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Determination of thiolic compounds as mercury complexes by cold vapor atomic absorption spectrometry and its application to wines

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Abstract

We report on the application of a commercially available mercury analyzer, which is based on vapour generation of Hg^0 by $NaBH_4$ reduction and atomic absorption detection, to the quantification and characterization of –SH groups and its application to wine samples. The behaviour of Hg(II) and thiol–Hg(II) (RS–Hg) complexes at nanomolar level (RS = L-cysteine, DL-penicillamine, *N*-acetyl penicillamine, glutathione, cysteinylglycine, homocysteine) has been studied following their reduction with alkaline $NaBH_4$ to give Hg^0. In the absence of thiol–Hg(II) is quantitatively converted to Hg^0 by stoichiometric amount of $NaBH_4$ (reaction ratio 1/4 mole $NaBH_4$/mole Hg), while the complete reduction of Hg(II)–thiol complexes to Hg^0 requires molar excess of $NaBH_4$ up to six orders of magnitude, depending on the type of complex and on the pK_a of the thiolic group. Under an appropriate excess of reductant, Hg(II) and its thiol complexes are not distinguishable giving the same response. These properties allow the discrimination of Hg(II) from Hg(II)–thiol complexes without any preliminary separation and the quantification of thiol groups. Instrumental detection limits are as low as 2.5 pg, permitting sample dilution, therefore, minimizing the risk of possible interferences occurring with complex real matrices.

The method has been applied to quantification of thiol groups in wine samples. Comparison with results obtained by HPLC coupled to atomic fluorescence detection confirmed the promising potentialities of the method.

Keywords: Chemical vapor generation; Atomic absorption spectrometry; Mercury–thiol complexes; Sulfhydryl groups in wine; Tetrahydroborate

1. Introduction

Thiolic compounds are low molecular or high molecular, ubiquitous molecules widely studied in many research areas, such as geochemistry [1], biochemistry [2,3] and metabolomics [4], nanotechnology [5], medicine [6] environmental [7] and food chemistry [8–10]. They are well known to play fundamental structural and functional roles in protein chemistry, being located mainly within the active site of many enzymes and directly involved in catalysis [11].

Heavy metals have a high affinity for –SH groups and the affinity of mercury, in particular, is known to be very high in the pH range of 1–13 for low molecular weight compounds (such as gluthatione, cysteine, homocysteine) and for macromolecules (proteins, humic matter) [12]. The interaction of heavy metals and, in particular, mercury with essential thiol groups of enzymes and proteins is the basis of the mechanism of their toxic activity [13–19].

The high affinity of inorganic (Hg^{2+}) and organic mercury cations (RHg^+, where R = CH_3, phenyl, e.g.) for –SH groups

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has been used for analytical and diagnostic purposes. Hg^{2+} has been proposed for amperometric [20] and conductometric [21] titration in order to determine thiols in a concentration range of 10^{-3} to 10^{-5} mol L^{-1}; RHg^{+} has been used as biochemical molecular probe for active sulfhydryl groups [12].

A new sensitive and selective method for determination of thiol groups at nanomolar level has been developed in our laboratory [22]. It is based on the cold vapour (CV) generation technique coupled with a laboratory assembled atomic fluorescence spectrometric (AFS) detector for the characterization of Hg(II)–thiol and Hg(II)–protein complexes [22,23]. The selective reduction of inorganic Hg(II) and its thiolic complexes by varying the concentration of alkaline $NaBH_4$ combined with AFS detection of the evolved Hg^0 revealed an interesting and sensitive analytical tool for the investigation and characterization of Hg(II)–thiol complexes such as the thiolic proteins ovalbumin, hemoglobin, glyceraldehyde-3-phosphate-dehydrogenase, aldolase, pyruvate kinase, hexokinase, lactate dehydrogenase, alcohol dehydrogenase, creatine phosphokinase, lysozyme and cytochrome *C* [23].

In this paper, the cysteine–, glutathione–, penicillamine–mercury complexes, previously studied by CV-AFS, and cystein–glycine–, homocysteine– and *N*-acetyl penicillamine–mercury complexes has been investigated by flow injection cold vapor atomic absorption spectrometry (FI-CV-AAS), which integrates flow injection mercury cold vapour generation with a very sensitive atomic absorption detector. FI-CV-AAS has been adapted for the characterization of Hg(II)–thiol complexes by reduction and titration curves and it has been applied to the determination of total thiolic compounds in wines.

2. Experimental

2.1. Chemicals

Working solutions of inorganic Hg(II) in a form of $Hg(NO_3)_2$ were prepared by serial dilutions of the stock solution (1000 ± 5 μg mL^{-1}, BDH Laboratory Supplies, Poole, England) just before use. Stock solutions of thiols (L-cysteine, CYS 30119, reduced glutathione, GSH G4251, DL-penicillamine, PSH 76410, *N*-acetyl-D-penicillamine, NAP 01423, cysteinyl-glycine, Cys-Gly C0166, homocysteine, HCys H4628, ethyl-2-mercaptopropionate, Et-2-PrSH W327905 from Fluka–Sigma–Aldrich, Chemical Co., Milan, Italy), were prepared as previously reported [22,23].

Stock and working solutions of $NaBH_4$ were prepared as previously reported [22,23].

Water purified with a Milli-Q system (Millipore Filter Co., Bedford MA, USA) was used in all the operations.

2.2. Working solutions

All the working solutions containing Hg(II) and/or thiols at nanomolar level ($(5–300) \times 10^{-9}$ mol L^{-1}) are prepared just before use by proper dilutions with a solution containing 10^{-2} mol L^{-1} sodium phosphate buffer pH (7.4, 5) $\times 10^{-2}$ mol L^{-1} NaCl (indicated as PBS/NaCl). The addition of NaCl prevents the formation of insoluble mercury hydroxides through the formation of mercury–chloride complexes.

Solutions containing Hg(II) and thiol, at the desired molar ratio, lead to the formation of Hg(II)–thiol complexes. Since the reaction of inorganic Hg(II) with sulfhydryl groups is instantaneous at room temperature no incubation time is required before measurements. The solutions were analyzed within 1 h from preparation.

2.3. Hg(II)–thiol titrations

Titrations were performed for thiols by processing separate solutions each containing the same concentration of Hg(II), typically 4×10^{-8} mol L^{-1}, and increasing amounts of the thiol, typically in the range of $(0–2) \times 10^{-7}$ mol L^{-1}, in order to realize different thiol/mercury molar ratios. Solutions were prepared in PBS/NaCl in 10 mL test tubes, loaded in the autosampler and analyzed within 15 min. A total of 10 mL were enough for three replicates measurements and the entire titration required about 15 min.

2.4. Sample collection and storage

A total of 10 mL of each wine sample ($N = 8$ wine samples examined) were sampled from original glass bottles, kept tightly closed in glass test tubes in the dark at 4 °C, and analyzed within 48 h from sampling. Mercury titrations for total –SH determination were performed with wine samples diluted 50 times with PBS/NaCl. All analyses were performed in November 2006. Reduction curve were performed by analyzing a 200 mL PBS/NaCl containing 5×10^{-8} mol L^{-1} Hg(II) and 480 μL of wine.

2.5. FI-CV-AAS analyzer

The Perkin-Elmer Flow Injection Mercury System (FIMS-100) equipped with an AS-91 autosampler, was employed in the conventional configuration for mercury–thiol titrations (Fig. 1, A configuration) with an injection loop of 200 μL, the peristaltic pump operated at 40 rpm, corresponding to a measured flow rate of 3.5 mL min^{-1}. $NaBH_4$ in 0.1 mol L^{-1} NaOH was employed as reducing agent at a concentration ranging between 10^{-8} and 3×10^{-4} mol L^{-1} (see results). Sample medium and carrier was PBS/NaCl.

For reduction curves FIMS-100 configuration was modified connecting the autosampler output to the reductant line (Fig. 1, B configuration). In this configuration the autosampler delivers a sequence of 15 solutions of $NaBH_4$, 0.1 mol L^{-1} NaOH, $NaBH_4$ concentrations ranging between 3×10^{-9} mol L^{-1} and 1 mol L^{-1}. The sample loop is loaded in continuous in the "fill" position of the injection valve. In this configuration the sample solution after loop loading could be recycled, limiting the sample consumption only to the injected quantity multiplied for the number of injections. A 30 cm × 0.75 mm i.d. reduction coil has been employed [22].