Radiation Technologies: Processes and Products

M. Luísa Botelho

The **Radiation Technologies: Processes and Products** activities focus on the research, development and demonstration of interaction of ionising radiation with matter for further application in Industry or other entities. Since 1989 these activities have been closely related to the gamma radiation facility (UTR), whose main applications are the sterilization of medical devices and pharmaceuticals and the decontamination of other products. These activities have led to an incremental interest by Industrials (CHIP) that have in turn led to a joint venture in 2003 for the management of UTR by CHIP, with scientific and technical support provided by ITN researchers.

Nowadays the group supervises the sterilization and decontamination procedures being carried out at the UTR. The group also develops work with National and International normalization, standardization and certification bodies (IPQ, CEN and ISO). UTR was upgraded by CHIP and future work will be carried out in compliance with ISO and European standards in the perspective of obtaining accreditation.

In order to develop the procedures at UTR, based on the Quality System, studies of dose distribution and determination of D_{min} and D_{max} are performed taking into account the Safety Assurance Level and the safety of the product.

The main Research and Development activities are focused on new processes for further application of the ionising radiation to Food, Pharmaceutical and Polymer Industry and others.

The main purpose of the **microbiological** work is to develop and implement validation technologies for inactivation procedures for micro-organisms, mainly by ionizing radiation (e.g.: γ and e-beam).

These technologies are based on microbiological studies on the bioburden in/on the products, and aim to improve quality in this field.

Thus, hazard analysis and the control of critical points in the production lines of the studied products are part of the validation studies, carried out for the **Pharmaceutical** and **Food** Industries. Environmental control in surgical operation theatres at hospitals is also carried out.

In order to improve our understanding of the Radiation Procedures the influence of dose rate and the type of radiation are studied using materials and micro-organisms.

The **Synthetic** and **Natural Polymers** studies aim to use radiation both for developing new materials and for improving the quality of existing materials. These studies also aim to improve understanding about the interactions between radiation and these materials in order to avoid damaging the final product. Copolymerisation, reticulation and other effects induced by radiation are tested (carried out and characterised) to improve the properties and biocompatibility of new materials to be used for biomedical and environmental applications.

The Group collaborates with several Universities and Polytechnic and Research Institutes.

New Projects are presently being developed to apply **ionising radiation techniques** to **Wastewater treatment.**

This technology could contribute to the correction and control of contamination by anthropogenically produced pollutants of water resources.

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Radiation Technologies: Processes and Products

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Funding (€)

Research Projects:	4.115,08
Services:	23.916,04
Total:	**590.000,00**

Publications[1]

Books:	0
Journals:	1 and 1in press
Proceedings:	4
Conf. Communications:	4
Other publications:	5
Theses:	Graduation 2

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Food Irradiation

S. Cabo Verde, M. L.Botelho, R. Tenreiro1, P. Pinto2, R. Ribeiro2, L. Sousa2, M.G. Lima2, A. Santana2 and M. J. Trigo3

Objectives

The use of ionising radiation, from radionuclides such as ^{60}Co, in conjunction with good manufacturing practices, is an effective technology to improve safety of food supplies. In this field two projects are being developed:

1 – "Application of Ionising Radiation to Eggs" – The aim of this project is to develop and validate methodologies for applying irradiation technology to eggs in order to ensure safe products, free of pathogenic microorganisms such as *Salmonella* spp. and *Campylobacter* spp., without significant adverse effects on egg quality.

2 - "Application of Ionising Radiation to Vegetables" – The aim of this project is to evaluate the effectiveness of irradiation on microbial, colour, texture and sensorial parameters in some produce.

Results

1 – "Application of Ionising Radiation to Eggs" –

The continuation of irradiation studies was developed in a cobalt-60 facility (UTR – ITN), with 47kCi activity, at room temperature and at a dose rate of 1 $kGyh^{-1}$. The range of applied doses was 0.2 up to 5 kGy. In the determination of the D-values of pathogenic microorganisms, eggs were artificially contaminated (*ca*. $10^7 – 10^8$ cfu/egg) externally (shell) and internally (yolk+white: y+w), with reference strains of *Salmonella typhimurium* (ATCC 14028), *Salmonella enteritidis* (ATCC 13076), *Campylobacter jejuni* (DSMZ 4688^T) and an environmental strain isolated from egg-products, identified as *Salmonella arizonae*. The 90% inactivation level of natural contaminants of eggs and egg-products was also determined. The study of the inactivation response of *S. enteritidis* and *S. arizonae* in egg-products was performed. The D_{values} (kGy) for each strain, for egg shell and yolk+white, and total microrganisms in eggs and egg-products, were calculated based on the number of survivors: *S. typhimurium* 0.33 ± 0.01 kGy (shell) and 0.37 ± 0.01 kGy (y+w); *S. enteritidis* 0.204 ± 0.002 kGy (shell), 0.12 ± 0.01 kGy (y+w), 0.27 ± 0.01 kGy (egg white product) and 0.333 ± 0.003 kGy (egg yolk product); *S. arizonae* 0.44 ± 0.01 kGy (shell), 0.394 ± 0.002 kGy (y+w), 0.227 ± 0.001 kGy (egg white product) and 0.529 ± 0.005 kGy (egg yolk product); *C. jejuni* 0.073 ± 0.005 kGy (shell) and 0.082 ± 0.001 kGy (y+w); total microorganisms 1.29 ± 0.02 kGy (whole egg), 1.7 ± 0.2 kGy (egg white product) and 1.2 ± 0.1 kGy (egg yolk product). The D_{values} were found to not be significantly different ($p < 0.05$) for the strains analyzed when present in the eggs shell or yolk+white. The comparison of pasteurisation *vs.* irradiation in the treatment of egg-products was carried out. The results obtained show a slightly higher efficiency (99,9% *vs.* 99,7%) of irradiation treatment (at 2 kGy) in the inactivation of total microorganisms present in egg-products.

Viscosity, physical-chemical properties and nutritional value of eggs and egg-products before and after γ irradiation, were studied by two training students (Escola Agrária de Santarém). Irradiation of shell eggs and egg-products seems to have no significant effects on the protein patterns (Fig. 1), phospholipids and viscosity.

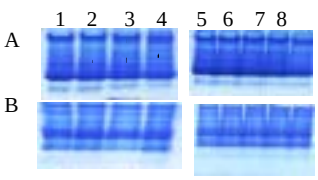


Fig. [1] – Protein analysis of whites (A) and yolks (B). 1-non-irradiated eggs; 2 to 4-irradiated eggs with doses of 0.5, 2 and 5 kGy; 5-pasteurized egg-products; 6-non

Based on the inactivation, functional and nutritional results obtained we could suggest that a radicidation dose of 1.5 kGy would be sufficient to eliminate the pathogenic microorganisms from the whole egg without significant adverse effects on egg quality.

The development of a protocol to detect pathogenic microorganisms directly from eggs and egg-products is also being studied, applying molecular biological methods in order to replace the laborious and time consuming standard methods (ISO 6579:2002 and ISO 70272:1995(E)).

2 - "Application of Ionising Radiation to Vegetables" – Several samples of minimal vegetables were irradiated in the gamma facility. Organoleptic and microbiological studies are being developed.

Published, accepted or in press work

1. S. Cabo Verde, R. Tenreiro, M. L. Botelho. "Hygienization of chicken eggs by ionizing radiation leads to safety eggs". Presented in FEMS 2003, June, Slovenia.
2. S. Cabo Verde, R. Tenreiro, M. L. Botelho. "Sanitation of chicken eggs by ionising radiation: HACCP and inactivation studies" Presented in IMRP 2003, September, Chicago. Manuscript submitted to *Radiat. Phys. Chem.*.
3. P. Pinto, R. Ribeiro, L. Sousa, S. Cabo Verde, M.G. Lima, M. Dinis, A. Santana, M. L. Botelho. "Sanitation of chicken eggs by ionising radiation: functional and nutritional assessment" Presented in IMRP 2003, September, Chicago. Manuscript submitted to *Radiat. Phys. Chem.*
4. M. JoãoTrigo M. Luisa Botelho *et al* 2nd Internal "Improving quality and safety minimally processed fruits and vegetables by gamma irradiation" Report to IAEA, POR-11682 CRP, September 2003

[1] Dept . Biologia Vegetal, Faculty Science/ Lisbon University; [2]- ESAS – Technical University; [3]– INIAP – Min. Agricultura e Pesca

Environmental Control

P. Matos, S. Xisto[1], P. Mazarelo[1], S. Cabo Verde, M. L. Botelho

Objectives

1 – Environmental control of surgical rooms in army hospitals and the impact on the incidence of cross-infections.
2 - The environmental control of production rooms at the pharmaceutical industry Aventis® was carried out in two phases: Steady state and Labouring state, leading to the classification of eight controlled areas.

Results

1 - This project focuses on the development and improvement of alternative techniques of controlling the environment in surgical rooms leading to the detection and identification of nosocomial microrganisms in a Portuguese Hospital. This will enable the construction of a database that could demonstrate the relationship between the improvement of the air born conditions and the hospital infection agents.
The first phase of the project was developed with the purpose of inter-calibration and validation of the two different air samplers as well as the validation of the collection techniques. The air samplers used were SAS® (surface Air System) and MAS-100® (Merck Air Sampler). Different volumes of air were collected nominally 90, 270 and 450 litres for assessment of the performance of the studied equipment (Fig. 1) and the results showed that the both equipments can be considered as being equivalent.

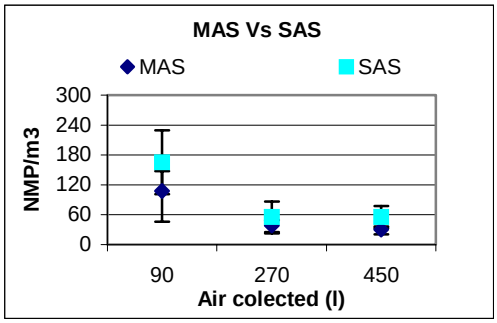


Fig. 1- graph showing the 3 different volumes collected, which shows that for the two air samplers used there is no significant difference between MAS-100 and SAS. Each point n=9 and confidence interval of 95%

When the two air samplers are compared at the same volume (Fig. 2) the results also show that both equipments are similar.
The second stage of this project includes continuous monitoring of the surgery room's natural airborne and nosocomial microrganisms, and the consequent actualisation of the database. The present study will be carried out based on molecular type of isolated trains, and this procedure could lead to a model for the detection of emerging microrganisms that could become hospital infectious agents

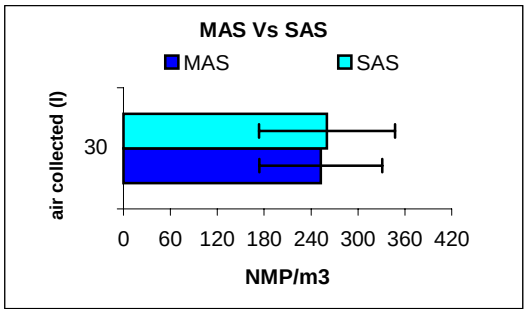


Fig. 2 - Results obtained with two air samplers for 30 litres of air collected. Each equipment n=21 and α=0.05.

2 - The sampling was performed using three methods: of air sampling by the air sampler MAS100 (0,1 m^3 of air), settle plates to detect airborne viable particles depositing from the air and contact plates to sample the surfaces. The results obtained in the two phases show that the biocontamination values (colony forming units – CFU) determined by the three techniques were below the acceptance criteria established by Aventis and the government ruled limits present in the "Portaria nº 42/92, 23rd of January": air samples < 500 CFU/m^3 of air; settle plates < 100 CFU/4 h and contact plates < 50 CFU/plate. Based on these results the eight production rooms studied could be classified as Category D areas. The morphological characterization of obtained colonies was carried out in order to detect major contaminant morphological types. In both phases the predominance of one morphological type was detected, gram-positive catalase-positive cocci (Fig. 3), which is frequently spread in the environment.

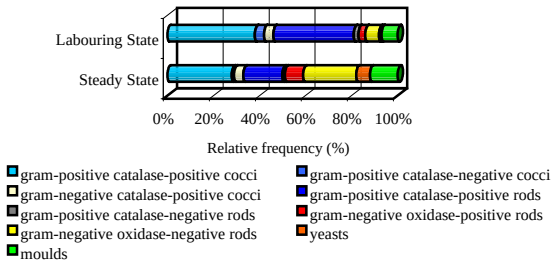


Fig. 3 – Morphological types of the obtained colonies by the three techniques, in the Steady State (n = 1148) and Labouring State (n = 1558).

NOTE: under this field this Group was also participating in the validation of URSA. This work is described in "Elemental characterization and speciation using ion beams" Group

Published, accepted or in press work

1. P. Matos, S. Cabo Verde Final Report: "Environmental control study on production rooms of the pharmaceutical industry Aventis". Oct. 2003.
2. P. Matos, M. L. Botelho. 1st Report: "Preliminary studies for environmental control in Portuguese Army Hospital". October 2003.

Effluents

M. L. Botelho, Paula Matos, Alexandre Costa, Rita Melo, M. C. Freitas and J. Branco

Objectives

Implementation of ionizing radiation technology in the wastewater treatment in Portugal. The response of microbiological and physical-chemical factors has been studied, according to the type and main characteristics of irradiation equipment, on natural effluent samples.

Results

Studies on two kinds of wastewater effluent samples from municipal effluent showed that total wastewater microrganisms inactivation and chemical dynamics at lower dose rates could have different responses than at higher dose rates. To compare the impact of γ and β radiation at the dose rate of 1 kGy/h was applied on effluent samples from a vegetable processing agro-industry. Physical-chemical and microbiological studies were developed before and after irradiation.

Physical-Chemical Studies

To study the dynamics of chemical elements between solid and liquid phases, Instrumental Neutron Activation Analysis (INAA) was used as was FTIR analysis. Samples were irradiated at doses of 5 and 10 kGy, at a dose rate of 1 kGy as well as at doses of 3 and 6 kGy at an electron beam facility and at dose rate of 1.1 kGy. The results obtained using INAA showed that there is no difference between γ and β radiation. In Fig.1 the response of the two kinds of radiation on Cl element can be seen, as an example response. FTIR analysis results suggested that the functional groups presented in both phases did not show changes after irradiation at 10 kGy. The functional groups formed are essentiality due to the nature of the effluent, namely from vegetables (green pepper and aubergine) and include aromatic hydrocarbons and aromatic rings, and are also from the water used during the process, such as Cl.

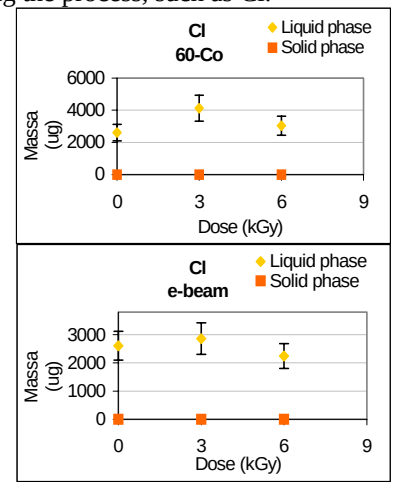


Fig. 1 - Example of behaviour of the chemical elements (Cl) presented at both phases.

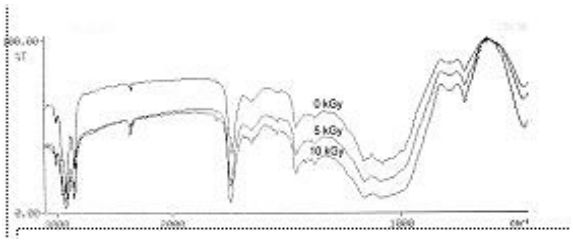


Fig. 2 - FTIR spectrum of the solid phase of an Agro-industry before irradiation (0 kGy) and after γ irradiation (5 and 10 kGy).

Microbiological Studies

The target doses were 0.3, 0.6 and 1.2 kGy for the determination of the total coliforms. For total count the doses were 3 and 6kGy.

To determine "total coliforms" the multiple-tube fermentation technique was applied to obtain the Most Probable Number (MNP) present in samples.

To determine the "total count" of microorganisms a direct plating technique was used. The growth conditions were aerobic incubation at 30ºC for 14 days on Tryptic Soy Agar (TSA) plates. Colony forming units were counted after 24h, 48h, 72h, 7 and 14 days.

Some of the results obtained for the inactivation of total microrganisms are presents on Figure 2.

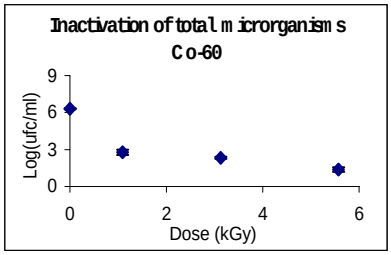
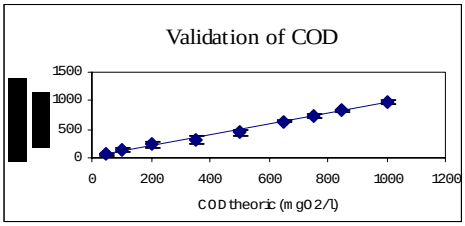


Fig. 2 – Inactivation of total microrganisms by gamma radiation (Co-60) D value found was 1,28 kGy.

In order to complement the physical-chemical analyses we are studying and validating another technique, the COD (Chemical Oxygen Demand). One of the main parameters to quantify is the organic matter in wastewater (Fig. 3).



Published, accepted or in press work

1. M.L.Botelho, *et al* Progress report of the research project "Impact of ε-beam and γ radiation on the wastewater and drinking water: Comparative studies" IAEA, July 2003.

Modification of Molecular Structures by Gamma Irradiation:

L.M. Ferreira, A.N. Falcão, M.H. Gil

Objectives

Preparation and/or modification of new polymeric and hybrid materials using gamma radiation for material properties tailoring.

Part I - New silica-based hybrid materials have been produced by irradiation of the precursors (tetraethylorthosilicate, TEOS, with addition of Zirconium propoxide, PrZr, and polydimethyl-siloxane, PDMS, silanol terminated). The influence of the molar fraction of PrZr, of the organic/inorganic volume ratio and of the irradiation dose, on the microscopic structure of the final materials was investigated.

Part II - The surface properties of low density polyethylene (LDPE) can be modified through the grafting of 2-hydroxyethyl methacrylate (HEMA) branches. In this way, new materials with promising applications as biomaterials can be produced. The influence of the sample preparation conditions on the final properties was studied. Different irradiation dose rates, HEMA concentrations and irradiation environment conditions were tested.

All irradiations experiments were carried out at the UTR ^{60}Co facility

Results

Part I – New silica-based hybrid materials

Inorganic mixtures of TEOS with addition of PrZr (x =0. 5 and 10 mol%) were used together with PDMS silanol terminated. Samples with volume ratios organic/inorganic of 4/1, 3/2, 1/1, 2/3 and 1 /4 were prepared. The irradiation dose for gel point was observed to depend on the PDMS/ INORG. volume ratio, increasing with decreasing volume ratio. Samples with PDMS/ INORG = 2/3 were irradiated to several irradiation doses above that corresponding to the gel point, which becomes higher for higher fractions of PrZr added. The resulting samples are homogeneous, transparent and flexible being able of swelling in a good solvent of the polymer. The microstructure of the samples was investigated by Small Angle Neutron Scattering. Samples were measured as prepared, and immersed in deuterated cyclohexane. Results indicate the presence of oxide dense clusters interconnected by polymer structures. The addition of PrZr increases the density of the clusters and decreases their size.

Part II – Modification of LDPE by Grafting Copolymerisation

Samples with $[HEMA]_i$=5, 10 and 15% were prepared at irradiation dose rates of 0,3 kGy/h and 0,5 kGy/h, both in the presence and absence of air.

Results show that: (i) the dose rate is determinant for the final grafting yield, low dose rates being preferable; (ii) in the presence of oxygen, grafting continues beyond irradiation, reaching a saturation level; (iii) the grafting yield depends on $[HEMA]_i$ in an almost linear way; (iii) in the presence of oxygen, after an induction period, all curves seem to fit a typical 1st order kinetics.

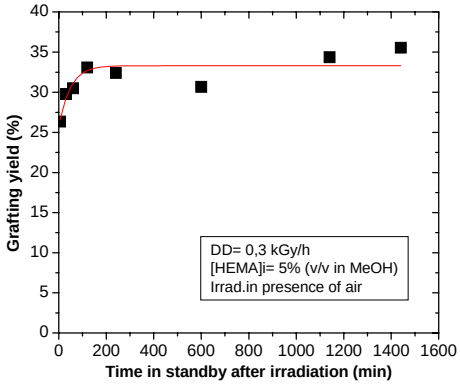


Fig.1: Grafting yield obtained after different waiting times pos irradiation. All samples have been irradiated for 10h in continuo.

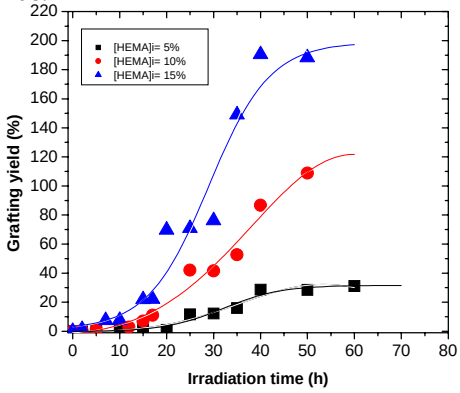


Fig.2: Effect of $[HEMA]_i$ on the grafting yield of HEMA onto LDPE. (dose rate of 0,3 kGy/h, irradiated in the presence of air).

Complementary DSC and TGA data show a decrease in cristalinity and thermal stability upon grafting. The decrease is enhanced by the presence of oxygen.

Published, accepted or in press work

1. A. N. Falcão, M. Carrapiço, J. Santos Sousa, F.M.A. Margaça, L.M. Ferreira, F.G. Carvalho, I.M. Miranda Salvado, J. Teixeira, Investigation of Organic-Inorganic Hybrid Materials Prepared by Irradiation, *Journal of Sol-Gel Science and Technology 26, 349-352,2003.*

Study on grafting of 2-hydroxyethyl methacrylate onto chitosan

M.H. Casimiro, J.P. Leal[1, 2], M.H. Gil[3]

Objectives

Our attention has been focused on efficient and well-controlled grafted copolymerisation methods of 2-hydroxyethyl methacrylate (HEMA) onto chitosan in order to obtain a biocompatible vehicle for sustained released of drugs.

Results

Of the possible physical-chemical modifications of chitosan, graft copolymerisation can provide materials with the desired properties through the appropriate selection of the molecular characteristics of side chain to be grafted. In the present study, 2-hydroxyethyl methacrylate (HEMA) has been grafted onto chitosan by different grafting methods. In this specific case the graft copolymerisation reaction was carried out by using gamma radiation (from UTR's ^{60}Co source; dose rates of 3.8 and 7.4 KGy.h^{-1}), by photo-induction with UV light (80 W.cm^{-1}) and by chemical initiation ($9.12\text{x}10^{-4}$M≤[Initiator]≤$1.82\text{x}10^{-2}$ M).

Evidence of grafting has been provided by characterization of chitosan and its graft copolymers by FTIR spectroscopy and thermal analysis. The grafting parameters are obtained by the following expression:

$$\text{Percent grafting} = \frac{\text{wt of monomer grafted}}{\text{wt of chitosan}} \text{x } 100$$

Results suggest that gamma irradiation is the method that leads to higher yields of grafting. They also show that the grafting yield increases with the increase in absorbed dose (*v.d.* figures1 and 2).

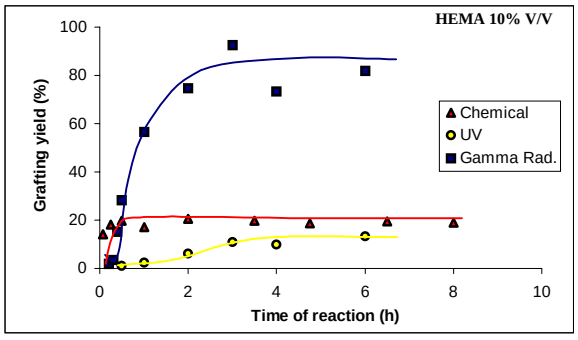


Figure 1. Profile of HEMA graft copolymerisation onto chitosan induced by chemical, UV and gamma radiation (dose rate 7.4 KGy.h^{-1}).

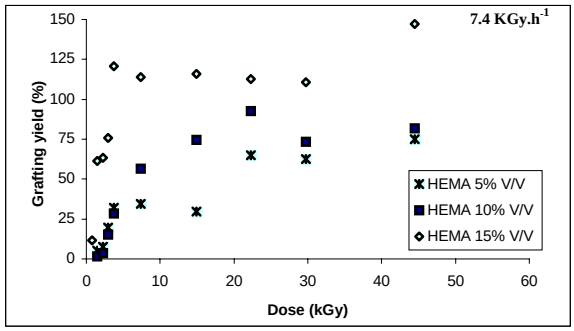


Figure 2. The effect of monomer concentration on grafting yield of chitosan induced by γ radiation.

The results from TGA curves (Figure 3) do not indicate a decrease of thermal stability by copolymerization. Although the profile of copolymer curves shows an increasing similarity with the poly(HEMA) curve with the increase in grafting yield.

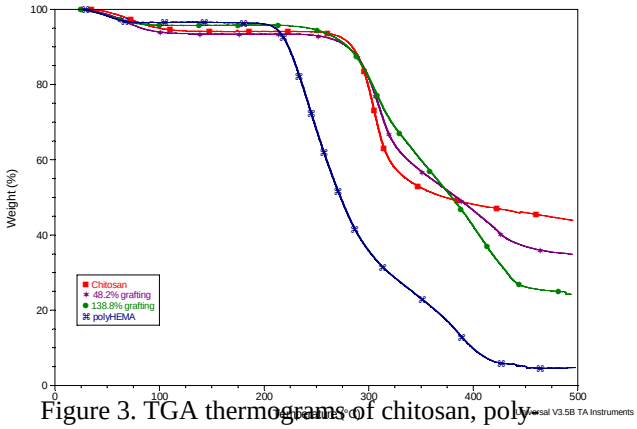


Figure 3. TGA thermograms of chitosan, poly-HEMA and copolymers with different grafting yields ([HEMA]$_i$=10% V/V; DR=3.8 kGy.h^{-1}).

Published, accepted or in press work

1. M.H. Casimiro, J.P. Leal, M.H. Gil, "Chitosan Graft Copolymerisation Induced by Chemical, UV and Gamma Radiation" in *Proceedings of 2003 Europe/Africa Regional Meeting of Polymer Processing Society*, Athens, Greece, September 2003.

[1] Dept. of Chemistry, Nuclear and Technological Institute, 2686-953 Sacavém, Portugal
[2] Dept. of Chemistry and Biochemistry, Faculty of Sciences, Lisboa University, 1749-016 Lisboa, Portugal
[3] Dept. of Chemical Engineering, Faculty of Science and Technology, Coimbra University, 3030-290 Coimbra, Portugal

Pharmaceuticals

L. Alves[1], P. Matos, H. Marcos, M. L. Botelho

Objectives

This work aims to simulate production *in situ*, in particular the production of one batch and the determination and validation of minimum doses for sterilization by gamma radiation. This work must be carried out in hygienic conditions. Therefore the environment conditions as well as the workers were monitored and validated. The bioburden in the worst conditions was determined.
Preliminary studies to radiosterilize a new product by MedMat Enterprise.
This product is named Bonelike® and is a highly bioactive bone substitute material with a chemical composition that has been formulated to closely resemble human mineral bone using innovative processing routes. Bonelike® is a synthetic hydroxyapatite that incorporates specific ionic species that enhance new bone formation rate. Bonelike® is available in different morphologies, such as dense and porous granules, blocks with specific shapes according to the implantation site and coatings on metallic implants and prostheses. It is used in dentistry, maxilofacial surgery and orthopaedics. Due to the general characteristics described it must be sterilized using ionizing radiation.

Results

Preliminary studies show that the natural bioburden was present in small quantities, thus the Most Probable Number (MPN) technique the accord with ISO 11137 "Sterilization of health care products – Requirements for validation and routine control – radiation sterilization". The clean room and the laminar flow were validated with the air sampler MAS – 100 and settle plates with Tryptic Soy Agar, for the qualitative e quantitative evaluation of the possible surface contamination by airborne viable particles depositing from the air.

Results from validation of the clean rooms were represented at figure 1

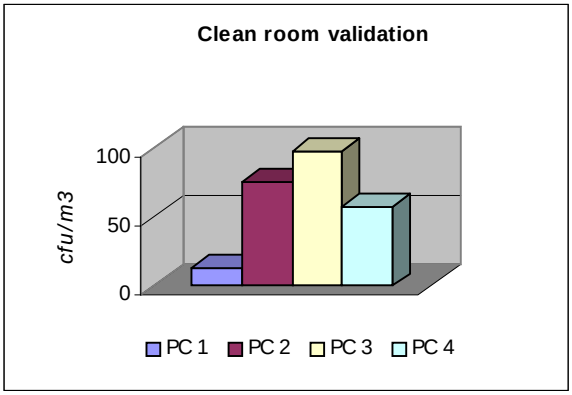


Figure 1 – Results obtained for the clean room validation. The clean room classification was made with 4 critical points at the area (PC) and classificated as Category C

The laminar flow was validated as well and the results show that is category was A as we can se on figure 2.

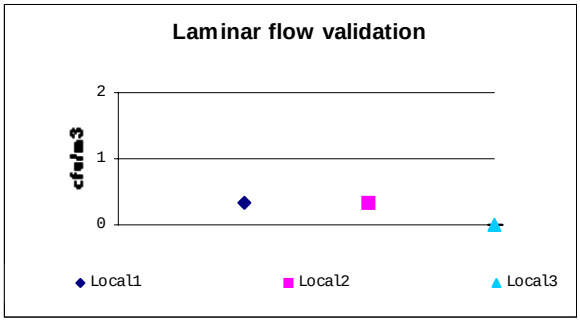


Figure 2 – Results obtained for airflow validation. The average of u.f.c./m^3 present were below than 1, than the classification to air flow equipment were of A

Published, accepted or in press work

1. L. Alves, P.Matos, H.Marcos, ML. Botelho, Internal report, 1st progress report Bonelike, October 2003.

1 on leave from the Upsalla University, Sweeden

Gamma Radiation Facility (UTR)

M. Luisa Botelho, P. Pereira, J. Venâncio, V. Damas

Objective

Since 1989 the Gamma Radiation facility works as a pilot plant to gamma irradiation services such as radiosterilisation of medical devices and pharmaceuticals, and decontamination of raw materials, herbs, etc, to institutions and companies.

UTR also supports R&D projects in the field of gamma radiation interation with matter in order to develop new applications of this technology for the Industry and other instituitions.

The last years the facility turns at difficulties due to the low activity of the facility (50 kCi in 2002) its laboring limitations have been increasing. In this way, its continuing performance decrease leads to very large irradiation times, which are very difficult to sustain face the real industry necessities. On other hand, for some products, large irradiation times can be dangerous for the final product quality.

In Agoust 2003 a joint venture with an enterprise named CHIP was performed and the replenishment of Cobalt – 60, up to 1,1 x 10^{16} (300 kCi), and the upgrading of the main systems were made with the tscientific and technical support provided by ITN researchers.

The future work in UTR will be carried out in compliance with ISO and European standards in the perspective of obtaining accreditation.

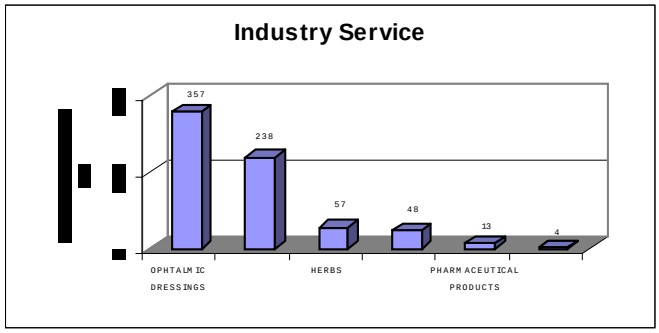
In order to develop the procedures at UTR, based on the Quality System, studies of dose distribution and determination of Dmin and Dmax will be performed taking into account the Safety Assurance Level and the safety of the product.

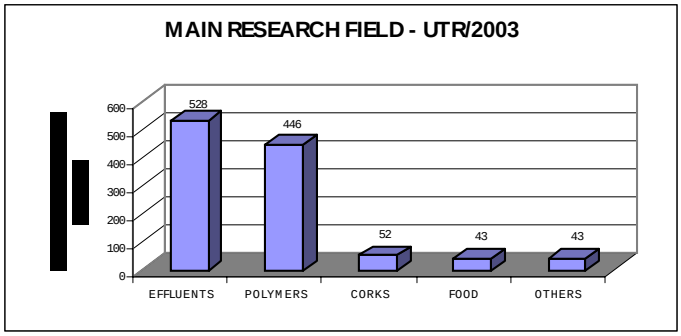
Results

During this year the facility has supplied gamma irradiation services for several national and foreigner companies, namely, *Oftalder S.A.*, *Hovione S.A.*, *Lecifarma Lda*, *Hikma Farmacêutica S.A.*, *Falcão Teles Lda*, *Lorca Marin* (Spain), and a few others with low economical expression.

The R&D irradiation services for internal and external projects were performed in order to made radiation technology transfer a reality.

The figures show a resume of the irradiation services distribution during until September 2003.





Published, accepted or in press work

1. A Carlos Oliveira, Hélio Yoriyaz, M. Carmo Oliveira, L.M. Ferreira, Monte Carlo simulation for dose distribution calculations in a CT-based phantom at the Portuguese gamma irradiation facility, *Nucl. Inst. and Meth. B*