detector in a gas chromatograph, which is used to continuously receive mobile phase from the column and detect passage of an analyte).

(5) Instruments require calibration to ensure proper functioning, and so do methods sometimes. Whereas calibrating a *relative method* is usually feasible (e.g., by constructing a calibration curve for a photometric determination), calibrating an *absolute method* such as a gravimetry is impossible (see Chap. 5).

(6) Finally, instruments can be classified as *qualitative*, *quantitative*, *mixed* and *structural* according to their analytical purpose. Most instruments are of the mixed type because they are flexible enough for adaptation to a variety of purposes; such is the case, for example, with mass spectrometers. Some, however, only have a single, specific purpose. Thus, a pH-meter can only be used for quantitative measurements; also, transmission infrared absorption spectrophotometers are basically used for identification (qualitative) purposes by routine analysis laboratories even though they also afford quantification.

4.1.7 Data Acquisition and Processing (2 Slides)

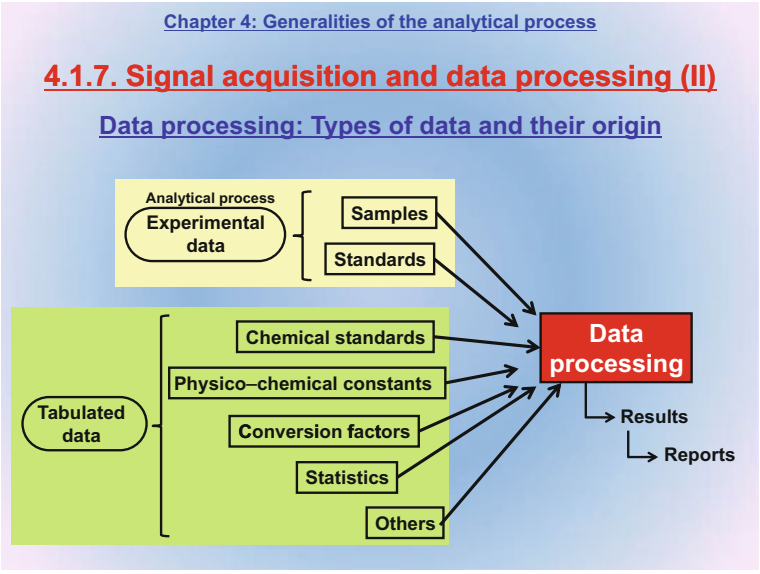
Slide 4.33



The third, last step of the CMP involves the acquisition of analytical signals and their processing to obtain the results needed. This step is commonly designated "data acquisition and processing" (DAP) and comprises the four sub-steps shown in this slide, which are closely related to the primary data—information—knowledge hierarchy of Slide 1.20.

(1) The first DAP sub-step is *signal acquisition*. Signals can be acquired manually (e.g., by reading a digital display and recording the readout on a laboratory notebook), semi-automatically (e.g., by measuring retention times, and peak heights and areas, in a chromatogram) or completely automatically.
(2) The next sub-step is *data processing*, which is dealt with in the next slide.
(3) The third step involves *expressing the results* (information), whether qualitative or quantitative, in accordance with legal requirements or the client's needs.
(4) The last step is *producing a report* (knowledge) based on the results for their placement in context and comparison with the information requirements and legal limits in order to, for example, facilitate well-grounded, timely decision-making.

Slide 4.34



This slide expands on the description of the second DAP sub-step given in the previous one, namely: *data processing*, which leads to obtainment of the results and production of a report.

The data to be processed can be of two different types:

- *Primary experimental data* obtained by analysing samples and tangible standards in the analytical process.
- *Tabulated data in printed or on-line form* for chemical standards (e.g., purity, hygroscopicity), physico-chemical constants (e.g., gravimetric factors, Slides 5.17 and 5.18), conversion factors (e.g., those for incomplete recoveries) or statistics (e.g., Student's *t*), among others.

4.2 Annotated Suggested Readings

BOOKS

Principles of Analytical Chemistry

M. Valcárcel

Springer-Verlag, Berlin, 2000

This was the first book to start the teaching of Analytical Chemistry with its foundations before dealing with methods and techniques in order to provide students with an accurate notion of what Analytical Chemistry is and means.

This chapter overlaps to a great extent with Chap. 4 in Valcárcel's book ("The measurement process in Chemistry") except that the text has been simplified in some parts and expanded in others to better illustrate the present and future of Analytical Chemistry 15 years later. Valcárcel's book can be used as a reference for direct consultation of the contents of this chapter.

Sampling for Analytical Purposes

Pierre Gy

Wiley, New York, 1999.

This is a short book (150 pages) exclusively devoted to the first operation of the analytical process. In addition to providing an integral, comprehensive definition, it summarizes all sampling choices dealt with in this chapter and a few others. The book is very useful for designing sampling processes and can help students expand their knowledge of specific sampling modes.

Non-chromatographic continuous separation techniques

M. Valcárcel & M.D. Luque de Castro

Royal Society of Chemistry (UK), Cambridge, 1991.

This was the first book to introduce the concept "non-chromatographic continuous separation systems", based on the principles of flow injection analysis. Such systems are used not for discriminate separation of analytes but rather to facilitate automation of sample preparation. The book deals with gas–liquid (diffusion, distillation), liquid–liquid (extraction) and solid–liquid systems (ion exchange, sorption).

4.3 Questions on the Topic (Answered in Annex 2)

4.1. What question does the development of an analytical process essentially answer regarding extraction of (bio)chemical information from an object: what, how, when or where?

4.2. Why do analytical processes invariably use measurement standards? How are they used?

4.3. A manufacturing process leads to an error in the quality-related parameters for the product that requires analytical control. What kind of sampling should be done in this situation?

[ ] Intuitive
[ ] Statistical
[ ] Directed
[ ] Protocol-based

Explain why.

4.4. What is the difference between "dissolution" and "disaggregation" of a solid sample?
4.5. Give four definitions of "sampling" or "sample collection".
4.6. How does the availability of materials and equipment (reagents, solvents, apparatuses, instruments, etc.) influence the choice of an analytical process for a specific sample–analyte pair? Use one or more examples.
4.7. What factors dictate the choice of an analytical method? What is usually the most important?
4.8. Tick the true statements about the preliminary operations of the analytical process:

[ ] They are equivalent to so-called "sample treatment"
[ ] They typically account for 50–70% of the length of a CMP
[ ] They come after measurement and transducing of the analytical signal
[ ] They have little impact on the quality of the final result

4.9. Why is sampling important in chemical measurement processes? Tick the correct answers.

[ ] Because it influences selectivity and sensitivity
[ ] Because it is essential to assure representativeness in the final result
[ ] Because it is a key to robustness in CMPs
[ ] Because it has a direct impact on the accuracy of the results of CMPs

4.10. Are equipment and method calibration part of a CMP? Why?
4.11. Name at least five features of the preliminary operations of CMPs. Is any of them positive?
4.12. What are the positive contributions of the preliminary operations of CMPs?
4.13. How are instruments classified according to the nature of the raw signals they provide?
4.14. What are the two information sources for the third step of the analytical process (data processing and result delivery)?
4.15. Name the five factors governing the development of an analytical measurement process.
4.16. What are the two main purposes of the preliminary operations of CMPs?
4.17. Why is variability a negative connotation of analytical processes?
4.18. How is automatability related to the preliminary operations of the analytical process?
4.19. What is the most sluggish, labour-intensive and error-prone step of an analytical process?

4.20. What should be balanced in designing a sampling plan?
4.21. What are the four types of sampling arising from the overall sampling plan?
4.22. What names are samples given according to size and nearness to the object?
4.23. Distinguish “object” and “sample”.
4.24. When and why must organic matter in a sample be destroyed in the preliminary operations of the analytical process?
4.25. What basic properties are favourably affected by separation techniques? What capital property is also favoured? What basic property can be adversely affected?
4.26. How are instruments classified according to the nature of the analytes to be determined?
4.27. How are sampling and representativeness related?
4.28. What are the main types of analytical separation systems?

4.4 An Abridged Version of the Chapter

The contents of this chapter can be shortened by about 25% for teaching Analytical Chemistry to students not majoring in Chemistry. The following 8 slides can be omitted for this purpose:

- Section 4.1.1: Slide 4.3
- Section 4.1.2: Slide 4.4
- Section 4.1.4: Slide 4.8
- Section 4.1.5.1: Slide 4.10
- Section 4.1.5.2: Slides 4.16, 4.17, 4.23 and 4.27