

Radiation Technologies: Processes and Products

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Radiation Technologies: Processes and Products is an interdisciplinary group that uses the holistic approach as the key to conceptualize a research or a service. This interdisciplinarity, using Biology, Chemistry and Physics science, allows the study of a subject from various angles and methods unified by a common goal: the validation of methodologies to understand the subject of study.

The group *modus operandi* permits a constant connection with Industries, Universities and other Research groups applying its “way of knowing” in response to requested services, as a collaborator in a research project or in the transmission of knowledge. The group activities focus on the delineation, development, validation and application of technologies and processes in various fields, such as Environment, Food and Pharmaceuticals. As a fundamental part of the validation studies, Risk Analysis is being applied as a process management tool either in production lines of studied products (*e.g.* food, devices and pharma-ceuticals) or in environmental control (*e.g.* hospitals rooms and pharmaceutical industries).

In the scope of ITN mission the group is solicited by the authorities or private industries to undertake a consultant role on sterilization and decontamination procedures mainly applying ionising radiation. The group also develops work with the National and International normalization, standardization and certification bodies (IPQ, CEN and ISO).

Being aware of society’s current needs and the demand of Quality, Innovation and Development, the upgrading and renewal of facilities are being carried out in the scope of project REEQ/996/BIO/2005. In the course of this project, modelling tools (Monte Carlo simulations) have been applied to the pre-upgrading phase of ionizing radiation equipments (*e.g.*

gamma experimental facility). Other domain of this project has been the design of a renewed layout of an existing building transforming it in an interdisciplinary laboratory with controlled environment in order to assist new applications for radiation technology, among others. These facilities together with the inclusion of automation/robotic systems, in a further stage, have as main purpose to allow researchers of National and International Institutions and Industries to develop radiation technologies and/or to suppress the need of environmental control areas (clean areas) for their work.

The Group’s main R&D activities are focused at employing ionising radiation technologies to new processes and applications in Agriculture, Food, Pharmaceutical, Wastewater Treatment and other areas. In order to improve our understanding of the Radiation effects in products integrated methodologies composed by Analytical Methods of Biology, Microbiology, Chemistry and Physics are being used. Molecular Biology new trends based on PCR technique are being developed as a diagnostic tool (*e.g.* potential pathogenic micro-organisms) and as well as fingerprinting methods to assess the bio-diversity profile of environmental samples.

Training and “know-how” diffusion are one of the main issues of this Group reflecting in the attainment of academic degrees (Graduation, M.Sc. and Ph.D.) and in the dissemination of obtained results in the scientific community (publications, workshops and conferences).

The financial support of the group is based on projects, sponsored by National (*e.g.* FCT, AdI) and International (*e.g.* IAEA) science foundations and expertise services to Industrial Companies.

Researchers

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Applied Research a link to Industry

S. Cabo Verde, L. Alves, A. Belchior, T. Silva, R. Melo, H. Marcos, M. L. Botelho

Objectives

Applied research is related with the practical application of knowledge, material, and/or techniques directed toward a solution to a specific requirement. In this scope, the Radiation technologies: processes and products Group has been requested to apply its experience to respond to Industry either as in contracted service or in a partnership.

1) One of the performed study objective was to validate the radiation sterilization process for a product denominated “Globone®” (requested service by Ceramed). This product is a medical device intended to be used as bone implant shielding. 2) As member of a partnership with the TradeLabor several studies were developed aiming the evaluation of air quality of National Hospitals and Offices.

Results

1) In order to accomplish the sterilization level aimed for the product Globone®, some previous studies were needed in order to develop techniques that validate the irradiation process. This validation procedure was divided in two parts: I – Establishment of the Sterilization Dose for the product (D_{min}) and II - Evaluation by FTIR of product material compatibility to γ radiation (D_{max}). The establishment of the Sterilization Dose (D_{min}), *i.e.* gamma radiation dose necessary to achieve a Sterility Assurance Level (SAL) of 10^{-6} , was based on Method VD_{max}^{25} - substantiation of 25 kGy described in the International Standard EN ISO 11137-2:2006. To ascertain the maximum radiation dose (D_{max}) to be applied to the product without changing significantly its chemical composition (*e.g.* hydroxyapatite and tricalcium phosphate properties) was used the technique Fourier Transform Infrared Spectroscopy (FTIR). The product sampling plan was representative of that subjected to routine processing and conditions. The whole irradiation process was completed in the Co-60 facility (UTR) under exploitation of CHIP and located in ITN. The irradiation was performed in a calibrated place (dose rate = 2.9 kGy/h) that is comparable to the whole irradiation process in the irradiation chamber. The irradiation geometry was planned in a way that minimized the Dose Uniformity (D_{max}/D_{min}). Routine dosimetry (Perspex, Harwell) were used to monitorize the product’s absorbed dose. The validation of the bioburden determination method was performed by artificial contamination of product samples with known concentrations of a *Bacillus pumilus* E601 culture and allowed the calculation of a correction factor of 1.4. The validated bioburden determination method was applied to 10 product samples from 3 different production batches. The obtained results pointed out to a higher dispersion in product bioburden values intra than inter batches that could be explained by the found contamination peaks. The analysis of results obtained from the division of bioburden values into contamination classes suggested that bioburden frequency values for the product Globone® do not follow a normal distribution. The natural microbiota of the product was morphologically and biochemically characterized. The

most frequent morphological types of microorganisms (genus *Staphylococcus* and *Bacillus*) found in the Globone® product point out to personnel and environmental contamination. These results highlights the importance of the identification of production line critical control points and of the implementation of preventive actions based on a chart control, in order to lower and homogenizes the product’s bioburden. The verification dose experiment was based on the estimated product bioburden and the Sterilization Dose of 25 kGy was found to be adequate to guarantee a probability of non sterile Globone® product and its inner package less than one per million ($SAL=10^{-6}$). The FTIR analysis of irradiated product samples ($n = 3$) at four distinct doses (25, 35, 50 and 100 kGy) indicated that there was no significant difference between the profiles of Globone® samples. This study performed to Ceramed was audit by an international entity. A similar work is being developed to other bone product (Bonelike®) from Medmat Innovation Industry. In a near future it is intended by the group to certify the validation procedure of sterilization process of health care products. This will be possible due to a new multidisciplinary laboratory of controlled air environment that is being projected under the REEQ/996/BIO/2005, and will be accredited by ISO 17025.

2) In the evaluation of air quality, namely airborne contamination, the experimental methodology relied on air sampling methods. The quantification of the number of colony forming units (cfu) was performed by a biocollector (MAS®100) and by sedimentation plates. These two air sampling methods were used with the goal of estimating the number of viable particles that remains suspended in the air (MAS®100) and to detect microorganisms of large dimensions ($> 5 \mu m$), which tend to settle by gravity (sedimentation plates). The sampling plan consisted in several air collections, in different places of each analysed room, in order to assure the representativity of the studied parameters. The results analyses are based on the correlation between the counts of viable microorganisms and particulate matter (physical parameters measured by TradeLabor) in indoor air. Due to the lack of legislation and limits for microbiological air contamination in health care facilities, for each client (*e.g.* Hospitals, clinics) are suggested recommendations in order to improve de indoor air quality. In terms of R&D a database is being developed that reflects typical microbial levels in a variety of indoor air environments. This microbial data will be correlated with measurement of physical parameters, HVAC system information and cleaning and disinfection procedures. The development of such a database would allow legislative bodies to begin to make prudent regulations regarding microbiological quality of indoor air.

Published work

Internal Reports to Ceramed – Part I and II, Sep. 2007.

Internal Reports to: IBMC, Hospital da Luz, June 2007; Hospital dos Covões June 2007, Clínica dos Poetas July 2007, Escritórios Mercer, Hospital Fernando Fonseca.

R&D in Physical and Chemical Field

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Concerning the use of ionising radiation, dosimetry represents an important tool in the physical field. In order to evaluate the absorbed dose values, as well as the evaluation of the dose distribution (uniformity) in a product being irradiated, several dosimetric studies have been carried out. Ideally, the product should be uniformly irradiated, however, in practice this uniformity is difficult to achieve. Regarding this, Monte Carlo codes PENELOPE and MCNPX have been used for simulating the dose rate distribution in a ⁶⁰Co gamma irradiator, in order to carry out the spatial dose distribution in a certain irradiation position. Simulated results were validated comparing them to dose measurements performed with a Fricke solution and thermoluminescence dosimeters which are standard dosimeters widely used in radiation processing for calibration purposes. The agreement between the simulated values and the measurements indicates the effectiveness of both codes in performing dose measurements. In the chemical field, the effect of ionising radiation on the structure of different compounds was evaluated. The influence of the chloroanisoles on the wine cork taint is well known and it is difficult to reduce its effect. Physical-chemical pre-treatments, before and after gamma irradiation, were applied on cork samples to understand its impact on chloroanisoles concentration. Sensorial and analytical studies were undertaken. Preliminary results point out that physical-chemical pre-treatment with different solvents leads to the decrease of chloroanisoles concentration on the cork samples. Analytical techniques have to be optimized to increase the precision of the detection methods.

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Microbiological application of ionising radiation

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The ability of ionising radiation to inactivate microorganism is being researched and applied in the sterilisation/disinfection of several products. The establishment of a sterilisation/disinfection dose is based on a validated procedure that relies on the follow up of the line production up to the end of the process. In this context the sterilisation dose of medical devices was estimated for a bone implant shielding and is being carried out for a synthetic bone graft. This integrated approach is also being applied in a research project with the University of Coimbra aiming a disinfection process of a book archive using the gamma radiation. The population of fungi isolated from the books seemed to follow exponential inactivation kinetics in a surrogate substrate. In the food technology field it was studied the applicability of gamma radiation as an alternative treatment for raw milk under the scope of a graduation thesis. The results suggested that a range of doses between 1.5 and 10 kGy could be applied in the hygienisation of raw milk without significantly affect its protein profile. The environmental sustainability is other research subject of continuous interest, namely the use of ionising radiation as an optimisation tool of wastewater treatment. The influence of dose rate in the inactivation of microbial populations was studied in domestic wastewater samples during an IAEA training of a Morocco PhD student. The data obtained pointed out to an absence of a dose rate effect probably due to the high levels of radiosensitive microbiota such as the coliforms. At the genetic level, the microbial biodiversity at several radiation doses is being assessed by molecular methods under the scope of an IAEA training at Copenhagen University/Denmark. The gamma inactivation response of enteric viruses in wastewater samples are being also investigated in the development of a post-doc project. These studies are under progress.

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Risk Analysis Studies

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The study developed under the project LPM/MDN PIDDAC "Study of microbiological environment in operating rooms of HMP for the prevention of cross infection" aims the typification of strains isolated from surgical room environment in order to construct a data base. The methodology used focused the microbiological evaluation of the environment of a surgical room of HMP quantitatively (colony forming units) and qualitatively (phenotypic and genotypic characterization of isolates). The sampling plan involved collections of air with a biocollector MAS100 and sedimentation plates (before, during and after surgeries), metal surfaces and dermic and washing solutions. The average air bioburden were 10² cfu/m³ for MAS100 and 10 cfu/plate for sedimentation plates with a slight increase of air bioburden values after surgery. The microbiological contamination of metal surfaces and dermic and washing solutions point out to be negligible (<1 cfu), except for a hand washing solution (10³ cfu/ml). From all types of analyzed samples 76 isolates were phenotypically characterized and the most frequent (> 40 %) isolated morphological type was the gram positive cocci. The majority of isolates (> 44 %) were found to be resistant to iodopovidone and alcohol solutions used in the disinfection of patients. A simple and rapid chloroform DNA extraction protocol was evaluated and found out to be efficient in 51% of the isolates. For the other isolates lysozyme DNA extraction method is being applied. Molecular biology methods, namely PCR typing techniques will be applied to all isolates in order to assess their genetic similarity.

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