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POINT OF VIEW

Volatile Species Generation for Trace Element and Speciation Analysis – Current State and Future Perspectives

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The current concept of trace element analysis relies mainly on liquid nebulization to atomic spectrometric detectors characterized by a low sample introduction efficiency, typically reaching 5–8%. This is the bottleneck of all the common nebulizers regardless of the detector employed. As a consequence, more efficient approaches to analyte introduction into element-specific detectors, including atomic absorption (AAS), atomic fluorescence (AFS) and inductively coupled plasma (ICP) with either optical emission (OES) or mass spectrometry (MS) detection, have been sought.

One of the strategies is volatile species generation (VSG) – a group of techniques based on analyte derivatization in order to form a volatile compound prior to spectrometric detection.[1] Selective analyte conversion from liquid to gas phase results not only in enhanced analyte introduction efficiency but also in separation of the analyte from the sample matrix, leading to a reduced risk of interference.[1] Additionally, VSG can employ substantially higher sample introduction flow rates than nebulization, further improving the resulting detection power. In principle, conversion of an analyte to the corresponding volatile compound can be achieved in three ways: chemically (C-VSG),[1,2] electrochemically (Ec-VSG)[3] or photochemically (P-VSG).[4]

Presently, hydride generation (HG) is the dominant and most explored VSG technique. However, HG is restricted to hydride-forming elements only, including thus *ca* eight analytes such as As, Se, Sb, Bi, Pb, Sn, Ge and Te.[1] C-VSG, *i.e.* chemical reduction by means of $NaBH_4$, is the most common approach to HG. Under the optimized conditions, the efficiency of chemical hydride generation (C-HG) reaches 100%, making this approach attractive for routine measurements. Owing to the benefits of the HG technique, effort has been made to expand the number of elements detectable by means of VSG to include volatile compounds other than binary hydrides. Generation of cold mercury vapors,[1] *i.e.* free Hg atoms, is another example of a routinely used VSG technique, the popularity of which is comparable to that of HG. VSG-based approaches have been intensively explored in the last 15–20 years in order to make use of the benefits offered by VSG for elements other than hydride-forming elements and mercury. C-VSG and P-VSG have been employed as the most dominant strategies.[2] Presently, successful VSG of more than 40 elements including transition and noble metals and even non-metals (S, P, Si, F, Cl, Br, I) has been reported.[2] The volatile species generated are of different chemical structures including, *e.g.*, carbonyls (Fe, Co, Ni, Mo, W), alkyl-halides (Cl, Br, I), free atoms (Cd), nanoparticles (Ag, Au, Cu, Pd), chelates (Pd) and oxides (Os).

The recent challenges in the field of total element content determination at ultratrace levels by means of VSG lie in: 1) extending the VSG technique to new elements; 2) identifying the structure of the volatile species generated; 3) understanding the mechanisms of the VSG step; and 4) reliably quantifying the generation efficiency. The importance of understanding the VSG mechanisms must be highlighted. The insights into VSG processes not only allow further optimization of the VSG step if necessary but also lead

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to trouble-free applications of VSG-based methods including interference control in real sample matrices. Efficiency of the VSG step is a crucial parameter characterizing VSG-based methods. Its accurate and reliable quantification is absolutely crucial for assessing the performance and competitiveness of the VSG method. There are several approaches to quantifying VSG efficiency.[5] The simplest one is based on determination of residual analyte in the liquid waste after the VSG step. However, it must be emphasized that this method can significantly overestimate the results since it is assumed that all the analyte not found in the waste liquid has been converted to the gas phase. This is not true if significant losses (deposits) of analyte occur on the inner surfaces of the volatile species generator, waste tubing, *etc.* It has been proven many times that the deposited fraction might reach tens of %, thus bringing huge uncertainty to the VSG efficiency results and leading to overestimation of VSG step performance. For that reason, other approaches are recommended to quantify VSG efficiency correctly. One of them is the comparison of VSG with liquid nebulization, both to be coupled to the same detector, most commonly ICP-MS. Providing the same sample loop volume is used and plasma conditions are identical for both approaches, *i.e.* VSG and nebulization are run simultaneously, one being used for analyte introduction while the other one operates with a blank, reliable data can be obtained. Using this approach, the efficiency of the nebulizer must be determined at first. Subsequently, a sensitivity enhancement factor is determined for the VSG step compared to liquid nebulization. VSG efficiency can be easily calculated as the product of these two values. The use of a radioactive indicator is another way to quantify VSG efficiency reliably. It not only allows the determination of VSG efficiency but also brings a detailed insight into the distribution of the fraction of analyte not converted into a volatile species between the liquid waste and apparatus components. Recently, VSG of cadmium was optimized using $NaBH_4$ as a reductant in the presence of Cr^{3+} and KCN as additives. Efficiency of the VSG step reached 60% as proven by comparison of VSG and liquid nebulization as well as using a ^{115m}Cd radioactive indicator.[6]

Apart from determination of the total element content, VSG in combination with atomic spectrometric detectors can also be employed for speciation analysis, providing the individual species are separated from each other prior to their introduction into the detector. There are three ways to employ VSG for speciation analysis.[1] Since atomization of the analyte-containing molecular structures is dictated by the principle of the atomic spectrometric detectors, followed by excitation (AFS, ICP-OES) or even ionization processes (ICP-MS), it is obvious that the information on the analyte's molecular structure is lost when these detectors are used. As a consequence, either prior separation of the species has to be performed or the selectivity of the VSG step has to be ensured.

The latter condition can be met when using selective generation of volatile species.[1] In this case, separation of the analyte species is achieved by the selectivity of their conversion from ionic species present in the sample solution to the volatile products in the gas phase. This strategy is widely used for oxidation state speciation analysis, making use of the fact that only lower oxidation state species are active for VSG whereas the higher oxidation state species can be converted to a volatile compound after pre-reduction. The content of the lower oxidation state is determined in the first sample aliquot while total element content is determined in the second, after pre-reduction. The concentration of analyte species in the higher oxidation state is subsequently calculated from the difference. Another example is the determination of toxic inorganic arsenic species (iAs, *e.g.* sum of As^{3+} and As^{5+}) in rice by employing HG with ICP-MS detection without any chromatographic separation.[7] This method uses 5 mol L^{-1} HCl and reduction by $NaBH_4$ to selectively convert iAs to AsH_3 while the non-toxic As species such as dimethylarsinic acid are not converted to volatile As species under the generation conditions used.[7]

In contrast to the selective generation of volatile species discussed above, speciation information is preserved in and can be deduced from the retention time of individual species with the other two approaches.

Post-column VSG serves to convert analyte species to corresponding volatile species after their chromatographic separation by high-performance liquid chromatography (HPLC) and prior to their spectrometric detection. A VSG step is included in the procedure solely to increase analyte introduction efficiency into the detector as well as to decrease the risk of interference due to separation of the analyte

from the mobile phase. Post-column hydride generation coupled to an atomic fluorescence spectrometer (HPLC-HG-AFS) has been successfully applied to determine toxicologically relevant arsenic species,[8] equal generation efficiency being obtained from arsenite, arsenate, methylarsonate and dimethylarsinate. As a consequence, just one standard is required to quantify all four species and the time for calibration is shortened.[8] A similar strategy but with an ICP-MS detector (HPLC-VSG-ICP-MS) has been applied to speciation analysis of mercury including inorganic mercury (Hg^{2+}), methylmercury ($MeHg^{+}$) and ethylmercury ($EtHg^{+}$).[9] Owing to the post-column VSG step in the HPLC-VSG-ICP-MS method, the sensitivity is enhanced 30–40 times and limits of detection improved by a factor of five in comparison to the setup without a VSG step (HPLC-ICP-MS). Additionally, the use of a VSG step substantially reduces the effect of the organic mobile phase on ICP-MS sensitivity if gradient elution is required.[9]

The last approach is generation of substituted (usually alkylated) volatile species.[1] It is based on the conversion of all analyte forms to volatile species that are subsequently separated by gas chromatography. The procedure typically employs a cryogenic trap (CT) as a simple example of a GC column. The CT consists of a glass U-tube either empty or packed with chromatographic support. Trapped analyte species are separated according to their boiling points and transported to the detector. The CT serves not only as a separation unit but also as a preconcentration device. This approach to speciation analysis is widely used with AAS and AFS as well as ICP-MS detection.[1] A method for the determination of the toxicologically important species of arsenic (iAs, mono- (MAs) and dimethylated arsenic (DMAs)) in whole blood or plasma, from 50–100 µL of sample without extraction, has been developed and validated, being based on HG-CT-ICP-MS. This is the only existing method capable of As speciation analysis at pg mL^{-1} concentrations corresponding to the normal level of exposure.[10] A method for the speciation analysis of the three main species of germanium, namely inorganic germanium (iGe), monomethyl germanium (MMGe) and dimethyl germanium (DMGe), in environmental waters has been developed using the same HG-CT-ICP-MS/MS approach and reaches the limits of detection at fg mL^{-1} Ge concentration levels.[11] The applicability of the method has been demonstrated on speciation analysis of Ge in lake water.[12] The benefits of this approach, based on VSG coupled to CT separation/preconcentration and atomic spectrometric detection, include the possibility of direct speciation analysis in samples with a complex biological matrix (cell lysates, whole blood, *etc.*) as well as minimum sample pretreatment. Extraction of the species which is typically unavoidable with the techniques based on HPLC separation is not required when using a VSG-CT approach. The absence of a species extraction step reduces the risk of undesired species transmutations during sample treatment (extraction) performed prior to subsequent HPLC separation. However, the VSG step itself can also be a source of unwanted changes in speciation information. This behavior is strongly analyte-dependent and related to the stability of the volatile species generated. Clear evidence of severe inter-species changes during VSG of Hg from HCl/$NaBH_4$ and Tris buffer/$NaBH_4$ environments due to de-alkylation has been reported.[13] This shows a serious limitation when applying the approach using the generation of substituted hydrides (VSG-CT-ICP-MS) in the case of Hg. For illustration, methyl mercury hydride (MeHgH) was de-methylated to Hg^0 by 45% and EtHgH hydride de-ethylated to Hg^0 by 71%; only Hg^0 as a volatile product was observed when generating species from phenylmercury. These artifacts could only be overcome by using post-column VSG instead of generation of substituted volatile species. Employing the HPLC-VSG-ICP-MS approach has thus been found to be applicable to precise and accurate Hg speciation analysis[9] as already discussed above. On the contrary, no risk of inter-species changes is observed in the case of VSG-CT-based speciation analysis of As[10] and Ge.[11]

It can be concluded that VSG is a useful tool also in the field of element speciation analysis. With post-column VSG, the separation of analyte species is realized by chromatography (HPLC) while the subsequent VSG step improves the sensitivity of the detector and decreases the risk of matrix interference. The experimental arrangement is quite simple and user-friendly, with low risk of analytical artifacts. Selective VSG and generation of substituted volatile species are “non-chromatographic” approaches to speciation analysis. Careful selection of VSG conditions leads to selective generation of just one analyte species from the mixture of species of the same element present in the sample. The use of a CT is responsible

for separation of the species and their release one by one into the detector in the case of generation of substituted volatile species. Unlike post-column generation, both of these approaches place higher demands on the operator's experience. Selectivity of the VSG conditions employed must be secured and inter-species changes during VSG step have to be avoided, respectively.

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