Laboratorio Acceleratori e Superconduttività Applicata – L.A.S.A.

NASAR: A PROJECT ON NANOSAFETY RESEARCH BY RADIOCHEMICAL AND NUCLEAR TECHNIQUES

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Rationale

- **Nanotechnology** is perceived as one of the key technologies of this century and is synonymous with things that are innovative and highly promising.
- **BUT** little attention has been dedicated to the research on toxicological effects and on the relations with factors that can affect the nanosefety on human health and on the environment.
- **Research** on the impact of engineered nanoparticles (NPs) is strongly hampered by a lack of reliable tools to detect, visualize and quantitatively trace particles movement and transfer in complex environmental and biological system.

The Obiective

- Understanding the toxicity mechanisms, that underpin some peculiar NPP-induced toxic responses, requires an integrated use of different analytical techniques of a more traditional type like spectroscopic, bioanalytic, molecular biology and more sophisticated ones like highly specialized microscopy, nuclear and radiochemical techniques.

Nuclear and Radiochemical Techniques

Radiotracers technique labelling metallic NPs by:
- **direct radioattivation of the NPs** by neutrons in nuclear reactors or by particles accelerated with cyclotrons;
- **radiochemical synthesis of NPs starting from radioactive ionic precursors of inorganic salts:** the radio-labelled NPs maintain the chemical- physical characteristics (distribution, size, Z potential) of the "cold" ones.

Instrumental Neutron Activation Analysis – INAA or Neutron Activation Analysis with Radiochemical separation - RNAA: this is a multielemental thechnique, with high sensibility for the determination at the same time of many elements with high accurancy and precision and with the minimum effect of the matrix.

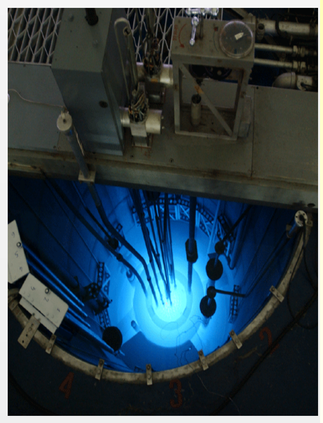
Gamma spectrometry at high resolution: by HPGe detectors it is possible the qualitative and quantitative analysis of the induced radionuclides.

Radiochemistry and Nuclear Physics Measurement Laboratory at LASA
UNIMI and INFN sez. Milano

LENA Laboratory
University of Pavia

Hot cells for radiochemical separations

Physics Measurement Laboratory



Research Nuclear Reactor, TRIGA MARC II, Pavia
Neutron thermal flux: 0.5-1x10^{13} n cm^{-2} s^{-1}

External Partners
CeSI (Chieti); UniInsubria (VA); UniSapienza (Roma); UniSiena

Accumulation in plantule of zerovalent Fe in nano form, to be used in the treatment of various form of soil pollution

There are very few studies in this regard concerning the impact of NPs on plant systems. We studied the **effects of iron particles (micron and nanoscopic) and iron ions on the germination** of monocotyledonides (*Lepidium sativum*) and dicotyledons (*Sorghum saccharatum*), using Fe-radiolabelled compounds with ^{59}Fe to have information on the uptake in the aqueous environment and on the distribution in the different parts of the two plantule types. **Conclusion**: The radiolabeling of Fe particles with ^{59}Fe allowed the quantitative measurement of newly accumulated Fe (from iron particles) in plantules versus Fe already normally present, and its distribution between leaves, buds and roots.

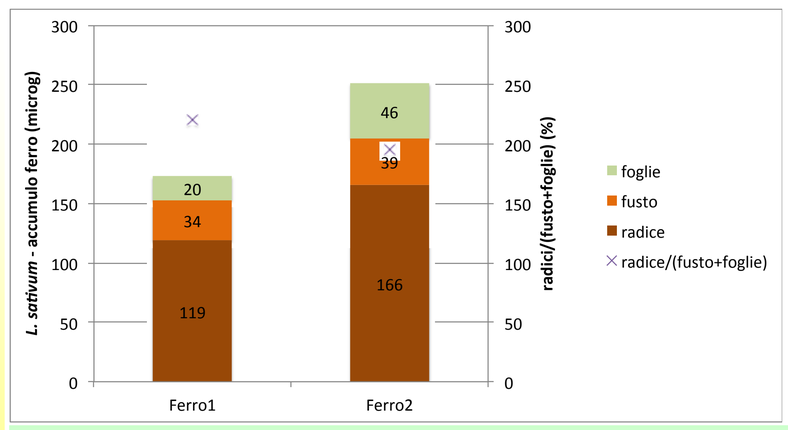


Figure 1 Iron accumulation in *Lepidium sativum* exposed to micro (Ferro2) and nano (Iron 1) form radiolabelled with ^{59}Fe and relative distribution for the part of interest. From bottom to top: roots, stems and leaves

Study the influence of NPs on the human reproductive system

INAA was applied to study the **in vivo migration of AgNPs and AuNPs through rabbit Blood-Testicular Barrier (BTB)**. The Ag and Au were determined in semen harvested two times after intravenous administration of AgNPs and AuNPs. The analysis showed the presence of Ag and Au in total semen after the first 3 days (of which about 70% associated with sperm) and a significant reduction at 7 days (**Table 1**). If Ag and Au were in the form of NPs or ion released by them it was not established. **Conclusion**: The presence of traces of Ag and Au in sperm has a significant toxicological relevance in relation to the experimental evidence of sperm count reduction and inhibition of their motility.

Sampling time	rabbit 1	rabbit 2	rabbit 3	rabbit 4
	Ag	Ag	Au	Au
		Concentration (ng·10^{-6} sperms)		
t = 0 (ctrl)	<0,13	<0,09	0,0009±0,0001	<0,003
3d	1,20 ±0,04	0,96 ±0,11	0,0180 ±0,0010	<0,156
7d	0,52 ±0,01	0,29 ±0,01	0,0011 ±0,0001	0,017 ±0,003
		Concentration (10^{6} sperms·mL^{-1})		
t = 0 (ctrl)	237	328	163	296
3d	88	48	37	37
7d	284	259	56	46

Table 1 Concentration of Ag and Au in the sperma samples of 4 rabbits at different sampling time. There is the evidence that Ag and Au transit through the BTB with an accumulation during the first three days and a rapid decrease in the next period.

Experimental studies on biokinetics, bioaccumulation and biotransformation of NPs in food

INAA was applied for the determination of the elements constituent of metal-based NPs of potential interest in the food applications. Using the Rabbit channel of Triga Mark II, that allows the rapid and sensitive determination of very short lived radionulides induced by irradiation of the samples for seonds/minutes, were studied:

- the quantitatively **amount of nanoAg in plastic food containers**, measuring the ^{108}Ag ($T_{1/2}$=2.4 min) by irradiation of solid disks, taken from the containers;
- the **degree of dispersion of nano-clay in polymeric films for food contact applications** by the simultaneous determination of main constituents of the nano-clay (cloisite-Na$^+$), via ^{28}Al ($T_{1/2}$=2.246 min), ^{27}Mg ($T_{1/2}$=9.46 min), ^{51}Ti ($T_{1/2}$=5.8 min) and ^{29}Al ($T_{1/2}$=6.6 min);
- the **uptake of gold NPs and ions into quail eggs** to determine if and how they pass through eggshell, membranes, arriving till the yolk. The eggs were boiled for different interval time in a solution containing radioattivated Au ions and Au NPs and the results are reported in **Figure 2**. For the boiling time of 40 min, the penetration in the different part of the eggs was also studied: was demonstrated that NPs were adsorbed into all the part of the eggs till the yolk with amount that is ten time more of ion species even if the greater amount remains attached to the shell.

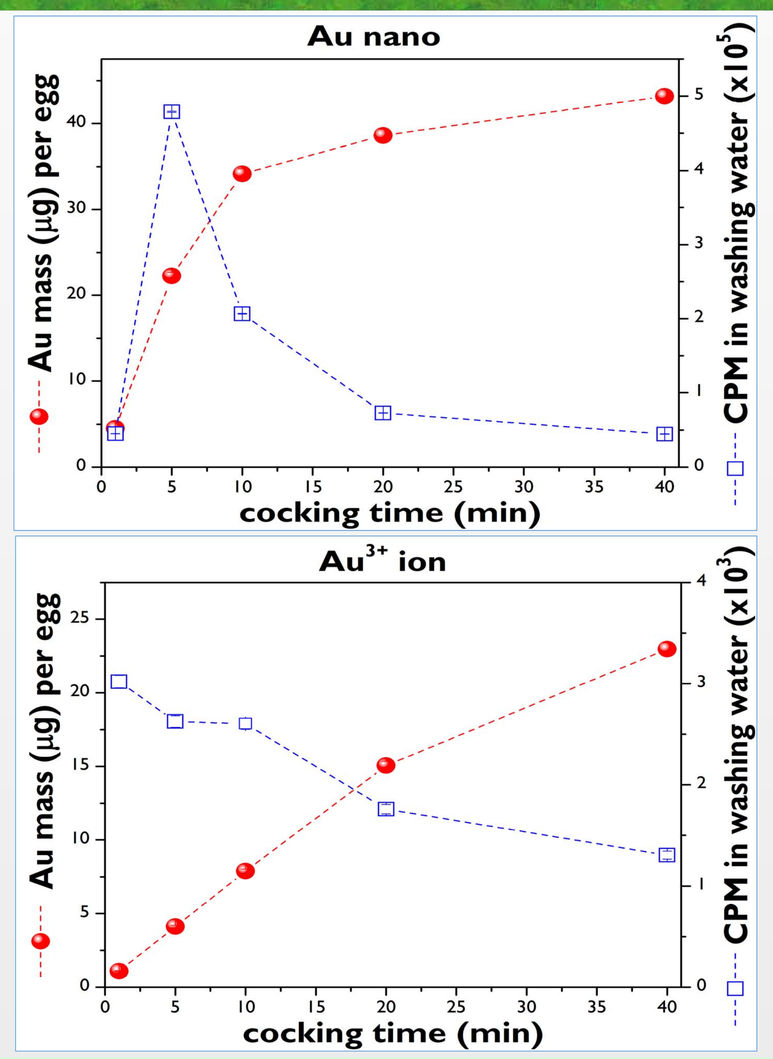


Figure 2 Penetration of the radiolabelled ^{198}AuNPs and ^{198}Au^{3+} ions ($t_{1/2}$ = 2.7 d) into quail eggs as a function of the cooking time.

Conclusions

- **Nanosafety** requires new specialists in the context of analytical chemistry, such as nuclear and radioanalytic techniques of ***radionochemistry***.
- The real crucial point is the **cultural and operative preparation** of **new operators** in these fields that implies the necessity that courses like **health physics, nuclear chemistry, radiochemistry and related subjects must be more present in the university curricula**.
- Key indicators of the nature of this problem are: **declining university enrolment, closure or dilution of university departments offering nuclear education and training, demographics of the workforce resulting from retirement over a relatively short period with little or no replacement planned, major reductions in research capacities as the industry matures, reducing funding for experimental research and closure of dedicated experimental facilities, which has been accelerated by growing social distrust of experiments involving radioactive materials**.
- It is important to take in mind that the subjects related to these fields require a constructive collaboration between Physics, Chemistry, Biology, Medicine that are only different chapters of the only one great book of the life science.

ONLY WITH EDUCATION AND TRAINING IT IS POSSIBLE TO TRY TO STEM THE SITUATION

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