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In vitro measurement of UO_2/phosphate ion interactions at the particle surface: preliminary results using electron dispersive energy (EDS) and ^{32}P adsorption assays

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Abstract

We have developed qualitative and quantitative methods to characterize in vitro UO_2/phosphate interaction using either energy dispersive X-ray spectrometry or adsorption measurement of ^{32}P. $U_2O(PO_4)_2$ was used as reference particles with homogeneous phosphorus distribution. EDS analysis at different accelerating voltages in the range of 10–30 kV has confirmed by a similar P/U weight ratio, the homogeneous chemical composition of $U_2O(PO_4)_2$, whereas after in vitro incubation of UO_2 in a medium containing 10 mM phosphate the P/U weight ratio was about twice as high at 10 than at 30 kV. The formation of uranium phosphate at the surface of particles was suspected. Preliminary results suggest that these individual particle analyses could be combined to a global radioactive measurement of ^{32}P-labelled phosphate adsorption in a suitable range of phosphate concentrations and particle weights. © 1998 Elsevier Science S.A.

Keywords: Uranium; Phosphate; Particle; Microanalysis X

1. Introduction

After inhalation, the dissolution parameters of actinide oxide control the actinide transfer to the main extra-pulmonary retention organs [1]. These mineral particles are mostly phagocytosed by alveolar macrophages and are confined to the phagolysosomal compartment [2]. An acid pH occurs in this compartment and the presence of phosphate ions contributed to the formation of poorly insoluble actinide phosphate. This has been demonstrated for uranium oxide on thin section by electron microscopy microanalysis [3]. Thus, in this case, the U dissolution rate appears slower in macrophage than in the extra-cellular medium [4]. We have recently reported the interaction of phosphorus with inhalable cerium oxide in vivo, after intratracheal instillations, and in vitro, after incubation in a phagolysosomal simulating medium containing different amounts of phosphate ions [5]. This was performed by energy dispersive X-ray spectrometry (EDS) after analysis of entire particles. Potentially, such a method could quantify the time evolution of the global P/metal ratio during the particle dissolution process [6].

In this study, we have developed two methods to improve qualitative and quantitative analysis of phosphate/uranium oxide interactions for inhalable particles: (1) microanalysis at different acceleration voltages to distinguish between surface and global chemical composition; and (2) radioactive measurement of ^{32}P adsorption after in vitro incubation.

2. Experimental details

UO_2 particles were provided by COGEMA/FTCA and contained more than 99.78% of ^{238}U. $U_2O(PO_4)_2$ particles were provided by Nuclear Physical Institut (Orsay) and were obtained as previously described [7]. Inhalable particles were prepared by sedimentation in 2% collodium solution for 15 min. X-ray diffraction was used to characterize the crystalline form of these compounds (Philips PW 3020 spectrometer). The specific area and density of the particles were determined using a Gemini system 2360 and a Accupyc 1300 (Micrometrics), respectively.

Electron microscopy analysis was performed on carbon-coated particles deposited on filters (Nucleopore, HTTP, 0.4 μm) using a Stereoscan 120, Cambridge Instrument with an accelerating voltage from 10 to 30 kV. EDS spectra

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were recorded after 500 s acquisition time. For each accelerating voltage, the P/U weight ratio was calculated using a Kevex, Quantex 2.02 software.

In vitro incubation of UO_2 and $U_2O(PO_4)_2$ powders were performed for 24 h in 0.1 M Tris–HCl buffer at pH 5 containing different amounts of phosphate ions (0–10 mM) with a trace amount of ^{32}P added as phosphoric acid. At the end of the assay, the particle suspensions were centrifuged at $3000\times g$ for 15 min and the amount of ^{32}P in the supernatant was compared to that of a medium without particles by liquid scintillation.

3. Results

The X-ray diffraction analysis confirmed that the powders corresponded to crystalline forms of either UO_2 or $U_2O(PO_4)_2$. The specific area and density were 0.155 and 3.076 $m^2\ g^{-1}$ and 6.4 and 14.24 $g\ cm^{-3}$ for $U_2O(PO_4)_2$ and UO_2, respectively. Fig. 1 shows scanning electron microscopy images of $U_2O(PO_4)_2$ reference particles, UO_2 native particles and UO_2 particles after in vitro incubation. Most of the $U_2O(PO_4)_2$ and native UO_2 particles were aggregates, and a larger surface area was clearly observed for $U_2O(PO_4)_2$ than for UO_2. After the in vitro incubation, the UO_2 particles appeared smoother than the native powder.

EDS analysis was confined to particles of 2–3 μm diameter. Fig. 2 shows X-ray spectra of $U_2O(PO_4)_2$ particles obtained at 10 and 30 kV, and the corresponding calculated P/U weight ratio remained nearly constant at 0.17 and 0.20, respectively. The mean values observed after analysis of six particles was 0.18 (SD=0.02, $n=3$), 0.22 (SD=0.01, $n=3$) for 10–15 and 25–30 kV, respectively. Such a ratio was observed for each particle analysed. After in vitro incubation of UO_2, the presence of the specific P Kα-ray was observed for each particle analysed. By contrast to $U_2O(PO_4)_2$ particles, the calculated P/U weight ratio varied depending on the accelerating voltage. For example, from the spectra shown in Fig. 3, this ratio was 0.019 at 10 kV and 0.012 at 30 kV. Such a two-fold decrease of the P/U weight ratio was observed at 10 and 30 kV on three particles, but the P/U weight ratio varied from 0.02 to 0.4 at 10 kV.

Fig. 4 shows adsorption of ^{32}P on $U_2O(PO_4)_2$ and UO_2 particles after the in vitro incubation using a similar powder weight (1 $mg\ ml^{-1}$ of incubating medium). For both powders, the fraction of ^{32}P adsorbed on particles decreased when phosphate ion concentration increased. For UO_2 an almost saturation value of ^{32}P adsorption was reached at 1 mM, whereas this plateau was observed at 0.1 mM phosphate for $U_2O(PO_4)_2$. Preliminary results showed that, in the range of 0–2 mM phosphate concentration, adsorption of ^{32}P gradually increased with increasing mass of particles in the range of 0–1 $mg\ ml^{-1}$ of incubating medium.

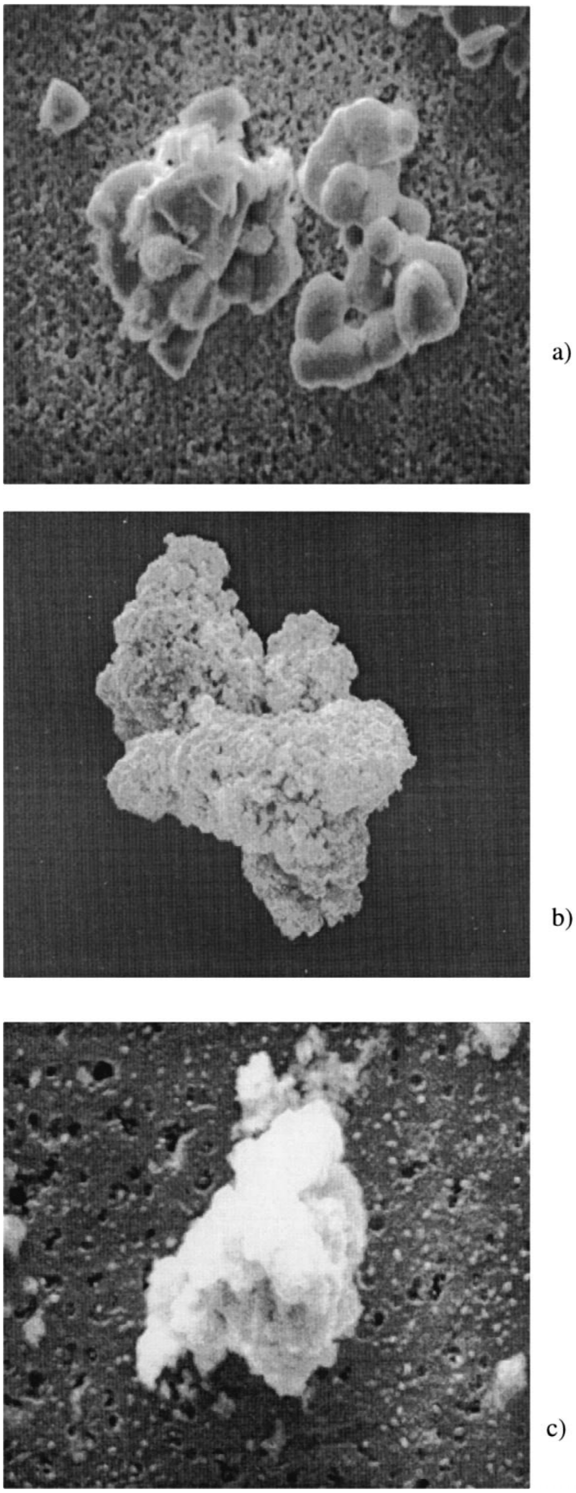

Fig. 1. Scanning electron microscopy images of (a) $U_2O(PO_4)_2$ reference particles (2 μm), (b) UO_2 native particles (2 μm) and (c) UO_2 particles (2 μm) after a 14-day in vitro incubation in Tris–HCl 0.1 M buffer at pH 5.

4. Discussion

This study shows that using defined reference powders, such as $U_2O(PO_4)_2$, it is possible to characterize the

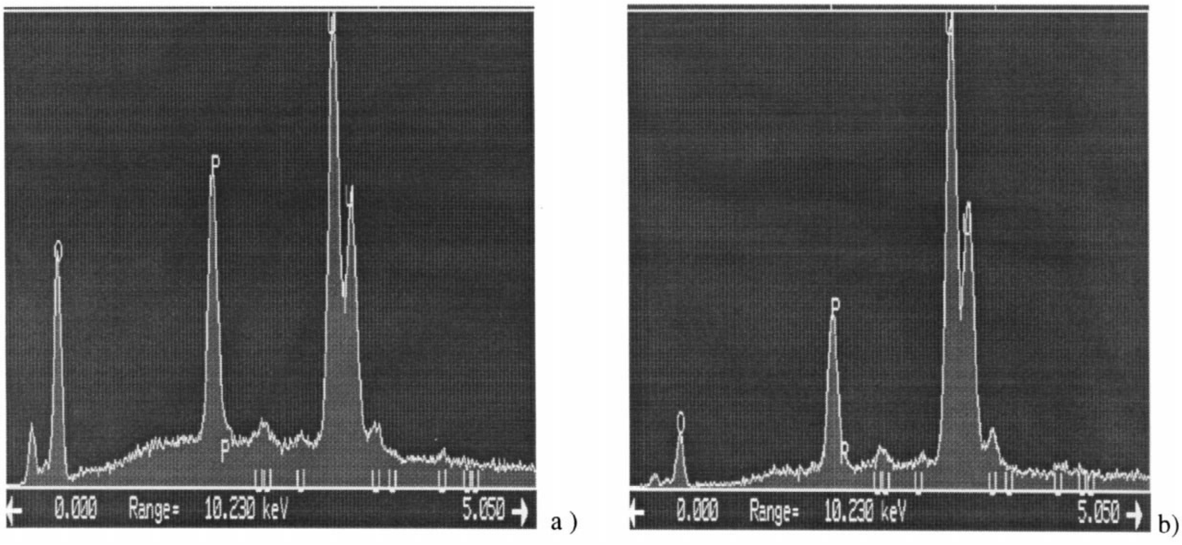

Fig. 2. X-ray spectra of $U_2O(PO_4)_2$ particles at (a) 10 and (b) 30 kV.

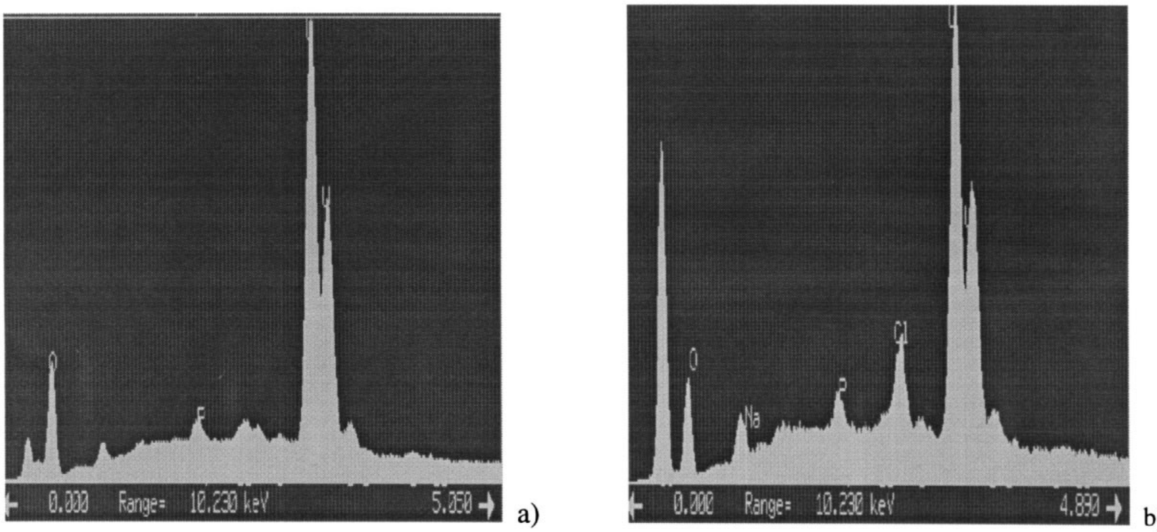

Fig. 3. X-ray spectra of UO_2 particles after a 14-day in vitro incubation in Tris–HCl 0.1 M buffer at pH 5 at (a) 10 and (b) 30 kV.

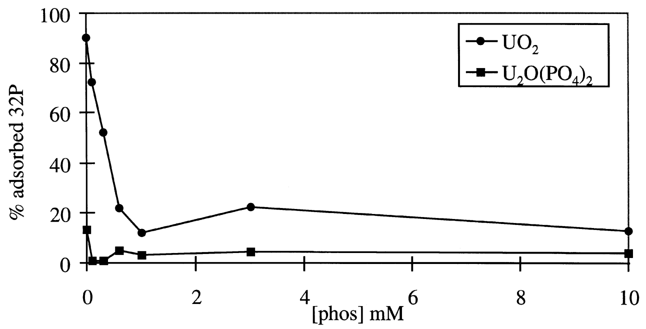

Fig. 4. Adsorption of ^{32}P on $U_2O(PO_4)_2$ and UO_2 particles after a 24-h in vitro incubation in Tris–HCl 0.1 M buffer versus phosphate ion concentration at pH 5.

interaction of phosphate ions with the surface of inhalable UO_2 particles. EDS analysis can be performed at different depths within the particle by varying the voltage in the range of 10–30 kV. These depths depended on the physico-chemical composition of the particles, and Fig. 5 shows the variation of the projected range of electrons in the particle that was calculated for the U compounds at different accelerating voltages, taking into account their density which we have experimentally measured [8]. Thus, we assumed that, for the 2–3-μm diameter particles studied here, a depth less than 0.2 μm was analysed at 10 kV, whereas this depth was 6 times deeper at 30 kV.

In the case of $U_2O(PO_4)_2$ particles, we have observed that the P/U weight ratio remained nearly constant in the range of 10–30 kV. This confirmed the homogeneous chemical composition of $U_2O(PO_4)_2$ particles. By con-

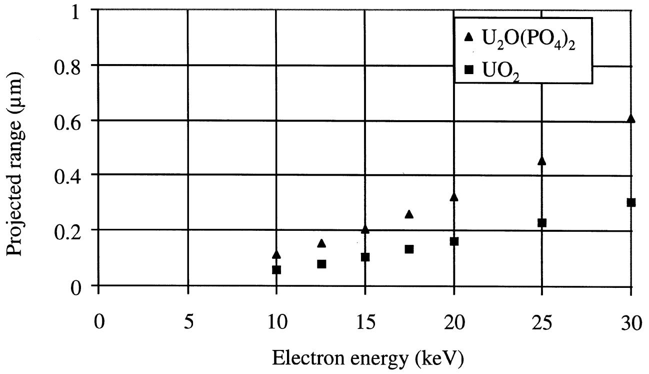

Fig. 5. Variation of the projected range of electrons in the particle calculated for the U compounds, UO_2 and $U_2O(PO_4)_2$, as a function of the electron accelerating voltage.

trast, after 14-day incubation of UO_2 in a media containing 10 mM phosphate ions, the P/U weight ratio seemed to gradually decrease as the acceleration voltage increased. This suggests the heterogeneous chemical composition of the particle, and that mostly uranium phosphate is formed at its surface. This will be confirmed by using a wider range of accelerating voltage. Studies are in progress to verify the occurrence of such surface interactions in vivo, after intratracheal instillations of UO_2 in the rat.

Because this work involves individual particles, EDS-analysed global measurement of the phosphate/UO_2 interaction needs a lot of measurements which are very time consuming. We have performed preliminary experiments on the use of ^{32}P as a radioactive tracer for global phosphate/particle interaction measurement. For the same particle volume, in spite of a 9-times lower specific area, ^{32}P adsorption of more than a factor of 10 seemed to occur for UO_2 and $U_2O(PO_4)_2$, respectively. Other experiments are needed to establish the quantitative estimate of the P/UO_2 interaction using ^{32}P.

In conclusion, the two approaches used appear potentially useful tools to characterize phosphate/UO_2 interactions at the particle surface. They will be used to characterize this interaction as a function of the form of U oxide, UO_2, U_3O_8, in different simulating media.

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References

[1] G.M. Kanapilly, C.H.T. Goh, Health Phys. 25 (1973) 225.
[2] S.Y. Helfinstine, R.A. Guilmette, G.A. Schlapper, Environ. Health Persp. 97 (1992) 131.
[3] M.H. Hengé-Napoli, E. Ansoborlo, M. Donnadieu-Claraz, J.P. Berry, R. Gilbert, B. Pradal, Radiat. Prot. Dosim. 53 (1994) 157.
[4] M.H. Hengé-Napoli, E. Ansoborlo, M. Donnadieu-Claraz, J.P. Berry, M.C. Cheynet, Cell. Mol. Biol. 42(3) (1996) 413.
[5] S. Matton, P. Massiot, H. Le Naour, C. Lizon, G. Rateau, P. Fritsch, Ann. Occup. Hyg. 41(1) (1997) 99.
[6] P. Massiot, H. Le Naour, S. Matton, C. Lizon, G. Rateau, I. L'Huillier, G. Grillon, P. Fritsch, Ann. Occup. Hyg. 41(1) (1997) 77.
[7] P. Benard, D. Louër, N. Dacheux, V. Brandel, M. Genet, Ana. Quim. Int. Ed. 92 (1996) 79.
[8] ICRU Report 37, 1984, p. 1.