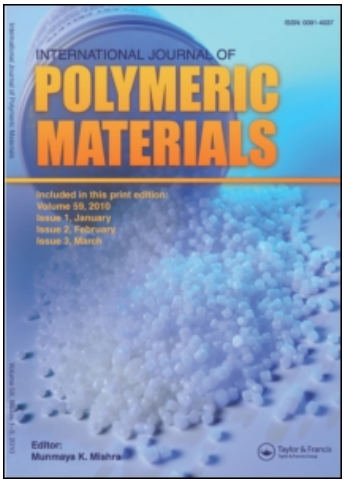

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A bioplastic material based on glucopolysaccharides and protein matrix sterilized by gamma radiation, ethylene oxide, moist and dry heat agents was studied.

Biodegradability, measured by evaluation of carbon dioxide production and followed by electronic microscopy, was tested only in the irradiated and ethylene oxide-treated material because of the physical alterations experienced by the material submitted to moist and heat processes. The sterilized material showed excellent biodegradation in periods shorter than sixty days. Both sterilized and unsterilized materials showed excellent biodegradation. After gamma radiation and ethylene oxide treatment, the material maintained its mechanical properties of tensile strength and ultimate elongation without significant modifications. On the contrary, dry heat diminished elongation capacity by 80% and moist heat diminished tensile strength by 36%. Water absorption capacity of the material remained unchanged after all sterilization processes.

Keywords: biodegradability, biopolymers, mechanical properties, sterilization, water absorption

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INTRODUCTION

The fundamental differences between natural macromolecular systems and man-made polymers reside in the latter having a very simple molecular structure, being highly durable and very aggressive to the environment. Therefore, the use of natural polymers as a replacement for synthetics represents an interesting possibility because it allows natural elimination, opening the doors to bio-recycling.

The biodegradation of a polymeric material comprises chemical, physical and biological processes that carry it to a state where every residual molecule can be assimilated by soil micro flora and micro fauna [1, 2].

Biodegradation, being a self-regulated process, has the advantage that it does not usually require complicated material processing. Important possibilities for industrial applications arise for a biodegradable plastic material that can be successfully sterilized without losing its biodegradability. Successful sterilization of a product implies the assurance that the sterilization process will not affect the material properties, so that the product will maintain its functionality [3–5]. With these considerations, an evaluation of the new material aptitude to with stand different sterilizing methods was undertaken.

Sterilizing methods consist of treatments that by chemical action or by application of energy can eliminate all living forms in a determined environment. Industrial sterilizing methods are mainly ethylene oxide treatment and radiation processing. In-hospital sterilization is usually fulfilled by ethylene oxide or dry or moist heat treatment.

Ionizing radiation, in the form of gamma radiation or electron beams, is employed in the sterilization of thermosensitive products since the middle of the 20th century. Its biocidal effect is attributed to the damage done to the contaminant micro-organisms DNA as a consequence of the radiation effects.

It is known that polymers can undergo two main effects upon irradiation: chain scission leading to molecular degradation, and crosslinking with formation of C-C intra- or intermolecular bonding. The predominant effect depends on the total applied dose, on the irradiation conditions such as dose rate, temperature, presence or absence of oxygen, and on the chemical and structural nature of the polymer [6].

Ethylene oxide gas (EtO) is widely used as a sterilizing agent because of its excellent diffusion capacity and adsorption. The sterilizing effect is based on alkylation reactions on proteins, nucleic acids and enzymes of micro-organisms. The efficacy of this treatment

depends on interrelated factors such as concentration and temperature of EtO and moisture content of micro-organisms; these factors determine the minimum contact time to attain the desired effect. Usual conditions for EtO sterilization are temperature about 55°C and relative humidity between 60 and 70% HR.

Dry heat sterilization is based on desiccation to death of micro-organisms; it is a slow process requiring high temperatures that exceed those tolerated by most synthetic polymers, therefore, this process is not suitable for those materials.

In the moist heat sterilization process, the saturated water vapor destroys the vital protein structures of the bacterial cytoplasm causing the micro-organisms' death. In this case, lower temperatures and less time can be used, assuring elimination of spores with a minimum temperature of 121°C.

The objective of the present paper is to investigate the biodegradability behavior of a bioplastic material prepared without toxic substances and previously described [7–9], after being submitted to industrial and in-hospital sterilization processes. The sterilizing agents applied were gamma radiation, ethylene oxide, dry heat and moist heat. To demonstrate the biodegradability degree a methodology that quantifies the CO_2 production was used, as well as the observation of structural changes using electron microscopy. Mechanical and physical properties such as tensile strength, ultimate elongation and water absorption capacity were evaluated.

Biocompatibility and toxicity assays of the sterilized materials will be reported in a future presentation.

EXPERIMENTAL

Plastic Preparation

The composite material, produced in this laboratory by compression molding, consists of a matrix formed by α (1,4) α (1,6) glucopolysaccharides and protein, both isolated from vegetal resources [7–9].

Once the bioplastics were constituted, test specimens were prepared according to ASTM standard D 638–03 [10] and submitted to the sterilization processes, as indicated below.

Mechanical Properties

Sterilized and control specimens were conditioned at 25 ± 1°C and 50 ± 5% RH for 48 h before testing. Five specimens were tested for each measurement.

Tensile strength and ultimate elongation were measured according to ASTM D 638–03, by means of a computerized INSTRON 1122 testing machine.

Water Absorption Capacity

The ability of molded products to absorb water was measured according to ASTM D-570 [11].

Specimens of 1 cm^2 were conditioned in an oven for 24 h at 50 ± 3°C, then cooled in a dessicator and weighed to the nearest 0.001 g. They were immersed in water at 23°C for 2 h, removed and weighed immediately.

Sterilization Methods

Irradiation

Irradiation was carried out in the Co-60 Semi Industrial Irradiation Facility of the Ezeiza Atomic Center, at an irradiation site where the dose rate was 5.56 Gy s^{-1}. The temperature in the irradiation chamber was 30°C. The total applied dose was 25 kGy.

Specimens were irradiated in 80-μm thick polyethylene packages and sealed without eliminating the air in the package.

Dosimetry was performed by means of high-dose dichromate dosimeters. Dose homogeneity attained was 30%.

Dry Heat

Dry heat sterilization was performed by heating in an electric oven at 150°C for 3 h, according to the Argentine standard IRAM 37100–1 [12].

Moist Heat

For moist heat sterilization, the samples were kept in an autoclave at 101, 33 kPa and 121°C for 30 min, according to IRAM 37005 [13].

Ethylene Oxide

Sterilization conditions consisted of: 1) chamber temperature at 50–55°C; 2) relative humidity RH 40%; 3) gas concentration in the range of 600 mg l^{-1}; and 4) sub-atmospheric pressure 40 kPa, with a contact time of 20 h.

Biodegradability

The degradation analyses were carried out in aerobic conditions as previously described [8,14] using 500 mg fragments of material.

The samples were placed in a microcosm consisting of 1500 ml flasks with 100 g of soil and compost, reproducing in the laboratory the natural environmental conditions according to the NF U52–001 and ASTM D5988–96 [15] standards. The soil conditioning includes sieving of 70% recently collected soil and 30% mature compost, the total organic carbon content not exceeding 2% [16], and the initial pH of 6.0 [17].

The relative humidity was maintained at 28% w/w in accordance with the NF EN ISO 846 [18] standard, at a constant temperature of $23 \pm 2^{\circ}C$.

The ratio of carbon: nitrogen 10:1 in the microcosm was preserved with an appropriate volume of $(NH_4)_2\ HPO_4$ and nitrogen was determined by Kjeldahl.

The degradation rate was determined by the production of CO_2. The evolved CO_2 was retained in an alkaline medium (0.5 N KOH) and then measured by titration with 0.25 N HCl and quantified as a function of the time elapsed from the beginning of the process, in relation to the organic carbon mass [19, 20].

The initial total organic carbon content of each sample was 40%. The carbon content analysis was performed using a microanalyzer Carlo Erba EA 1108 equipment.

The microbiological activity was controlled in parallel studies: 1) pure starch was used as control of positive biodegradation, 2) the sample in the presence of starch was used as control of the incidental inhibition process in the microcosms, and 3) the autoclaved microcosms' components as a negative control. Microcosm without sample was used as blank.

The percentage of biodegradation (%B) is expressed as follows:

$$\%B = 100 \times (M - M_{blank}) \times 12/(mi \times 44 \times w) \quad (1)$$

$M = CO_2$ mass (mg)
$M_{blank} = CO_2$ mass of blank (mg)
mi = initial mass of sample (mg)
w = total carbon content of samples (%)

In parallel experiments fragments of the samples were buried in the microcosm soil and scanning electronic microscopy was used to observe their degradation at different stages.

Statistical Analysis

Mechanical Properties

The Student test was employed to analyze the results. Differences between means were considered significant when $p \leq 0.05$. Average

values and standard deviation of three replicates of each sample were calculated in the water absorption capacity studies.

Biodegradability

Standard deviation was calculated with a 95% of confidence following ASTM standard D5988–96 [15].

RESULTS AND DISCUSSION

Mechanical and Physical Properties

The mechanical properties of sterilized biopolymeric plastic were tested. The results of tensile strength tests are shown in Figure 1, where it can be observed that the plastic material submitted to irradiation, ethylene oxide, and dry heat does not show changes or significant differences with reference to its original condition. However, when treated by moist heat the material presented a 36% reduction in tensile strength.

The results of elongation to breakage tests are shown in Figure 2. It can be seen that neither gamma radiation nor ethylene oxide sterilization significantly modify this property. However, dry heat sterilization produced a reduction of about 80% in the material elongation capacity.

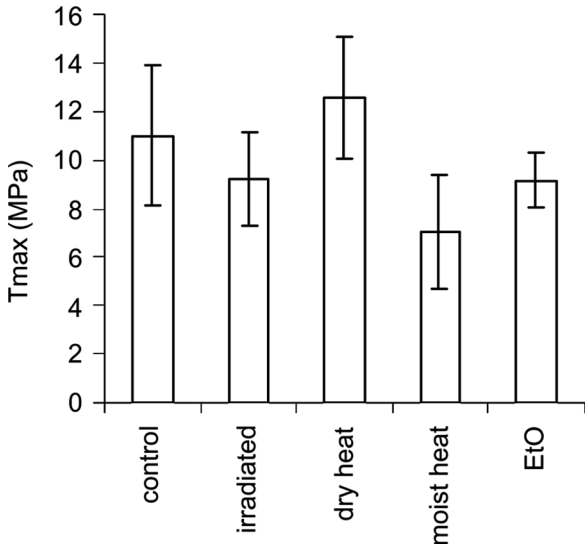

FIGURE 1 Tensile strength of control and sterilized materials.

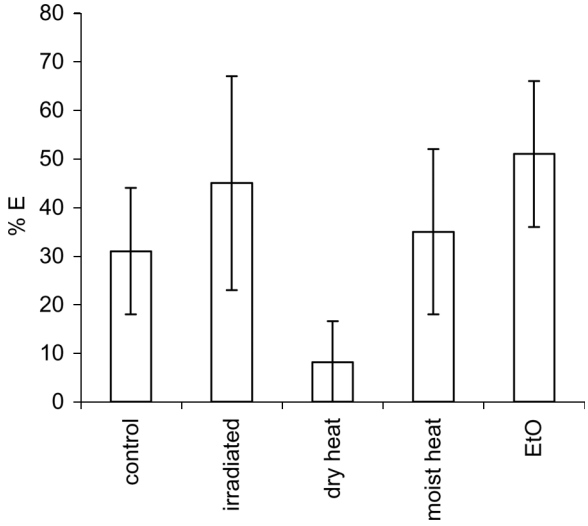

FIGURE 2 % Elongation at break of control and sterilized materials.

In Figure 3 the stages of the plastic material formation are shown. Between pH 5–5.3 the polar induction on the proteins would be produced to achieve the molecular arrangement. The polar end would

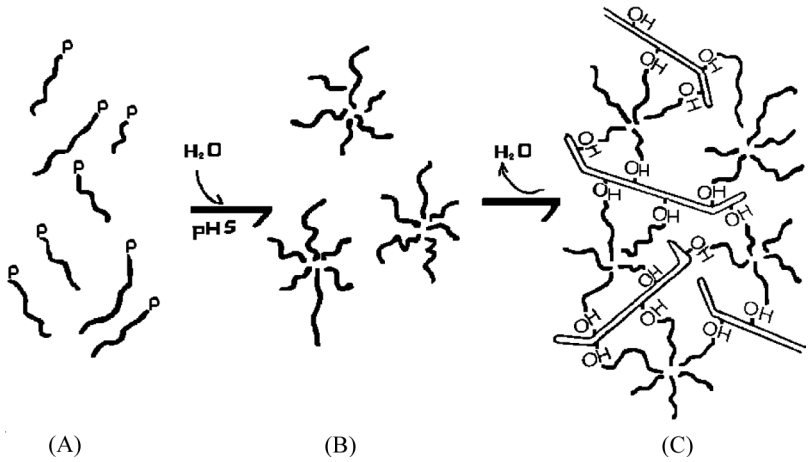

FIGURE 3 Stages of material conformation: (a) isolated and purified proteins, selected as previously described [9], depending on solution ionic force, PH 5–5.3; (b) induced molecular adjustment in polar medium in presence of connecting hydroxyl groups; (c) cohesion of nano structures by the incorporation of carbohydrates. *p: polar.

remain exposed and in the presence of hydroxyl groups would favor intermolecular attraction; by further treatment at appropriate temperature and pressure, the material conformation is definitively made permanent.

The dry heat treatment at 150°C for 3 h turned the samples into a highly brittle material. This effect could be explained by the heating at about the conformation temperature, favoring a total loss of moisture acting as plasticizer in the original material and modifying the molecular plastic structure.

On the other hand, the moist heat given to the material, consisting in autoclaving at 121°C for 30 min at 101.33 kPa, subjected the material to the action of water vapor in addition to high temperature. In this case the strong reduction of tensile strength suggests a structural degradation, the material becoming very soft and deformable, with almost no capacity for recovering its original shape.

The radiation dose applied in these assays lies in the range of the sterilizing doses usually employed in radiation sterilization and is lower than the doses required to modify material properties. As can be seen in Figures 1 and 2, the mechanical properties tested did not change further than the error range as a consequence of the gamma radiation sterilization process.

This plastic material absorbs water in the range of $49 \pm 2.5\%$. Table 1 shows the results obtained from the measurement of water absorption capacity after each sterilization treatment. It can be noticed that there are no significant changes after any of the treatments.

Biodegradability

The samples that could with stand the sterilization processes without physical damage and maintained the plastic properties were

TABLE 1 Water Absorption Capacity of Sterilized and Unsterilized Plastics

Sterilizing agent	(%) Water absorption* ± sd
Unsterilized	49.00 ± 2.5
Gamma radiation	48.16 ± 3.46
Moist heat	49.40 ± 2.35
Dry heat	50.00 ± 2.60
Ethylene oxide	50.08 ± 3.01

*Measured according to ASTM D-570

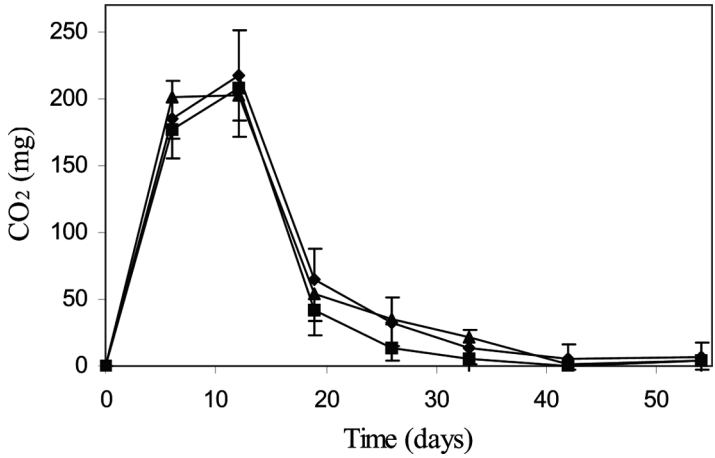

FIGURE 4 Carbon dioxide evolution produced during 54 days. Plastics submitted to sterilization processes: (-■-) irradiation; (-▲-) ethylene oxide; (-◆-) unsterilized. (95% confidence limit errors bars were simplified for clarity).

subjected to the biodegradability assay as was stated in the Experimental section.

The amount of net carbon dioxide evolution produced by sterilized and unsterilized samples are presented in Figure 4. As can be observed, during the first two weeks the metabolic activity increases steadily.

In Table 2 the degree of degradation is indicated, expressed as a percentage of biodegradation until the end of the 54-day experiment.

The degree of biodegradation achieved was over 60% in the unsterilized and both sterilized materials, considering 733.3 mg as the total amount of CO_2 to be produced from 500 mg of material containing 40% total carbon content.

TABLE 2 Degree of Biodegradation (B%) of Sterilized and Unsterilized Materials

Treatment	CO_2 (mg)	(%) B*
Unsterilized	524.5+/−57.4	71.5
Gamma radiation	448.8+/−41.9	61.2
EtO	519.3+/−56.3	70.8

*B% exposure after 54 days, calculated from Eq. (1).

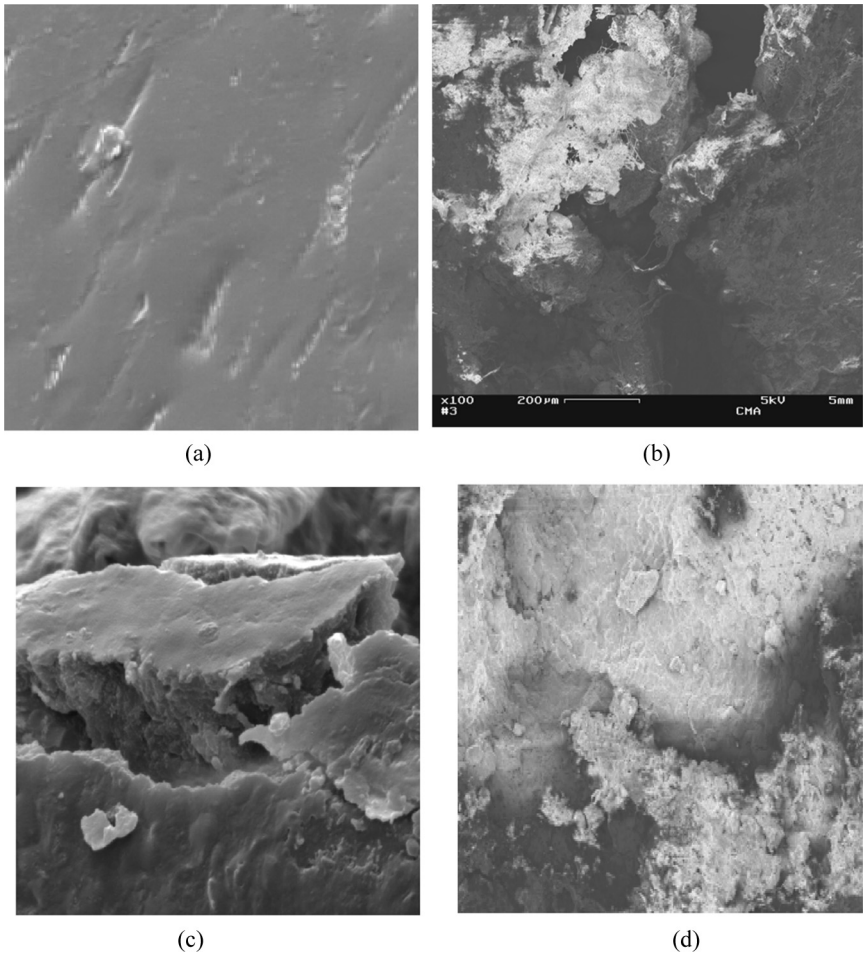

FIGURE 5 Scanning electronic microscopy. At the beginning of the biodegradation assay: (**a)** unsterilized (400X). At the 15th day of the biodegradation assay: (**b**) unsterilized (100 X); (**c**) radiation sterilized (1600 X); (**d**) EtO sterilized (100 X).

The electron microscopy results of the plastic surface before and after the sterilizing processes is presented in Figure 5. As can be appreciated, surface deterioration begins between 15 to 20 days after the beginning of the experiment. No differences were noticed between sterilized and unsterilized samples in the same observation period. The sample pieces underwent a complete disintegration at the end of

the test, which constitutes an essential requirement for a plastic to be considered biodegradable.

It was found that gamma radiation and ethylene oxide sterilization do not affect the material behavior towards biodegradation in a microcosm of soil and compost since both samples produced equivalent amounts of CO_2 under the action of micro-organisms. Both sterilized samples as well as the unsterilized exceeded 60% biodegradation degree after 54 days of experiment, thus complying with the requirements of the international standards definition of biodegradability that states that a biodegradable material should surpass the 60% biodegradation degree after 180 days.

All samples underwent an intense metabolic activity within the first 15 days of the experiment. The positive control with pure starch showed that starch experienced a slower degradation rate with CO_2 evolution that continues to be important during the following days.

At the same time, the samples in the presence of starch showed an additive pattern regarding the CO_2 production, proving that no inhibitory effects took place in the biodegradation of the plastic due to other carbon sources similar to starch.

CONCLUSIONS

The results of mechanical and physical properties proved that the material under study could be sterilized without significant structural damage by gamma radiation and ethylene oxide treatment.

In both cases it was also proved that these treatments do not affect the biodegradability of the material, which can be deduced from the comparison of the material sterilized by both sterilization processes and unsterilized, because all of them experienced a similar CO_2 production. This was proved by experimental assays complying with the requirements of international standards, and independently by electron microscopy.

However, moist heat and dry heat are not advisable as sterilizing processes for the material under study because of the damage they produce in tensile strength and elongation properties, respectively.

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