Development of a SIMS compatible internal standard spiked resin for quantitative bio-imaging of biological samples by laser ablation ICP-MS: an application to uranium contaminated kidney samples

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Context

It would be desirable that both samples and calibrations standards could be shared by different imaging techniques. The aim of this work is to develop a sample/standard preparation methodology compatible with laser ablation and Secondary-Ion Mass Spectrometry (SIMS) analyses for a complementary analysis of uranium distribution in kidney with both techniques. On the other hand, a novel approach based on internal standard doped resin was carried out in order to incorporate a more feasible IS than ^{13}C (1) . Therefore, thulium spiked pure EPON resin was synthetized and employed to embed dehydrated samples and doped kidney homogenates based on the chemical sample preparation protocol of biological samples for SIMS analysis (2, 3). Nevertheless, the chemical dehydration protocol was adapted to the viscous and liquid state of the homogenates. In parallel, in order to replace the use of organ homogenates for calibration standards (laborious task and required access to animal facilities), uranium spiked resin was also tested. Then, serial thin and ultra-thin sections were cut with microtome and the homogeneity of internal standard was evaluated by randomly analysing small selected areas by LA-ICP-MS and SIMS. Laser ablation conditions were optimized to achieve complete sample consumption of the tissue whilst minimizing the penetration into the glass slide.

Experimental procedure

- ☺ Localisation
- ☺ Quantification
- 😐 Resolution > 5 µm
- ☺ Quantification
- ☺ Non-Specific sample prep.
- 😐 Sample size ≈ mm up to cm

Complementary analytical techniques

Improvement: Sample sharing

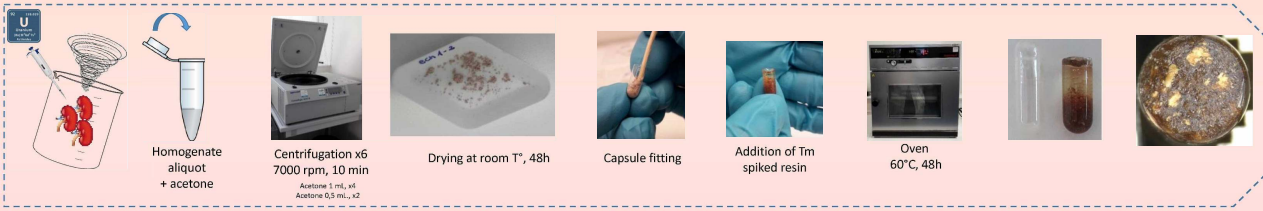
- ☺ Micro localisation
- ☺ Resolution ≈ 1 µm
- 😐 Quantification relative
- 😐 Sample size ≈ mm

😐 Specific sample prep. (dehydration + resin embedding)

Indispensable condition

Given the constraints imposed by these both systems, the preparation of LA-ICP-MS standards was adapted to the technical requirements of SIMS.
For this, the first step is the dehydration of uranium spiked kidney homogenates (see poster Nagore GRIJALBA et al.).

Test 1 = dehydration and resin embedding of uranium spiked kidney homogenates (laser ablation standards). The resin is spiked with Tm as IS.*

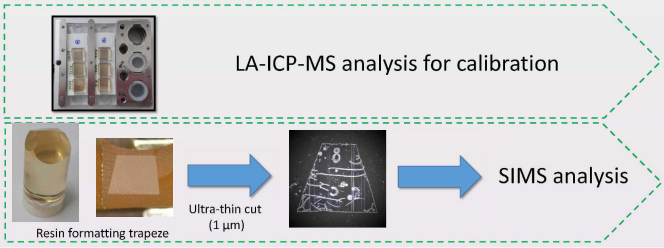


Resin is wet
Non homogeneous distribution
No flat surface

* SIMS chemical dehydration protocol was adapted to the physical properties of the kidney homogenates

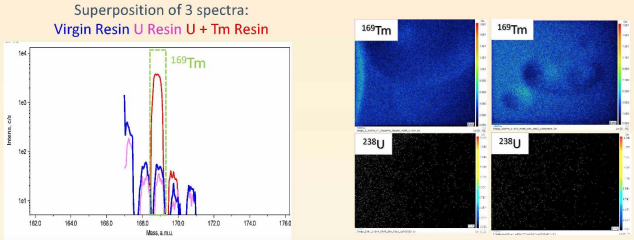
Test 2 = Alternative to dehydrated kidney homogenate. The resin is spiked with Tm as IS and U as external calibrator.

Resin is dry
Suitable for SIMS



Step 1: Tm and U homogeneity by SIMS

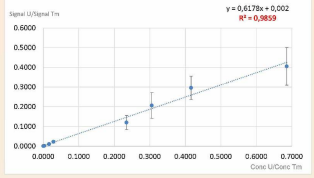
Superposition of 3 spectra:
Virgin Resin U Resin U + Tm Resin

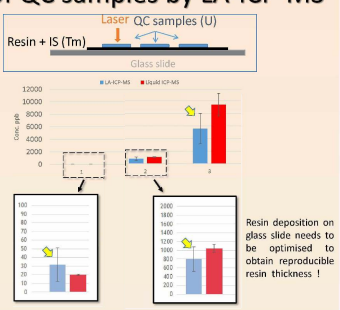


Resin U (20 ppm) + Tm (35 ppm): Tm is detected, homogeneus distribution (3000 cps)
Virgin resin et Resin U : Tm is detectable at background level (10 cps)

Step 2: Calibration and analysis of QC samples by LA-ICP-MS

Ablation of 10 surfaces of 0.5x0.5 mm²
Energy 100%
Frequence 75 Hz
Spot 35 µm
Speed 35 µm/s





Resin deposition on glass slide needs to be optimised to obtain reproducible resin thickness !

Conclusions

We developed an analytical methodology based on internal standard spiked resin which will allow the quantification by LA-ICP-MS the resin embedded samples used for other imaging techniques like SIMS microscopy. The advantages of this new methodology are 1) the addition of a suitable internal standard to both matrix-matched standards and biological samples without altering their original uranium distribution, 2) an appropriate sample preparation compatible with several imaging techniques (SIMS, TEM, LA), 3) ease to prepare and 4) room temperature storage as a solid material which would facilitate its transport. Some optimisations are still needed, in particular to control the resin thickness. This methodology will positively contribute to the collaboration among bio-imaging techniques users.

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