Radiation Technologies: Processes and Products

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Radiation Technology: 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 the response to a requested service, 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 Pharmaceutics. 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 pharmaceutics) 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 the 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 on employing ionising radiation technologies to new processes and applications on Agriculture, Food, Pharmaceutical, Wastewater Treatment and other areas. In order to improve our understanding of the Radiation effects on the 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 microorganisms) and as well as fingerprinting methods to assess the biodiversity 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 two IAEA projects, one ADI/FCT project, one LPM/MDN PIDDAC project, one re-equipment FCT project, Industrial contributions and one IEFP/FSE grant.

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Processes and Products Validation

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Objectives

As defined by the FDA, validation" means "Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes." Based on these guidelines two studies were developed: 1) one had as main objective the **validation** of a **sterilization process** of antibiotic liposome formulations for future implementation in the end of the production line. This work was performed under a consortium research project – Biostrat – between Bluepharma S.A. and INETI to develop new formulations of drugs with an antimycobacterial agent. 2) one other study, a service requested by a Pharmaceutical Industry (Delta Laboratórios), aimed at the **validation** of a finished **pharmaceutical product** through the quantitative determination of the active substance, Bleomycin Sulfate, and the evaluation of the uniformity of content of single dose.

Results

1) In order to find out if gamma radiation could be applied as a **sterilization process of antibiotic liposome formulations** was developed an experimental design integrating physical, microbiological and chemical methods. The physical studies relied on dosimetric systems according to ISO/ASTM standards, namely Fricke and Polymethilmetacrilato dosimeters for calibration (local and irradiation geometry) and routine purposes. The microbiological studies focused the detection of the critical points in the future production line in order to minimize the contamination and in the determination of the sterilization dose (D_{min}) based on ISO standards to delineate future chart controls for the bioburden. The physical-chemical studies were developed in INETI to find out the maximum acceptable radiation dose (D_{max}) that preserves the functions of the liposomes, and were based on the following parameters: antibiotic and lipid concentrations, mean size diameter and polydispersity index of the vesicles and zeta potential. The whole irradiation process was completed in the ^{60}Co facility (UTR) under exploitation of CHIP, S.A. and located in ITN. This validation consisted in the test and documentation of protocols that could guarantee not only the efficiency of the irradiation process but the maintenance as well of the functions of the irradiated product. The products used and tested in this study are still in development and in an experimental phase. Therefore, even though the ISO 11137:2006 lays as reference and guideline to all the irradiation procedures, none of the methods described in ISO were applied outright. Instead, the overall study for the foreseen radiation sterilization of the product was done using the main parameters that underlie ISO 11137:2006 such as, maximum acceptable dose (D_{max}) for product, possible sterilization dose (D_{min}) following the Good Manufacturing Practice in the production line and the foreseen dose uniformity in the process (D_{max}/D_{min}). The evaluation of bioburden in every step of liposome production lines point out to the identification of the extrusion and the columns passage as critical points. These results linked with the ones obtained in bioburden characterization led us to suggest a periodical control of the water system as a corrective action. The bioburden determination before and after irradiation at incremental doses (1 up to 5 kGy) suggested that product's microbial population resistance follow the standard distribution described in ISO 11137:2006 and can attain a low bioburden average. Consequently, future batches produced in the studied production lines that present a bioburden of ≤ 3 cfu per product unit could be irradiated at doses (D_{min}) between 12 and 15 kGy to achieve a Sterility Assurance Level of 10^{-6}. The maximum acceptable dose (D_{max}) in the whole irradiated batch (23 up to 49 kGy) has to be lower than 23 kGy to guarantee the product properties, having a dose uniformity cautious approach of $D_{max}/D_{min} \leq 1.5$ or 1.9, respectively, valid for the current irradiation geometry in UTR.

2) The experimental procedure used in the **validation of Bleomycin Sulphate for injection** was based on the specifications and control methods referred in European Pharmacopeia. The diffusion method using as test organism the strain *Mycobacterium smegmatis* ATCC 607 was performed as a microbiological assay to quantify the concentration (potency) of the active substance, Bleomycin Sulphate. Based on the estimated product potency it was calculated the content of active ingredient in each of the product units (n = 10) by subtracting the weight of the container from the respective gross weight. The followed methodology also included the calibration and control of the rooms and equipments used, namely by: 1) the biomonitorization of the air in the assay room and in the Biohazard safety cabinet equipment; 2) the control of the released pharmaceutical product in the assay room and in the Biohazard safety cabinet equipment and 3) the verification of the air flow velocity of the Biohazard equipment. The results validity was checked by statistical parameters (Shapiro-Wilk test, Bartlett test and Analysis of Variance). The obtained results demonstrated that the analysed samples of Bleomycin Sulphate satisfy the specifications required for the product release in the national market.

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Chemical Evaluation

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The application of ionising radiation as a tool to optimize the wastewater treatment is foreseen. The efficiency of the radiation impact was measured based on Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD) parameters. The samples used for the study were untreated swine and dairy wastewater, and were undertaken punctually, representing the worst scenario. An increase of the COD and a decrease of TSS were observed after irradiation for the two kinds of wastewater tested. These could be explained by the radiation-induced effect that degradates the organic pollutants that leads to an increase of the chemical species in solution (decrease TSS), resulting in a higher COD for the same absorbed doses. The overall results point out to a decrease of BOD with the increase of the irradiation doses. However, at 7 kGy, for the swine wastewater, there was a punctual increase of BOD which could be connected with the survived microbiota presented in the sample. The Kjeldahl Nitrogen and total phosphorous were also measured as they are potential toxics to the ecosystem. The obtained results are inconclusive, more studies are in progress. A lab scale agricultural wastewater treatment system will be build up, in a near future, to simulate an overall picture of an integrated treatment to foresee better the technical and economical benefits of the design.

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Microbiological evaluation

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The impact of ionizing radiation on microbiota and cells, is studied using different approaches. The range of the irradiation action is measured by inactivation curves where the samples are submitted to several doses and dose rates. The survivors' strains are phenotypical and biochemical characterized and compared with the strains retrieved from non irradiated samples. This permits to establish an integrated view of the changes in the microbial community associated with the absorbed dose applied to the sample as well as for the use of ionizing radiation as tool in the sterilization process. The developed work in this context are the validation of the radiation sterilization of a kettle filter for the industry *Lobo International, Ltd*, Cambrigde/ England and other projects under the title: "Processes and products validation". Untreated swine and dairy wastewater studies were carried out aiming to use ionizing radiation as an optimization tool of the wastewater treatment. We studied the impact of the ionizing radiation on the effluents' microbiota. Studies were also carried out to a genotypic level applying molecular biology fingerprinting techniques (MSP-PCR, RADP-PCR and the 16S- PCR digestion) to isolates bacterial DNA in order to access their genetic similarity and possible genetic alterations induced by gamma radiation. These studies are under progress.

Physics evaluation

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Under REEQ/996/BIO/2005, the upgrading of an irradiation facility (PRECISA 22 ML) is foreseen. To assess the dose distribution inside the irradiation facility, Monte Carlo methods were applied. These methods in radiation transport calculations and particle transport simulations allow a comprehensive knowledge of the spatial dose distributions and the optimization of the irradiation process. The computational tools used to perform these studies were MCNPX and Penelope codes. Taking into account the construction of a pilot plant for wastewater treatment, the studies were performed regarding three different levels inside the irradiator. The results showed dose rate uniformity in the level more distant to the source. The level near the source showed significant differences between dose rate values, including the maximum dose rate of approximately 35 Gy/h. The validation of the simulation results obtained was performed by chemical dosimetry methods, namely by Fricke solution. Studies are underway in order to estimate the uncertainties and to further improve the agreement between experimental and simulated values, including studies using physical dosimetry. Since, dosimetry plays an important role in an irradiation process, a continuous collaboration with CHIP, S.A. is of crucial importance. As result, it was performed a routine dosimeter calibration, Red-Perspex, batch JB 4034, using ceric cerous as a reference dosimeter.

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