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Evaluation of Plasmid DNA purification for studies of radiation damage

Andreia Nunes Gouveia, Ana Clara Guerrini Schemberg, Elisabete José Vicente, Andrea Balan, Jorge de Oliveira Echeimberg, João Dias de Toledo Arruda Neto, Airton Deppman, Katherin Shtejer Diaz, Vladimir Petrovich Likachev, Fernando Guzman

This work is related to biological concern about the project for studying radiation DNA interactions, from both experimental and theoretical points of view, which has been carried out by groups at the Institute of Biomedical Science and at the Institute of Physics, at University of São Paulo. These studies have strong interdisciplinary interfaces, forcing us to face new problem that are rather unusual when purely Physical or purely Biological research are developed. In this regard, the plasmid DNA purification for studies of radiation damage present some difficulties. Most of then are related with samples purity level, which is based in presence of enzymes, rest of bacterial lyses, genomic DNA, RNA, and other substances. Many protocols can be found in literature, which may be chosen according to the application for which the DNA will be used. The cesium gradient protocol, although is considered obsolete by many researchers, is still used by some groups because it is considered the only one to achieve the high level of purification needed for the studies of radiation induced damages. Other protocols exist, mainly based on commercial kits, which are rather practical and relatively cheap. We tested some protocols for plasmid DNA purification. After this study we will be able to choose the best procedures for purification level we need to get reliable analysis of the radiation-DNA interaction.

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The potentialities of coherent X-radiation for macromolecular crystal research

V. P. Likhachev, V.B. Gavrikov, A.M. Figueiredo Neto, A.L. Bonini, J. D. T. Arruda-Neto, A. Deppman, S. Simionatto

The potentialities of coherent X-radiation for investigations of structural features of macromolecular crystals were studied using Monte Carlo techniques. We developed a Monte Carlo (MC) code to simulate coherent X-ray radiation (CXR) [1-3], produced by relativistic charged particles. The code calculates both the number of coherent photons under CXR peaks, as a function of the crystal target orientation with respect to the beam direction, and the shape of a CXR line at fixed crystal orientation. The calculations are carried out in the Laue geometry for observation angles exceeding γ^{-1}, where γ is the projectile Lorentz factor. The MC simulation takes into account multiple scattering process, attenuation of the photon flux in the target and detector window, finite detector acceptance and energy resolution of a detector. The developed code can be used to study the potentialities of CXR applications and to the planning of further experiments with CXR. The radiation spectra were simulated for the case of interaction of 20 MeV electrons with a liquid crystal, and the influence of the various crystal parameters was studied. The simulations show that coherent X-radiation spectra are almost free of background, and that such measurements could be considered as an alternative to those where Synchrotron sources are used. References: 1. Study of incoherent X-radiation induced by relativistic electrons in crystals, V.B.Gavrikov, V.P.Likhachev, J.D.T. Arruda-Neto, and A.L. Bonini, Phys. Rev. A65(2001)022903 2. Incoherent X-radiation produced by relativistic electrons in crystals, V.B.Gavrikov, V.P.Likhachev, J.D.T. Arruda-Neto, and A.L. Bonini, Eur. Phys. J. A12(2001)487-493 3. Interference effect in coherent X-radiation, V.B.Gavrikov, V.P.Likhachev and V.A.Romanov, Nucl. Instrum. and Methods, A 457(2001) 411-414

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XRF and NAA Analysis of Trace Elements in Poultry Bone

Ana C. Cestari, João D. T. Arruda-Neto, ~~Ana C. Cestari~~, Cibele B. Zamboni, Laura C. Oliveira, ~~João D T Arruda-Neto~~, A Carlos Perez, Odair D. Gonçalves, Stenio D. Magalhães

The techniques of X-Ray Fluorescence and Neutron Activation Analysis have been used to measure the trace elements concentration in poultry bones and assess its dependence on the administration of uranium- and phytase-doped ration. Phosphated rocks, well known as uranium-rich, are extensively used as a source of phosphorus in fertilizers, and also in livestock food supplements. Phytase (and some other enzymes) is also present in poultry nutrition to improve the availability of phosphorus, minerals, and metal ions like calcium. We then studied if the administration of phytase would also improve the availability of uranium, resulting in a higher accumulation of this radionuclide in bones with consequential interference in skeleton mass [1] . To investigated this situation an experiment involving several groups of Cobb broilers were performed at the facilities of Veterinary Medicine from UNESP University [2] . One hundred and fifty, seven days old Cobb broilers were separated into three groups, which were fed with variably doped rations: a) uranium (20 ppm -U); b) U-doped food (20 ppm) plus phytase (120 ppm) and c) U-doped (20 ppm) food plus phytase (180 ppm). The tibia bones were analyzed by X-ray fluorescence

(XRF), using a synchrotron radiation source, to detect and quantify several trace elements and the calcium content was measured by neutron activation analysis. Our preliminary results suggest that the biokinetics of U does not change by administration of phytase, and the skeleton mass grows faster than the corresponding uranium content.
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Monitoring of Environmental Contamination Through the Analysis of Teeth by Means of Nuclear Techniques

M.C.C.Oliveira, J.D.T. Arruda-Neto, A.Deppman, V.P. Likhachev, F.M.Luzardo, L.L Cazorla, D.R. Medero, R.Semler

Radionuclides (mostly uranium and thorium) and heavy metals (lead and cadmium) are environmental contaminants produced by agricultural and industrial activities, or are simply the result of soil and water pollution by non regular human activities (e.g. disposal of garbage in rivers and water ponds). Humans incorporate these contaminants via the food chain, where bones are the most important target-organ. The incidence of health risk and hazards would depend, obviously, on the time length and intensity of such incorporation. However, while in vivo monitoring of human bones is difficult, the analysis of teeth is a promising possibility, particularly for the quantification of lead and cadmium in deciduous tooth (milk tooth), and uranium in adult tooth.
This study will be focused, initially, on the Guarapiranga dam (the most important reservoir of drinking water for the great São Paulo region) and on the human settlements located in its surroundings. Their tooth will be collected and classified by age and social-economical status, with the collaboration of the Dentistry School from UNISA, which is developing several social tasks in the Guarapiranga region. Water, plants and fishes will be collected and analyzed too, aiming at biokinetical study of contaminants, particularly the transfer dynamics among the species of the dam.
The main analysis techniques to be employed are neutron activation analysis (NAA) and high performance liquid chromatographer (HPLC). For the NAA use will be made of the IPEN Research Nuclear Reactor, and the Cuban collaborators are in charge of running the HPLC. The preparation of targets and data analysis will be carried out at the Physics Institute, University of São Paulo.
Preliminary results obtained with HPLC show that the concentrations of lead and cadmium is considerably higher for deciduous teeth, comparatively to those from adults, as possibly due to the equally higher blood flux through the non-mineralized tooth structures.

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GAMMA RADIATION APPLICATION TO PRESERVE AMERICAN BULLFROGS LEGS (Rana catesbeiana)

Luciano de Azedias Marins, Edgar Francisco Oliveira de Jesus, Robson Maia Franco, Eliana de Fatima Marques de Mesquita, Monica de Queiroz Freitas

Depending on the dose of radiation energy applied, foods may be pasteurized to reduce or eliminate pathogens, or they may be sterilized to eliminate all microorganisms, except for some viruses (1). For example, low (up to $1kGy$) to medium doses ($1 - 10kGy$) kill insects and larvae in wheat and wheat flour and destroy pathogenic bacteria and parasites. Low to medium doses also inhibit sprouting of potatoes and other foods and slow the ripening and spoilage of fruit. Higher doses ($10 - 50kGy$) sterilize foods for a variety of uses such as for astronauts during space flight and imune-compromised hospital patients who must have bacteria-free food (2). Gamma radiation effects on American bullfrogs legs treated with dosages of $2kGy$, $5kGy$ and $7kGy$ were evaluated. Although the primary objective of irradiation of muscle foods is destruction of pathogenic bacteria, substancial reduction of spoilage microorganisms also occurs.(3) reported that levels of aerobic and anaerobic bacteria were reduced by over four logs and almost five logs, respectively, in chilled ground beef irradiated at doses to $2,5kGy$. Bacteriological and sensorial analyses were done. The study was carried out in two stages: the first one , bacteriological and sensorial analyses of radiated and no-radiated frozen meat at -18^oC; the second one, bacteriological analyses of the meat frog kept under refrigeration at the first, fifth, tenth and fifteenth days of storage. Sensorial evaluation showed up that meat frog does not demonstrate sensorial changes after being irradiated. Counting of heterotrophic aerobic mesophilic bacteria (CHAMB) and of heterotrophic aerobic psychrotrophic bacteria (CHAPB) showed up a significant reduction of microbiota in irradiated frog meat. Due to deterioration, meat frog presents organolepticals changes, after those 10 days.