Radiation Technologies: Processes and Products

The Radiation Technologies Processes and Products activities are directed to the development and demonstration of radiation processing applications, namely radiation sterilisation of medical devices and pharmaceuticals, preparation and characterisation of graft copolymers and detection of irradiated food, privileging gamma radiation. These activities are closely related with the gamma radiation facility UTR, unique in this country.

The ***Group of Radiation Sterilisation*** is supporting the sterilisation and decontamination procedures being made in gamma radiation facility - UTR and others Industrial Procedures.

The main purpose of this group is to develop and implement the validation technologies for Procedures of inactivation of microorganisms mainly by radiation (e.g.: γ and ε-) and by others lethal agents (e.g.: steam under pressure). These technologies are based on microbiological studies on bioburden in/on the products in order to improve the quality in this field; as so, the hazard analysis and control of critical points in the production lines of the products make part of the validation studies.

The mechanisms of action of the lethal agents in microorganisms are studied in order to improve the knowledge of the Procedures.

This Group is in straight connection with Vegetal Biology/Faculty of Science, University of Lisbon, teaching sterilisation procedures in Microbial Biotechnology Class.

The Group develops work to Enterprises involving Food, Medical Devices and Pharmaceutical Industry and is in connection with the National and International bodies of normalisation, standardisation and certification (IPQ, CEN and ISO). To achieve these goals we have had various projects running.

The main purpose of ***Polymer Modification and Methods of Characterisation Group*** is to develop and optimise the techniques of copolymerisation and/or modification of the molecular structure of natural (Chitin and Chitosan based polymers) and synthetic polymers (Polyethylene based polymers) using gamma radiation as the energetic source for the chemical changes involved in those processes.

These studies are oriented in order to obtain new materials with biocompatibility or with the proper properties to be used for the immobilisation of drugs and biocatalysts.

These studies are carried out at the *Polymers Characterisation Laboratory (LCP)*/Physics/ITN in close collaboration with Prof. M. Helena Gil from University of Coimbra. Irradiations are done at the ITN radiation facility (UTR).

We are now giving special attention to natural polymers. Most of these materials are naturally biocompatible, but in general with no adequate mechanical properties. Gamma irradiation may be the solution for some of the most desirable modifications (copolymerisation, reticulation, etc).

Another purpose is to answer several problems presented by the national industry, particularly concerning with the treatment of plastic devices by gamma radiation. This problem has two faces: *1)* the gamma resistance of the materials and, *2)* the possibility to improve some properties in the materials with gamma radiation or during gamma sterilisation.

Detection of Irradiated Food using analytical methods is an important tool to identify foodstuffs treated by radiation. The publication of EU Food Irradiation Directives during 1999 has boost up the need for the implementation of those methods.

Two physical methods are studied: the ESR method (using the IST equipment) and the Viscosimetry method. Concerning the ESR method our studies have found that the stability of radiation-induced radicals depends not only on the sample and storage conditions but also on the kind of radiation used to treat the product.

The aim our work using the Viscosimetry method is to establish a comparable and reliable protocol to get approval this method as a CEN standard.

Radiation Sterilization

BIOSTER Project*

M. L. Botelho; H. M. Marcos; A. Coelho[1]; M. Chumbinho[1]; F. M. Águas[1]; C. Teles[2]; M. Baptista[2]; S. Cabo Verde[3]; R. Ribeiro[3]; I. Ribeiro[4]; A.P. Marques[4]; I. Oliveira[5]

Objectives

The purpose of this project was develop and validate the methods to determine the biological parameters in medical devices prior to sterilisation and transfer these procedures to the Industry. It was a consortium Project whose partners were the Medical Devices Industry (BASTOS VIEGAS, FAPOMED, OFTALDER), ITN as R&D lab, INFARMED as Certification Entity and APORMED as Normalisation Entity. The BIOSTER Project-AdI/FEDER/PRAXIS XXI - L-1347 began in February of 1997 and finished in June 1999.

Results

During 1999 the chart controls for initial contamination of each product were build up based on validated protocols. Studies in the resistance of natural bioburden in/on products to the lethal agents: γ radiation, ε- beams and steam under pressure lead to validation of the Dose (Dmin) or time sterilization to a SAL of 10^{-6}, based in Method 2 of ISO11137/1995.

Summarising, the "Pre-proposal" was presented in September 1995, accepted in May 1996 by AdI/PRAXIS XXI. "Proposal", and "Contract Consortium" presented in July 1996 accepted by AdI/PRAXIS XXI in September 1996. Definitive contract was signed with AdI/PRAXIS XXI in June 1997. The IMPLEMENTATION PHASE started in February 1997 with 5 months of training of personal and preliminary studies lead out to the bioburden estimation for three "parameters" family based on validated protocols. The seasonal influence on the numbers and type of microorganisms in /on the products were studied based on the temperature and humidity records along the year. Microbiological studies in the line production of each factory were performed. Education of the factories' workers for improving the hygienic conditions were carried out Corrections in line productions were undertaken in order to obtain a better microbiological quality of the products.

Four scientific as well as economic reports were presented and audits were successfully passed. The implementation phase finished in June 1999 and the final report from the scientific auditors was in favour of BIOSTER Project. Part of examples of the results obtained in the 1st PHASE of the project (initial contamination of the products) is to be presented as an example in ISO 11737-3 *Guidance on the evaluation and interpretation of bioburden data.* The experimental results obtained from dose distribution studies, during the project were compared and worked out with simulation by Monte Carlo [1] (see published worked -1999, Oliveira *et al*). Education graduated students to the Industries in the University was accomplished.

Reference

1. Oliveira, C., Salgado, J., Botelho, M.L., Ferreira, L.M., Monte Carlo Application for Irradiation Processes Planning at the Portuguese Gamma Irradiation Facility. *IVth Topical Meeting on Industrial Radiation and Radioisotopes Measurements Applications*, Raleigh, North Caroline, USA, 3 - 7 Oct. 1999, (oral communication).

Further work

Some controversial results obtained under the 1st and 2nd phase of the project, mainly the initial contamination values and the application of Method 1 of ISO11137/1995 for determination of sterilisation dose are still under study.

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* Budget: PTE 33 × 10^6 PTE (EURO 164 056,1) for two years, salaries not included.

[1] BMCT/PRAXIS fellowship-University Degree.

[2] oftalder/Bioster & ITN fellowship–University Degree.

[3] ITN fellowship – University Degree.

[4] ITN fellowship – Technician.

[5] Bastos Viegas – Pharmacist.

Microbiological Validation in Products to Gamma Sterilized in UTR and Analysis of Control Critical Points in line production"

M. L. Botelho; H. M. Marcos; C. Teles[1]; A. P. Marques[2]; Nuno Medeiros; Ana Marques[3]

Objectives

R&D are applied under contract with Industry. The group developed work in the field of biological validation in a pharmaceutical product to gamma sterilised in UTR as well as in the field of hazard analysis and control of critical points in the production line in a water Industry.

These technologies are based on microbiological studies on bioburden in/on the products in order to improve the quality in this fields as so, the hazard analysis and control of critical points in the production lines of the products it take part of the validation studies.

The mechanisms of action of the lethal agents in microorganisms are studied in order to improve the knowledge of the Procedures.

Determination of sterilisation Dose (Dmin) based on bioburden estimates, before and after irradiation to sub-lethal doses in UTR, and the analysis of natural contaminants resistance of LYOMER product was performed.

Some particles were detected in glass bottles of water in summer season. Studies were developed on the line production in order to detect the origin of the problems and control the critical points in related connection with team of EMPOA.

Results

A preliminary sterilisation dose of 18,5 kGy was found out to Lyomer Product. The final Report was presented to JABA for being audit by TUV.

Some simulation studies of the principal phases of the line production which were made in ITN point out the washing procedure of glass bottles as a probable origin of the particles, although further studies is under development.

Further work

Peaks of contamination were detected in study of Lyomer Product so a chart control must be worked out based on an initial contamination validated procedure, taking into account the contamination's peaks and its frequency in order to lead to a definitive validation Dmin.

The EMPOA work is in development as so to confirm if the simulation results obtained at ITN lab are in agreement with the particles detected experimental work at EMPOA line production is foreseen.

[1] oftalder/Bioster & ITN fellowship–University Degree.
[2] ITN fellowship - Technician.
[3] EMPOA – Responsible for Quality Control.

Polymer Modification and Methods of Characterisation

Preparation and characterisation of polyethylene based copolymers with controlled hydrophilicity

L.M. Ferreira, M.H. Casimiro, M.E. Andrade, M.H. Gil[1],

Objectives

Modification of low density polyethylene (LDPE) surface properties through the grafting of 2-hydroxyethyl methacrylate (HEMA) branches by gamma radiation, in order to adequate the final support for the immobilisation of biocatalysts, pharmaceuticals and enzymes.

To achieve these objectives we have to:

1. .Prepare LDPE based copolymers using hydrophilic monomers (HEMA) linked to the polyethylene (PE) backbone by gamma radiation. Kinetic studies of the grafting reaction are required.
   The physical properties of the new materials (porosity, surface area, density, hydrophilicity and thermal properties) must be evaluated such as the chemical composition.
2. Immobilise biocatalysts through covalent linkage to the polymeric backbone.
3. Study of the operational behaviour and activity of the biocatalysts immobilised to the different copolymeric supports prepared.

Results

The grafting reaction kinetic date obtained have shown that independently of the dose rate (0,3 and 0,5 kGy/h), the irradiation environment (presence and absence of air) and the monomer concentration (5, 10 and 15% V/V MeOH) the reaction shows a first order behaviour.

The reactions kinetics conducted in the absence of air do not show initial induction period and the final grafting yield is higher.

ΔH_f and T_f decreases with the radiation doses. This decrease is higher in the samples irradiated in the presence of air and with the lower monomer concentration.

The irradiation of samples in the absence of air and with a higher monomer concentration results in final copolymers with higher grafting yields and with not so lower decrease of the backbone cristalinity.

RMN data suggest for a better surface distribution of HEMA branches when the copolymers are prepared in the absence of air with the lower dose rate.

Further work

Finish the kinetic studies for the well knowledge of the reactional copolymeric system PE/HEMA. Further more we must improve the knowledge of the copolymers surface coating (i.e. the dimensions of the hydrophilic branches).

Optimisation of a method for chemical backbone activation, in order to promote the covalent linkage of proteases and phosphatases to the copolymeric supports.

Finish the characterisation of all PE graft copolymerized with HEMA (PE-g.co-HEMA) samples obtained using gamma radiation.

Define the thermal behaviour profile of this material in the different final conditions of grafting.

[1] Department of Chemical Engineering, Faculty of Science and Technology, University of Coimbra, Portugal.

Preparation and characterisation of chitosan based controlled drug delivery systems

M.H. Casimiro, L.M. Ferreira, M.H. Gil[1]

Objectives

Modification and characterisation of a natural, biodegradable and biocompatible polymer (chitosan) in order to prepare an hydrogel capable of work as polymeric matrix in controlled drug delivery systems.

Results

Initial chemical modifications of chitosan (with acetic and anidride maleic) were performed in order to promote the acetilation and vinylic introduction in the molecule.

After we have submitted the chitosan to this chemical treatment, DSC and FTIR data shown that we are in presence of modified chitosan with vinylic groups.

Previous gamma irradiations of chitosan in acetic acid were done (10 and 15 kGy). The results have shown that a slightly degradation had occurred due the irradiation.

Irradiations with gamma rays of the modified polymer together with a reticulant agent (metilene-bis-acrilamid) as well as the graft copolimerisation of chitosan with acrylic acid were done with a 8 kGy doses. Results show that we are in presence of new polymers with swelling properties.

Further work

Finish the characterisation of this first serie of experiments and try a second one in order to study the influence of the irradiation dose on the hydrophilicity and swelling capacity of the new materials. In a near future we intend to have a chitosan based hydrogel with the proper characteristics to allow the immobilisation by occlusion of drugs. Further research on drug release rate will also be needed.

[1] Department of Chemical Engineering, Faculty of Science and Technology, University of Coimbra, Portugal.

Study of gamma sterilisation effects onto starch based polymers

L. M. Ferreira, R. L. Reis[1]

Objectives

Study of gamma radiation effect onto three different starch based polymers (Starch with vinylic ethylene alcohol, starch with cellulose acetate and starch with polycrapolactone).

These polymers have good biocompatibility and good mechanical properties to be used as human implants (backbone implants). Irradiation with gamma rays could join in one single process two different and desirable effects in the materials: *1)* sterilisation and, *2)* improvement of some properties.

Results

The dosimetric studies allow to define a proper geometry for polymeric samples irradiation.

The irradiation of 396 samples was done with a good dose uniformity (1.01), at three different doses of gamma radiation (10, 20 and 25 kGy).

The obtained data about their mechanical properties, degradation, water absorption and sterilisation efficiency (with biological indicators), are not yet enough to conclude about the real changes promoted in the materials as a consequence of gamma radiation.

Further work

Finish the characterisation of the first batch of samples in order to identify the real molecular changes introduced in the materials.

[1] Department of Polymers Engineering, Engineering School, University of Minho, Braga, Portugal.

Study of thermal and mechanical properties vs. gamma irradiation in industrial polyethylene films

L.M. Ferreira, M.H. Casimiro, E. Cunha[1]

Objectives

Improvement of mechanical and thermal properties in industrial bi-oriented polyethylene films, through the increase of reticulation degree by gamma irradiation. The reason of this study comes from the necessity of use this industrial film in special applications, where a high elasticity and resistance to heat (60ºC to 80ºC) without film rupture is required.

Results

The data coming from the irradiation of several samples in two different environment (presence and absence of air), show that the film is very stable. The reticulation increase is not appreciable even at high dose (40 kGy). This first serie of irradiations have been done at a high dose rate (13 kGy/h).

Nevertheless we observed a slight decrease in ΔH_f and T_f with dose. This effect is much more evident when the film is irradiated in the absence of air. That was an unexpected result for a polyethylene based film.

Further work

We must continuos with the irradiations, increasing the final gamma dose at the same dose rate, in order to despite the unexpected results. In other way a lower dose rate of irradiation should be assayed, trying to turn the reticulation the leader process during the film irradiation.

[1] FIBOPE, Filmes Biorientados S.A., Esposende, Portugal.

Food Irradiation

Detection of Prior Irradiaton Spices and Dried Fruits

M. P. Esteves[1], J. Empis[2], M.E. Andrade

Objectives

Identification of prior irradiated spices and dried fruits: almonds, raisins, dates and pistachio using two analytical methods: electron spin resonance (ESR) and viscosimetry (VISC).

Results

ESR method

Dried almonds, raisins, dates and pistachio were irradiated at room temperature with a dose of 5 kGy using either gamma radiation from cobalt-60 source or accelerated electron beam. The ESR measurements were carried out 2-3 months and 6 months after irradiation.

The characteristic "cellulose" radical, described by several authors, was recorded for gamma irradiated almond (skin) and pistachio (shell). "Sugar-like" signal was recorded for raisins (dried pulp) gamma and electron irradiated and "complex" signal was recorded for dates (stones) gamma irradiated.

The stability of radiation-induced radicals was found to depend not only on the sample and storage conditions but also on the kind of radiation used to treat the product. It was found that the characteristic "cellulose" radical observed in pistachio shells could not be recorded after 6 months when electrons irradiation was used, while it was still observed for gamma irradiated samples. Some slight differences were evident between spectra recorded form gamma irradiated and electron irradiated samples of almonds (skin) and dates (stones) [1].

Viscosimetry method

The apparent viscosity of 15 % (w/v) heat-treated aqueous suspensions of different batches of pepper (black and white) was studied to establish the best conditions for viscosity readings to detect the irradiation treatment in the interval of measurable apparent viscosity (Esteves, M. P., Polónia, I., Andrade, M. E., Empis, J., Alteration of Apparent Viscosity of Irradiated Pepper – A Tool for Semi-quantitative Estimation of Irradiation Dose, *Z Lebensm Unters Forsch* 201 (1995) 351-354). The maximum measurable apparent viscosity found under the conditions of our work (spindle no.4 and 50 rpm) was rather smaller (i.e.12 Pa s) comparing with the data described in the literature (20 Pa s, spindle no.6 and 50 rpm).The results of different batches of peppers gamma irradiated with the absorbed doses 3, 5, 7 and 10 kGy have showed a clear decreasing of the apparent viscosity with the absorbed dose.

References

1. Esteves, M. P., Andrade, M. E., Empis,J., Detection of Prior Irradiation of Dried Fruits by ElectronSpin Resonance (ESR), *Radiation Physics and Chemistry* 55 (1999) 737-742.

Further work

ESR method

The differences between gamma and electron irradiation must be confirmed and object of further studies. Mechanisms related with the fruit itself and its post-irradiation storage conditions could be involved.

Viscosimetry method

The standardisation of the method is far to be concluded as it is necessary to harmonise the equipment/technique used to obtain comparable and reliable data.

[1] Instituto Nacional de Investigação Industrial.
[2] Instituto Superior Técnico/Universidade Técnica de Lisboa.

Upgrade of the operating and security systems of the Radiation Technology Unit (UTR)

L.M. Ferreira, L. Portugal, C. Cruz, P.Pereira , J. Epiménio[1]

Objectives

To endow the UTR with up-dated technical operating means in order to assure a more safety operation and a bigger rentability.

Results

During the last year (1999) UTR was submitted to an intense technical revision. This effort included:

- Replacement of all electric cables inside the irradiation camera, including the i/o radiation detectors cables
- Reparation of the radiation detectors controlling unit
- *Storehouse* electrification
- Climatization of the *Command Room* and of the *Dosimetry Laboratory*
- Calibration certification renewing of the dosimetry readout equipment
- Y2k compliance certification of control operation software
- Replacement of the PC for the facility control operation with a Y2k compliant one
- Certification renewing of the Lyomer Radiosterilisation Process, by TUV auditory
- Work related with automation and robotics involving Omron PLC and peripheral controllers. The PLC was programmed to replace and old panel with tenth of solenoids, contactors and starters to automate and make easy all operations for ventilation, cooling systems and safety related to the irradiator system.

Furthermore, some sensory and temperature systems controllers modifications were done as well as diagnostics of pneumatics and air cooling equipments, to improve functionality and make easy the yearly maintenance. Meanwhile the development system for the PLC software was upgraded and a new control program was written matching better specifications.

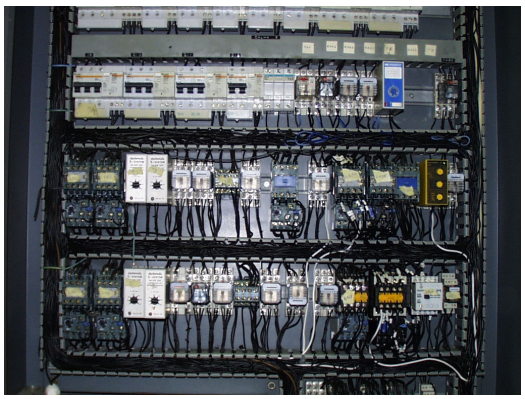

Fig.1: Partial view of the old electric panel.

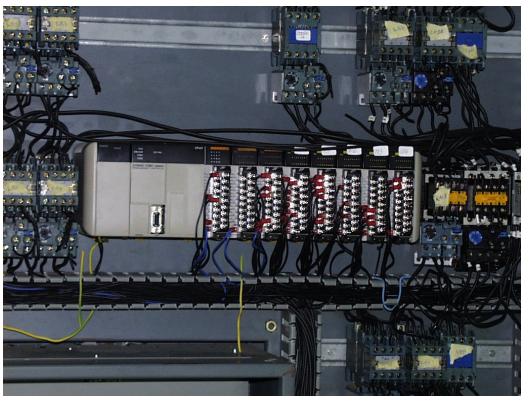

Fig.2: Electric panel with the new PLC installed.

Further work

- Electrification of the workshop area
- Modification /reparation of the facility fight-fire system
- Identification and reparation of the problems detected in the Cobalt-60 source water-cooling system
- Educational program for cobalt-60 facility operators

[1] EDP, Centro Operacional de Évora.