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Review

The role of ultrasound in analytical derivatizations☆

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ABSTRACT

Ultrasound is a type of energy that until recently was rarely used for analytical purposes. In recent years, work in chemical and industrial fields alerted analytical chemists to the great potential of ultrasonic energy to accelerate or improve different steps of the analytical process. One of these steps is derivatization: depolymerization, redox, hydrolysis, esterification, alkylation and complex formation are examples of derivatization reactions, all of which are significantly improved with the aid of ultrasound. This review discusses the valuable characteristics of ultrasound and its influence on a number of derivatization reactions is discussed in this review.

Contents

1. Introduction

Ultrasound (US) is simply sound pitched above human hearing. Humans can hear frequencies from about 16 Hz to 18 kHz, while US spans frequencies from 20 kHz to the GHz range, which can be split into two distinct regions, namely: a power region and a diagnostic region. The former, in the low-frequency end, provides enough acoustic energy for the production of cavitation. High-frequency US, which possesses low amplitude, around 5 MHz onwards, produces no cavitation, so it is used for medical scanning, chemical analysis and the study of relaxation phenomena, among other applications.

Being a sound wave, US is transmitted through any substance, solid, liquid or gas possessing elastic properties. The movement of the sound wave is communicated to the molecules of the medium, creating expansion and compression cycles travelling through the medium. In a liquid, the expansion cycle produces negative pressure that pulls molecules away from one another and can create bubbles or cavities in the liquid when the negative pressure exerted exceeds the local tensile strength of the liquid. The process by which bubbles form, grow and undergo implosive collapse is known as

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"cavitation". Collapse occurs when a bubble can no longer absorb the energy efficiently from the US so it implodes. Rapid adiabatic compression of gases and vapours within the bubbles or cavities produces extremely high temperatures and pressures, estimated to be about 5000 °C and 2000 atm, respectively. The size of the bubbles is very small relative to the total liquid volume, so the heat they produce is rapidly dissipated with no appreciable change in the environmental conditions [1,2].

There are a number of variables influencing the cavitation phenomenon, which can be taken into account for proper optimization of US action on a given system subjected to this type of energy. The most important of these variables are:

- The *presence of gas* and *particulate matter*: both decrease the cavitation threshold.
- *External applied pressure*: increased external pressure raises the rarefaction pressure required to initiate cavitation.
- *Solvent viscosity*: the cavitation threshold increases with increased viscosity.
- *Solvent surface tension and vapour pressure*: solvents of low surface energy and/or low vapour pressure decrease the cavitation threshold.
- *Applied frequency*: more power is required at a higher frequency to maintain the same cavitational effects.
- *Temperature:* the cavitation threshold increases with decreased temperature, in part due to an increase in either the surface tension or viscosity of the liquid as the temperature decreases, or to a decrease in the liquid vapour pressure).
- *Ultrasound intensity*: this variable is directly proportional to the square of the vibration amplitude of the ultrasonic source. As a rule, increasing the intensity increases the sonochemical effects; however, the ultrasonic energy a system can take is limited, as cavitation bubble creation and collapse depend on the duration of the rarefaction cycles, the collapse time, temperature and pressure on collapse, all mutually depended.
- *Field type:* Induction of acoustic cavitation is much more efficient and reproducible in a standing wave field than in a progressive one.
- *Attenuation:* As it progresses through the medium there is an attenuation of the US intensity. Part of the energy is dissipated in the form of heat, which is hardly appreciable in the bulk medium during sonication. The extent of attenuation is inversely proportional to the sonication frequency.
- *Types of ultrasound cavitation*: there are two types of cavitation – *transient* and *stable*. The former is known as inertial cavitation, of short lifetime, so mass flow by diffusion of gas into or out of the bubbles is not allowed; by contrast, evaporation and condensation of liquid occur freely. Stable or *non-inertial cavitation* was at one time thought to be of little significance in terms of chemical effects, but new, more sophisticated measurement tools enable new and contradictory results to be obtained [3,4].

The main cause of the effects of US radiation on chemical reactions is the high temperature and pressure created within a collapsing cavitation bubble, which produces the formation of free radicals and various other species. Thus, sonication of pure water causes its thermal dissociation into H and OH radicals, the latter forming hydrogen peroxide by recombination. These radicals constitute one of the essential pieces of evidence for the phenomena classified as sonochemistry, and they produce a number of reactions, mainly caused by OH radicals. There is less evidence of the behaviour and detection of H•, even though, in principle, it is produced in amounts equivalent to those of OH• in the primary degradation step. The two radicals, however, are rather different in chemical nature. Thus, the OH radical is known to initiate a number of reactions in solution; by contrast, the H radical can be rapidly captured by molecular oxygen. If the water contains some salt such as potassium iodide [5] or copper sulphate [6], then sonication produces other free radicals.

Effects of ultrasonic irradiation seem to have been developed on a practical rather than on a theoretical basis (critics of US regarded it as a super-agitation tool). The three rules derived from published material on sonochemistry are as follows [7]:

- Rule 1 applies to homogeneous processes, and states that the reactions which are sensitive to sonochemical effect are those that proceed via radical or radical–ion intermediates. This means that sonication can affect reactions proceeding through radicals and that ionic reactions are not likely to be modified by such irradiation.
- Rule 2 applies to heterogeneous systems, which are more complex and where reactions proceeding via ionic intermediates can be stimulated by the mechanical effects of cavitational agitation.
- Rule 3 applies to heterogeneous reactions with mixed mechanisms (i.e. radical and ionic). They will have their radical component enhanced by sonication, even though the general mechanical effect from Rule 2 may still apply.

There are two possible situations for heterogeneous systems involving two different mechanism paths. If the two mechanisms lead to the same product(s) (i.e. the process is "convergent"), the sole effect will be an increase in the overall rate. On the other hand, if the radical and ionic mechanisms lead to different products, then sonochemical switching will be possible through a favoured pathway. In such "divergent" processes, sonication actually changes the nature of the reaction products.

Concerning the subject matter of this review, we define derivatization as any chemical modification of a target compound to obtain other with suitable properties for subsequent steps of the analytical process. This modification can affect all steps of sample preparation and not only the formation of products that enhance response at the detector. Accordingly, this review contains two sections concerning the use of US in matrix modification to release analytes and those facilitating detection.

The nature of reactions in matrix modification can be varied (e.g. hydrolysis – enzymatic or non-enzymatic – depolymerization, redox), but the aim is mainly to liberate the analyte (or a part of it) for subsequent derivatization, if required, to facilitate detection analysis including derivatization to enhance sensitivity. The latter can involve more or less complicated reactions, and even only to lead the analyte to the atomic state, as is the case with organometallic compounds. Thus, the subsequent sections are divided into these two types of US-assisted reactions. All them can involve inorganic, organic and organic–inorganic species, and are implemented in discrete or continuous systems. Most exploit existing experience in non-analytical areas.

2. Ultrasound for derivatization in the analyte isolation phase of sample preparation

Reactions for matrix modification have a common denominator that they are not immediately related detection. Table 1 includes some examples of these reactions to show the applicability of ultrasound to enhance and accelerate them.

2.1. Depolymerization reactions

The literature shows a very long experience with the depolymerization effect of US on high polymers such as starch, gelatine and arabic gum [8–10] with non-analytical purposes. One of the few examples of US-assisted analytical depolymerization of organic compounds is the conversion of polysaccharides into monosac-

Table 1
Representative examples of derivatization reactions no related with detection assisted by ultrasound.

Type of reaction	Sample	Notes	Reference
Organic depolymerization	Environmental and food samples	Depolymerization of polysaccharides for determination of monosaccharides	[11,12]
Inorganic depolymerization	Aqueous samples	Depolymerization of polymeric molybdate for determination of phosphate by the Molybdenum Blue method	[13]
Redox reaction	CCl_4-saturated aqueous media	Formation of oxidizing species for degradation of CCl_4	[14–16]
Redox reaction	Water samples	Conversion of organomercurials in inorganic mercury for total and inorganic determination by CV-AAS	[17]
Redox reaction	Urine samples	Conversion of organomercurials in inorganic mercury for total and inorganic determination by CV-AAS	[18–20]
Redox reaction	Wastewater samples	Estimation of the chemical oxygen demand	[21,22]
Redox reaction	Vegetable oils	Fast oxidation of oils for correlating the time required with long-time oxidative stability	[23]
Redox reaction	Steel and alloys	Oxidation of Ni(II) prior to photometric determination of nickel by complexation with dimethylglyoxime	[24]
Redox reaction	Aqueous samples	Oxidation of Co(II) prior to photometric determination of cobalt by complexation with salicylaldehyde thiosemicarbazone	[25]
Hydrolysis reaction	Suppositories	Hydrolysis of paracetamol and reaction with *o*-cresol for photometric determination as Indophenol Blue	[30]
Hydrolysis reaction	Strawberries	Hydrolysis of conjugated phenolic compounds for LC–DAD analysis	[31]
Enzymatic hydrolysis	Yeast, oyster and mussel tissues	Determination of trace and ultratrace levels of Se	[34]
Enzymatic hydrolysis	Mussel tissues	Multielement determination with ICP-AES	[35]
Enzymatic hydrolysis	Antarctic krill	Quantitative extraction of Se organic compounds	[36]
Enzymatic hydrolysis	Biological samples	Sample preparation prior to LC–ICP-MS analysis for mercury speciation	[37]
Enzymatic hydrolysis	Rice	Isolation of starch for structure analysis by SEC and scanning electron microscopy	[38]
Enzymatic hydrolysis	*Jatropha curcas* L. seed kernels	Enhancement of oil isolation	[39]
Enzymatic hydrolysis	Proteins in solution or gel	Digestion of proteins for MS analysis of peptides	[40]
Enzymatic hydrolysis	Human hair	Determination of illicit drugs after pronase E hydrolysis	[41]
Enzymatic hydrolysis	Urine	Analysis of conjugated female hormones	[42]
Enzymatic hydrolysis	Fish plasma	Acceleration of protein denaturation, reduction, alkylation and enzymatic digestion	[43]

CV-AAS, cold-vapour atomic absorption spectrometry; LC–DAD, liquid chromatography diode array detection; ICP-AES, inductively coupled plasma atomic emission spectrometry; LC–ICP-MS, liquid chromatography inductively coupled plasma mass spectrometry.

charides prior to the determination of total carbohydrates, which requires very drastic conditions and long reaction times in the absence of US, but is dramatically shortened with the aid of this energy [11]. Nevertheless, more in depth research is required to clarify the influence of the US-frequency to boost depolymerization and the relationship between this parameter and the acidity of the medium [12].

Also in inorganic reactions, US has been used to boost reactions involving a slow, limiting depolymerization step. This is the case with the determination of phosphate using the Molybdenum Blue method, in which the coloured complex is formed in two steps: (1) reaction of *o*-phosphate with molybdate ions in an acid medium to give molybdophosphoric acid; (2) reduction to the blue heteropolyacid by a suitable reductant (usually ascorbic acid). Application of US in both steps showed that ascorbic acid was degraded via an oxidation reaction promoted by free radicals formed during irradiation. Application of US for 15 min during the formation of the heteropolyacid was found to increase the absorbance of the solution by about 20%; however, US applications to the molybdate solution for 1 min provided the same improvement, so the limiting step was depolymerization of molybdate ions, which occurred rapidly in the presence of US [13].

2.2. Redox reactions

The formation of OH and H radicals in sonicated aqueous media accelerates or facilitates redox reactions, which are slow or unlikely in the absence of US. Four major applications of oxidation reactions widely used in analytical chemistry demonstrate the gains in using US. These include increased efficiency and shortened reaction times. These are the oxidation of inorganic species in CCl_4-saturated aqueous media [14–16], the degradation of organometallic compounds prior to the determination of the target metal [17–20], oxidation of organic matter for the determination of the chemical oxygen demand (COD) [21,22], and the fast oxidation of oils for correlating the time required with long-time oxidative stability [23]. Processing times were dramatically shortened in all instances.

A representative example of oxidation reaction prior to the formation of a detector-active product is the one that involved in the photometric determination of nickel by complexation with dimethylglyoxime, which requires the oxidation of Ni(II) by bromine, iodine, hydrogen peroxide or persulphate. The oxidant is mixed with the Ni(II) solution before adding the chelating agent; however, replacing the oxidant with US irradiation under reproducible conditions substantially increases the absorbance and precision relative to the strongest oxidant among those used for this purpose (viz. persulphate). In addition, the absence of an oxidant reduces interferentes [24]. Similar behaviour was observed in the oxidation of Co(II) to Co(III) prior to complexation with salicylaldehyde thiosemicarbazone in a continuous manifold [25].

2.3. Hydrolysis reactions

Although the earliest examples of the use of US as a substitute for phase transfer catalysis in organic addition, reactions were reported more than two decades ago and a number of such reactions have since been improved as a result [1,2,26–29], very few analytical applications exploiting this potential have been reported. One of them is a method for the determination of paracetamol where the drug is hydrolyzed to *p*-aminophenol, which reacts with *o*-cresol in alkaline medium to form Indophenol Blue [30]. The method was developed for determining the analyte in suppositories, so extraction from a toluene solution to an aqueous phase was required prior to hydrolysis and the addition reaction. All these steps were performed in a continuous manifold as that shown in Fig. 1A for liquid–liquid extraction without phase separation, which provides the multipeak recording shown in Fig. 1B. It is worth

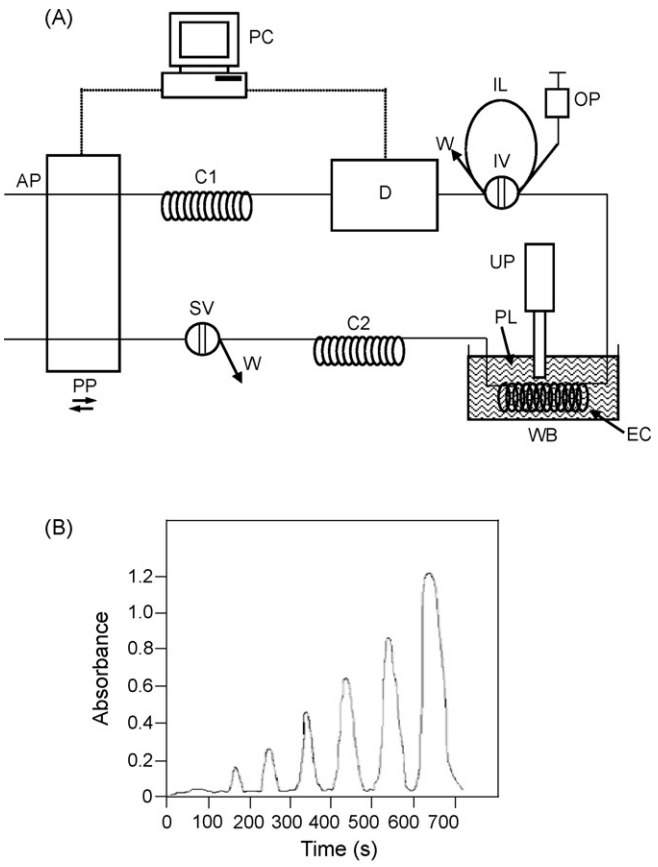

Fig. 1. (A) Flow injection manifold for continuous US-assisted liquid–liquid extraction without phase separation. AP, aqueous phase; C, coil; D, detection system; EC, extraction coil; IL, injection loop; IV, injection valve; OP, organic phase; PC, personal computer; PL, propagating liquid; PP, peristaltic pump; SV, selection valve; UP, ultrasonic probe; W, waste and WB, water bath. (B) Multi-peak recording obtained from continuous US-assisted liquid–liquid extraction of paracetamol into a basic aqueous phase containing *o*-cresol.

emphasizing that one of the main advantages of the use of US for enhancing processes implemented in a continuous fashion over that of microwave energy is the small temperature rise involved in the former case, which avoids the presence of undesirable air bubbles in the dynamic system and hence production of non-specific signals at the detector.

The US-assisted leaching of phenol compounds from strawberries with an acetone solution containing 1 M HCl and *ter*-butyl-hydroquinone facilitates the hydrolysis of the target phenols and their dissolution in the leachant, thus accelerating their removal from the matrix. A titanium probe was used to develop three 30-s cycles; the operating conditions included 50% US amplitude and 0.8 s pulses over 1 s for an overall time of 30 s per cycle. The resulting yields were similar to those obtained by maceration at 85–90 °C for 20 h, with no appreciable degradation [31], which is one of the major shortcomings of long leaching times.

2.4. Ultrasound-assisted enzymatic hydrolysis reactions

One of the most important, recent contributions of US is to accelerate hydrolysis reactions are those involving enzymes. The long-incubation time required for these reactions divides them into short incubation time (4–6 h), medium (10 h) and long (24 h); the last being the most frequent choice [32]. The enzymatic hydrolysis step can lead to either a product requiring derivatization for proper detection or an atomic state for direct detection by any atomic technique.

The earliest uses of sonication to improve enzymatic hydrolysis revealed both enchanced activity and inactivation of the enzyme action depending on the amplitude of sonication, but a clear explanation of this behaviour has not been found yet. Enhanced activity was explained by Bracey et al. [33] by a reduction in particle size of enzyme agglomerated from 51 to 2 μm after 30 min of sonication.

Enzymatic hydrolysis assisted by US has most times been used for the determination of inorganic analytes, one of the most interesting uses being in metal–metalloid speciation, where enzymatic hydrolysis in combination with ultrasonication enables the determination of trace and ultratrace levels of metals and metalloids, and their species, while preserving their chemical integrity. Enzyme probe sonication has proved a powerful choice for accelerating the hydrolysis of yeast material, oyster and mussel tissues with proteolysis enzymes for the determination of Se [34]. Total Se was released within 15 s and complete dissolution of Se species in the yeast material (viz. Se–methionine) took 30 s. No buffered medium was required to use an ultrasonic probe, and no chemicals other than water were needed. One other typical application is the US-assisted enzymatic hydrolysis of mussels for multielement determination with ICP-AES, where the use of US energy shortened the long hydrolysis time of conventional thermostatic devices (30 min as compared to 12–24 h in conventional devices) [35]. Also Siwek et al. have shown an increase in enzymatic activity in the hydrolysis of Antarctic krill with US, allowing to quantitatively extract Se organic compounds in 15 min compared with 24 h in the absence of sonication [36]. Recently, acid and enzymatic hydrolyses – both assisted by ultrasound – of biological samples before introduction into LC–ICP–MS for mercury speciation analysis have been reported. A 2-mm microtip was used to irradiate small (5 mg) samples, which required only 5-min irradiation for total removal of both organic (CH_3Hg^+) and inorganic (Hg^{2+}) mercury using either acid or enzymatic hydrolysis. Longer irradiation times caused analyte losses by volatilization [37].

Organic applications of US-assisted enzymatic hydrolysis have been focused on the past years on the digestion of solid samples for isolating target fractions. One example is the isolation of rice starch; analysis of starch structure by high-performance size-exclusion chromatography and scanning electron microscopy, which revealed no damage to the molecular structure or the starch granule surface [38]. One more example is the isolation of oil from *Jatropha curcas* L. seed kernels, where US-assisted enzymatic hydrolysis took about 2 h, while Soxhlet extraction required 24 h [39]. The application of US for a short time (60 s or less) has also facilitated proteolytic hydrolysis in both solutions and gels, thereby greatly reducing the operating time relative to conventional overnight incubation. In addition, it has enabled the identification of individual proteins by MALDI-TOF and HPLC–MS/MS [40]. Illicit drugs in human hair have also be determined by GC–MS after accelerating the hydrolysis catalysed by pronase E by ultrasonic irradiation using a common cleaner ultrasound bath [41]. The drastic shortening of the hydrolysis time (from 14 h to 30 min) clearly shows the action of ultrasound on this sample preparation step.

We used US probe assistance for the analysis of female steroid hormones as conjugated forms (mainly, glucuronides and sulphates) [42]. The method has been applied to female urine samples to assess the metabolism of these compounds. The method implements an enzymatic hydrolysis (β-glucuronidase with sulfatase activity) kinetically enhanced by ultrasonic energy (duty cycle 35%, radiation amplitude 50% of the converter applied power – 450 W) in order to generate the free steroid forms. This enables a drastic shortening of the time required for this step as compared with conventional protocols (from 12–18 h to 30 min). The reaction kinetics of the ultrasound-enhanced hydrolysis was characterized in comparison to that of the conventional protocol (Fig. 2). After hydrolysis, the free steroid hormones

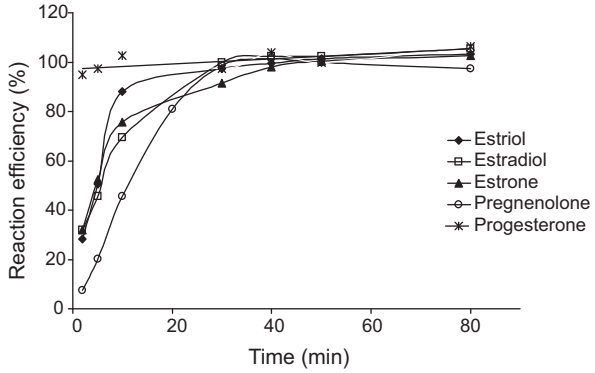

Fig. 2. Kinetics of the ultrasound-enhanced enzymatic protocol for analysis of conjugated steroids in a urine pool.

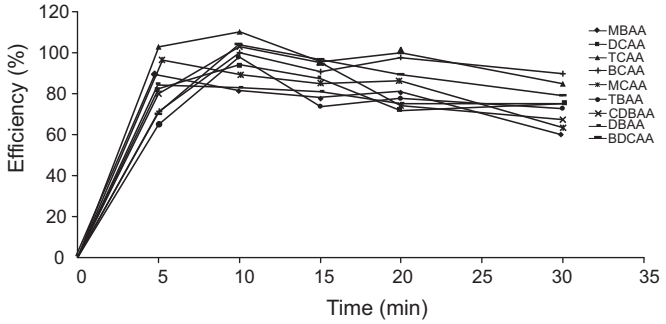

Fig. 3. Kinetics study of the extraction–derivatization step of nine haloacetic acids (HAAs). MCAA, monochloroacetic acid; MBAA: monobromoacetic acid; DCAA, dichloroacetic acid; DBAA, dibromoacetic acid; BCAA, bromochloroacetic acid; TCAA, trichloroacetic acid; TBAA, tribromoacetic acid; CDBAA, chlorodibromoacetic acid; BDCAA, bromodichloroacetic acid.

were isolated and preconcentrated by automated solid-phase extraction and the eluate was subsequently analysed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The target analytes were confirmed and quantified by multiple reaction monitoring (MRM).

A recent method with ultrasound assistance of up to three consecutive reactions has been proposed by Carreira et al. for fast protein quantification in freshwater fish plasma [43]. An ultrasonic reactor was used, first to speed up protein denaturation; then reduction (using DL-dithiothreitol) and alkylation with iodoacetamide were drastically accelerated by irradiation for 1 min each reaction. Finally, aliquots of few μl of the treated sample were taken, diluted with a trypsin solution and sonicated for two 2.5-min intervals. Formic acid was added to stop the enzymatic reaction and the solutions were dried by vacuum centrifugation prior to MALDI quantification.

3. Ultrasound in derivatization to enhance detection

In addition to enzymatic hydrolysis reactions for liberation of inorganic species led directly to atomic detectors or proteins determined by MALDI-TOF or HPLC–MS/MS, the following reactions produce derivatives that allow determination of the original analytes with or without prior individual separation.

3.1. Esterification reactions

Although the literature on enhancement of esterification by US dates back 20 years, the earliest authors gave no information about the sonication conditions. The instrumentation available was usually an ultrasonic bath designed for cleaning and degassing and with no capability for changing such conditions.

One example is derivatization of α-hydroxy acids with (+)-1-(9-fluorenyl) ethyl chloroformate for resolving enantiomers. Fransson and Ragnarsson used US to enhance this derivatization preparatory to reverse-phase high performance liquid chromatography (HPLC) to separate the analytes. Their US bath, however, was a simple model and so they did not provide any details about the gains in using this type of energy [44].

The conversion of amino acids into *N*(O,S)ethoxycarbonyl amino acid ethyl esters is significantly improved by US assistance. The derivatization reaction, developed at a microscale, constitutes the step prior to single-drop microextraction (SDME), which is followed by GC–MS [45]. The derivatization step involves mixing the sample (urine) with the reagents (ethanol–pyridine–ethylchloroformate) and sonication for 10 min. Comparative tests of the derivatization reaction of 12 amino acids assisted by stirring at room temperature, at 70 °C and under ultrasonication only, can be summarized as follows: (1) The reaction involving stirring at room temperature took a long time to complete and barely levelled off after 80 min. (2) Heating and ultrasonication considerably accelerated the reaction, the latter clearly being a better choice for fast completion of reaction. (3) Ultrasonication can expose subtle interactions and special effects of entropic and enthalpic origin. (4) The efficient removal of bubbles from the bulk solution by US is of paramount importance as bubbles are detrimental to the SDME process – by attaching the drops, they reduce the surface available for extraction and facilitate separation. (5) Ultrasonication for 10 min following 2 min vigorous stirring increased yields of the corresponding derivatives by 20–35%, depending on the particular amino acid.

More recently US probe-assistance has been used to enhance the isolation of haloacetic acids (HAAs) from vegetables with *in situ* derivatization to methyl esters prior to introduction into a gas chromatograph [46]. The target analytes were isolated by ultrasound-assisted leaching in a dynamic system, while converted into methyl esters. This dual ultrasound assistance enables displacement of the leaching equilibrium by esterification of the leached compounds together with a considerable shortening of the time for sample preparation. A plot of the kinetics study of the simultaneous ultrasound-assisted steps is shown in Fig. 3, which demonstrates that the process was completed in only 10 min (it is worth noting that the EPA method for analysis of these compounds requires at least 1 h only for quantitative derivatization). After this treatment, the esterified HAAs are transferred to a hexane phase by liquid–liquid extraction for subsequent GC–ECD analysis.

3.2. Alkylation reactions

These reactions are widely used prior to gas chromatography, mainly to decrease polarity and/or increase pressure vapour of the target analytes. Our group applied US probe-assistance for extraction and then silylation prior to gas chromatography–mass spectrometry for the characterization of the triterpenic fraction in olive leaves [47]. The target analytes were erythrodiol, uvaol, oleanolic acid and ursolic acid, although identification and relative determination of maslinic acid were also possible. Quantitative leaching was obtained with ethanol as leachant and ultrasonic assistance – duty cycle 0.5 s, output amplitude 50% of the converter applied power (450 W) – for 20 min, a very short time as compared to conventional procedures by maceration, which usually requires at least 5 h. After isolation, an aliquot of the ethanolic leachate was silylated to derivatize the analytes prior to gas chromatography–mass spectrometry analysis. Silylation as derivatization step is considered a limiting factor for the analysis of

triterpenes owing to the time required for the quantitative development of the reaction – from 30 min to 3 h. Application of ultrasound energy (duty cycle 0.4 s, output amplitude 70% of the converter, applied power 450 W) enhanced silylation kinetics and accelerated the reaction. As a result of the optimization of this step, a shortening of the derivatization time from 2 h to 5 min was achieved. The resultant method enables a considerable reduction of time to succeed in the isolation and determination of triterpenic compounds in olive leaves. Another example corresponds to a method for simultaneous determination of sterols and fatty alcohols in olive leaves and drupes [48]. This was based on ultrasound-assisted extraction and derivatization prior to individual identification–quantification by chromatographic separation and mass spectrometry detection (single ion monitoring mode). The sample preparation procedure involved five steps. Three of these steps were assisted by ultrasound, namely: leaching of the raw material – duty cycle 10% (viz. ultrasound application 0.1 s/s), output amplitude 10% of the converter, applied power 50 W, position of the ultrasonic probe-tip 1 cm from the bath bottom, and irradiation time 10 min, saponification of the leachate – output amplitude 45% of the converter, applied power 200 W, duty cycle 50% an irradiation time 10 min – and silylation of the target analytes – output amplitude 40% of the converter, applied power 180 W, duty cycle 50% and irradiation time 10 min. Ultrasonic assistance achieves: (i) to accelerate the leaching step from 24 h to 10 min, (ii) to shorten the time for the saponification step from 2 h to 10 min with no degradation of analytes, and (iii) to decrease the derivatization time from 120 to 10 min [49].

Estrogenic compounds can be derivatizated in a cup horn booster and determined by GC–MS. Derivatization of estrogens is usually carried out at temperatures between 60 and 75 °C, in sand, water or oil baths, in heater blocks or even in the ovens of gas chromatographs [50–53]. However, these derivatization procedures are time-consuming (30–90 min). To accelerate this derivatization step, Zuo et al. [54] used microwave energy and obtained optimum derivatization after 1 min. The ultrasonic-assisted derivatization was performed with 125 μL of pyridine and 25 μL of BSTFA + 1%TMCS for 10 min under 80% of power and nine cycles in a cup booster ultrasound bath [55]. Sonication time had a positive effect on the derivatization step, better LODs were obtained after 10 min sonication (0.35–1.66 ng/L). However, the LODs obtained after only 1 min of sonication (0.37–6.10 ng/L) were similar to most of the LODs found in the literature, except when compared with the results obtained by Zuo et al., who reported even lower LODs for GC–MS (0.02–0.1 ng/L). The procedure time was also decreased using a cup horn sonication device that allows simultaneous derivatization of at least three samples.

Phenols can be involved in the formation of a number of derivatives such as ethers, esters, and/or bromine and silyl derivatives to improve their chromatographic characteristics. One simple, efficient derivatization reaction is acetylation by acetic anhydride in an alkaline medium, which has been used for automatic determination of phenolic compounds (viz. phenol and *o*-, *m*- and *p*-cresol). The procedure, developed in the approach shown in Fig. 4, involves three main steps, namely: (a) US irradiation to accelerate the derivatization reaction; (b) pervaporation [56] to remove the products of the target analytes from the aqueous matrix; and (c) GC to separate the individual products, followed by flame ionization detection (FID) [57]. The sample was pumped into the reaction chamber together with the reagent and kept in it for US irradiation for the required time; then, the mixture was moved to the lower chamber, from which a He stream transfers the volatile products to the separation column. The multivariate optimization design used in step (a) showed the probe distance to the reaction chamber to be the most important factor, followed by the pulse duration and radiation amplitude. The reaction

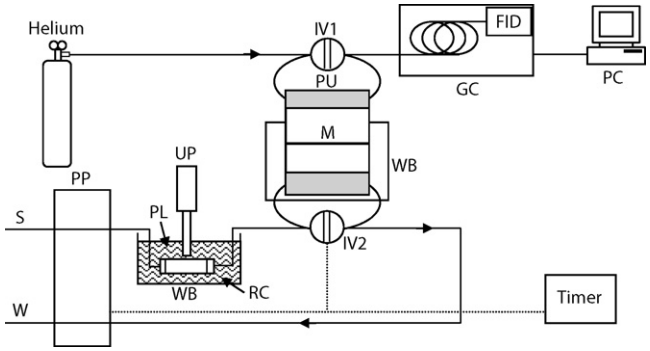

Fig. 4. Experimental set-up for the fully automated continuous determination of phenols. FID, flame ionization detector; GC, gas chromatography; IV1, HPLC injection valve; IV2, low-pressure injection valve; M, membrane; PC, personal computer; PL, propagating liquid; PP, peristaltic pump; PU, pervaporation unit; RC, reaction chamber; S, sample; UP, ultrasonic probe; W, waste; and WB, water bath.

time was more than halved relative to the absence of US irradiation and to the use of microwaves under the optimal working conditions.

3.3. Complex formation

Although US seemingly facilitates complex formation reactions (e.g. in the method for the determination of Ni by the formation of Ni–DMG complex, where US favours Ni(II) oxidation [24], or that for phosphate based on the formation of the heteropolyacid complex, where US accelerates depolymerization [13], the potential effect of US on this type of equilibrium has not yet examined. The only reported example to the authors' knowledge deals with the liquid–liquid extraction of Fe(II) from an aqueous sample to an *o*-phenanthroline–dichlorometane phase, which is not significantly improved by US assistance [58].

4. Conclusions

Ultrasound is a ubiquitous form of energy known and used, albeit to a rather disparate extent, in many areas of chemistry. Ultrasound can facilitate almost every step of the analytical process or even preliminary operations.

Ultrasound is a well- and long-established technique both in basic research and a wide range of applications. As a result much of the equipment is readily available, and this makes it attractive to analytical chemist in the many disciplines of the science. At present, the use of US in analytical derivatization reactions is scarce. There is considerable potential for this technique given both achievements in this field and the understanding of basic characteristics of US energy. The latter knowledge involves the following between the most significant aspects:

(1) *Ultrasound frequency*: It has been widely demonstrated that low frequencies, close to 20 kHz, enhance cavitation, which is the source of the dramatic effect of ultrasonic power on chemical reactivity. However, higher frequencies are advantageous when radical formation is the key to facilitating, accelerating or making possible a given reaction.
(2) *Ultrasound power and intensity*: The first requirement for attaining the level of US required to cause chemical effects on a reaction is that sufficient acoustic energy be supplied to overcome the cavitation threshold of the medium. Once the threshold has been exceeded, the region of cavitation around the radiating source, the "cavitational zone" will increase with increasing intensity; also one might expect to sonochemical

rate to increase in parallel. Nevertheless, a limiting value can be reached, beyond which the sonochemical rate will decrease by increasing power [59,60]. The explanation for these effects of US power lies in the production of a large number of cavitation bubbles that act as a "cushion" to dampen the efficiency of energy transmission into the medium.

(3) *Solvent, temperature and pressure*: The choice of solvent and the bulk working temperature are two significantly important, often interrelated variables. An increased solvent vapour decreases the maximum bubble collapse temperature and pressure. Hence, for reactions where cavitational collapse is the primary source of sonochemical activation, a low-bulk temperature is preferred. Conversely, for a reaction requiring elevated temperature, a high-boiling solvent will be more appropriate. Application of an external pressure to a reaction system, which increases the hydrostac pressure of the liquid, increases the energy required to initiate cavitation. In practical terms, if such a threshold energy can be exceeded with the available irradiation source, then raising the hydrostatic pressure will increase the sonochemical effect as the maximum temperatures and pressures experienced during bubble collapse will be higher under these conditions.

Ultrasonication dramatically accelerates the derivatization reactions. A few examples include: hydrolysis times reduced from 18 h to 30 min for hydrolysis [41]; esterification complete in 10 min; rather than 80 min to 10 min for esterification [45]; a 24-fold reduction in time for silylation [47] and a 12-fold reduction in time for saponification [48]. This field merits further study by investigators interested in reaction mechanisms and in enlarging the scope of sonochemistry.

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