FAST SYNTHESIS AND SEPARATION OF THE ARSENOGLUTATHIONE COMPLEXES

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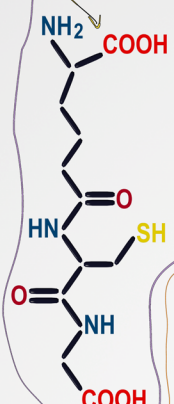
IMPORTANCE OF GLUTATHIONE

Glutathione (GSH) in its reduced form is a tripeptide consisting of L-glutamine, L-cysteine, and glycine. It plays an important role in protecting cell components from free radicals, peroxides, lipid peroxides and heavy metals. The molecule is present in every living organism where it acts as a central antioxidant. Glutathione has a key role in the cycle of the transport of an acyl group (transport of the aminoacids in kidney) and is a reducing agent in some enzymatic processes. It supports enzymatic methylation of inorganic arsenic (iAs^{III}) to monomethylarsonous acid ($MMAs^{III}$), monomethylarsonic acid ($MMAs^{V}$), dimethylarsinous acid ($DMAs^{III}$), and dimethylarsinic acid ($DMAs^{V}$)[1]. Complexes of arsenic compounds and glutathione are very unstable and tend to undergo a decomposition to simple organoarsenic species and free glutathione during chromatographic separation.[2]

REACTIONS

The free thiol group (-SH) in the cystein of this unique tripeptide makes the glutathione a trapper for the arsenic and organoarsenic compounds.



According to Feldmann et al.[2] the complexes are generated during the reaction of the trivalent arsenic species: iAs^{III}, $MMAs^{III}$ and $DMAs^{III}$ with glutathione. As the pentavalent $MMAs^{V}$ and $DMAs^{V}$ are used for the reaction the excess of the GSH is applied.

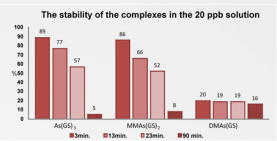
$$iAs^{III} + 3GSH \rightarrow As^{III}(GS)_3 + 3H_2O$$

$$MMAs^{V} + 4GSH \rightarrow GS\text{-}SG + MMAs^{III}(GS)_2 + 3H_2O$$

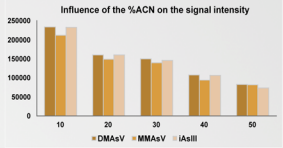
$$DMAs^{V} + 3GSH \rightarrow GS\text{-}SG + DMAs^{III}(GS) + 2H_2O$$

PROBLEMS

The resulting complexes are very unstable at room temperature. They undergo fast decomposition even at 5ºC when diluted and even during the chromatographic separation.[2]



The gradient reverse phase separation is not perfect as the content of the organic solvent influences the signal of arsenic compounds.



SOLUTION

FAST SYNTHESIS TO OBTAIN STANDARDS

A 1000 ppm solutions of iAs^{III}, $MMAs^{V}$ and $DMAs^{V}$ in deionized water (DIW) were used to prepare the stock reaction solutions with defined concentrations: 100 ppm, 200 ppm, 300 ppm As of each specie in DIW. The glutathione solution was prepared in 5 mM TCEP in DIW. The both solutions were mixed in ratio 1:1 achieving final and desired concentration of As: 50 ppm, 100 ppm and 150 ppm, and molar ratio GSH to As: 1:50, 1:100, 1:300. The reactions were carried out at 25 ºC for 24 hours and then stored at 8 ºC in a fridge.

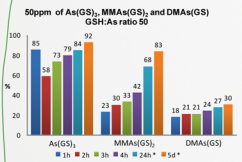
DETECTION was carried out using Agilent 7700x inductively coupled plasma mass spectrometer (ICP-MS) with ASX-500 autsampler, equipped with a Micro-Mist concentric nebulizer and High Matrix Interface.

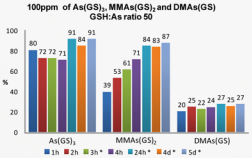
ISOCRATIC SEPARATION

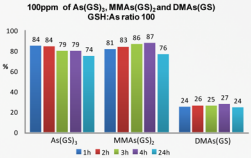
The separation was carried out on the Agilent Infinity HPLC System equipped with thermostated autosampler and column compartment. The Aeris widepore 3.6u XB-C18 precolumn and separation column (250 x 2.10 mm) were used to separate the arsenocomplexes in isocratic mode. The injection volume was 25 µl. The column compartment and the autosampler temperature was set: 5ºC. Mobile phases (MP) used: MPA: 2% acetonitrile (ACN), 0.1% formic acid (FA) in DIW; MPB: 80% ACN, 0.1% FA in DIW. Isocratic mode: 0-10 min: 5% B.
The reaction samples were diluted to 2 ppm and then to 20 ppb of each arsino-glutathione specie prior to the analysis. The dilutions were carried out in the MPA.

Arsenic was detected at m/z 75, in collision cell mode (He 3.5 ml/min). As the organic solvent was used to elute fractions an appropriate torch (ID 1.5 mm) was installed. The torch position, gas flows and lens voltages were optimized to give maximum signal to noise ratio. 200ppb Tellurium solution in 2% HNO_3 was mixed to the column effluent at ratio 1:2.5 used as an internal standard.

RESULTS



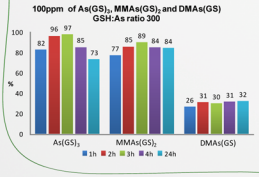


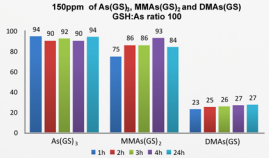


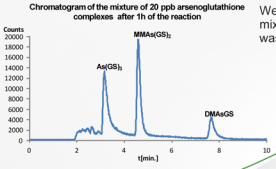
* One measurement only

The isocratic conditions of separation of the complexes carried out in a column with the reverse phase appeared to be perfect to achieve elution of $As(GS)_3$, $MMAs(GS)_2$ and DMAs(GS). The signals of the analysed arsenoglutathione complexes are very well separated within 10 minutes.

The synthesis of the complexes of arsenic species with glutathione was tested with several GSH:As ratios and As concentrations. The results suggest that the highest yield of the reaction for $As(GS)_3$, $MMAs(GS)_2$ can be obtained with the 100 ppm of iAsIII, $MMAs^{V}$ and $DMAs^{V}$ with the ratio GSH:As of 100.
In all cases the yield of the synthesis of DMAs(GS) was at the lowest level between 18% and 32%.







We established that the recovery of the mixture of the arsenic species from the column was between 80-95%.

CONCLUSION

The synthesis of the complexes using 100 ppm and 150 ppm organo-arsenic species with GSH:As ratios 100 and 300 are efficient within just 1 h in the case of $As(GS)_3$ and $MMAs(GS)_2$. The complexes in the stock solutions are stable even after one day in all cases.

We succeeded in establishing fast and reliable method for the separation and estimation of the particular complex content using isocratic mode. The big advantage is the fact that we eliminated the influence of organic solvent on the intensity of arsenic signals in ICP-MS.

Do you know that?

Arsenic troxide is used as a drug in the chemotherapy treatment for a type of acute promyelocytic leukaemia (APL).
Glutathione is combined with certain chemotherapy drugs because it decreases the toxic side effects of these drugs.
In cosmetics the glutathione injections are used to whiten up a skin.

LITERATURE

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ACKNOWLEDGEMENT

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