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References


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Strategies for solid sample treatment

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An overview of the different ways to accomplish pretreatment of solid samples is presented. The use of (a) auxiliary energies such as lyophilisation, ultrasound, microwaves and laser radiation, (b) commercial modules including simple units such as Soxhlet and more sophisticated supercritical fluid extractors, and (c) robotic stations working individually or coupled to other automated alternatives, is looked at critically.

1. Introduction

Automation of the preliminary steps of the analytical process is one of the aims of today’s analytical chemistry, particularly in the field of environmental, clinical and industrial analysis, due to both the variety of samples and the number of analyses required daily [1].

The different approaches proposed for this task in the last few years can be classified into three main groups: (A) different energy sources which, when applied, dramatically shorten the treatment step and which also enable its automation; (B) modules for solid sample treatment, which can be simple manual units for batch treatment, or other, more complex systems whose characteristic variables can be altered depending on the type of target sample or analyte; (C) robotic stations, which can work as dedicated modules for dissolution or leaching, as analytical black-boxes for development of the overall analytical process, or as equipment interfaced to other automated alternatives.

2. Use of auxiliary energy sources

Among the energy sources commonly used to aid sample pretreatment, some of the most promising at present are lyophilisation, ultrasound, microwaves and laser sources (Fig. 1).

2.1. Lyophilisation

The term lyophilisation refers to an operation in which water (or other components) is separated by sublimation from a frozen system or phase. The passage from solid to gas phase occurs without the appearance of water or the solvent in liquid state. This technique has been traditionally used in the analytical laboratory for storage of biological samples, standards and reagents [2], particularly in clinical laboratories. Two additional benefits make lyophilisation attractive to analysts: as the removal of water allows subsequent reconstitution in a minimal volume of liquid, a preconcentration effect is inherent to this step [3]. The other, which can be more important, is the texture of lyophilised materials, which are very easily attacked by solvents [4]. This structure can be optimised by the use of the appropriate temperature, pH, reagent addition and lyophilisation time. A major disadvantage of lyophilisation is that volatile compounds can be removed from the matrix during the process.
A method for the evaluation of the stability of lyophilised samples was proposed and applied to evaluate this parameter of ten elements (Fe, Zn, Cu, Mn, Co, Cd, Hg, Pb, As and Se) during lyophilisation of rat liver [2]. Fe was found to be extremely stable during the process. The total amount of a given element and the total amount of Fe in each sample were determined, then the ratio of the target element to Fe for the wet sample and the lyophilised one was calculated and used to estimate the stability of the element. No differences in the amounts of Fe in the wet and lyophilised samples were observed, so there were no losses of Fe during this step; therefore, Fe was selected as a reference element for the evaluation of the others. The target element to iron ratio between wet and lyophilised samples for Zn, Cu, Mn, Co, Cd, Hg, As and Se remained constant, but there was a difference for Pb; only 92% of the Pb was retained during the lyophilisation step. Lyophilisation storage was also found to be suitable for preserving the speciation pattern of butyltin and phenyltin compounds in sediments. The stability of organotins in these matrices is strongly dependent on the treatment applied, particularly on both drying and storage procedures. Several drying procedures have been tested and compared: air-drying (for two weeks), in a desiccator (in the darkness for one week), oven-drying (at 100°C for 4 h), under an IR lamp (for 1.5 h), and lyophilisation. The use of lyophilisation and drying-desiccation kept butyl species stable for at least 12 months, and phenyl species for 3 months, whereas the other treatments caused a significant decrease in the concentration of both types of compounds [3].

In addition to no losses of the trace element, sample contamination is virtually avoided when a preconcentration step is aided by lyophilisation [4]. This assertion was tested by adding radioactive tracers for 21 elements (including many volatile ones) to river and sea water; the solutions were lyophilised and the tracer residues were finally counted. The activity from each isotope was measured in the reference sample and compared to that in the lyophilised sample. The results confirmed that at least 95% of all elements were retained in the residue, and lyophilisation proved to be a quantitative preconcentration technique for the majority of inorganic trace elements in water. No consistent retention value for the most volatile elements (Hg and I) was obtained. Both positive and negative effects of lyophilisation on the determination of lipoproteins in serum have been reported [5].

A lyophilisation step preserves and preconcentrates samples but, moreover, favours the digestion process. The features of the lyophilised samples can be optimised by the use of the appropriate experimental conditions (namely temperature, pH, reagent addition and lyophilisation time) thus facilitating subsequent treatment for destruction of organic materials. Izquierdo et al. [6] checked the change in texture after optimisation of the lyophilisation step, in order to facilitate digestion for further determination of Cu, Cd, Pb and Zn in certified reference materials and fresh bovine liver.
samples. The lyophilised samples were treated with an acid mixture (10:1 HNO₃-H₂SO₄) and heated to favour both digestion and the release of acids. There were no losses or gains of the target analytes as the results were within the confidence levels of the proposed determination method. Significant differences in the time and working conditions required for digestion were observed depending on the conditions of the lyophilisation step.

2.2. Ultrasound

Although analytical chemists have shown little interest in the use of ultrasound, its potential usually surpasses that of the other conventional auxiliary energies. Thus, ultrasound is of great help in the pretreatment of solid samples as it facilitates and accelerates steps such as dissolution, fusion and leaching, among others. The propagation of ultrasonic waves characterised by a minimum frequency of 16 kHz results in rapid fluid movement through compression and rarefaction: free radicals are generated, chemical layers are dispersed and the contact between the ingredients of the reaction is accelerated. Usually, ultrasonic effects are much more intense in heterogeneous than in homogeneous chemical systems, because emulsification is favoured and mass heat transfer in two-phase systems is increased [71]. These effects have been exploited for sample preparation in agricultural, biological and environmental chemistry.

One of the main uses of ultrasound is for accelerating leaching, particularly when this step is accomplished in a continuous fashion [8]. One of these approaches, such as that shown in Fig. 2, was designed for the determination of iron in plant material by acid treatment followed by complex formation with 1,10-phenanthroline. After recirculating the leaching solution through the sample chamber for a preset time, valve SV was switched and the solution was driven to a continuous manifold where merging with the derivatization reagents, reaction and detection were accomplished. The proposed method is applicable to any pulverizable sample: plant material, soil, coal, filtration solvents, etc. The leaching time for the samples assayed could be reduced from over 40 to 5 min, for removal of 97% of the iron in the sample. Complete recovery was achieved in 10 min. Comparison of four leaching techniques (Soxhlet, sonication, mechanical shaking and homogenisation-sonication) applied to the determination of munition residues in soil [9] showed that ultrasonic radiation is the most recommended, as it can be used in an unattended fashion for the simultaneous processing of a number of samples and, in addition, it is relatively inexpensive. The ultrasonic device (bath or probe) does not suffer from interferences as is the case with Soxhlet devices. Aromatic compounds from must and wine have been extracted using ultrasound. This technique has provided recoveries between 94 and 99%, good linearity and reproducibility (R.S.D. less than 9%) for most of the compounds, thus proving to be better than extraction on C₁₈ (reproducibility ranging between 10 and 14%, expressed as R.S.D.) [10]. Nevertheless, some shortcomings of ultrasonic agitation should be pointed out: (i) the dependence of the efficiency of analyte extraction on the vigor of agitation and (ii) the influence of the characteristics of the sol-
vents used (i.e. viscosity, vapor pressure and dissolved air concentration) on the acoustic impedance. However, as long as experimental conditions are constant, the ultrasonic device is an excellent way to perform solid–liquid extractions.

Ultrasonic treatment can also be applied in order to reduce the sample preparation time when tedious processes such as fusion have to be carried out. The determination of Ca in aluminosilicate rocks was accelerated with the use of a 22 kHz ultrasonic vibration, during alkaline fusion and acid leaching (they took 1 min each). The results obtained agreed with the certified values [11], thus verifying the absence of side-effects caused by this type of energy.

The chemical effects of ultrasound were proved during the leaching of airborne particulate matter with H₂O or acetone. The sonication was carried out at 40 kHz with 30 ml solvent at 20°C for periods of 15, 30 and 60 min prior to ion chromatography coupled to a Dionex AMMS-1 suppressor column. Tests with the analytes in pure H₂O showed that the sonication resulted in the formation of nitrite and nitrate due to the oxidation of nitrogen, an increase in acetate concentration and a decrease in formate concentration. The tests were repeated with quartz fibre filters impregnated with airborne particulate matter [12]. Both the conversion of dithiocarbonate fungicides (e.g. Zineb) in foods into CS₂ by SnCl₂–HCl treatment, and the separation of the latter in a capped headspace flask have been helped by ultrasound, thus accelerating both steps dramatically and shifting them to completion [13].

In addition and as a reinforcement of the use of ultrasound in the analytical laboratory, other processes which can also be aided by ultrasonic energy, are the following: (i) liquid–liquid extraction, particularly when conventional methods require long extraction times (up to 2 h to obtain a recovery of 90% versus 10 min for recoveries over 92% using ultrasonic irradiation [14]); (ii) enzyme-catalysed reactions, which can improve the yield of the products up to 200% [15]; (iii) electrochemical processes, whose reproducibility can be dramatically enhanced (sonochemical stripping voltammetry provides R.S.D.s of 6% after sonication for 10 min versus 20% obtained with the conventional mode [16]); (iv) atomic spectroscopic techniques where ultrasonic nebulization is preferred to pneumatic nebulization, on account of the smaller and more uniform particle sizes produced, which allow introduction into the instrument of virtually any sample concentration [17].

2.3. Microwaves

A number of commercial devices based on microwaves are currently used on a laboratory scale, since their implementation results in the improvement of existing processes that involve solid samples, such as digestion, leaching, gas removal and distillation, by dramatically decreasing the time required for their completion. The activation of these processes by exposure to microwaves gives rise to faster and cleaner reactions when compared to conventional heating, as this
Microwaves for analytical purposes have found widespread use in very different areas including clinical, food and environmental analysis, so the presence of different commercial microwave devices from domestic ovens to sophisticated continuous-treatment apparatuses and focused microwave digesters in both routine and research laboratory is very common at present. Some researchers working in this field have patented their approaches. Microwave-assisted processes (MAP) [19], and microwave-assisted extraction (MAE) [20] are some of the modes of using microwaves for aiding these analytical steps. Solid samples such as artichokes, diets and sewage sludges, were digested with a mixture of concentrated HNO₃ and H₂O in a microwave device for a few minutes. The results obtained for the determination of Cu and Mn in these samples were compared with those provided by wetashing batch digestion, and proved to be of a similar order. The same procedure was applied to the determination of Pb and Zn in certified reference material. Good recoveries were reported for Pb whereas the accuracy of the results for Zn was low [21]. This was also the case for the robotic determination of traces of Zn and other metals such as Fe and Cu in soils based on the use of the DTPA (diethylenetriamine pentaacetic acid) leaching method assisted by a focused microwave digester. The proposed method provided higher extraction efficiencies for Fe and Cu, and similar values for Zn as compared with the manual method. The time required for this step was dramatically shortened from 2 h to 3 min.

A new generation of methods for speciation analysis in solid samples, in which the sample throughput is controlled by the duration of the GC run and not by the sample preparation step, is available in the present literature thanks to the use of low-power focused microwaves. Two approaches have been developed in this respect for the speciation analysis of organotin compounds: selective microwave-assisted leaching of the target analytes from the matrix and microwave-assisted matrix dissolution; both of them leave the organotin moiety intact. The technique has been validated by the analysis of environmental certified reference materials [22].

Both leaching and gas removal can be assisted by microwaves as is the case with a novel analytical separation technique: pervaporation from solid samples [23,24]. A method for speciation of mercury in solid samples based on microwave-assisted pervaporation was proposed in which the suitable leaching solution was added to the solid sample situated at the donor chamber of the pervaporation unit, as shown in Fig. 3. Focused microwaves were then applied to this chamber in a sequence of increased power, thus enabling the selective removal of the different inorganic and organic mercury species from the matrix. Finally, the volatile analytes were evaporated and diffused through a hydrophobic membrane, to be accepted in a gas stream, and led to an atomic fluorescence detector [25].

Another process successfully assisted by microwave energy is distillation; in this case a significant reduction of sample preparation time compared to that required for conventional distillation is achieved. A precise and accurate procedure was proposed for the determination of inorganic As in seafood, making use of this technique [26]. Under the working conditions established, it was verified that the organo-arsenic bonds were not broken during the microwave-assisted distillation of AsCl₅. Significant differences were observed in the distillation volume required (15 ml) and the time necessary for the total distillation of AsCl₅, which is reduced to 12 min, as compared to the 40 min needed by the conventional procedure.

A microwave-based rotary evaporator, furnished with a glass or a transparent polypropylene vessel, that can be used at temperatures up to 180°C while being rotated under vacuum has been recently commercialised and applied successfully to dairy products, foods and pharmaceuticals [27].

Other microwave-assisted processes such as liquid–liquid extraction, headspace, synthesis and a number of chemical reactions including saponification, redox, complexation, amplification, etc. are examples of the wide field of application of this form of radiant energy.

### 2.4. Laser

Laser radiation is commonly used as a radiation source in spectroscopic techniques. However, its application in solid sample pretreatment steps such as extraction, desorption, etc. is possible after characterization of several physical parameters of the source, such as laser power, wavelength, etc. Laser-assisted leaching methods for Cu, Zn, Ti, Mn and Mo from metal or alloy samples...
have been developed with the sample immersed in an aqueous solution of EDTA or NH₄Cl and irradiated with a pulsed Nd laser, operated at 1060 nm with pulse energy of 5 J. Several μg of the analytes are removed under these working conditions.

Laser-induced desorption has proved to minimize decomposition of target analytes. For the identification of PAHs in different types of meteorites [29], the constituents of the sample were desorbed with a pulsed infrared laser with a low power to avoid decomposition. The results obtained allowed the identification of different classes of meteorites on the basis of their trace organic compounds. The proposed method minimized the contamination due to sample handling, laboratory atmosphere, virtual leaks, usual in sample preparation by conventional analytical procedures.

Laser ablation solid sampling has been mainly used prior to ICP-AES, and a decrease in the rate of change, or roll-off in mass removed, has been observed with increasing incident laser power density; so the use of internal and external standardization is mandatory when high accuracy is required [30].

A recent application of laser-induced breakdown spectroscopy is for quantitative analysis of colloidal and particulate iron in water [31]. The results obtained show the capability of this technique for the analysis of this type of samples.

3. Modules for solid sample treatment

The apparatuses commercially available for the pretreatment of solid samples can be classified into two main groups: simple, cheap and time-consuming units such as Soxhlet modules, and more complex, expensive and fast devices such as supercritical fluid extractors. Very simple and time-consuming modules have been used for years for the pretreatment of solid samples, particularly for the accomplishment of leaching steps. The most common approaches in this area are as follows.

A very cheap setup for analytical leaching is a vessel in which the solid sample is immersed in a leaching liquid phase. Most frequently, the vessel is open and furnished with a condensation system in order to avoid solvent losses through vaporisation. The process is usually accelerated by heating, and also efficient agitation favours analyte removal. Because the leaching phase is not renewed during the process, a displacement of the separation equilibrium to the liquid phase is not seen, so the extraction efficiency may be rather low if the analytes are not very soluble and the liquid molecules do not compete advantageously with those of the analyte for the active site of the matrix.

A well-known system in this context is the Soxhlet extractor in which the initial solid phase (or a solid–liquid mixture) is placed in a cavity that is gradually filled with leaching liquid phase by condensation of vapours from a distillation flask. When the liquid reaches a preset level, a syphon aspirates the whole content of the cavity and unloads it back into the distillation flask, but carrying the extracted analytes in the bulk liquid. This operation is repeated many times until virtually complete separation is achieved and the analytes are all in the flask. Inasmuch as the solvent acts stepwise, the assembly can be considered a batch system; however, since the solvent is recirculated through the sample, the system also bears a continuous character. The most salient advantages of Soxhlet are the fact that the sample is repeatedly brought into contact with fresh portions of the solvent, thereby aiding displacement of the separation equilibrium, and that no filtration is required. The drawbacks involved in the use of this conventional leaching technique are the inability to provide agitation, which would be of help to accelerate the process, and the constant heat applied to the leaching cavity, which is a function of the solvent boiling point and can be insufficient to break some matrix–analyte bonds. Microwave assisted Soxhlet extractors have been proposed and commercialized without noticeable success. Nevertheless, the literature shows applications in which a saving in time (1 versus 4 h) is achieved using Soxtec instead of conventional Soxhlet. Such is the case with the leaching of alkylketene dimer from pulp and paper matrices for subsequent determination by GC–MS [32].

One of the most significant contributions to solid sample treatment in the last few years has been supercritical fluid extraction (SFE) [33] or, better, supercritical fluid leaching. This technique emerged in the past decade as the panacea to all the shortcomings concerning solid sample treatment. Despite the promising features of this technique, it has not fulfilled the expectations so far, perhaps due to the non-ideal features of the supercritical fluids at present used as leachers. Thus, the main shortcoming of supercritical CO₂ is its low dielectric constant, which hinders or makes the leaching of polar and ionic analytes difficult. The use of supercritical
H₂O yields very diluted analytes due to its liquid state under normal pressure and temperature; while other leachers such as ammonia, freons, organic solvents (ethanol, methanol, etc.) are far from affording clean methods.

The disadvantages of CO₂ as supercritical extractant can be minimized either by adding cosolvents, which increase the dielectric constant of the medium, or by decreasing the polarity of the target analytes [34]. The SFE of dioxins from sediments [35] is a good example of this assertion: virtually 100% of the analyte was extracted within 30 min from a sediment using supercritical CO₂ containing 2% MeOH; meanwhile, the standard Soxhlet extraction of the same sample for 18 h provided 65% recovery only.

Water is an almost ideal leaching agent as its dielectric constant can be changed in a wide range by changing the temperature and pressure. The drawback of analyte dilution can be circumvented by a concentration step after leaching, which can consist of either an in-line retention on a solid support packed in a column or in the flow-cell of a non-destructive detector, thus enabling monitoring of the kinetics of the extraction process [36], or a liquid–liquid extraction, evaporation, etc. Thus, the determination of organic pollutants from environmental solids has been developed using water to extract polar, moderately polar and non-polar organics, increasing the extraction temperature from 50°C (subcritical water) to 400°C (supercritical water). Although polar organics are easily extractable with water, quantitative extraction of low-polarity organics such as PAHs can be achieved in 15 min with recoveries significantly higher (89–112%) than those reported by NIST based on 48 h Soxhlet extractions [37]. The use of water under subcritical conditions has also received the name of high-pressure solvent extraction [38] and accelerated solvent extraction [39].

A recent comparison of extraction techniques such as supercritical fluid, Soxhlet and high-pressure solvent extraction (HPSE) for leaching of organophosphorus from soil shows that HPSE provides extraction efficiencies comparable with those obtained with Soxhlet and SFE but with substantial saving of time and cost [40].

4. Laboratory robotics

Undoubtedly, the only way to enable full automation of the overall analytical process when solid samples are involved is with the use of a robotic station as there is no other automated alternative at present capable of some basic unitary operations (namely, weighing, leaching, digestion or other type of sample pretreatment for separation of the target analytes, centrifugation, etc.) without human intervention. Nevertheless, it is fair to emphasize that when a task can be developed by both a robotic station and another automated alternative, usually the latter is faster, cheaper and sometimes more precise than robotics. Taking into account this fact, three general uses of laboratory robotics can be distinguished:

(a) Dedicated robots are required when a laboratory step is either very time-consuming or must be routinely repeated many times, as is the case with weighing, dissolution, dilution, etc., which are very common in industrial and clinical laboratories. These tasks are frequently performed by dedicated robots [41].

(b) Robotic stations, in which a number of peripherals aid the robot in the overall development of the analytical process. This type of application is justified only when all (or at least a major part) of the steps involved cannot be developed by other automatic alternatives, as is the case in the determination of the oxidative stability of olive oil [42], in which the removal and replacement after weighing of the sample tubes from and back to a heating block have to be repeated at preset time intervals for at least one week. The information is acquired and treated by the computer after weighing; thus, the treatment of each sample finishes when a pre-established change in weight is reached.

(c) The most rational use of a robotic station is its coupling to other continuous or discontinuous automated alternatives in order to develop each step of an analytical process by the most friendly system. In this respect and concerning solid samples, the robotic station is always entrusted to sample weighing and pretreatment by the most suitable operation, such as digestion, leaching, fusion, etc.; all of them can be easily developed by the robot by a simple change in both peripherals and control program. After sample dissolution or leaching, the solution containing the target analyte can be 'handled' by, for example, a continuous system into which the robot can insert the solution for subsequent operations such as an on-line continuous separation step, dilution, derivatization, etc. Finally, determination of the analytes can be done by driving the treated sample solution to a suitable detector [43].
5. Final remarks

The number of approaches currently available for the pretreatment of solid samples allows the most suitable method to be selected for each application, depending on both the type of analyte, and the subsequent steps to be developed in order to complete the analytical process. There is a reasonable amount of literature on each alternative, providing the novice with a good background. In addition, new developments are published in journals allowing the researcher to keep abreast with the most recent advances.

The present situation can be summarised as follows: the development of automated methods for treatment of solid samples without human participation can only be carried out with the use of a robotic station. Nevertheless, a number of auxiliary energies and commercially available modules can facilitate and/or accelerate one of the most time consuming steps of the analytical process, i.e. to obtain the analyte(s) from a solid sample in the form of a solution.

It is fair to point out that sample pretreatment must not be looked at as an isolated step, but integrated in the analytical process. Its automation, less developed than the other two steps, makes special emphasis in R&D in this field mandatory in order to equilibrate it with the other two subsequent steps: signal measurement and transducing, and data acquisition, treatment and results delivery.

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References

Predicting environmental fate parameters with infrared spectroscopy

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One of the principal uncertainties associated with risk assessments of organic chemicals in the environment is the lack of chemical-specific values that quantify the many processes determining the chemical’s transport and transformation. Because it is not feasible to measure the many constants that are required when thousands of chemicals must be considered, reliable predictive methods are required. A precise theory-based method is now available for many processes whose mechanisms are understood well enough to model theoretically. However, a void exists for processes that are not well understood. We have been developing an infrared spectroscopy-based method to fill that void. Herein, we summarize previously published progress to date, and present new results that strengthen the feasibility of the method. Specifically, we show that the method is capable of predicting constants for ten physical/chemical properties and distribution coefficients for a diverse set of organic esters, typically to within an error percent of ten. The predictive models are built using full infrared spectra and partial least-squares (PLS) modeling. A critical evaluation of the approach, and a brief description of ongoing developments, is presented.

1. Introduction

Environmental exposure and risk assessments of an organic pollutant require in-depth knowledge of the chemical’s behavior in the environment of concern. When a chemical is released in the environment, a large number of chemical transport, transformation, and distribution processes will determine exactly what (and how much) a person or an ecosystem will be exposed to at some time and distance from the point of introduction. The mechanisms of some of these processes, such as volatilization and hydrolysis, are quite well understood and have been coded into computer-implemented engineering models for chemical exposure analysis. To execute these models, both environment-specific parameters (such as pH and temperature) and chemical-specific parameters (such as physical properties, rate and equilibrium constants) are required.

Unfortunately, there are problems that severely limit both the accuracy and applicability of these