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The use of SIMS for uranium localization in biological research

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Abstract

Secondary ion mass spectrometry (SIMS) enables the localization and isotopic determination in microvolume of all elements from the Mendeleyev table. Based on the ablation of samples by ion bombardment, the SIMS method allows rapid assessment of trace elements in biological samples. In this work, studies in vitro and in vivo have been carried out using, respectively, rat macrophages and lung tissue sections containing uranium oxides. Following the localization studies, the isotopic ratio between the ^{235}U and ^{238}U was measured. In the present work, analytical procedures and the potential of the SIMS in biological research are discussed. © 1998 Elsevier Science S.A.

Keywords: SIMS; Uranium; Radiotoxicology; Internal dosimetry

1. Introduction

The increase of nuclear applications has been followed by the growth of public attention on the potential associated risks. The studies concerning the actinides, typically found at low concentrations, remains of particular interest, especially in the case of radiotoxicological research analyses. The new recommendations of the International Commission on Radiological Protection (ICRP 66) [1] give emphasis to biokinetic models for particles incorporated via the respiratory tract. In this model, the parameters related to the blood absorption play an important role in the clearance process. In this case, the alveolar macrophages represent the interface in both accumulation and degradation of these particles. Thus, the knowledge of the phenomena taking place in this interface is crucial to establish all absorption parameters, in particular those related to the bound fraction.

Uranium oxide particles have already been widely studied. The presence of these particles in the fuel cycle represent an occupational risk of exposure for workers. Recent studies have shown either morphological or chemical transformations of uranium particles in lung macrophages after different times of intake [2–4]. In general, for these investigations, the detection of uranium cellular distribution is based on electronic microscopy techniques, mainly transmission electronic microscopy (TEM) associated with energy dispersive spectrum (EDS) analysis. However, despite their performance, these techniques do not permit isotopic ratio analyses, very important data to evaluate the internal dose delivered to tissues. In this context, we have proposed the use of secondary ion mass spectrometry (SIMS) as a complementary method.

SIMS is a method based on the bombardment of (usually) solid specimens with positive or negative ions. As a result, a progressive erosion of the specimen from which atoms and groups of atoms are sputtered occurs. Among these particles, some are ejected as charged ones. These latter, so-called secondary ions, are accelerated towards an electrical sector, used as an energy filter, and analyzed by mass spectrometry according to their mass-to-charge ratio.

For a given element, the yield of secondary ions depends not only on its ionization potential but also depends on the physico-chemical properties of the specimen, such as its thickness and chemical composition. Hence, the SIMS is a specimen-dependent method where the preparation of the sample is an important step to avoid either complicated operational conditions or even impossibility of analysis. Another important advantage is related to its application as an ion microscope, which allows the localization of a given

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nuclide at microvolume level. Due to this capability, SIMS is sometimes known as analytical ion microscopy (AIM).

We have previously shown the use of SIMS as a pure mass spectromety for quantitative analyses of uranium from urine samples [5]. In this work, we have investigated the application of secondary ion mass spectrometry for localization of uranium in tissue sections. The feasibility studies using lung alveolar macrophages were aimed at determining the conditions allowing localization and determination of the isotopic ratio of uranium. Based on these results, lung tissue sections were used in order to localize and identify the origin of uranium particles. Specimen preparation as well as instrumental conditions are discussed.

2. Material and methods

2.1. Animals

The experiments were conducted on Sprague-Dawley Ico OFA-SD (IOPS-Caw) rats, weighing about 300 g during the experiments. The animals were fed ad libitum. All procedures concerning the rats were carried out according to Animals (Scientific Procedures) Act 1986. The animals were sacrificed by exsanguination after an intraperitoneal injection of 500 mg kg^{-1} of sodium pentobarbital (Sanofi).

2.2. Uranium compounds

Industrial dust containing uranium oxides was collected at two different workplaces. UO_2 was obtained from an industrial site where part of the research on a new uranium-enrichment process, based on atomic vapor laser isotopic separation (AVLIS) is performed. UO_4 is present in the enrichment uranium fuel cycle during the treatment and recycling of production waste. A particle fraction of small size was obtained using a micronizing mill. After sedimentation in ethanol in order to eliminate clusters of particles, a particle fraction of 0.5 μm in geometric diameter was retrieved. The chemical composition was determined by X-ray spectrometry (Philips PW 1730) and infra-red spectroscopy (Unicam IRTF Genesis). Two types of uranium oxide were identified, UO_2 and UO_4, composed, respectively, of natural and enriched uranium.

2.3. Specimen preparations for SIMS

2.3.1. In vitro

The alveolar macrophages were harvested from the rats by bronchoalveolar lavage, with physiological saline solution. The liquid was centrifuged (800×*g* for 10 min). The cells were plated with cell culture medium (199-SIGMA) containing 2 mM of L-glutamine, kanamycin, penicillin and streptomycin (Biomemerieux), and enriched with 5% of Approsera (MCR-UK). A deposit of about 40 μg of uranium particles (UO_2) per million cells was added into this medium. The particles in suspension were previously homogenized for 10 min by ultrasound. Then, 1.5 h later, the culture medium containing either no adherent or no phagocytized cells was removed and replaced by a new one. The plates were placed in a cell culture chamber at 37°C with an atmosphere saturated in water and containing 5% CO_2. After 24 h, the floating matter was removed from the culture medium and the adherent cells were recovered from the plates and centrifuged for 10 min at 800×*g*. The pellet was fixed with glutaraldehyde, treated with osmium and embedded into epon resin. Thin sections, 1 μm in thickness, were then placed on gold supports.

2.3.2. In vivo

2.3.2.1. Instillation

The instillation was processed with approximately 400 μg of UO_4 particles in suspension in saline. This suspension, having a concentration of 2.5 g l^{-1}, was homogenized for 10 min by ultrasound before instillation in rats, in order to break clusters formed after dust sedimentation.

2.3.2.2. Lung sections

Forty-eight h after instillation, the animals were sacrificed and the lungs were fixed by intratracheal injection of 1% glutaraldehyde before being removed from the lung cavity. The lungs were then cut off in small pieces and fixed in epon resin as previously described.

2.4. Mass spectrometry apparatus

A SIMS type IMS 4F (Cameca, France), schematically shown in Fig. 1, was employed. The primary ion voltage was set at 10 kV, while the specimen voltage was held fixed to 4.5 kV. The nature of the primary ion beam can be chosen from among O_2^+, O^-, Cs^+ and Ar^+, depending on the element to be analyzed, as well as on the specimen matrix. For the determination of uranium, O_2^+ has been chosen because its electronegativity enhances the sputtering of these elements as positive secondary ions.

The cell distribution of the selected secondary ions was obtained using a large primary beam. The image detection is processed according to the beam position to build the digital image. For the spectrometry mode, for isotopic ratio determination, this device is equipped with a dual detection system: one electron multiplier (EM) and one Faraday cup (FC). This combination permits a dynamic range for counting secondary ions from 10^{-1} to 5×10^9 counts per second (cps).

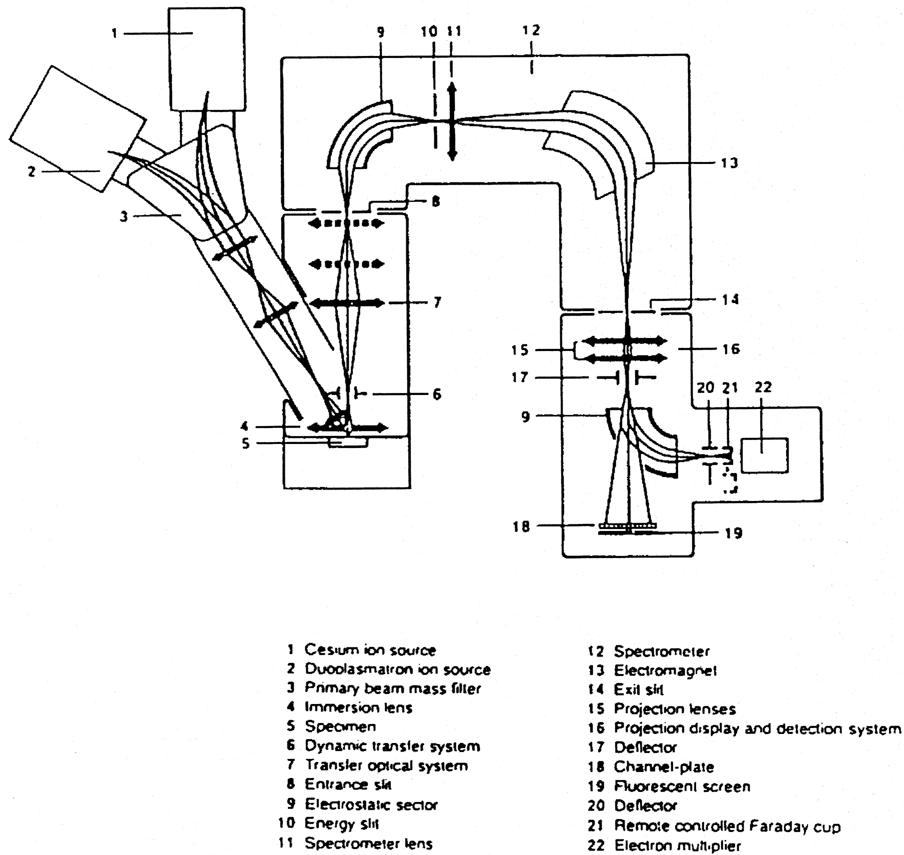

Fig. 1. The Cameca IMS 4F (with the permission of Cameca).

3. Results

The culture of alveolar macrophages was analyzed under O_2^+ bombardment using spots of 150 μm in diameter. To obtain an overview of the cell distribution, a primary current of 1 μA was employed, and the direct ion image of $^{40}Ca^+$ was recorded for 60 s of acquisition time. This image, shown in Fig. 2A, gives us an idea of the cell surfacial density. Once the morphological aspect was recorded, the SIMS system was set for uranium analysis. In this case, the uranium distribution was reached by recording the cluster $(^{238}U^{16}O)^+$, as shown in Fig. 2B,

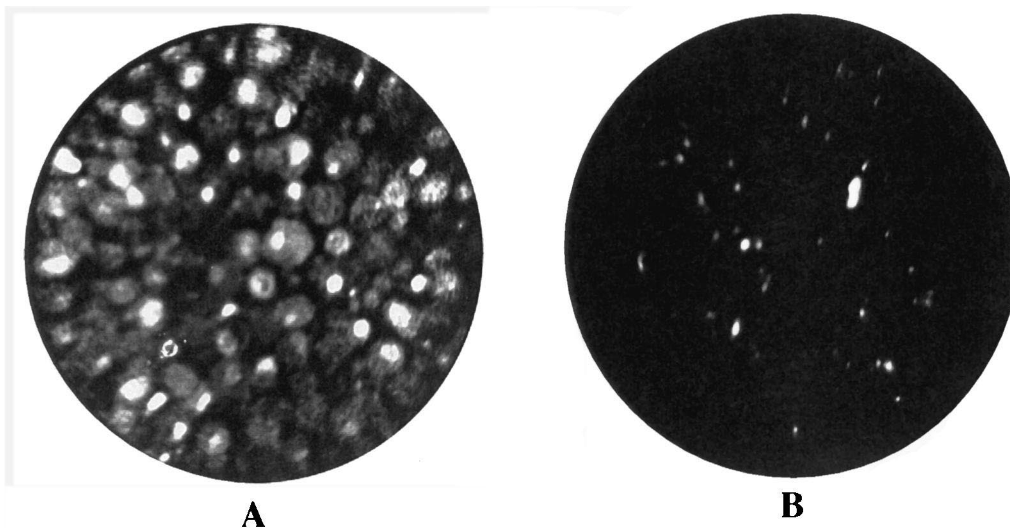

Fig. 2. Direct analytical images obtained from cultured alveolar macrophages incubated for 24 h. The calcium image (A) gives the spatial distribution of alveolar macrophages. The uranium particle distribution (B) was obtained by analysis of $^{238}U^{16}O$. Surface diameter, 150 μm.

which has a better sputtering yield as a positive secondary ion than the $^{238}U^{+}$ alone.

To visualize the lung tissue section, the $^{40}Ca^{+}$ ion was also chosen in order to permit a better visualization of tissue morphology. This is possible due to the presence of calcium all over the alveolar cells, combined with its high concentration in the nuclei. Fig. 3 shows three images obtained from a lung tissue section. The first image (Fig. 3A) is the result of bombardment by O_2^{+} to obtain the distribution of calcium sputtered as positive ions, for 100 s acquisition. The second image (Fig. 3B) shows the uranium distribution, here analyzed by the recording, for 8 min, of the mass of 254 u, corresponding to the cluster $(^{238}U^{16}O)^{+}$. The third image (Fig. 3C) is obtained by superposition of the previous two images, allowing the localization of uranium particles in the section. For this specimen, the analyzed zone had a diameter of 250 μm, and the primary ion current was in the order of 1 μA.

Table 1
Comparison of $^{235}U/^{238}U$ isotopic ratios derived from SIMS measurements and the expected value from two specimens: alveolar macrophages cells and lung tissue section

Specimen	$^{235}U/^{238}U$ isotopic ratio (%)	
	Expected	SIMS ($X \pm 2\sigma$)
Alveolar cells	0.72	0.72±0.09
Tissue section	3.30	3.32±0.44

In both experiments, for the isotopic ratio determinations, a spot of 60 μm of diameter was bombarded, and the depth profile analysis was used. During the bombardment, the analysis system scans the mass range of interest twice, i.e. ^{235}U and ^{238}U masses, each scan corresponding to one cycle. For these experiments, five series of such 10 cycles were performed for a given bombarded spot. The values reported in Table 1 were calculated as an average

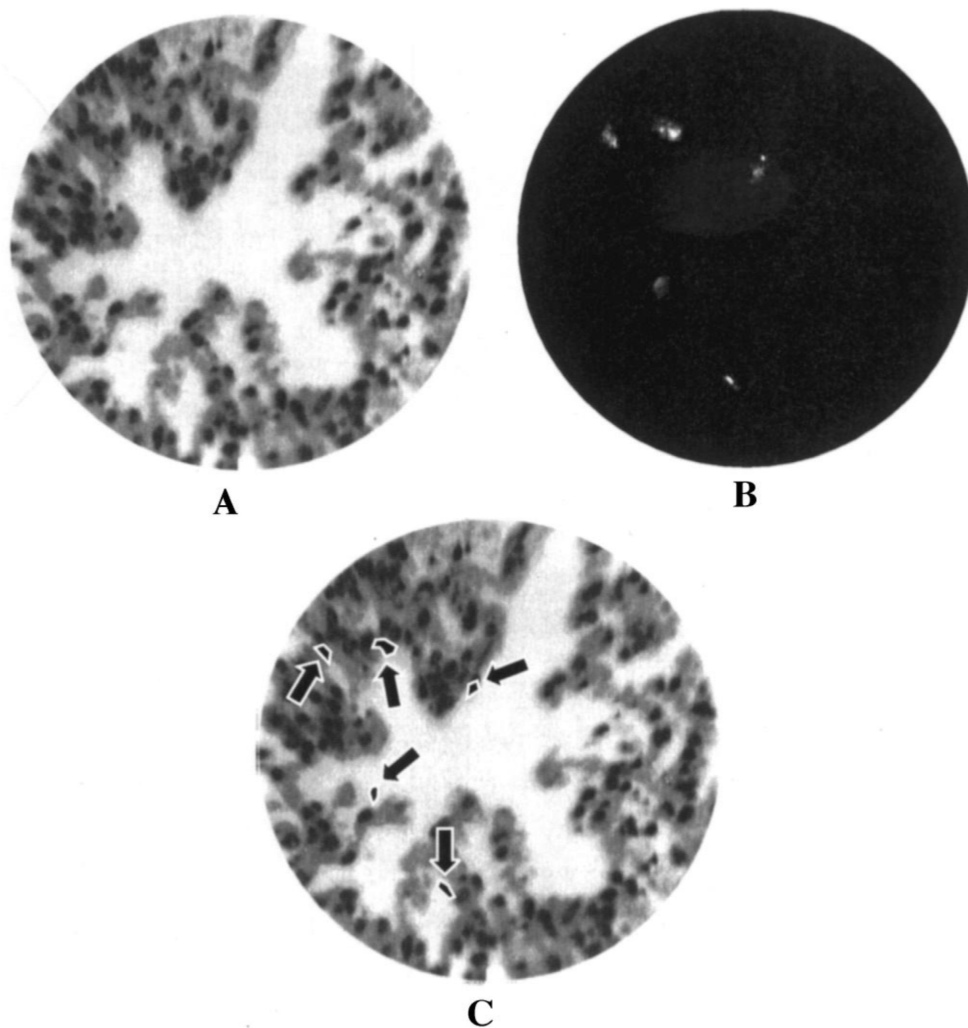

Fig. 3. Direct images obtained from a lung tissue section. The calcium ($^{40}Ca^{+}$) image (A) permits the visualization of cartographic distribution of cells and the structure of respiratory bronchioles. The uranium detection is obtained by the UO^{+} cluster (^{238}U plus ^{18}O) image (B). The superposition of the two images, as shown in (C), allows the localization of uranium in the tissue section. Surface diameter, 250 μm.

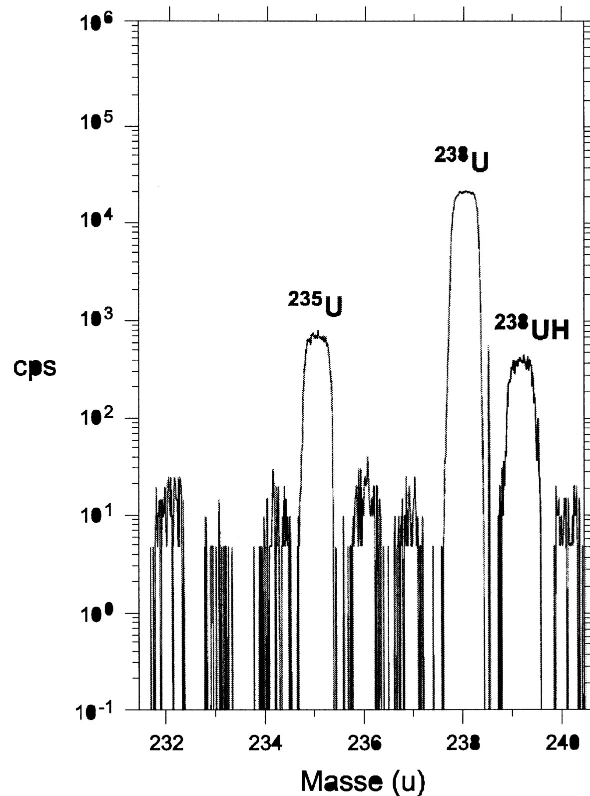

Fig. 4. Mass spectrum obtained from lung tissue section exposed to enriched uranium.

over all series. The presence of uranium can be obtained by the mass spectrum. Fig. 4 presents a typical spectrum obtained, always under oxygen bombardment, from the tissue section shown in Fig. 3, containing enriched uranium.

In parallel with these specimens, a specimen containing alveolar macrophages, without uranium, was prepared to assess the background in the uranium mass region. As a result, using the same analytical procedures, an average background of 5 cps was measured in the region of mass between 238 and 254 u (diameter of the analyzed zone, 250 μm).

4. Discussion

The present results have demonstrated a good agreement between the expected values and those obtained by SIMS for the determination of the isotopic ratio of ^{235}U and ^{238}U, providing knowledge of the origin of the uranium (i.e., from natural, depleted, enriched or reprocessing uranium). However, the low concentration of ^{234}U did not permit obtaining its concentration relative to ^{238}U.

The high mass resolving power (defined as $M/\Delta M$) of SIMS permits separation of neighbouring masses and reduction of problems of mass interference. The SIMS-4F can reach a mass resolution of 10^4. But, there is a trade-off between detection limit and mass resolution. The increase in mass resolution implies a greater of detection limit due to the reduction of ion transmission. In this work, we have noticed that a mass resolution of ca. 600 was good enough for mapping analyses as well as quantitative ones.

As shown in the Fig. 4, in all specimens containing uranium, a peak in the region of mass 239 u was always observed. Since there was no ^{239}Pu in these specimens, this peak was related to the presence of the polyatomic species $^{238}U^{1}H$. The contribution of ^{238}UH was measured for each specimen, and a value of 2% was found for the $^{238}UH/^{238}U$ ratio. This value is in agreement with the one previously presented [5]. For analytical studies, on the basis of this protocol, such a result could be used to analyze specimens containing both uranium and plutonium by subtracting the ^{238}UH component.

As pointed out before, the SIMS is a specimen-dependent method. Despite the employment of specimens 1 μm thick, no significant charge effects were observed. In other words, the drawbacks related to the increase of capacitance for bad conductor specimens was negligible.

The choice of $^{40}Ca^{+}$ was seen to be adequate in our approach to obtain the morphological distribution in a tissue before uranium localization. Despite a spatial resolution of about 1 μm, it is possible to localize the uranium particles within a macrophage, as shown for lung tissue sections.

5. Conclusions

These results confirm the feasibility of the use of SIMS for analytical studies in bioassays, in particular for the detection of long-lived α emitters. Combined with electronic spectroscopy, SIMS makes possible the improvement of microlocalization analyses, by the assessment of isotopic ratio. In terms of time, the SIMS presents a great advantage due to its rapidity in detection.

The observations made on alveolar tissue sections, 48 h after instillation, emphasize the potential of the SIMS for studies of the clearing of lung alveolar space, and actinide localization after internal exposure.

Whereas further studies are required, on the basis on these results secondary ion mass spectrometry seems to be a very important method with regard to cellular mapping studies for radiotoxicological research, mainly in the field of internal dosimetry.

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