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IUPAC Technical Report

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Abstract: Approaches for sample preparation are developing rapidly as new strategies are implemented to improve sample throughput and to minimize material and solvent use in laboratory methods and to develop on-site capabilities. In majority of cases the key step in sample preparation is extraction, typically used to separate and enrich compounds of interests from the matrix in the extraction phase. In this contribution, the topic of analytical scale extraction is put in perspective emphasising the fundamental aspects of the underlying processes discussing the similarities and differences between different approaches. Classification of extraction techniques according to the mass transfer principles is provided.

Keywords: analytical chemistry; extraction; technical report.

CONTENTS

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1 Introduction: Perspective on sample preparation

The fundamentals of sampling and sample preparation are substantially different from those related to chromatographic separations or other traditional disciplines of analytical chemistry. Sampling and sample preparation frequently resemble corresponding engineering processes on a smaller scale. Within the analytical workflow, the sample preparation step typically consists of extracting components of interest from a sample matrix. This procedure can vary in degree of selectivity, speed, and convenience depending on the approach and conditions used, as well as on the geometric configuration of the selected extraction phase. Optimisation of this process enhances overall analytical performance. Proper design of extraction devices and procedures facilitates rapid and convenient on-site implementation, integration with separation and quantification steps, and/or automation. The key to rational choice, optimisation, and design is an understanding of the fundamental principles that govern mass transfer of analytes in multiphase systems. There is a tendency to name extraction techniques according to random criteria. The objective of this report is to emphasise common principles among different extraction techniques, describe a unified theoretical treatment, and develop a corresponding nomenclature of terms used in extraction.

The analytical procedure for complex samples consists of several steps; these typically include sampling, sample preparation, separation, quantification, statistical evaluation, and decision making (Fig. 1). Each step is critical for obtaining correct and informative results. The sampling step, often the first step within the procedure, entails deciding where to get samples that properly define the object or problem being characterised, and choosing a method to obtain samples in the right amount. The objective of the sample preparation step, on the other hand, is to isolate the components of interest from a sample matrix, as most analytical instruments cannot handle direct introduction of untreated matrices. Sample preparation, in turn, usually involves extraction procedures, and can also include "clean up" procedures for very complex, "dirty" samples. As analytes often need to be brought to a concentration level suitable for detection, the sample preparation step may also include enrichment of analytes. During the separation step of the analytical process, the isolated complex mixture containing the target analytes is divided, often by means of a chromatographic or electrophoretic technique, into its constituents, which are then subsequently identified and quantified. Identification can be based on retention time or migration time combined with selective detection, *e.g.*, mass spectrometry (MS). Statistical evaluation of the obtained results provides an estimate of the concentration of the target compound in the sample being analysed. The resulting data is used to make appropriate decisions, which might include additional sample collection and analysis for further investigation of the object or problem.

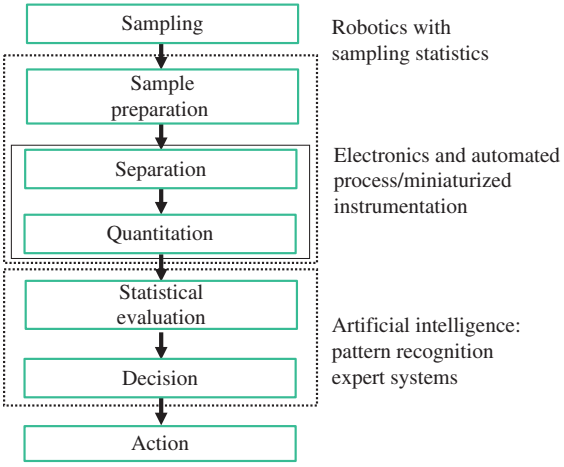

Fig. 1: Integration of steps in the analytical process.

As emphasised in Fig. 1, analytical steps take place in succeeding order; as such, a subsequent step cannot begin until the preceding one has been completed. Therefore, the slowest step determines the overall speed of the analytical process, and improving the speed of a single step may not necessarily result in an increase in throughput; to increase throughput, all steps need to be considered. Also, errors introduced in any preceding step, including sampling, will result in overall poor procedure performance.

In recent years, major breakthroughs in the development of improved instrumentation, such as miniaturisation of analytical devices and integration of different steps into one system, have been possible due to scientific advancements in robotics, sampling statistics, electronics, and artificial intelligence (see Fig. 1). An ideal instrument should be able to perform all analytical steps with minimal human intervention, preferably directly on the site where an investigated system is located rather than moving samples for laboratory analysis, as is common practice at the present time. This approach would eliminate errors and reduce the time associated with sample transport and storage ("green" features), and therefore, result in faster analysis and more accurate and precise data. Although such a device has not yet been built, today's sophisticated instruments are already capable of separating and quantifying complex mixtures, as well as automatically applying chemometric methods to statistically evaluate results. It is more difficult to hyphenate sampling and sample preparation steps, primarily because current state-of-the-art sample preparation techniques employ multi-step procedures involving organic solvents. This trait hinders the development of methods capable of integrating sampling and sample preparation with separation methods for the purpose of automation. As a result, over 80 % of analysis time is currently spent on sampling and sample preparation steps for complex samples [1].

One of the reasons behind the slow progress in the area of sample preparation is that the fundamentals of extraction involving natural, frequently complex samples are much less studied compared to the physicochemically simpler systems used in separation and quantification steps such as chromatography and mass spectrometry. This situation creates an impression that rational design and optimisation of extraction systems is not possible. Consequently, the development of sample preparation procedures is frequently considered more of an "art" than a "science."

Figure 2a provides a classification of extraction techniques and unifies the fundamental principles behind the different extraction approaches, while Fig. 2b provides a schematic representation of the three fundamental operations. In principle, exhaustive extraction approaches do not require calibration, as the majority of the analytes are transferred to the extraction phase by employing an overwhelming volume of extraction phase. In practice, however, confirmation of satisfactory recoveries is implemented in the method by using surrogate standards. To reduce the amount of solvents and time required to accomplish exhaustive removal, batch equilibrium techniques (for example, liquid-liquid extractions, LLE) are frequently replaced by flow-through techniques. For example, a sorbent bed can be packed with extraction phase dispersed on a supporting material; when sample is passed through, the analytes in the sample are retained on the bed. Large sample volumes can be passed through a small cartridge, allowing the well-packed bed to facilitate efficient mass transfer. The extraction procedure is followed by the desorption of analytes into a small volume of solvent, resulting in substantial enrichment and concentration of analytes. This strategy is used in sorbent-trap techniques and in solid phase extraction (SPE) [2].

Solid-phase extraction was initially developed as a complement or replacement for liquid-liquid extraction. Facilitated by the manufacture of convenient sorbent-filled cartridges (Fig. 3) as disposable laboratory supply items, SPE emerged as a widely used laboratory technique in the early 1980s. Over time, various sampling formats and sorbents have been developed to facilitate the processing of different sample types and to extend the scope of the method (Figs. 3–5, [3–5]). A high level of automation has also been made possible with the use of robotics and online interfaces to separation and spectroscopic instruments [3, 6].

Typical cartridge devices consist of short columns (for example, a packed syringe barrel) containing a sorbent with a nominal particle size of 50–60 μm (to facilitate sample processing using gentle suction) packed between porous plastic or metal frits. The disc format was developed to provide higher sample processing rates for large sample volumes, such as environmental water samples, and to minimize plugging by suspended particles and matrix components by increasing the surface area of the sorbent bed while minimizing

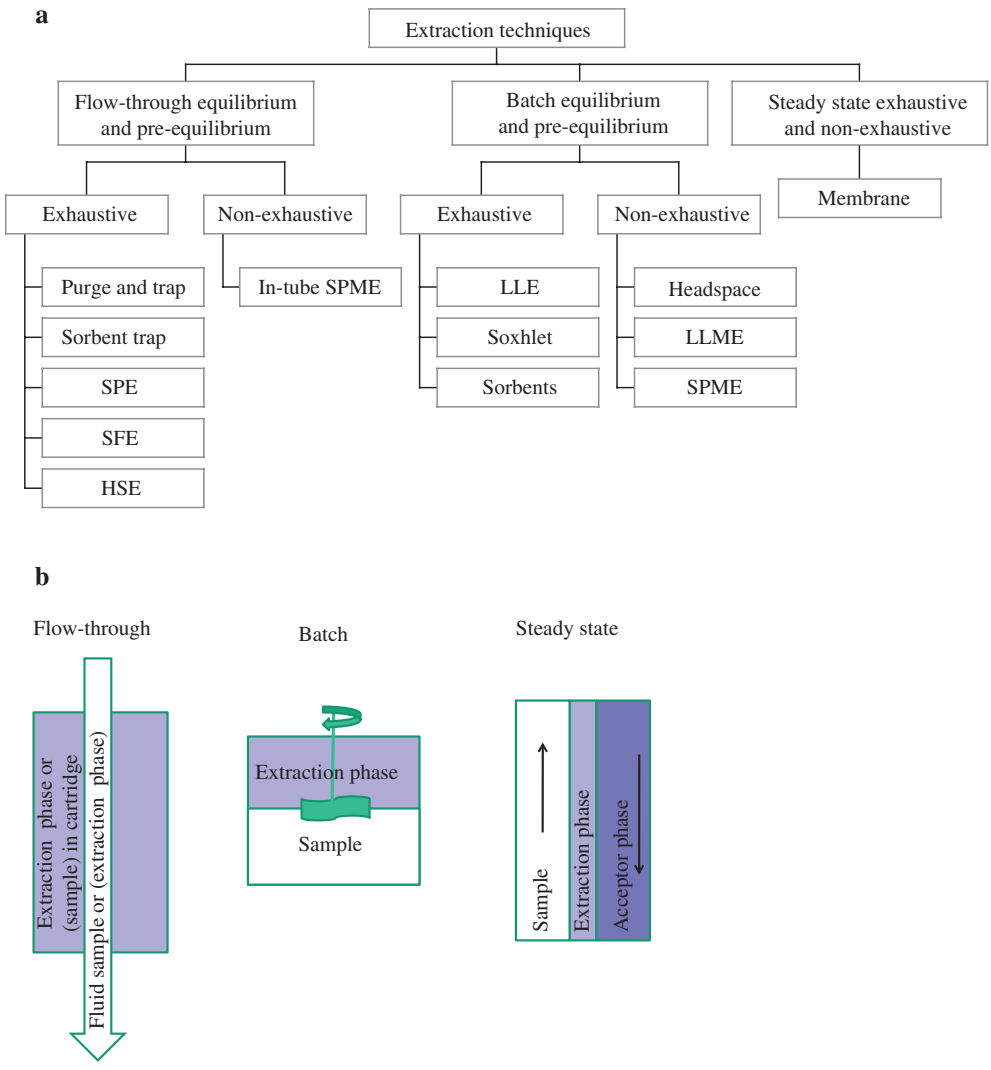

Fig. 2: (a) General classification of extraction techniques. (b) Schematics showing the principles of the extraction process behind classification.

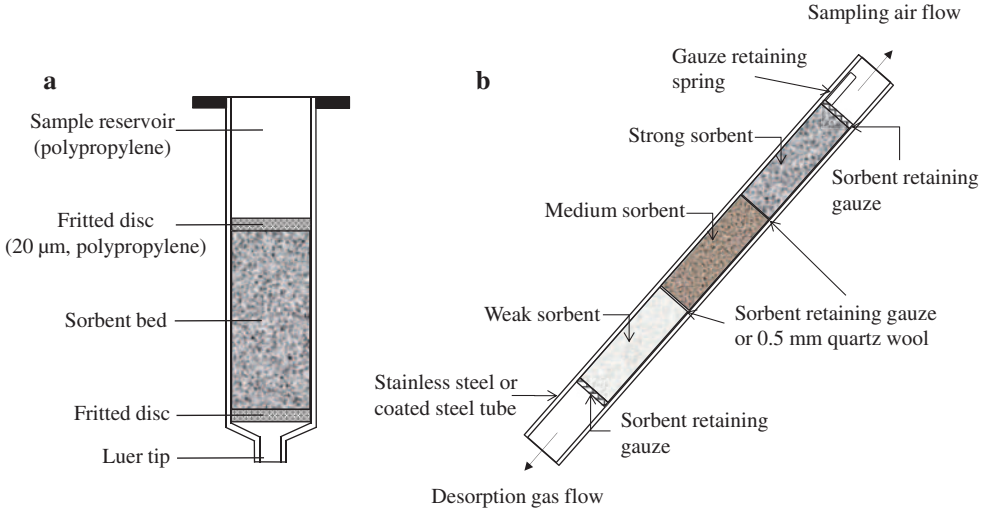

Fig. 3: Solid-phase extraction using a cartridge device for liquid samples (a), and gas-phase samples (b).

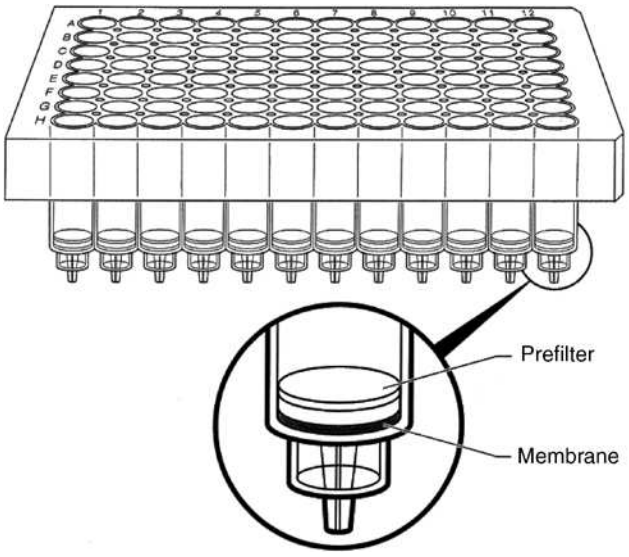

Fig. 4: Multiwell extraction plate for solid-phase extraction.

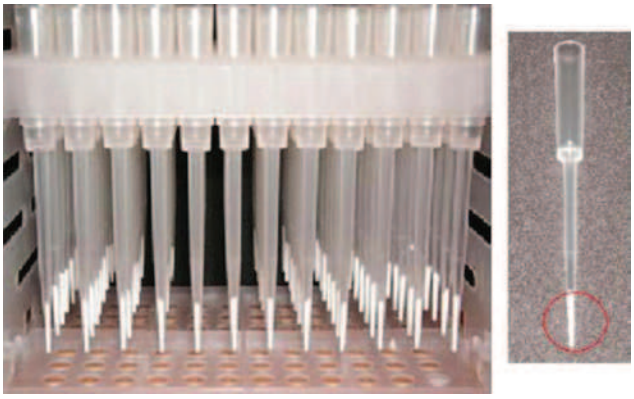

Fig. 5: Modified pipette tips for simultaneous solid-phase extraction.

the bed height. Since it is easier to miniaturise discs than cartridges, discs in the form of microdiscs or pipette tips that contain only a few milligrams of sorbent are also favoured for handling small sample volumes. Two disc formats dominate solid-phase extraction applications: particle-loaded membranes containing sorbent particles of 8–12 μm diameter immobilized in a web of short PTFE fibres formed into 0.5 mm-thick discs with various diameters, and particle-embedded glass fibre discs containing sorbent particles of about 10–30 μm diameter woven into a glass fibre matrix. Particle-loaded membrane discs are flexible and superficially resemble filter-paper discs requiring a supporting structure, such as a porous glass or plastic support for use during sampling. Small particle-embedded discs are semi-rigid and self-supporting, while large diameter discs require a support similar to particle-loaded membranes. Disc technology has contributed directly to the automation of solid-phase extraction through the development of multiwell extraction plates (Fig. 4), which are used for isolation and sample clean-up in high-throughput screening procedures. Monolithic sorbents are frequently used in miniaturised sampling devices as well, for example, in modified pipette tips (Fig. 5), since these sorbents overcome problems with the packing of very short homogenous particle beds. For convenience in handling, the micro extraction by packed sorbent (MEPS) configuration employs a sorbent-packed syringe instead of a typical cartridge (Fig. 6) [7]. Core-shell microspheres with a magnetic core comprise the most recent format introduced for solid-phase extraction used in the dispersive batch mode rather than the

flow-through approach [8]. The small particles have a high surface-area-to-volume ratio and can be readily dispersed throughout the sample volume, then simply isolated by the application of a magnetic field.

Sorbents used for solid-phase extraction can be broadly classified into three groups: (i) inorganic oxides, such as silica gel and alumina; (ii) low-specificity sorbents, such as silica-based chemically bonded phases and porous polymers; and (iii) compound- and class-specific sorbents, such as immunosorbents, molecularly imprinted polymers, and restricted access media. The wide range of available retention mechanisms is important for expanding the scope of solid-phase extraction and providing compatible sorbents for use with different sample matrices. Sorbent selection facilitates single compounds, groups of similar compounds, or general extraction conditions to be employed. As analyte concentrations are usually low in solid-phase extraction, the breakthrough volume of the sampling device essentially determines the amount of analyte that can be isolated under frontal analysis conditions. Typical solid-phase extraction devices with short sorbent beds are not expected to provide high plate numbers and breakthrough volumes; consequently, they depend on the kinetic properties of the sampling device as well as its retention characteristics [9]. The volume of strong solvent that preserves minimum retention for the least-retained analyte of interest determines the volume and composition of the rinse solvent that can be used for matrix simplification. Recovery of the analytes in a minimum solvent volume for subsequent analysis generally requires a solvent in which the analytes have low retention on the sorbent, unless thermal desorption into the gas phase is employed for recovery. The rinse and recovery steps are described by elution models similar to those for column chromatography. All sampling conditions can be modelled by a few simple experiments in which the sorbent is used as a column packing material for liquid chromatography to generate initial data for simulation [3, 9].

Solid-phase extraction techniques have their own problems, although these are different, from those of classical liquid-liquid extraction. The surface chemistry of sorbents, and therefore their retention properties, are not as reproducible as the solvation properties of solvents. Solid sorbents, in addition, tend to have higher levels of contamination that may interfere in the determination of analytes. The lower sorption capacity of sorbents can result in poor and irreproducible analyte recovery as a result of analyte displacement or plugging of sorbent pores by matrix components.

For (mainly) solid samples extracted by the flow-through extraction technique (Fig. 2b), the sample can be packed in the bed and the extraction phase can be used to remove and transport the analytes to the collection point. In supercritical-fluid extraction (SFE), compressed gas is used to wash analytes from the sample matrix (Fig. 7); an inert gas at atmospheric pressure performs the same function in purge-and-trap methods (Fig. 8). In dynamic solvent extraction, *e.g.*, in a Soxhlet apparatus, the solvent continuously removes the

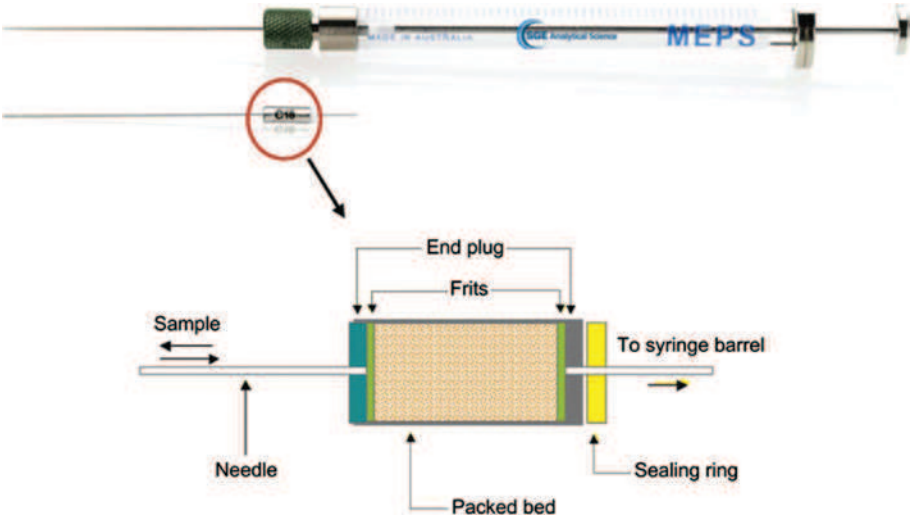

Fig. 6: Typical sampling device for microextraction by a packed sorbent bed.

analytes from the matrix at the boiling point of the solvent (Fig. 9). In more recently introduced pressurised fluid extraction (PFE) techniques, smaller volumes of organic solvent or even water are used to achieve greater enrichment at the same time as extraction (Fig. 10), which is made possible due to increased solvent capacity and elution strength at high temperatures and pressures [10].

Alternatively, non-exhaustive approaches can be designed on the basis of the principles of equilibrium, pre-equilibrium, and permeation techniques [11]. Although equilibrium non-exhaustive techniques (*e.g.* static headspace) are fundamentally analogous to equilibrium-exhaustive techniques (*e.g.* purge-and-trap), the capacity of the extraction phase is smaller and is usually insufficient to remove most of the analytes from the sample matrix. This is due to the use of a small volume of extraction phase both absolutely and relative to the sample volume, such as is employed in microextraction (solvent microextraction [12] or solid-phase microextraction SPME [13]), or in the case of a low sample matrix–extraction phase distribution constant, as is typically encountered in headspace techniques [14]. Pre-equilibrium conditions are accomplished by breaking the contact between the extraction phase and the sample matrix before equilibrium with the extracting phase has been reached. Although the employed devices are frequently identical to those used for microextraction systems, shorter extraction times are employed. The pre-equilibrium approach is conceptually similar to the

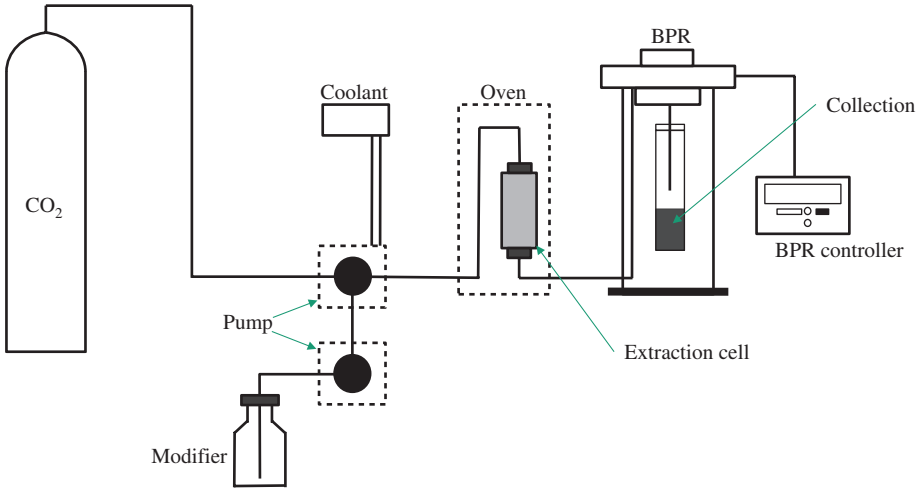

Fig. 7: Apparatus for supercritical fluid extraction (BPR = back-pressure regulator).

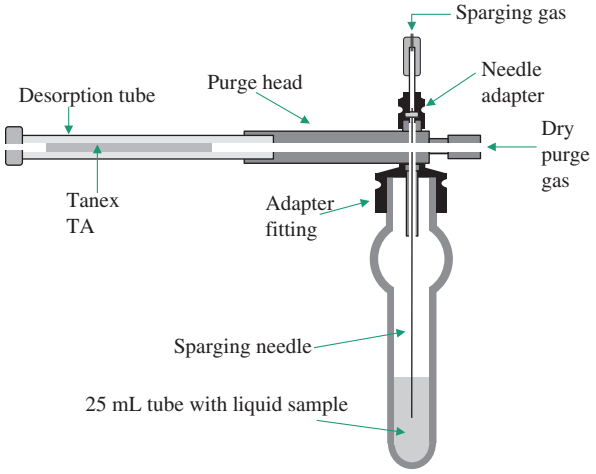

Fig. 8: Typical apparatus for dynamic headspace analysis (purge-and-trap).

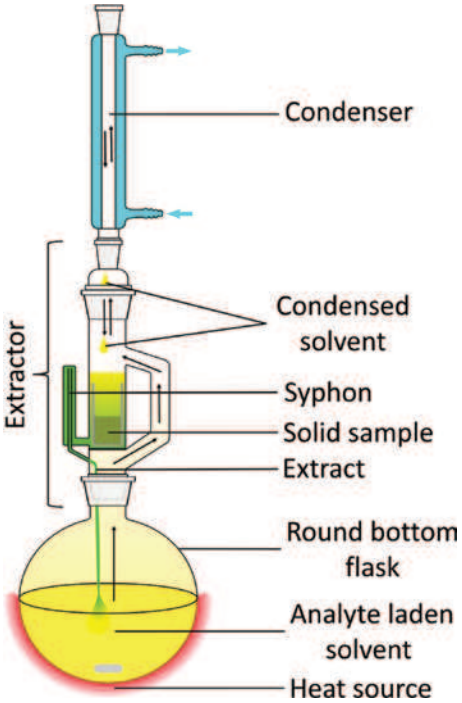

Fig. 9: Apparatus for classical Soxhlet extraction.

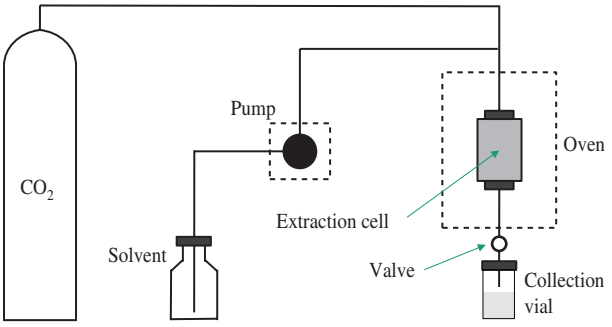

Fig. 10: Apparatus for pressurised-liquid extraction.

flow-injection analysis approach (see below) [15], in which quantification is performed in a dynamic system, and equilibrium is not required to obtain acceptable levels of sensitivity, reproducibility, and accuracy.

An important advantage of microextraction techniques when compared with traditional exhaustive approaches lies in its flexibility of configurations, which is made possible as restrictions related to high recovery requirements are eliminated. Figure 11 illustrates several implementations of SPME [16]. These include mainly open-bed batch extraction concepts, such as coated fibres, vessels, and agitation mechanism disks, as well as in-tube flow-through approaches. Some devices better address issues associated with agitation, while others are more effective in facilitating ease of sample introduction for analytical instruments. The fibre technique remains, to this date, the most used SPME approach. It should be noted that solid-phase microextraction was originally named after the first experiment using an SPME device, which involved extraction on solid fused silica fibres [17]. Subsequently, the name was retained as a reference to the appearance of the extracting phase (relative to a liquid or gaseous donor phase), even though the extraction phase is not always technically a solid.

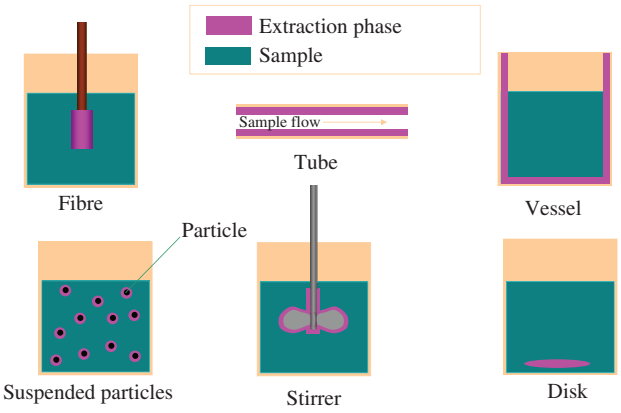

Fig. 11: Solid phase microextraction (SPME) configurations.

Similarly, different liquid-phase microextraction (LPME) techniques have been developed. In one batch configuration, termed single-drop liquid-phase microextraction (SD-LPME), the extraction of target analytes is performed from an aqueous sample into a droplet of organic solvent suspended from the tip of a micro-syringe (see Fig. 12a) [18].

After extraction, the organic droplet is retracted into the syringe, and subsequently injected into a gas chromatograph for separation and detection. In another batch configuration, termed hollow-fibre liquid-phase microextraction (HF-LPME), target analytes are extracted from aqueous samples through a thin film of a supported liquid membrane (SLM) sustained in the pores in the wall of a porous hollow fibre and into a small volume of acceptor solution located inside the lumen of the hollow fibre (see Fig. 12b) [19].

In a third batch configuration, termed dispersive liquid-liquid microextraction (DLLME), a mixture of extraction and dispersing solvents is injected into an aqueous sample [20]. The extraction solvent is dispersed in the sample and, after extraction, this solvent is recovered by centrifugation. High enrichment of target analytes can be obtained with the use of SD-LPME, HF-LPME, and DLLME, while the consumption of hazardous organic solvents is reduced to a few µL per sample. In addition, since the techniques are based on liquid-liquid extraction principles, efficient sample clean-up can be achieved. Due to the small volume of extracting phase used in the different LPME techniques, the extractions are typically non-exhaustive. Other LPME-type approaches have also been proposed, including electromembrane extraction (EME) [21], solidified floating organic drop microextraction (SFODME) [22], and parallel artificial liquid membrane extraction (PALME) [23].

In steady state permeation techniques (see Fig. 2b), *e.g.* membrane extraction [24], continuous transport of analytes through the extraction phase is accomplished by simultaneous re-extraction of analytes.

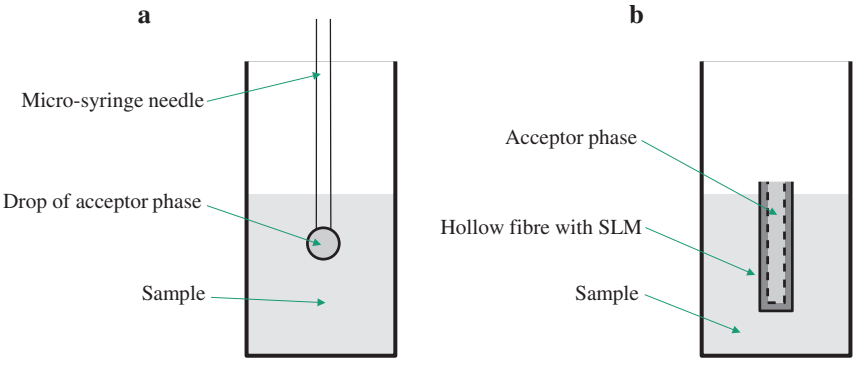

Fig. 12: Principles of liquid phase microextraction (LPME) configurations: (a) Single-drop liquid-phase microextraction (SD-LPME). (b) Hollow-fibre liquid-phase microextraction (HF-LPME).

Membrane extraction can be made exhaustive by designing appropriate membrane modules and optimising the sample and stripping flow conditions [25], or it can be optimised for throughput and sensitivity in non-exhaustive open-bed extractions [26].

An important direction in extraction was the introduction of needle-based extraction techniques (Fig. 13) as, with the help of an autosampler, such approaches facilitate the integration of sample preparation into the rest of the analytical process in the laboratory. In addition to fibre SPME and single drop microextraction (SDME) [27–29] that operate in the batch mode, flow-through needle extraction methods that use a piece of GC capillary column inside the needle have been proposed, such as In-tube SPME or in-needle capillary adsorption trap (INCAT) [30–32]. Similarly, an internally coated needle format, named solid phase dynamic extraction (SPDE) [33, 34], and a packed needle format, named needle trap (NT), have also been developed. The strength of needle-based techniques lies in their potential, not only for laboratory automation, but also for on-site sampling compatibility with convenient coupling to analytical instruments. A key feature that differentiates Needle Trap from the techniques illustrated in Fig. 13 is its exhaustive extraction nature. Exhaustive extraction simplifies Needle Trap calibration and allows particle collection, resulting in total concentration information, as opposed to the free concentration provided by the other techniques illustrated in Fig. 13. In this regard, needle trap is similar to the much larger sorbent traps [35, 36] commonly used in numerous analytical applications.

In addition to classifying methods based on more fundamental principles, it is also instructive to divide techniques according to particular characteristics. For example, recently a new trend has emerged towards the development of solvent-free techniques (Fig. 14) [37]. This is an important direction for analytical chemistry, not only because it addresses health and pollution prevention issues, but also because such approaches tend to be easier to implement for high throughput in laboratory and on-site monitoring in field conditions, saving energy and time ("green" technology). This direction has generated a lot of interest and research opportunities recently, and will likely continue to be a very active area in the near future. The most promising solventless techniques include headspace, membrane, and sorbent approaches.

Another recently observed trend in analytical chemistry regards the simplification and automation of extraction processes using flow analysis and related techniques (Fig. 15). Advanced flow-through methodology was first launched in the 90s to supplement traditional continuous-flow, flow injection (FI), and μFI setups by the second generation of FI, that is, sequential injection analysis (SIA) [38–40] based on discontinuous programmable flow, and multicommutation approaches based on continuous-flow networks furnished with commutation (solenoid valve) microdevices [41, 42], respectively. In 2000, these approaches were extended by the third generation of FI, named the lab-on-a-valve (LOV) platform [43, 44] (See Fig. 15 for a schematic diagram of the various generation of flow analysis approaches). LOV was initially developed as a mesofluidic approach for downscaling reagent-based assays to the micro- and submicroliter level. Yet, concurrently, it has shown a vast potential to accommodate a wide variety of sample processing steps in a micro-scale format (see Fig. 16). It should be noted that the development of the three generations of FI, resulting in miniaturisation of

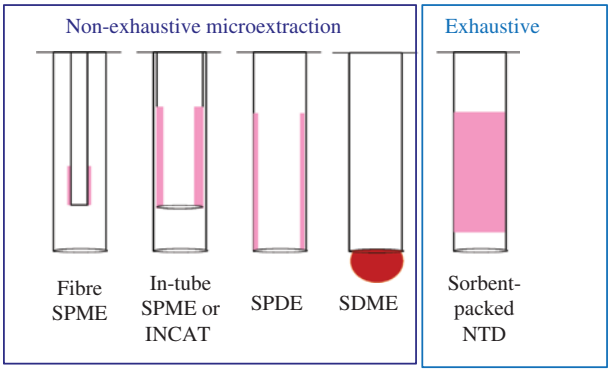

Fig. 13: Needle-based extraction techniques.

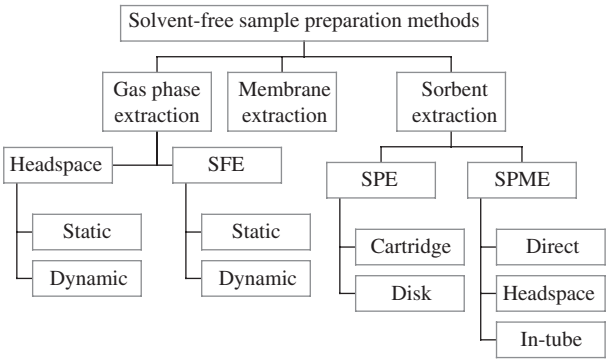

Fig. 14: Classification of solvent-free extraction techniques.

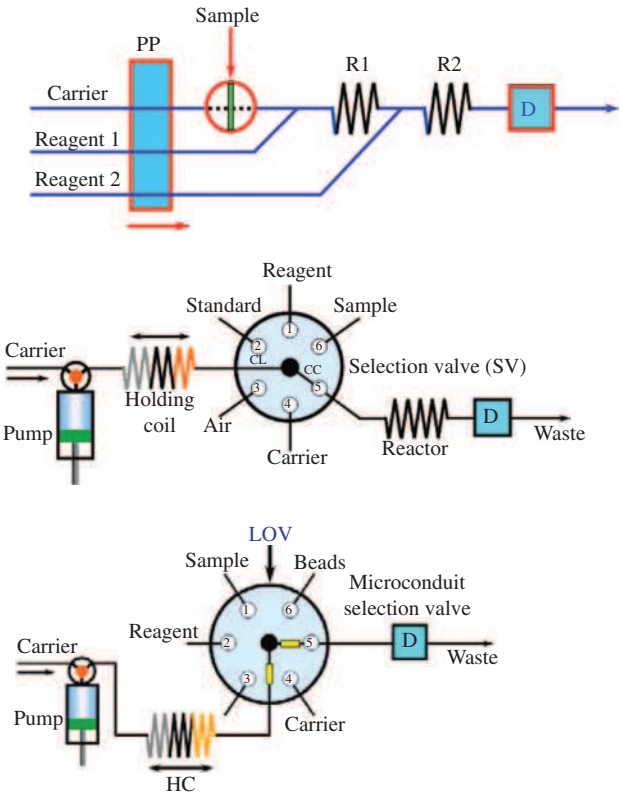

Fig. 15: Schematic illustration of the distinct generations of flow analysis. Top: flow injection analysis; Middle: sequential injection analysis; Bottom: lab-on-a-valve.

the manifolds, was made by chemists in response to evolving demands. Over the last two decades, a number of research centres have, in parallel, intensively focused on the miniaturisation of flow systems, which has resulted in the development of the so-called micro total analysis systems (μTAS), or, as it is more recently termed, lab-on-a-chip (LOC) [45, 46]. The channel network, which is made by various procedures such as micro-drilling, wet-etching, photolithography, laser erasing, and more recently by 2D and 3D printing, is impressively exact and reproducible, allowing different channel profiles to be obtained. In many instances, they can be prepared from inexpensive materials, such as silicon, glass, polymethyl methacrylate, and polydimethylsiloxane, and mass-produced at a low cost; in fact, at a significantly lower cost than LOV platforms. However, microfluidic devices are usually dedicated; that is, they have a fixed architecture for predetermined

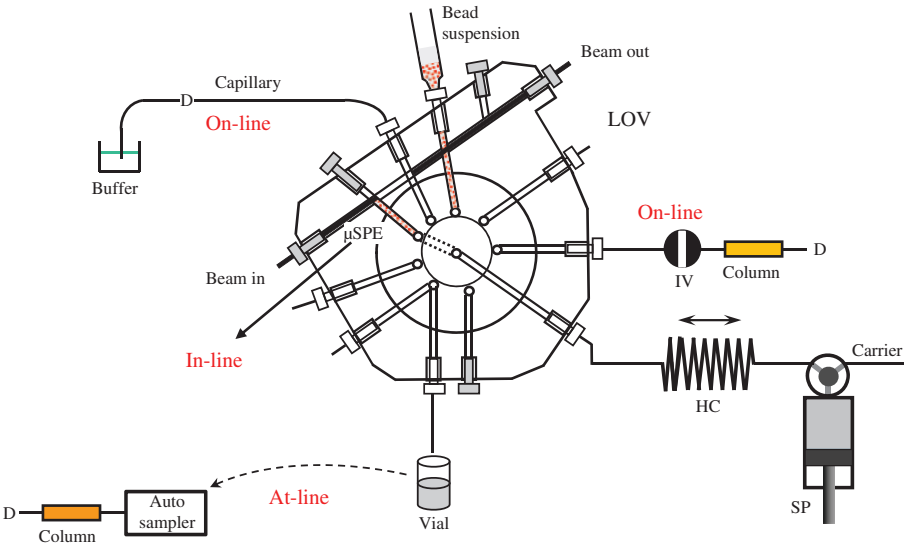

Fig. 16: Illustration of the LOV mesofluidic platform incorporating on-chip μSPE as a front end to the column separation system.

chemistries and sample extraction protocols unless moving components (*e.g.*, magnetic beads) are integrated into the microfluidic network [47]. The most severe limitation of microfluidic devices for real-life applications is the difficulty of handling complex matrices due to the ease of channel clogging or cross-contamination [48]. Sample processing in LOC devices has of yet focused mostly on sample stacking in on-chip electrophoretic separations, for example in field amplified stacking methods and isolectric focusing [49].

SIA and LOV have emerged as convenient front-end tools to facilitate automated μSPE procedures, which yield high concentration factors [11, 50]. Flow-based systems usually operate under non-exhaustive pre-equilibrium due to the precise timing and exactly repeatable experimental conditions present in flow networks. The fundamental principles of flow-through SPE and μSPE are given below. In conventional flow-based column pre-concentration systems, the sorbent-packed column is employed as an integral component of the flow network. This hinders reliable long-term unattended operations as a result of the progressive tighter packing of the sorbent bed, cross contamination effects, and the malfunction of the reactive surfaces due to leakage of sorbent moieties and/or irreversible sorption of matrix components. These drawbacks can be alleviated by adapting the concept of bead injection [51], where the on-line packed microcolumns are renewed after each cycle. Current trends in the field are devoted to the replacement of non-selective ion-exchangers, chelators, or reversed-phase sorbents by selective molecularly imprinted polymers, magnetic core-shell microstructures, or carbon-based nanocomposites. LOV has shown itself to be a straightforward and cost-effective alternative to currently available robotic sample processors (*e.g.*, Prospekt-2 and Symbiosis from Spark Holland or OSP-2 from Merck) employing exchangeable cartridge modules for single use SPE columns prior to separation by liquid chromatography [52].

Miniaturisation of assays based on generation of hydrides or volatile species linked to the advent of miniaturised spectrometers, such as plasma on a chip, has led to the integration of gas-liquid separators (*e.g.*, Venturi-type, membrane-type, and gas-expansion separators) within the LOV module to achieve a portable analyzer for on-line sample processing [53, 54]. In addition to the benefits of chemical vapour generation – embracing the separation of analytes from complex matrices, analyte enrichment, and fast reaction speed – and those of miniaturisation via SI-LOV programmable flow, facilitating decreased sample and reagent consumption, interfering effects from transition metals ions to a large extent can be reduced by the judicious exploitation of kinetic discrimination schemes. That is, even subtle differences in reaction rates for chemical reactions can be used for analytical purposes [11]. Because of the precisely-controlled hydrodynamic conditions in the flow network and the short residence time of the sample plug within the system, possible side reactions can be discriminated against at the expense of the main reaction [55].

Liquid-phase microextraction (LPME) procedures in SIA-batch mode or mesofluidic format [56], commonly referred to as in-line cloud point extraction, single-drop microextraction, dispersive LPME, batch-type LPME, in-syringe LPME, and hollow fibre-assisted LPME, *etc.*, have received a great deal of attention during the last few years. Flow networks integrate the overall analytical process including the preliminary steps of derivatization of the analyte, if necessary, extraction, mixing, phase separation, and transfer of the extractant zone to a detector for measurement. The programmed backward-forward flow of well-defined stacked zones in open tubular extraction reactors ensures rapid and efficient mass transfer, which is assisted by the thin-film, tube-wetting characteristics of the extractant phase. The discontinuous flow pattern inherent to the SIA concept readily facilitates the separation of immiscible phases under steady-state conditions in lieu of the dynamic conditions in the Lab-at-valve configuration [57–59].

A further way to improve sample throughput is to perform the extraction step in parallel using commercially available autosamplers. This is typically realized in multi-well plate systems facilitating the simultaneous extraction of 96 or more samples at the same time. This is particularly useful when the extraction is slow compared with the following steps in the analytical process. LLE, SPE, and SPME processes have been automated in this way [60, 61].

2 Fundamentals

As the preceding discussion and Fig. 2a and b indicate, there is a fundamental similarity among extraction techniques used in the sample preparation process. In all techniques, the extraction phase is in contact with the sample matrix, and analytes are transported between the phases. In exhaustive techniques, the phase ratio is high and geometries are more restrictive to ensure the efficient quantitative transfer of analytes compared to non-exhaustive approaches, which do not have this requirement. The thermodynamics of the process are defined by the extraction phase/sample matrix distribution constant. It is instructive to consider in more detail the kinetics of processes occurring at the extraction phase/sample matrix interface, since this defines the time required for extraction. In many cases, the analytes are also re-extracted from the extraction phase; however, this step is not discussed here, since this process is analogous and much simpler in principle compared to removing analytes from a more complex sample matrix. The main objective of this section is to outline the common fundamental principles among various extraction techniques so as to facilitate a better understanding of selection criteria for appropriate techniques, device geometries, and operational conditions.

2.1 Thermodynamics

The fundamental thermodynamic principle common to all chemical extraction techniques involves distribution of an analyte between the sample matrix and the extraction phase.

2.1.1 Distribution constant

When a liquid is used as the extraction medium, then the distribution constant, K_{es},

$$K_{es} = a_e / a_s \approx C_e / C_s \tag{1}$$

defines the equilibrium conditions and ultimate enrichment factors achievable in the technique, where a_e and a_s are the corresponding activities of the analytes in the extraction phase (A_e) and matrix (A_s), and can be approximated by the appropriate concentrations. Figure 17 shows a schematic of an extraction system for liquid-liquid extraction. For extraction by a solid phase, the adsorption equilibrium can be described by:

$$K_{es}^{s} = S_e / C_s \tag{2}$$

where S_e is the solid extraction phase surface concentration of adsorbed analytes. The relationship above is similar to Eq. 1, except that the extraction phase concentration is replaced with the surface concentration. The S_e term in the numerator indicates that the sorbent surface area available for adsorption must also be considered. The limited surface area of solid extraction phases can complicate calibration at equilibrium conditions due to displacement effects and the non-linear adsorption isotherm [62]. The above equations can be used to calculate the amount of analyte in the extraction phase for equilibrium conditions [12]. For example, for equilibrium liquid microextraction techniques and large sample volumes, including direct extraction from the investigated system, the appropriate expression for the amount of analyte, n, is very simple:

$$n = K_{es} V_e C_s \tag{3}$$

where K_{es} is the extraction phase/sample matrix distribution constant, V_e is the volume of the extraction phase, and C_s is the concentration of the sample. This equation is valid when the amount of analytes extracted is insignificant compared with the amount of analytes present in a sample (large V_s and/or small K_{es}), resulting in negligible depletion of the analyte concentration in the original sample. In Eq. 3, K_{es} and V_e determine the sensitivity of the microextraction method, whereas K_{es} determines its selectivity. The sample volume can be neglected, thus integrating sampling and extraction without the need for a separate sampling procedure, as discussed in more detail later. The non-depletion property of the small dimensions typically associated with microextraction systems results in minimum disturbance of the investigated system, facilitating convenient speciation and investigation of multiphase distribution equilibria, as well as repeated sampling from the same system to follow a process of interest.

When significant depletion occurs, the sample volume, V_s, has an impact on the amount extracted and, therefore, on sensitivity [63]. This effect can be calculated by use of the equation:

$$n = \frac{K_{es} V_e C_0 V_s}{K_{es} V_e + V_s} \tag{4}$$

The distribution constants are dependent on various parameters, including temperature, pressure, and sample matrix conditions such as pH, salt, and the organic component concentration. All these parameters need to be optimised for maximum transfer of analytes to the extraction phase during the method development process. In practice, however, kinetic factors defined by the dissociation constants, diffusion coefficient, and agitation conditions frequently determine the amounts of extracted analytes from complex samples since the overall rates are slow, and therefore extraction amounts for time-limited experiments do not reach equilibrium values.

2.1.2 Matrix effects

Two potential complications are typically observed when extracting analytes from complex matrices. One is associated with competition among different phases for the analyte, and the other with fouling of the extraction phase due to adsorption of macromolecules such as proteins and humic materials at the interface. The

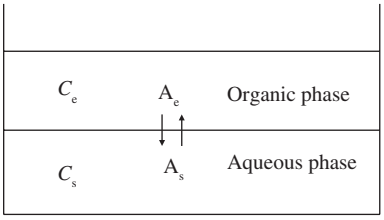

Fig. 17: Partitioning between an aqueous sample matrix and organic extraction phase.

components of heterogeneous samples (including headspace, immiscible liquids, and solids) partition in the multiphase system and are less available for extraction. This effect depends on the analyte affinity and the volume of the competing phases, and can be estimated if appropriate volumes and distribution constants are known. The mass of an analyte extracted by an extraction phase in contact with a multiphase sample matrix can be calculated by use of the equation:

$$n = \frac{K_{es} V_e C_0 V_s}{K_{es} V_e + \sum_{i=1}^{i=m} K_{is} V_i + V_s} \tag{5}$$

Where m is number of phases interacting with analyte present in the sample/investigated system, $K_{is} = C_i^{\infty}/C_s^{\infty}$ is the distribution constant of the analyte between the *i*th phase and the matrix of interest [13]. Equation 5 simplifies to Eq. 4 if there are no competing phases in the sample matrix.

The typical approach used to reduce fouling of the extraction phase involves introduction of a barrier between the sample matrix and the extraction phase to restrict the interaction of high molecular weight interferences with the sorbent surface (Fig. 18). The extraction phase can be surrounded by a porous membrane with pores smaller than the size of the interfering macromolecules (Fig. 18a), *e.g.* use of a dialysis membrane with the appropriate molecular weight cut-off. This approach is conceptually similar to membrane dialysis from complex matrices, in which the porous membrane is used to prevent large molecules from entering the dialyzed solution [64]. This principle has been used to design matrix-compatible extraction phases in which the extraction phase is overcoated with a biocompatible polymer [65]. Hollow fibre membranes have been used in solvent microextraction, both to support the small volume of solvent and to eliminate interferences when extracting biological fluids [66]. This concept has been further explored by integrating the protective structure and extraction phase in individual sorbent particles, resulting in restricted-access materials (RAM) [67]. The chemical nature of the small inner pore surface of the particles is hydrophobic, facilitating extraction of small target analytes, whereas the outer surface is hydrophilic, thus preventing adsorption of excluded large proteins. In practice, fouling of the hydrophobic interface occurs to a significant extent only when the interfering macromolecules are hydrophobic in nature.

A gap made of gas is also a very effective separation barrier (Fig. 18b). Analytes must be transported through the gaseous barrier to reach the coating, thus resulting in the exclusion of the non-volatile components of the matrix. This approach is practically implemented by placing the extraction phase in the gaseous headspace above the sample; it results in a technique such as headspace SPME, which is suitable for extraction of analytes from complex aqueous and solid matrices [68]. The major limitation of this approach is that

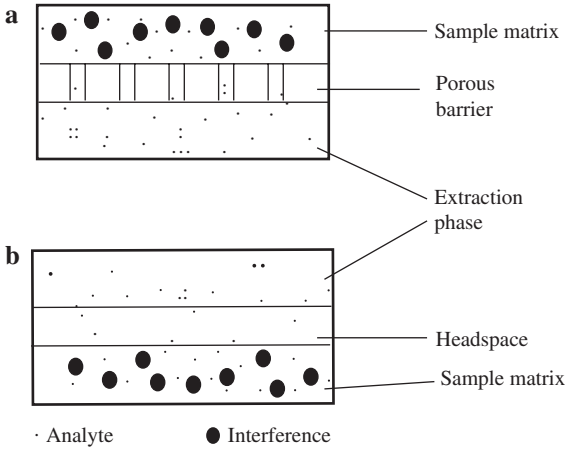

Fig. 18: Integrated clean-up and extraction using selective barrier approaches based on size exclusion with a porous membrane (a) and based on volatility with a headspace gap (b).

the rates of extraction are low for low volatility or polar analytes due to their small Henry's law constants. In addition, method sensitivity for highly volatile compounds can suffer, as these analytes have a high affinity for the gas phase, where they are concentrated. The effect of the headspace on the amount of analyte extracted and, therefore, on sensitivity can be calculated by use of Eq. 5, which indicates that reducing the volume of gas minimizes the effect.

Extraction at elevated temperatures enhances Henry's law constants and thus increases the analyte concentration in the headspace; this results in rapid extraction by the extraction phase. The coating/sample distribution coefficient also decreases with increasing temperature; however, this leads to a reduction in the amount of analyte extracted at equilibrium. To prevent this loss of sensitivity, the extraction phase can be cooled simultaneously with sample heating. This 'cold finger' effect results in increased accumulation of volatilised analytes on the extraction phase. The additional enhancement in the sample matrix–extraction phase distribution constant associated with the temperature gap present in the system can be described by the equation [69]:

$$K_T = K_0 \frac{T_s}{T_e} \exp\left[\frac{C_p}{R}\left(\frac{\Delta T}{T_e} + \ln\frac{T_e}{T_s}\right)\right] \tag{6}$$

where $K_T = C_e(T_e)/C_s(T_s)$ is the distribution constant of the analyte between the cold extraction phase on the fibre having a temperature T_e and the hot headspace at temperature T_s, C_p is the constant-pressure heat capacity of the analyte, $\Delta T = T_s - T_e$ and K_0 is the coating/headspace distribution constant of the analyte when both coating and headspace are at temperature T_e. Due to the enhancement of the sample matrix–extraction phase distribution constant, quantitative extraction of many analytes [70], including volatile compounds, is made possible with this method [71].

2.1.3 Characteristics of the extraction phase

The properties of the extraction phase should be carefully optimised, as they determine the selectivity and reliability of the method. These properties include both bulk physicochemical properties, *e.g.*, polarity, as well as physical properties, *e.g.*, thermal stability and chemical inertness. Solvents and liquid polymeric phases, such as poly(dimethylsiloxane) [72], for example, are very popular due to their wide linear dynamic ranges associated with linear absorption isotherms. They also facilitate "gentle" sample preparation, as chemisorption and catalytic properties, frequently associated with solid surfaces, are absent. No loss or modification of the analyte occurs during extraction and/or desorption. Despite these attractive properties of liquid extraction media, solid phases are frequently used due to their superior selectivity and extraction efficiency for some groups of compounds. For example, carbon-based sorbents are effective for extraction of volatile analytes.

The development of selective extraction materials often parallels that of the corresponding selective chemical sensors [73]. Manufacturing approaches and structures similar to those of sensor surfaces have been implemented as extraction phases. For example, phases with specific properties such as molecularly imprinted polymers [74] and immobilized antibodies [75] have been developed for extraction. These sorbents rely on differences between bulk properties of the extraction phase and the highly specific molecular recognition centres dissolved in it to facilitate high-selectivity extraction with minimal non-specific adsorption [76]. In addition, chemically tuneable properties of the extraction phase can be controlled during the preparation procedure. For example, polypyrrole has been used successfully for a range of applications, from ion-exchange extraction to hydrophobic extraction based on selective interaction between the polymer and the target analytes [77]. In addition, the tuneable properties of the polymer, *e.g.*, the oxidation/reduction equilibrium in conductive polypyrrole, can be explored to control adsorption and desorption [78].

Demands on the specificity of extraction phases are typically less stringent than for sensor surfaces, as powerful separation and quantification techniques such as GC–MS or LC–MS are usually employed after extraction to facilitate accurate identification of the analyte. More demand is placed, however, on the thermal stability and

chemical inertness of the extraction phase, as extraction materials are frequently exposed to high temperatures and different solvents during extraction and introduction to separation instruments. New coating chemistries, such as sol–gel polymerization, for example, have been developed to address these needs [79].

To optimise sensitivity, the choice of extraction phase is frequently based on its affinity towards the target analyte. In practice, however, kinetic factors defined by dissociation constants, diffusion coefficients, and agitation conditions frequently determine the amounts of analytes extracted from complex samples. As overall extraction rates are slow, the amounts of analytes extracted during experiments of limited duration do not reach equilibrium values.

2.2 Kinetics

2.2.1 Liquid-liquid extraction

It is instructive to consider the simple case of the static extraction of water by an organic solvent, as illustrated in Fig. 19, to demonstrate the effects of different parameters on the extraction kinetics. An appropriate equation showing concentration profiles in each of the phases can be obtained by solving Fick's Second Law differential equation for appropriate boundary conditions:

$$\frac{\partial C(x,\,t)}{\partial t} = D\frac{\partial C(x,\,t)}{\partial x^2} \tag{7}$$

If no convection is present in the system, the distribution constant is defined by Eq. 1, and the two phases are placed in contact with each other at $t=0$; then, the solution can be found using a Laplace Transform approach for the aqueous sample phase ($x<0$):

$$C_s(x,\,t) = C_0\frac{\dfrac{z}{K_{es}} + erf(z\sqrt{tD_s})}{1+\dfrac{z}{K_{es}}} \tag{8a}$$

For the organic extraction phase ($x>0$):

$$C_e(x,\,t) = C_0\frac{z\{1-erf(x/(z\sqrt{tD_e}))\}}{1+\dfrac{z}{K_{es}}} \tag{8b}$$

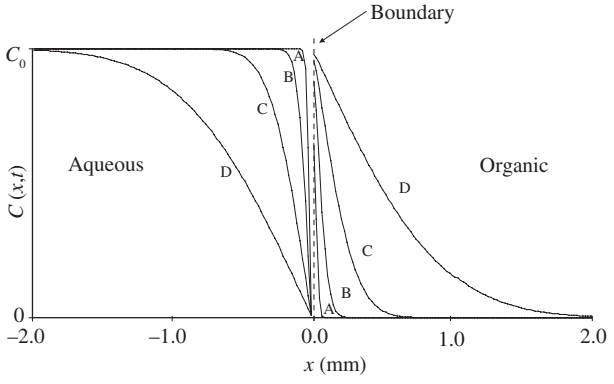

Fig. 19: Concentration profiles at the interface between infinite volume sample and extraction phases for analyte characterised by identical diffusion coefficients in the aqueous and organic phase (10^{-5} cm^2/s). The profiles correspond to A- 1 s; B- 10 s; C- 100 s, D- 1000 s, after merging both phases.

where C_0 is the initial concentration of the analyte in the aqueous phase, *erf* is the error function, D_e and D_s are the diffusion coefficients for the analyte in the extraction phase and in the sample, respectively, $z = D_e/D_s$, and K_{es} is an appropriate distribution constant defined by Eq. 1. The solution to the above equation is shown graphically in Fig. 19 for several extraction times when the diffusion of analytes in the aqueous and organic phases is 10^{-5} cm^2 s^{-1}. Figure 19 illustrates that the concentration gradient decreases and extends deeper into both phases as a function of time. The flux of analytes decreases proportionally as the gradient decreases. The concentration effect of analytes at the boundary on the organic side compared to the bulk aqueous concentration is not observed at the beginning of extraction due to the drop in concentration on the aqueous side. Therefore, decreasing the boundary layer thickness and diffusion length by applying agitation to one or both phases increases the rate of extraction dramatically. The effects of agitation can be calculated using the boundary layer model, which will be discussed later. One other way to improve mass transfer is to use thin films of sample matrix and/or extraction phase to decrease the diffusion length. In addition, a combination of sample agitation and use of a thin extraction phase can be used to facilitate even shorter extraction times. If the extraction and matrix phases are of different states of matter, then it is more critical to overall extraction kinetics to agitate or use the thin film format of the phase, since the solid state is typically characterised by a smaller diffusion coefficient. For example, when extracting gas or liquid samples with poly(dimethylsiloxane), it is critical to disperse the extraction phase as a thin film so the equilibrium between the phases may be rapidly reached. When extracting solid samples, grinding to reduce the size of the sample facilitates faster leaching processes.

2.2.2 Extraction of solids

The most challenging extractions involve the presence of a solid as a part of the sample matrix. This case can be considered as the most general example of extraction, since it involves a number of fundamental processes occurring simultaneously during the extraction. If we assume that a matrix particle consists of an organic layer on an impermeable but porous core, and that the analyte is adsorbed onto the pore surface, the extraction process can be modelled by considering several basic steps, as shown in Fig. 20. To remove the analyte from the extraction vessel, the compound must first be desorbed from the surface (A(M,S)), as exemplified in Fig. 20. Then, it must diffuse through the organic part of the matrix (A(M,L)) to reach the matrix/fluid interface (A(M,I)). At this point, the analyte must be solvated by the extraction phase (A(EP,P)), diffuse through the static phase present inside the pore to reach the portion of the extraction phase influenced by convection, be transported through the interstitial pores of the matrix, then eventually reach the bulk of the extraction phase (A(EP,B)). The simplest way to design a kinetic model for this problem is to adopt equations developed by engineers to investigate mass transport through porous media [80, 81].

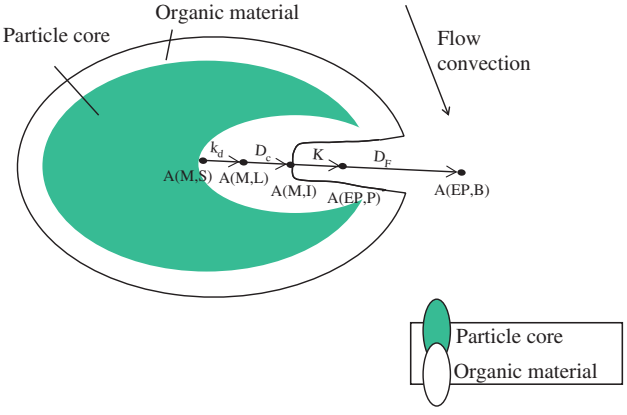

Fig. 20: Processes involved in the extraction of heterogeneous samples containing porous solid particles. The symbols/terms in the figure are discussed in the text.

For the purpose of this discussion, we consider the efficient and frequently applied experimental arrangement for removing solid-bound semivolatile analytes, involving the use of a piece of stainless steel tubing as the extraction vessel. The sample is typically placed inside the tube, and a linear-flow restrictor is attached to maintain pressure at the end of the vessel. During the process, the extraction phase continuously removes analytes from the matrix, which are then transferred to the collection vessel. This leaching process is very similar to chromatographic elution with packed columns using the frontal analysis method. The main difference is that in sample preparation, analytes are dispersed in the matrix at the beginning of the experiment, while in chromatographic frontal analysis, a long plug is introduced into the column at the initial stage of the separation process. The principal objective of the extraction is to remove analytes from the vessel in the shortest possible time, requiring elution conditions under which the analytes are unretained. In chromatography, on the other hand, the ultimate goal is to separate components of the sample, which requires retention of analytes in the column. Another major difference is that the packing matrix is usually well characterised in chromatography, whereas in sample preparation, it is often unknown.

One way to develop a mathematical model for this extraction approach is to establish the mass balance equation for the system after careful consideration of the individual mass transfer steps occurring during the extraction process (see Fig. 20), and specific boundary conditions [82]. Extensive investigations on similar topics have already been conducted by engineers who have studied mass transfer in porous media [30], as well as by chromatographers [30–32]. In these studies, the relationship between various matrix parameters and flow conditions on the elution profile were described mathematically and verified experimentally. In chromatography, this relationship is usually described as contributions from each of the mass transfer steps to the plate height (HETP). The overall performance of the system can be defined as the sum of the relevant individual components judiciously selected to reflect the most significant individual steps present in the elution process. For the purpose of this discussion, this approach is adopted to develop a model for extraction kinetics in flow-through techniques.

The effect of slow desorption kinetics of analytes from the matrix on the elution profile can be described as the contribution to the HETP [24], h_{RK}:

$$h_{RK} = \frac{2ku_e}{(1+k)^2(1+k_0)k_d} \tag{9}$$

where k is the partition ratio, k_d is the dissociation rate constant of the analyte-matrix complex of a reversible process, and k_0 is the ratio of the interparticle void volume to the interstitial void space, and is expressed as:

$$k_0 = \frac{\varepsilon_i(1-\varepsilon_e)}{\varepsilon_e} \tag{10}$$

where ε_i is interparticle porosity, ε_e is interstitial porosity, and u_e is the interstitial linear extraction phase velocity, expressed as:

$$u_e = u(1+k_0) \tag{11}$$

where $u = L/t_0$ is the chromatographic linear velocity, L is the length of the extraction vessel, and t_0 is the time required to remove one void volume of the extraction phase from the vessel. Chromatographic and interstitial linear velocities are identical if matrix particles have low porosity. This analysis can be extended to elution through a matrix having multiple adsorption sites characterised by different dissociation rate constants by using the approach described by Giddings [83].

The diffusion of the analyte in the liquid or swollen solid part of the matrix is important when polymeric materials are extracted, or the matrix has substantial organic content. Its contribution can be expressed as h_{DC}:

$$h_{DC} = \frac{2}{3}\frac{k}{(k+1)^2}\frac{d_c^2}{D_s}u_e \tag{12}$$

where d_c is the thickness of the matrix component permeable to analyte, and D_s is the diffusion coefficient of the analyte in the sample matrix.

The analytes migrate in and out of a pore structure of the matrix during the elution. This can be described as resistance to mass transfer in the fluid associated with the porous nature of the environmental matrices, giving rise to the following HETP component, h_{DP}:

$$h_{DP} = \frac{\theta(k_0 + k + kk_0)^2 d_p^2 u_e}{30k_0(1+k_0)^2(1+k)^2 D_p} \tag{13}$$

where θ is the tortuosity factor for the porous particle, d_p is the diameter of the particle, and D_p is the diffusion coefficient of the analyte in the material filling the pores, which in most practical cases will be an extraction phase; therefore $D_p = D_e$, where D_e is the diffusion coefficient of the analyte in the extraction phase. This contribution can be quite important considering the relatively large particle size (about 1 mm) of environmental matrices and becomes particularly important when the pores are filled with dense organic material, such as humic matter rather than the extraction phase.

In the flowing bulk of the fluid, an analyte experiences resistance to mass transfer associated with eddy diffusion (random paths of the analytes through the vessel filled with the particles), which is given by h_{ED}:

$$h_{ED} = 2\lambda d_p \tag{14}$$

where λ is a structural parameter, and close to 1 for spherical matrix particles. This contribution to band broadening is the most important factor in HPLC separations, and is expected to remain significant in extractions, as matrices typically have large particle sizes.

In addition, we should also consider analyte diffusion along the axis of the vessel (longitudinal diffusion), which can be defined as h_{LD}:

$$h_{LD} = \frac{\gamma_M D_e}{u_e} \tag{15}$$

where γ_M is the obstruction factor characteristic of the matrix structure. The contribution of this component is expected to be small. The analyte concentration profile generated during an experiment as a function of time $C(x, t)$ can be represented using an equation that describes the dispersion of a plug of finite width [25]:

$$\frac{C(x,t)}{C_0} = \frac{1}{2}\left\{\operatorname{erf}\left(\frac{\frac{L}{2} - x - \frac{ut}{1+k}}{\sigma\sqrt{2}}\right) + \operatorname{erf}\left(\frac{\frac{L}{2} + x + \frac{ut}{1+k}}{\sigma\sqrt{2}}\right)\right\} \tag{16}$$

where L is the length of the extraction vessel, C_0 is the initial concentration of analyte in the extraction vessel, and σ is the mean square root dispersion of the band, and expressed as:

$$\sigma = \sqrt{Ht\frac{u}{1+k}} \tag{17}$$

where H is equivalent to the HETP in a chromatographic system, and is the sum of the contributions discussed above, $H = H_{RK} + h_{DC} + h_{DP} + h_{ED} + h_{LD}$. The mass of analyte eluted from the vessel during a given extraction time t can be calculated from the following equation:

$$\frac{m(t)}{m_0} = \frac{\int_{-\infty}^{-\frac{1}{2}L} C(x,t)dx}{C_s L} \tag{18}$$

where, $m(t)$ is the extracted mass of analyte, and m_0 is the total mass of analyte in the vessel at the beginning of the experiment. We will refer to this function as the "time elution profile", emphasising the similarity of the extraction process in this simple case to chromatographic elution.

2.2.3 Convolution model of extraction

The above discussion applies only to situations where the analytes are initially present in a fluid phase, which in flow-through techniques corresponds to elution of uniform spikes from the extraction vessel, or when weakly adsorbed native analytes are removed from an organic-poor matrix such as sand. In other words, the above relationships are suitable for systems in which the partitioning equilibrium between the matrix and extraction fluid is reached quickly compared to the fluid flow. They are also suitable to model static/dynamic extractions under good solubility conditions ($k=0$), in which the sample is initially exposed to the static extraction phase (vessel is capped) for the time required to achieve an equilibrium condition prior to elution by the fluid flow. If dynamic extraction is performed from the beginning of the extraction, then in the majority of practical cases the system is not expected to achieve the initial equilibrium conditions. This is caused by the slow mass transport between the matrix and the fluid (for example, slow desorption kinetics or slow diffusion in the matrix). The expected relationship between the mass of analyte removed from the vessel versus extraction time can be obtained in this case by convoluting the function describing the rate of mass transfer between the phases $F(t)$ with the elution time profile $m(t)/m_0$ for example as derived above (Eq. 18) [84]:

$$\int_{\tau=0}^{\tau=t} \frac{m(t-\tau)}{m_0} F(\tau)\, d\tau \tag{19}$$

where t and τ are time variable used in convolution expression.

The resulting function describes a process where elution and mass transfer between the phases occur simultaneously. In this discussion, we will refer to this function as the "extraction time profile" to emphasise the point that in a majority of extraction cases, these two processes are expected to be combined. $F(t)$ describes the kinetics of the process, which defines the release rate of analyte from the sample matrix and can include, for example, the matrix-analyte complex dissociation rate constant, the diffusion coefficient, the time constant that describes the swelling of the matrix that facilitates removal of analyte, or a combination of the above. Detailed discussion, graphical representations, and applications of this model to describe and/or investigate processes in supercritical fluid extraction have been described elsewhere [85, 86].

The conclusion above can be stated in a more general way: convolution among functions describing individual processes occurring during the extraction describes the overall extraction process and represents a unified way to describe the kinetics of these complex processes. The exact mathematical solution to the convolution integral is frequently difficult to obtain, but graphical representation of the solution can be calculated using Fourier Transform or numerical approaches. Frequently, it is possible to incorporate mathematical functions that describe a combination of the unit processes. In the example of the flow-through system discussed above, the elution function describes the effect of porosity and analyte affinity towards the extraction matrix on the extraction rate. It should be emphasised that the convolution approach considers all processes equivalently. In practice, however, only a small number, and frequently, just one unit process controls the overall rate of extraction. As such, most equations can be simplified by considering this fact.

Determination of the limiting step is not possible exclusively by qualitative agreement with the mathematical model, since the effect on recovery of most of the unit processes has an exponential decay nature. To properly recognise them, quantitative agreement and/or the effect of extraction parameters need to be examined. Identification of the limiting process provides valuable insight on the most effective approach to optimise extraction.

3 Optimisation of the extraction process

A fundamental understanding of the extraction process leads to better strategies to optimise performance. In heterogeneous samples, for example, the release of solid-bound analytes from the sample matrix, through a reversal of chemisorption or inclusion, frequently controls the extraction rate. By recognising this fact, extraction parameters can be changed to increase extraction rates [87]. For example, dissociation of the

chemisorbed analytes can be accomplished either by using a high temperature or through application of catalysts. Examples of these applications include the development of high temperature supercritical fluid extraction [88], followed by the evolution of both the hot solvent extraction approach [89], and microwave extraction (Fig. 21), with more selective energy focusing at the sample matrix/extraction phase interface [90]. There is also an indication that milder conditions can be applied by taking advantage of the catalytic properties of the extraction phase or additives [91]; however, more research is still needed to gain insight into the nature of interactions between analytes and matrices. Benefits include not only improved speed, but also selectivity resulting from application of appropriate conditions. For example, this strategy of simultaneous extraction and clean-up has been applied successfully to a challenging case of extraction of polychlorinated dibenzo-p-dioxins from fly ash [92].

If the extraction rate is controlled by the mass transport of analytes in the pores of the matrix, then the process can be successfully enhanced by application of sonic and microwave energy, which induce convection even in the small dimensions of the pores. Frequently, diffusion through the whole or portion of the sample matrix that contain natural or synthetic polymeric materials control the extraction rate [93]. In this case, swelling the matrix and increasing temperature will result in increased diffusion coefficients, and therefore, increased extraction rates.

4 Flow-through techniques

For homogeneous samples and a flowing fluid extraction phase, the description of the extraction process is much simpler, and can be based directly on the chromatographic theory for liquid stationary phases. Let

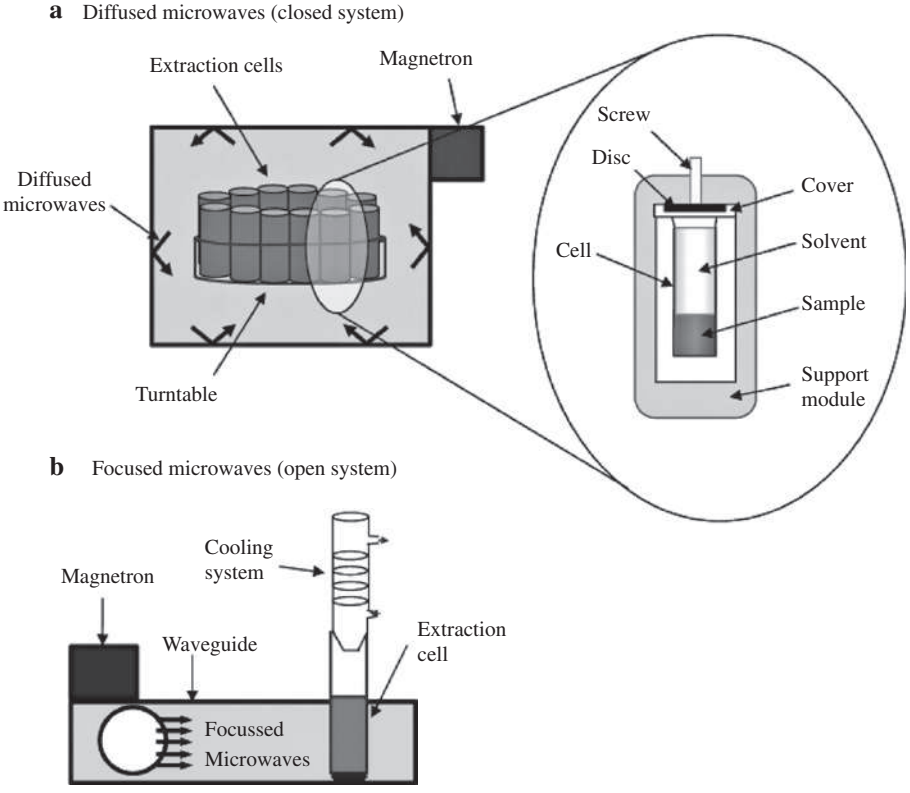

Fig. 21: Apparatus for microwave-assisted extraction in a closed system (a), and open system (b).

us consider another case of the flow-through system where the extraction phase is dispersed as a thin layer inside the extraction bed, and the sample flows through the cartridge. The bed can be constructed with a piece of fused-silica capillary which is internally coated with a thin film of extracting phase [94] (a piece of open tubular capillary GC column; in-tube SPME [95]), or packed with extracting phase dispersed on an inert supporting material (SPE cartridge). In these geometric arrangements, the concentration profile along the *x*-axis of the tubing containing the extracting phase, as a function of time *t*, can be described by adopting the expression for dispersion of a concentration front:

$$C(x,\,t) = 0.5C_s\left(1 - erf\frac{x - \frac{u_s t}{1+k}}{\sigma\sqrt{2}}\right) \tag{20}$$

where u_s is the linear velocity of the sample through the tube, and k is the partition ratio, defined as:

$$k = K_{es}\frac{V_e}{V_v} \tag{21}$$

where K_{es} is the extraction phase/sample matrix distribution constant, V_e is the volume of the extracting phase, and V_v is the void volume of the tubing containing the extracting phase. σ is the mean square root dispersion of the front, and is defined as:

$$\sigma = \sqrt{Ht\frac{u_s}{1+k}} \tag{22}$$

where H is equivalent to the HETP in chromatographic systems. This can be calculated as a sum of individual contributions to the front dispersion. These contributions are dependent on the particular geometry of the extracting system, as discussed previously.

Figure 22 illustrates the normalised concentration profiles produced in the bed during extraction [46]. Full breakthrough is obtained for the last curve, which corresponds to the breakthrough volume of the sample matrix. The time required to pass this required volume through the extraction system corresponds to the equilibration time for the compound with the bed.

Equation 20 and Figure 22 indicate that the analyte front migrates through the capillary/bed with a speed that is proportional to the linear velocity of the sample and inversely related to the partition ratio. For in-tube SPME and short capillaries with a small dispersion, the minimum extraction time at equilibrium can be assumed to be similar to the time required for the centre of the band to reach the end of the capillary:

$$t_e = \frac{L(1 + K_{es}\frac{V_e}{V_v})}{u} \tag{23}$$

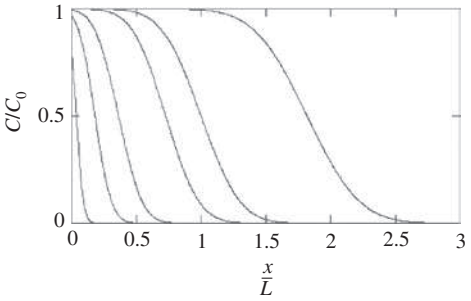

Fig. 22: Normalised concentration profiles for in-tube SPME, calculated using the equation discussed in the text.

where L is the length of the capillary holding the extraction phase. For packed bed extractors typically used in SPE techniques, analogous equations can be developed; in such cases, the calculated time corresponds to the maximum extraction time before breakthrough occurs. As expected, the extraction time is proportional to the length of the capillary and inversely proportional to the linear flow rate of the sample. Extraction time also increases with an increase in the extraction phase/sample distribution constant and with the volume of the extracting phase, but decreases with an increase of the void volume of the capillary.

5 Batch techniques

Coupling equations for systems involving convection caused by flow-through a tube, such as discussed above, are frequently not available for other means of agitation and other geometric configurations. In these cases, the most successful approach is to consider the boundary layer formed at the interface between the sample matrix and the extraction phase. Independent of the agitation level, fluid contacting the extraction phase surface is always stationary, and as the distance from the extraction phase surface increases, the fluid movement gradually increases until it corresponds to bulk flow in the sample. To model mass transport, the gradation in fluid motion and the convection of molecules in the space surrounding the extraction phase surface can be simplified as a zone of a defined thickness, in which no convection occurs, and perfect agitation in the bulk of the fluid occurs everywhere else. This diffusion boundary layer zone is called the Prandtl boundary layer (Fig. 23) [96].

6 Boundary layer model

A precise understanding of the definition and thickness of the boundary layer in this sense is useful. The thickness of the boundary layer (δ) is determined by both the rate of convection (agitation) in the sample and an analyte's diffusion coefficient. Thus, in the same extraction process, the boundary layer thickness will be different for different analytes. Strictly speaking, the boundary layer is a region where as the extraction phase is approached, analyte flux is progressively more dependent on analyte diffusion and less on convection. For convenience, however, the analyte flux in the bulk of the sample (outside of the boundary layer) is assumed to be controlled by convection, whereas the analyte flux within the boundary layer is assumed to be controlled by diffusion. The symbol δ is defined as the position where this transition occurs, or the point at which convection towards the extraction phase is equal to diffusion away from the extraction

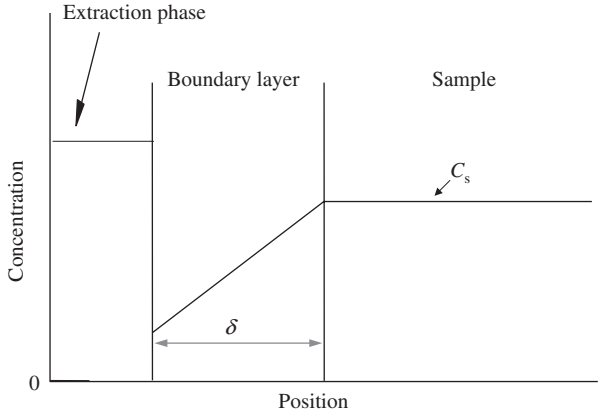

Fig. 23: Boundary layer model.

phase. At this point, the analyte flux from δ towards the extraction phase (diffusion-controlled) is equal to the analyte flux from the bulk of the sample towards δ, controlled by convection.

In many cases, when the extraction phase is dispersed well to form a thin coating, the diffusion of analytes through the boundary layer controls the extraction rate. Figure 24 indicates the resulting equilibration time profile [13, 97]. The profile consists of three regimes: linear, kinetic, and near equilibrium. Various applications require use of different regimes, as emphasised below.

The equilibration time, t_e, can be estimated as the time required to extract 95 % of the equilibrium amount, and calculated from the equation below [13]:

$$t_e = B\frac{\delta b K_{es}}{D_s} \tag{24}$$

where b is the extraction phase thickness, D_s is the analyte's diffusion coefficient in the sample matrix, K_{es} is the analyte's distribution constant between the extraction phase and the sample matrix, and B is a geometric factor referring to the geometry of the supporting material upon which the extraction phase is dispersed. The boundary layer thickness can be calculated for given convection conditions using engineering principles, which will be discussed in more detail later. Equation 24 can be used to predict equilibration times when the extraction rate is controlled by diffusion in the boundary layer, which is valid for thin extraction phase coatings ($b < 200$ μm) and high distribution constants ($K_{es} > 100$).

7 Solid and liquid sorbents

There are substantial differences in performance between liquid and solid coatings (Fig. 25). In the case of liquid coatings, the analytes partition into the extraction phase, where the molecules are solvated by the coating molecules. The diffusion coefficient in the liquid coating allows the molecules to penetrate the whole volume of the coating within a reasonable extraction time if the coating is thin (Fig. 25a). In the case of solid sorbents (Fig. 25b), the coating has a well-defined crystalline or amorphous structure, which if dense, substantially reduces the diffusion coefficients within the structure. Therefore, within the experimental time, sorption occurs only on the porous surface of the coating (Fig. 25b). During extraction by a solid phase, compounds with poor affinity towards the phase are frequently displaced at longer extraction times by analytes characterised by stronger binding, or those present in the sample at high concentrations. This effect is associated with the limited surface area available for adsorption. If this area is substantially occupied, then a competition effect occurs [14] and the equilibrium amount extracted can vary with concentrations of both the target and other analytes. On the other hand, in the case of extraction with liquid phases,

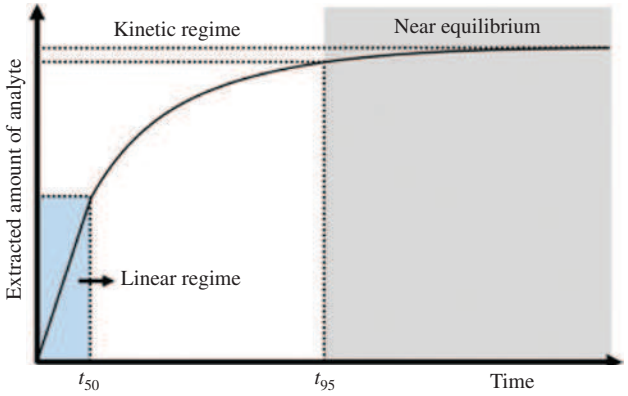

Fig. 24: Equilibration time profile in typical extraction systems.

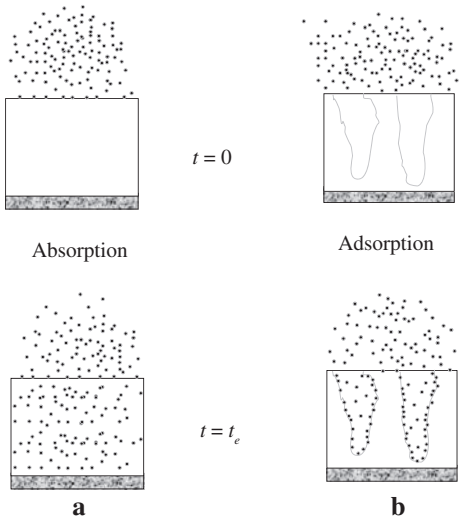

Fig. 25: Extraction using absorptive (a) and adsorptive (b) extraction phases immediately after exposure of the phase to the sample ($t = 0$), and after completion of the extraction ($t = t_e$).

partitioning between the sample matrix and the extraction phase occurs. In this case, equilibrium extraction amounts vary only if the bulk coating properties are modified by the extracted components, which only occurs when the amount extracted is a substantial portion (a few percent) of the extraction phase. This is rarely observed, since extraction/enrichment techniques are typically used to determine trace contamination samples. However, this outcome cannot be neglected as a possible cause of non-linearity when quantifying very complex matrices, or gaseous samples containing a high concentration of components with a high affinity towards the liquid polymeric extraction phase.

8 Diffusion-based calibration

The only way to overcome the fundamental limitation of porous coatings, as suggested in Fig. 26, is to use an extraction time much shorter than the equilibrium time, so that the total amount of analytes accumulated onto the porous coating is substantially below the saturation value. At saturation, all surfaces available for adsorption are occupied. When performing experiments with short pre-equilibrium extraction times, it is critical to precisely control extraction times and convection conditions, as they determine the thickness of the diffusion layer. One way of eliminating the need for compensation of differences in convection is to normalise (use consistent) agitation conditions. For example, the use of stirring at a well-defined rotation rate in the laboratory, or fans for field air monitoring, can ensure consistent convection [98, 99]. The short-time exposure measurement described above does provides an advantage, as the rate of extraction is defined by the diffusion of analytes through the boundary layer of the sample matrix and their corresponding diffusion coefficients, rather than by distribution constants. The diffusion coefficients are similar for small molecules, while the distribution constants can vary by orders of magnitudes for compounds with different polarities and/or affinities to the extraction phase. This situation is illustrated in Fig. 26 for cylindrical geometry of the extraction phase dispersed on the supporting rod.

The analyte concentration in the bulk of the matrix can be considered constant when a short sampling time is used, and a constant supply of analyte via convection is available. These assumptions are true for most sampling cases, where the sample volume is much greater than the volume of the interface, and the extraction process does not affect the bulk sample concentration. In addition, the solid coating can be treated as a perfect sink. The adsorption binding is frequently instantaneous and essentially irreversible. In a typical sample, the analyte concentration on the coating surface is far from saturation, and can be assumed to be negligible for

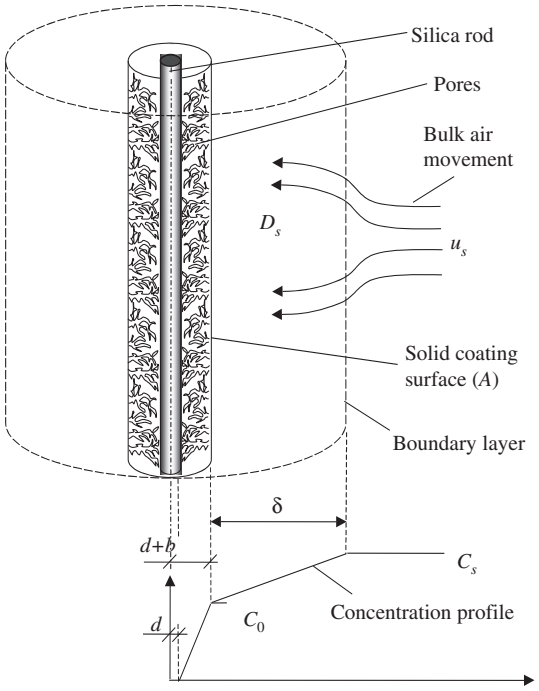

Fig. 26: Schematic of the diffusion-based calibration model. The symbols/terms are defined in the text.

short sampling times and relatively low analyte concentrations (linear regime in Fig. 24). The analyte concentration profile can be assumed to be linear from C_s to C_0. In addition, the initial analyte concentration on the coating surface, C_0, can be assumed to be equal to zero when extraction begins. The diffusion of analytes inside the pores of a solid coating controls mass transfer from the outer to the inner surface of the coating.

A function describing the amount of extracted analyte with sampling time can be derived [100], resulting in the following equation:

$$n(t) = \frac{B_1 A D_s}{\delta} \int_0^t C_s(t)dt \tag{25}$$

where n is the amount of extracted analyte over a sampling time (t); D_s is the gas-phase molecular diffusion coefficient; A is the surface area of the sorbent; δ is the thickness of the boundary layer surrounding the extraction phase; B_1 is a geometric factor; and C_s is the analyte concentration in the bulk of the sample. It can be assumed that the analyte concentration is constant for very short sampling times, and therefore, Eq. 25 can be further reduced to:

$$n(t) = \frac{B_1 D_s A}{\delta} C_s t \tag{26}$$

where t is the sampling time [101].

As can be seen from Eq. 26, the amount of extracted amount is proportional to the sampling time t and to D_s for each analyte and bulk sample concentration, as well as inversely proportional to δ. This is consistent with the fact that an analyte with a greater D_s will cross the interface and reach the surface of the fibre coating faster. Values of D_s for each analyte can be found in the literature, or estimated from their physicochemical properties [46]. This relationship allows for quantitative analysis. For rapid sampling with solid sorbents, Eq. 26 can be re-arranged to estimate the analyte concentration in the sample:

$$C_s = \frac{n\delta}{B_1 D_s A t} \tag{27}$$

The amount of extracted analyte (n) can be estimated from the detector signal. The thickness of the boundary layer (δ) is a function of sampling conditions. The most important factors affecting δ are the geometric configuration of the extraction phase, sample velocity, temperature, and D_s for each analyte. The effective thickness of the boundary layer can be estimated for the coated fibre geometry (Fig. 26) using Eq. 28, adapted from heat transfer theory:

$$\delta = 9.52 \frac{d}{\mathrm{Re}^{0.62}\mathrm{Sc}^{0.38}} \tag{28}$$

where Re is the Reynolds number $= 2u_s d/\nu$; u_s is the linear sample velocity; ν is the kinematic viscosity of the matrix; Sc is the Schmidt number $= \nu/D_s$; and d is the fibre diameter. The effective thickness of the boundary layer in Eq. 28 is a surrogate (or average) estimate, and does not take into account changes in thickness that may occur when the flow separates and/or a wake is formed. Equation 28 indicates that the thickness of the boundary layer will decrease with an increase in linear sample velocity (Fig. 26). Similarly, when sample temperature (T_g) increases, the kinematic viscosity also increases. Since the kinematic viscosity term is present in the denominator of Re and in the numerator of Sc, the overall effect on δ is small. A reduction of the boundary layer and an increase of the mass transfer rate for an analyte can be achieved in at least two ways: by increasing the sample velocity, and by increasing the sample temperature. However, an increase in temperature will reduce the efficiency of the solid sorbent. As a result, the sorbent coating may not be able to adsorb all molecules reaching the surface, and therefore, may stop behaving as a zero sink for all analytes.

9 Calibrants in the extraction phase

Internal standardization and standard addition are effective calibration approaches often employed when quantifying target analytes in complex matrices. They compensate for additional capacity or activity of the sample matrix. However, such approaches require delivery of the standard. This is incompatible in some sampling situations, such as on-site or *in vivo* investigations. The standard in the extraction phase approach is not practical for conventional exhaustive extraction techniques; since the extraction parameters are designed to facilitate quantitative removal of the analytes and standards from the matrix, the standards would remain in the extraction phase throughout the whole process. However, in microextraction, a substantial portion of the analytes and standard remain in the matrix during extraction and after equilibrium is reached [102]. This suggests that the standard could be added to the investigated system together with the extraction phase to effectively compensate for matrix conditions. This property of microextraction techniques has been explored to integrate addition of calibrants with the rest of automated high-throughput analysis [103]. Two calibration methods, kinetic calibration with standard [11, 13, 104], and standard-free kinetic calibration, have been proposed [105].

9.1 Kinetic calibration with standard in the extraction phase

The symmetry of absorption and desorption in microextraction systems can be expected, as both processes are controlled by the diffusion of compounds through the boundary layer (see Fig. 27). This symmetry is the basis of kinetic calibration, allowing to compensate for changes in the convection conditions in the investigated system [98]. The kinetic calibration method uses the desorption of standards, which are pre-loaded in the extraction phase, to calibrate the extraction of analytes.

For field sampling, where the sample volume is very large, the desorption of standard from an extraction phase can be described by:

$$\frac{Q}{q_0} = \exp(-at) \tag{29}$$

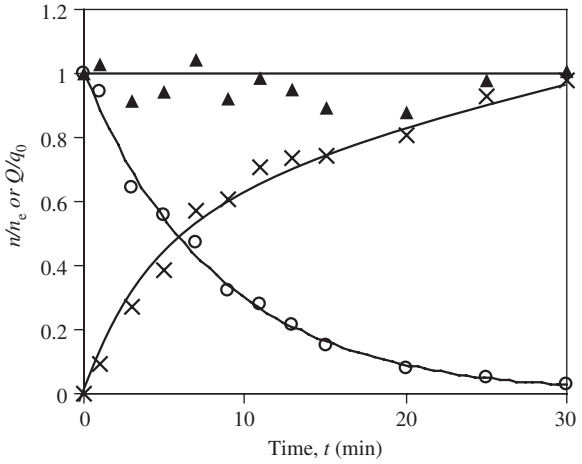

Fig. 27: Principle of kinetic calibration based on symmetry between the extraction profile of the analyte and the desorption profile of the standard for simultaneous absorption of toluene (x) and desorption of deuterated toluene (d-8) (o) onto and from a 100 μm PDMS fibre into water of 0.25 cm s^{-1} at 25 °C. (▲) represents the sum of n/n_e and Q/q_0.

where q_0 is the amount of pre-added standard in the extraction phase, and Q is the amount of the standard remaining in the extraction phase after exposure of the extraction phase to the sample matrix for the sampling time, t.

A diffusion-controlled mass transfer process can be used to describe the entire kinetic process [106, 107]:

$$n = [1 - \exp(-at)]\frac{K_{es}V_eV_s}{K_{es}V_e + V_s}C_s \tag{30}$$

where a is a rate constant that is dependent on the volumes of the extraction phase, headspace, and sample, as well as the mass transfer coefficients, the distribution coefficients, and the surface area of the extraction phase.

Equation 30 can be changed to:

$$\frac{n}{n_e} = 1 - \exp(-at) \tag{31}$$

where n_e is the amount of extracted analyte at equilibrium. When the constant a has the same value for the absorption of target analytes and the desorption of pre-loaded standards, the sum of Q/q_0 and n/n_e should be 1 at any desorption/absorption time [75]:

$$\frac{n}{n_e} + \frac{Q}{q_0} = 1 \tag{32}$$

Then, the initial concentrations of target analytes in the sample, C_0, can be calculated with Eq. 33 [108, 109]:

$$C_0 = \frac{q_0 n}{K_{es}V_e(q_0 - Q)} \tag{33}$$

where V_e is the volume of the extraction phase, and K_{es} is the distribution coefficient of the analyte between the extraction phase and the sample.

The change of environmental variables will affect the extraction of the analyte and the desorption of the pre-loaded standard simultaneously; therefore, the effect of environmental factors, such as biofouling, temperature, or turbulence, can be calibrated with this approach. The feasibility of this technique for time-weighted average (TWA) water sampling was demonstrated by both theoretical derivations and field trials [110].

This technique is a pre-equilibrium method, and can be used for the entire sampling period. The concentration determined before the sampling reaches equilibrium is a TWA concentration because the desorption of the pre-loaded standard calibrated the extraction of the analytes and the extraction is an integrative process. If the sampling reaches equilibrium, the determined data are the concentrations of the analytes in the sample at the time the samplers were retrieved. If the investigated system has a large capacity (large volume and/or high affinity towards the standard), then the amount of standard remaining in the extraction phase is expected to be insignificant, and calibration at equilibrium conditions would not work.

The standard in the extraction phase technique applies to any geometry, and facilitates use of a simple PDMS-rod or PDMS-membrane as a passive sampler to obtain TWA concentrations of target analytes in a sampling environment. Both PDMS-rod and PDMS-membrane samplers are simple and easy to deploy and retrieve. They have large sampling rates and can achieve sensitivity levels that are much higher than those achieved by fibre-retracted SPME devices (see Section 11), since the samplers are in direct contact with the sample matrix via a high surface area [111]. The concept of standards in the extraction phase has been extended to determine the concentrations of target analytes directly in the veins of animals, indicating that this approach is useful for in- *in vivo* studies as well [112]. Experiments demonstrated that this calibration method corrected for sample matrix effects, and minimized displacement effects associated with the use of pre-equilibrium extraction. The pharmacokinetic profiles of diazepam, nordiazepam, and oxazepam obtained by kinetic calibration based on deuterated standards are quite similar to those determined by more established calibration methods [113]. The applications of this technique for quantitative analysis using liquid-phase microextraction (LPME) were also reported [77].

In cases where the deuterated compounds are too expensive and/or not available, an alternative calibration method which employs the target analytes as internal standards by means of dominant desorption can be applied [114]. Dominant pre-equilibrium desorption not only offers a shorter sample preparation time, but also provides time constants for the purpose of quantitative analysis. This kinetic calibration approach was successfully applied to on-site sampling of polyaromatic hydrocarbons (PAHs) in a flow-through system, and toward *in vivo* direct pesticide sampling in the leaves of a jade plant [86].

When using kinetic calibration with the standard in the extraction phase method, the samplers require pre-loading of a certain amount of standards, either deuterated compounds or target analytes. Several standard loading approaches can be used, which include: a) headspace extraction of the standard dissolved in a solvent or pump oil; b) headspace extraction of pure standard in a vial; c) direct extraction in a standard solution; and d) direct transfer of the standard solution from a syringe to the extraction phase [115].

The SPME kinetic calibration technique, using desorption of pre-loaded standards to calibrate extraction of the target analytes, requires that the physicochemical properties of the standard be similar to those of the analyte, which limits the application of the technique. Alternatively, the one-calibrant technique, which uses only one standard to calibrate for all extracted analytes, can be used [116]. This one-calibrant technique makes the kinetic calibration method more convenient and applicable to a broader range of applications.

9.2 Standard-free kinetic calibration

Kinetic calibration with standard in the extraction phase can be used for both grab sampling and long-term monitoring. For fast on-site or *in vivo* analysis, the pre-loading of standards can be inconvenient. Also this calibration method may not work in some fast sampling situations, as the loss of standard can oftentimes be insufficient for detection. In such cases, a standard-free kinetic calibration approach can be used for fast on-site and *in vivo* analysis [77]. In this calibration method, metrological traceability is ensured by validation of analytical procedures used in the determination of target species at two different sampling times.

Equilibrium extraction results in the highest sensitivity, as the amount of analyte extracted with the extraction phase is at a maximum when equilibrium is reached. If sensitivity is not a major concern, then a reduction in extraction time is desirable. When the extraction conditions are kept constant, *e.g.*, fast sampling, Eq. 34 can be used to calculate n_e, the amount of analyte extracted at equilibrium:

$$\frac{t_2}{t_1}\ln\left(1-\frac{n_1}{n_e}\right)=\ln\left(1-\frac{n_2}{n_e}\right) \tag{34}$$

where n_1 and n_2 are the amounts of analyte extracted at sampling times t_1 and t_2, respectively. Then, the concentration of the analyte in the sample can be calculated with Eqs. 3 or 4 [77].

This calibration method can be used for the entire sampling period, without considering whether the system reaches equilibrium. This aspect of the technique is desirable for systems when the equilibrium time is unknown, and is particularly useful for instances where a number of compounds are measured simultaneously. The method is unsuitable for long-term monitoring of pollutants in the environment, since the method requires that the sampling rate remain constant and the determined concentration is therefore representative of a spot sampling.

10 Headspace extraction

The use of the headspace above the sample as an intermediate phase is often an effective approach to accelerate the extraction of analytes characterised by high Henry constants. When a thin extraction phase is used, the initial extraction rate, and hence, the extraction time, can be controlled by the diffusion of analytes through the boundary layer present in the interface between the sample matrix and the headspace. As the analytes reach the bulk of the headspace they are rapidly transported into the extraction phase because of the high diffusion coefficients in the gas phase. In order to increase the transport of analytes from the sample matrix into the headspace, the system can be designed to produce a large sample/headspace interface. This can be accomplished by using large diameter vials with good agitation, purge, or even spray systems. At room temperature, only volatile analytes are transported through the headspace. For low volatility compounds, heating of the sample is a good approach if the smaller distribution constant is acceptable. The most suitable approach, in such cases, consists of simultaneously heating the sample while cooling the extraction phase. Heating of the sample not only increases the Henry constant, but also induces convection of the headspace due to density gradients associated with temperature gradients present in the system, resulting in higher rates of mass transport. On the other hand, cooling the sorbent increases its capacity. The collection of analytes can be performed in the same vial [117], or can be separated in space, as occurs in the purge-and-trap technique. In heating-cooling experiments, both kinetic and thermodynamic factors are addressed simultaneously. A substantial enhancement in the extraction phase/sample matrix distribution constant occurs due to the temperature gap in the system. Headspace approaches can also be advantageous in analysis of complex matrices, since adverse effects associated with the presence of solids, oily, or high molecular weight interferences, which can cause fouling of the extraction phase, are eliminated.

11 Passive time-weighted average (TWA) sampling

Consideration of different arrangements of the extraction phase is always beneficial when selecting the most appropriate geometry for a given application. For example, extension of the boundary layer by a protective shield that restricts convection will result in a time-weighted average (TWA) measurement of the analyte concentration (Eq. 25). Various diffusive samplers have been developed based on this principle. For example, when the extracting phase in a needle trap (Fig. 28a) or an SPME device (Fig. 28b) is not exposed directly to the sample, but contained in a protective tubing (needle) without any flow of the sample through it, the diffusive transfer of analytes occurs through the static sample (gas phase or other matrix) trapped in the needle. The system consists of an externally coated fibre with extraction phase withdrawn into the needle. This geometric arrangement represents a very powerful method, capable of generating a response proportional to the

integral of the analyte concentration over time and space (when the needle is moved through space) [118]. In this case, the only mechanism of analyte transport to the extracting phase is diffusion through the matrix contained in the needle. During this process, a linear concentration profile (shown in Fig. 28c) is established in the tubing between the small needle opening, characterised by surface area A and the distance Z between the needle opening and the position of the extracting phase. The amount of analyte extracted, dn, during the time interval, dt, can be calculated by considering Fick's first law of diffusion [13]:

$$dn = AD_m \frac{dc}{dz} dt = AD_m \frac{\Delta C(t)}{Z} dt \tag{35}$$

where $\Delta C(t)/Z$ is an expression of the gradient established in the needle between the needle opening and the position of the extracting phase, Z, $\Delta C(t) = C(t)\text{-}C_z$, where $C(t)$ is a time-dependent concentration of analyte in the sample in the vicinity of the needle opening, and C_z is the concentration of the analyte in the gas phase in the vicinity of the coating. If C_z is close to zero for a high extraction phase/matrix distribution constant capacity, then, $\Delta C(t) = C(t)$. The concentration of analyte at the coating position in the needle, C_z, will increase with integration time, but be kept low compared to the sample concentration due to the presence of the extraction phase. Therefore, the accumulated amount over time can be calculated as:

$$n = D_m \frac{A}{Z} \int C_s(t) dx \tag{36}$$

As expected, the extracted amount of analyte is proportional to the integral of the sample concentration over time, the diffusion coefficient of the analyte in the matrix filling the needle, D_m, and the area of the needle opening, A, as well as inversely proportional to the distance of the coating position with respect to the needle opening, Z. It should be emphasised that Eqs. 35 and 36 are valid only where the amount of analyte extracted onto the sorbent is a small fraction of the equilibrium amount needed to ensure a constant rate at a given concentration (linear regime in Fig. 24). To extend integration times, the coating can be placed further into the needle (larger Z), the opening of the needle can be reduced by placing an additional orifice (smaller A), or a higher capacity sorbent can be used [119]. However, the first two solutions will result in low measurement sensitivity. An increase of sorbent capacity, on the other hand, presents a more attractive opportunity, and can be achieved by either increasing the volume of the coating, or the affinity of the coating towards the analyte. It is important to note, however, that an increase in coating volume will require an increase in the size of the device; as such, the optimum approach to increased integration time is to use sorbents characterised by large coating/gas distribution constants. In cases where the matrix filling the needle is different than

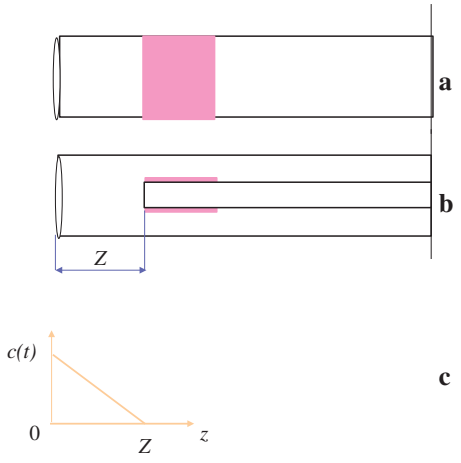

Fig. 28: SPME/TWA approaches based on sorbent in needle and in-needle fibre.

the sample matrix, an appropriate diffusion coefficient should be used, as discussed below in the case of membrane extraction.

There are a number of passive samplers that have been reported based on this principle [120, 121]. In many cases, selective permeation through membranes is used to not only slow down mass transfer, but also to introduce selectivity and eliminate matrix effects. A widely used approach in environmental aquatic passive sampling is the semipermeable membrane device (SPMD), which consists of a sealed membrane containing an extraction phase [122]. This sampling system was developed to mimic the accumulation of organic compounds by fish. A performance reference compound (PRC) dissolved in the extraction phase is sometimes used in SPMD to facilitate semi-quantitative field implementation of the devices to compensate for differences in exposure temperature, membrane biofouling, and flow velocity-turbulence at the membrane surface. PRC dissipation rate constants obtained for SPMD field exposure and laboratory calibration studies allow for calculation of an exposure adjustment factor, the ratios of which reflect changes in SPMD sampling rates (relative to laboratory data) [123]. More recently, it was demonstrated that polymeric sheets and SPE disks can be used for the same purpose, simplifying not only construction of the device, but also the desorption of analytes collected during sampling for quantification [124]. PDMS membranes and different sorbents embedded in PDMS membranes in combination with thermal desorption were applied successfully for this application [97, 125].

12 Extraction combined with derivatisation

The capacity of the extraction phase for analytes that are difficult to extract, such as polar or ionic species, is frequently enhanced by introducing a derivatisation step. The objective of this approach is frequently not only to convert native analytes into less polar derivatives that are extracted more efficiently, but also to label them for better detection and/or chromatographic separation. The most interesting implementation of this approach is simultaneous extraction/derivatisation. In this technique, the derivatizing reagent is present in the extraction phase during extraction. The major advantage of this approach is that two steps can be integrated into one. There are two limiting cases that describe the combination between extraction and derivatisation. The first occurs when mass transfer to the fibre is slow compared to the reaction rate. In this case, Eq. 25, as discussed above, can be used to describe the accumulation rate of analytes, assuming that the derivative is trapped in the extraction phase. In the second limiting case, the situation is reversed, in that the reaction rate is slow compared to the transport of analytes to the extraction phase. In other words, at any time during extraction, the extraction phase is at equilibrium with the analyte remaining in the well-agitated sample, resulting in a uniform reaction rate throughout the coating. This is a typical case for a thinly dispersed extraction phase, since equilibration time for well-agitated conditions is very short compared to a typical reaction rate constant. The accumulation rate of the product in the extraction phase, n/t, can then be defined by:

$$n = V_e k_r K_{es} \int C_s(t) dt \tag{37}$$

where C_s is the initial concentration of analyte in the sample, and k_r is the chemical reaction rate constant. In other words, when the sample volume is large, such as is the case for direct sampling in the field, the reaction and accumulation of analyte in the extraction phase proceeds with the same rate as long as the reagent is present in excess. It is worth noting that the rate is also proportional to the extraction phase/sample matrix distribution constant. If the concentration varies during analyte accumulation, then the collected amount would correspond to the integral over concentration and time, as discussed above in the case of TWA sampling. For a limited sample volume, however, the concentration of analyte in the sample phase decreases with time as it is partitioned into the coating and converted to trapped product, resulting in a gradual decrease of the rate. The time required to exhaustively extract analytes from a limited volume can be estimated using experimental parameters [13].

13 Membrane extraction techniques

Membrane extraction is an attractive approach for continuous monitoring applications. Permeation through a membrane is a specific extraction process where the sorption into and desorption out of the extraction phase occur simultaneously. The sample (donor phase) is in contact with the side of the membrane where extraction into the membrane material occurs, while permeated analytes are removed by the stripping phase (acceptor) from the other side of the membrane. When membrane extractions entail good flow (agitation) conditions at both the acceptor and donor sites as well as efficient stripping, the rate of mass transport through the membrane is controlled by the diffusion of analytes through the membrane material. The concentration gradient, which facilitates transport across the membrane, is formed by the difference in analyte concentration between the sample side ($K_{es}C_s$) and the stripping phase, which is close to zero for high flow rates of stripping phase (Fig. 29). The mass transfer rate through the membrane, n/t, can be estimated at steady state conditions using the following equation:

$$n/t = B_2 A D_e K_{es} C_s / b \tag{38}$$

where A is the surface area of the membrane, D_e is the diffusion coefficient in the membrane material, K_{es} is the membrane material/sample matrix distribution constant, b is the thickness of the membrane, and B_2 is a geometric factor defined by the shape of the membrane. The permeation rate through the membrane is proportional to both the diffusion coefficient (D_e) and the distribution constant (K_{es}), and inversely proportional to b. D_e determines the rate of analyte migration through the membrane, and K_{es} the magnitude of the concentration gradient generated in the membrane [126]. This information can be used to calibrate the extraction process *a priori* if these parameters are obtained from tables or experimental data [127].

The concentration of the analyte can be calculated by converting Eq. 38:

$$C_s = \frac{bn}{B_2 A D_e K_{es} t} \tag{39}$$

The membrane material/sample matrix distribution constant K_{es} determines the sensitivity of membrane extraction (Eq. 39), indicating that the membrane, although a physical barrier, is also a concentrating medium and analogous to the extraction phase in other configurations. However, the concentration in the stripping gas phase is lower compared to the sample, since a gradient needs to exist in the membrane to generate diffusive mass transfer through the membrane material (Fig. 29). Incorporating a concentrating component, such as a sorbent after the membrane, will facilitate analyte enrichment, and consequently, sensitive analysis. When the membrane is in direct contact with the aqueous phase, the mass transfer through the boundary layer surrounding the membrane can contribute to the overall mass transfer in the system. Therefore, for analytes characterised by high Henry constants, it is important to consider a headspace membrane extraction arrangement.

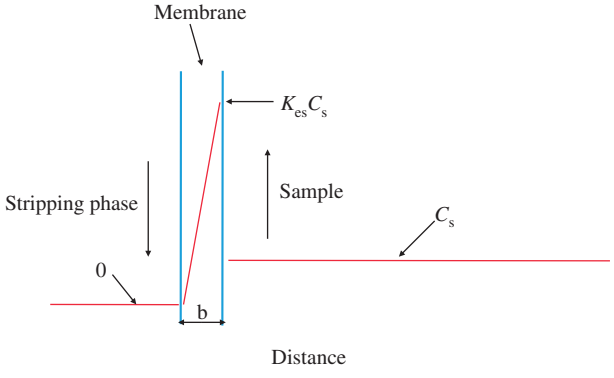

Fig. 29: Membrane extraction at good sample agitation and stripping conditions. The symbols/terms are defined in the text.

The strength of the membrane extraction approach is that it provides simple selective contact between sample and instrument; thus, it can be used with portable instruments. Quantification is a weak point in membrane extraction, as it is frequently impossible to relate correctly the amount of target analyte transferred through the membrane to its concentration in the matrix, since both the concentration in the matrix and the mass transfer conditions affect the extracted amount. Therefore, it is critical to characterize the mass transfer conditions in order to facilitate correct quantification. To address this challenge, an analytically non-interfering internal standard can be added to the receiving phase (stripping phase) [128]. This approach is analogous to the standard in the extraction phase technique that is often used in batch extraction, as discussed in Section 9.1. The membrane extraction with a sorbent interface (MESI) system was used to evaluate this approach. During the membrane extraction process, the internal calibrant present in the carrier gas, which acts as a stripping phase, and the target analyte present in the sample matrix will permeate simultaneously through the membrane in opposite directions. The changes in accumulated amounts (relative loss) of internal calibrant can be used as a means of calibration to correct variations in the extraction rate that occur due to variations in environmental factors, such as sample velocity and membrane temperature, which in turn determine the extraction conditions. Thus, during field analysis, this approach allows for more accurate estimates of concentrations of target analytes at various sampling or monitoring conditions [129].

In addition to the liquid polymeric membranes described above, there are numerous types of porous membranes and associated extraction processes available for use in analytical applications. Applications can vary depending on the needed porosity and selectivity, from microporous filtration to remove particles and ultrafiltration where larger macromolecules are retained, to reverse osmosis membranes that allow the separation of ions from water. The selectivity of a microporous membrane can be further improved by filling the pores of the membrane with organic solvent, resulting in a supported liquid membrane. In addition to applying high pressers in the ultrafiltration system, the separation of smaller molecules from larger particles can be accomplished by introducing a concentration gradient generated by the continuous flow of dialysate in a dialysis system. In addition, the separation of ions from neutral species can be facilitated with the use of an electrical potential, in a process called electrodialysis. The standard in the *in vivo* sampling technique called microdialysis, which is a miniaturised version of the dialysis system, is shown on Fig. 30. In a typical procedure, the probe is placed directly into the tissue with the help of a needle or cannula. The dialysis hollow fibre membrane makes contact with the tissue, allowing low molar mass analytes present in the tissue to permeate through the porous structure to the flowing extraction phase (perfusate). The extraction phase, together with the permeated analytes (dialysate), then leaves the device to be quantified and/or identified by a suitable instrument.

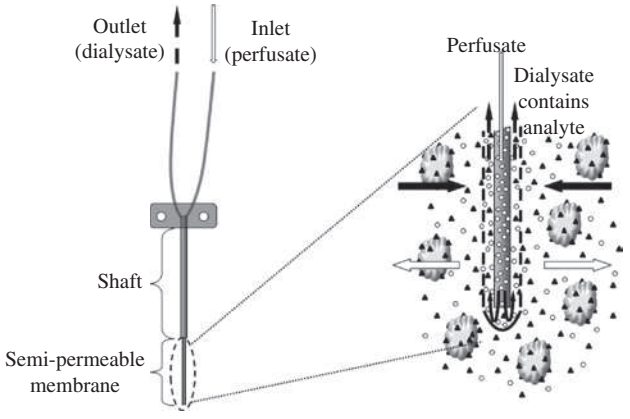

Fig. 30: Microdialysis probe.

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Annex I – Frequently used symbols and abbreviations

A	Area of extraction phase
B, B_1, B_2	Geometric factors
b	Thickness of the extraction phase
C_0	Initial concentration of analyte in sample
C_e	Concentration of analyte in extraction phase
C_s	Concentration of analyte in sample
D_e	Diffusion coefficient of analyte in extraction phase
D_s	Diffusion coefficient of analytes in sample matrix
d	Radius of the fibre core
d_c	Thickness of the matrix component permeable to analyte
d_s	Dimension of sample solid particulate matter
H	Height equivalent to theoretical plate (HETP)
K_{es}	Extraction phase/sample matrix distribution constant ($K_{es} = C_e/C_s$)
K^s_{es}	Sample matrix/solid extraction phase distribution constant ($K^s_{es} = S_e/C_s$)
k	Partition ratio
k_d	Dissociation rate constant
k_0	The ratio of the intraparticulate void volume to the interstitial void space
k_r	Chemical reaction rate constant
L	Length of extraction phase
n	Amount of analyte extracted onto the extraction phase
S_e	Surface concentration of analyte adsorbed on solid extraction phase
PDMS	Poly(dimethylsiloxane)
SFE	Supercritical fluid extraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
t_0	Time required to remove one void volume of the extraction phase
t_e	Equilibration time
u	Chromatographic linear velocity
u_e	Interstitial linear extraction phase velocity
V_e	Volume of the extraction phase
V_v	Void volume
V_s	Sample volume
Z	Distance between the sample and the extracting phase
z	D_e/D_s

δ Boundary layer thickness
ε_i Intraparticulate porosity
ε_e Interstitial porosity

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