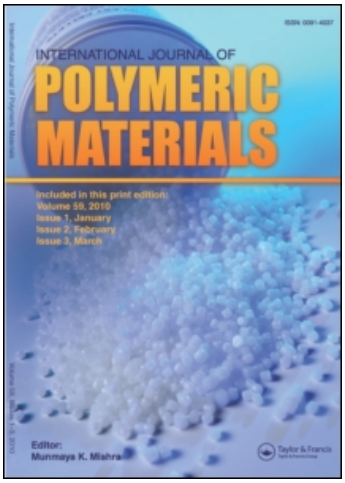

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Polyolefin Biodegradation

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Polyolefin Biodegradation

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Although polymer plastics found a broad application in different areas of industry, and particularly in medical industry, the behaviour of these materials in biological media still requires better understanding. Biodegradation and biostability of polyolefins are reviewed with reference to the solution of two important opposite problems, which are prediction of material durability and investigation of possible material recycling.

Keywords: Biodegradation; polyolefins; biocompatibility; polyethylene; polypropylene; microorganisms

1. BIOCOMPATIBILITY. BIOMEDICAL ASPECTS

1.1. Interaction of Polyolefins with Biomedical Media

Once implanted into the body medical polymers must show specific properties without interactions with surrounding tissues or with the body as a whole. So far there is no recognised definition of biocompatibility since there is no material parameters or biological tests which could be used as a quantitative characteristic of this property of the polymer [1].

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Biocompatibility generally means that the polymer can exist in contact with blood and enzymes without undergoing degradation or provoking thrombosis, breakdown of tissues, or harmful, immune, toxicological or allergenic effects.

Higher molecular weight polyethylene usually shows a low toxicity [2]. The degradation of the molecules results in formation of low-molecular weight fragments and even of monomers. Whereas the reciprocate dependence of the toxicity of polymers of a certain homologous series on their molecular weight have been reported [2].

The rate of degradation of the polymeric implant in the biological medium alters material biocompatibility. Generally, the compatibility of polymeric materials depends either on initial interactions with physiological components or on stability of implants in surrounding biological medium.

Degradation of polymers in biological medium is a complex physico-chemical process comprising diffusion of the medium components in polymer and transformation of chemically unstable bonds. Depending on the ratio between the rates of diffusion and of chemical reaction, the degradation process can be limited by different stages (diffusion or chemical reaction):

- The rates of diffusion and of chemical reaction are of the same order of magnitude: Reaction takes place in a specific reaction zone which size increases with time finally covering the size of polymer, *i.e.*, reaction takes place in the internal diffusion-kinetic zone.
- The rate of diffusion is one order (or more) of magnitude higher than that of chemical reaction: As solubility of low-molecular weight compounds in polymer is complete, degradation occurs in the whole volume of polymer, *i.e.*, in the internal kinetic area.
- The rate of diffusion is one order (or more) of magnitude lower than that of chemical reaction: In this case degradation occurs in the thin surface layer, *i.e.*, in the external diffusion-kinetic zone.

Having crystalline structure, polyolefins represent the materials which degrade in the external diffusion-kinetic zone [3]. The degradation occurs in the thin reaction layer of which size is generally impossible to find due to the absence of K_{eff} and D_{cat}. As generally recognised the thickness of this layer tends to zero, and the degradation actually occurs onto the material surface.

For the film of thickness l, the changes in thickness are derived from the equation

$$l = l_0 - K^s_{\text{eff}} C^s_{\text{cat}} t/\rho \tag{1}$$

where K^s_{eff} is the rate constant of degradation on the polymer surface, C^s_{cat} is the concentration of catalyst on the polymer surface, ρ is the polymer density.

The changes in weight are derived from the equation

$$m = m_0 - K^s_{\text{eff}} C^s_{\text{cat}}\, st \tag{2}$$

where s is the polymer surface to be in contact with medium.

For the fibre of the radius r, the following equations can be written

$$r = r_0 - K^s_{\text{eff}} C^s_{\text{cat}}\, t/\rho \tag{3}$$

$$m^{1/2} = m_0^{1/2} - K^s_{\text{eff}} C^s_{\text{cat}} (\pi l/\rho)^{1/2} t \tag{4}$$

1.2. Catalysts Derived from Biological Media

An analysis of the literature shows that the following substances should be considered as catalysts: water, salts and enzymes. The selection of these substances was made partly because they are the most widespread and partly because their catalytic activity is now recognised.

1.2.1. Water

The water content in biological media is large. For example, the human body contains approximately 75% of water, mainly in the intracellular fluid of the tissues and in the plasma. Thus polymers implanted into any part of the body must be in contact with water.

Abundant information on the sorption and diffusion of water in various polymers is available. The data acceptable for polyolefins application in medicine are shown in Table I.

TABLE I Water sorption (c_w°) value and water diffusion coefficient (D) in various polymers used in medicine

Polymer	$T/°C$	$c_w^\circ/(g/100\,g)$	$D/(10^{-9}\,cm^2/s)$
Polypropylene (PP)	25	0.007	2.4
Polyethylene (PE) $\rho = 0.923$	25	0.006	2.3

The table data show slow water diffusion in PE and PP thus indicating stability of carbon-chain polyolefins against water.

1.2.2. Salts

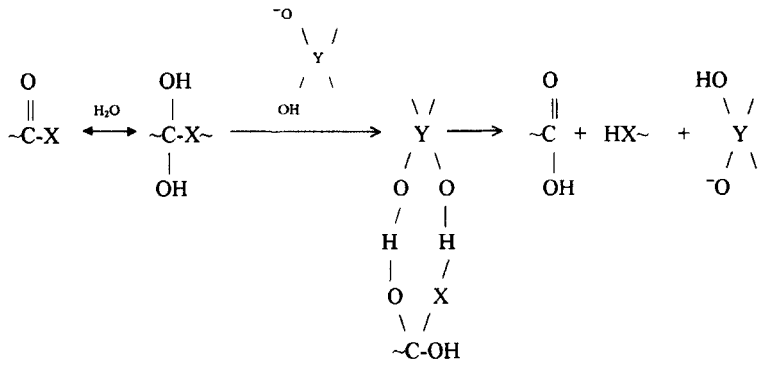
Salts are found in biological media in large amount. Table II shows the ionic composition of liquids in human body [4]. The plasma and intracellular liquid contain proteins in the anionic form due the presence of carboxylate groups.

Electrolytes diffuse in hydrophobic polymers with mechanism similar to the transport of gases and vapours. Therefore, in the case of electrolytes with a high vapour pressure (for example, hydrochloric acid) the c° and D values are close to the corresponding values for water in the same polymers. For electrolytes with a low vapour pressure (for example, chlorides and phosphates) we found very low values of c° and D, *i.e.*, hydrophobic polymers do not sorb these electrolytes to a significant extent.

The following general scheme can be suggested which shows how salts catalyse processes leading to the degradation of polymers containing carbonyl groups [3].

TABLE II Ionic composition of the liquids in the human (mequiv/l)

Ion	*Plasma*	*Liquid in tissues*	*Intracellular liquid*
Na^+	138	141	10
K^+	4	4.1	150
Ca^{2+}	4	4.1	40
Mg^{2+}	3	3	40
Cl^-	102	115	15
HCO_3^-	26	29	10
PO_4^{3-}	2	2	100
SO_4^{2-}	1	1.1	20
Organic acids	3	3.4	–



Thus salts (especially phosphates) have a strong catalytic effect on the degradation process in polymers containing carbonyl groups.

1.2.3. Enzymes

Enzymes play an active role in the degradation of polymers implanted in human body. However, only recently these effects received experimental justification.

Using the method of quantitative histoenzymology [5] Salthouse studied the activity of various enzymes in a capsule on the surface of materials after implantation for different times into rats (Tab. III). An increase in the enzyme activity takes place in all the materials after 14 days, associated with an increased phagocytosis in the implantation region. After the heating of the surgical incision and the formation of a

TABLE III Activity of enzymes in the capsule on the surface of various materials

Group of materials	*Time after implantation*	*Activity*		
		acid phosphates	*aminopeptidase*	*oxyreductase*[1]
Polypropylene	7	+[2]	±	+
Polyethylene	14	++	+	+
Polyurethane	28	±	±	±
Polytetrafluoroethylene	42	−	−	±

[1] Combined activities of succino-and lactohydrogenase and of cytochromeoxidase.
[2] List of symbols: − = no activity; ± = very low activity; + = moderately high activity; ++ = substantial activity.

capsule from the connective tissue an equilibrium concentration of enzyme is apparently reached on the polymer surface.

Enzymes diffuse easily in the capsule, and are absorbed on the surface of polymers in different concentrations. The surface concentration of enzyme catalysts depends either on the number of enzyme species in the bulk of the capsule or on the competitive adsorption of other proteins (provoking no catalysis), lipids, *etc.* The mechanism of the action of enzymes on polymers is extremely complex because the majority of polymers are not specific substrates for the enzymes.

1.3. Stability of Polyolefins in Living Body

As reported above, a number of polyolefin parameters allow to refer these materials to stable polymers.

1.3.1. Polyethylene

Polyethylene (PE) is applied in surgery since 1950s: that time a weak response of the body to implant was reported. For example, only formation of so-called granulated tissue around polymer was found after PE implantation under rat skin for 3 weeks following by vascularisation after 6 weeks. After 12 months no inflammation was found in the layer of the fibrous tissue whereas fibrous tissue had become thinner [6]. However, later Calnan [7] reported the hardening of such a sponge and formation of a dozen fibrous tissue that was not acceptable for flexibility of soft tissues. Calnan also found the reaction of inflammation around implant. The opposite results reported for PE in Refs. [6] and [7] are quite natural since so far no reliable quantitative criteria have been developed for biocompatibility of biomedical polymers. This is due to the complicated character of body-implant interaction in case of capsulated interface. Collection of data in this area defined the fields of PE application. Particularly, polyethylene is not recommended for substitution of the soft tissues whereas it is reasonable material to substitute bone tissues (*i.e.*, head of the hip bone and other elements of the pelvis bones). First application of low pressure polyethylene (LPPE) for substitution of the bone tissue showed large wear of the material [8]. Later improvement of the

synthesis technology allowed to get polyethylene of different molecular weight (*e.g.*, higher molecular weight polyethylene) thus enabling wide PE application to prosthesising pelvis.

Low pressure conditions and application of special catalysts allow to get super high molecular weight polyethylene of molecular weight of 4×10^6 g/mol. Using the method of hot pressure, slabs can be obtained which serve as initial material for the preparation of the elements of endoprosthesis. Such a treatment results in the excellent properties of polyethylene which basically depend on the temperature. Tables IV and V collect PE parameters as they depend on molecular weight whereas Table V also shows comparative properties of polyethylene and of bone cement.

During last 20 years super high molecular weight polyethylene (SHMWPE) presents itself in good light for endoprosthesising pelvis bones. However, this does not mean that SHMWPE application solves all problems. Although it shows excellent antifrictional properties and good ability of dry sliding, its weariness must be also mentioned. For example, for pelvis joint the couple metal/PE shows weariness of 0.2 mm/hour, and the couple ceramics/PE shows weariness of 0.1 mm/hour. The weariness provokes a formation of particles and consequent negative response of the living body such as formation of granules

TABLE IV Properties of polyethylene

Property	*Unit*	*Low pressure polyethylene. Low density. Developed network*	*High pressure polyethylene. High density. Non-developed network*	*Super high molecular weight polyethylene. Non-developed network*
Molecular weight	g/mol	5×10^4	2×10^4	4×10^6
Density at 23°C	g/cm^3	≤ 0.9200	≥ 0.9200	$= 0.9380$
Melting area	°C	105–110	130–135	135–138
Impact viscosity at 23°C	mJ/mm^2	6	13	140–160
Shape stability at 1.8 H/mm^2 according to ISO/R75.A	°C	= 35	= 45	= 95
Wear stability		–	+	+ +

TABLE V Properties of polyethylene in comparison with properties of bone cement

Property	*Unit*	*Bone cement, PMMA*	*Super high molecular weight polyethylene*
Density at 23°C	g/cm^3	1.20–1.25	0.9380
Water uptake	%	about 2	0.01
Temperature limit (short time interval)	°C	90	100
Elastic modulus at 23°C	H/mm^2	4400–5200	800–1000
Tensile yield stress:	H/mm^2		
• in air at			
23°C		38–44	41
40°C		about 38	about 37
• after exposure for 300 days in Ringer solution at 40°C (tested in air at 23°C)		33–36	?
Tensile stress at:	H/mm^2		
23°C		–	22
40°C		–	about 16
Extension at rupture	%	1	about 450
Compression yield stress:	H/mm^2		
• in air at 23°C		85–130	20–30
• after exposure for 300 days in Ringer solution at 40°C (tested in air at 23°C)		89–90	?
Impact viscosity	J/mm^2	1.5–2.0	no break point

around foreign body. Biological ageing of the material leads to the loss of its positive properties. Table VI shows positive and negative characteristics of polyethylene used as an endoprosthesis.

1.3.2. Polypropylene

Polypropylene (PP) is used in medicine due to its high chemical stability and favourable mechanical properties. In the early 1970s it was applied for lining the valves of artificial hearts, and for ball joint prostheses. It is now commercially available as suture threads from the Ethicon Company (USA).

Results of histology show that PP of "medically pure" grade provokes only a moderate response of the tissues of the living body [5, 9, 10] as compared with PP containing traces of catalysts and stabilisers.

TABLE VI Properties of super high molecular weight polyethylene as an implant material

Characteristics	*Properties to be improved*
Biocompatibility (implant and products of ageing)	Mechanical stability
Springing ability	Hardening (as it depends on geometry)
Antifrictional ability	Hardening (as it depends on geometry)
Viscosity and plastic characteristics	• weariness • resistance to biological ageing • inclination to brittle destruction • thermosterilisation

1.3.2.1. Ageing of Polypropylene Fibres in vivo PP fibres prepared from a complex thread (diameter of a thread unit = 0.033 mm) in a form of wicker braid have been investigated. The maximal period of tests *in vivo* in the skin cellular tissue of rabbit was 4 years. The samples exposed *in vivo* were taken of the rabbit at fixed time and washed to remove connective tissue. The samples of original material underwent the same treatment. Then the properties of original and exposed materials were compared.

The alteration of mechanical properties of the fibres by biological ageing are illustrated in Figure 1. After 4 years fibres loose about 60%

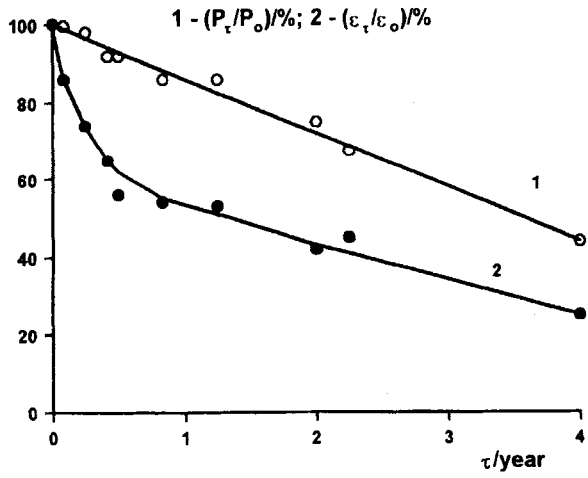


FIGURE 1 Stress (1) and extension (2) at rupture of PP fibres in the living body.

of their initial durability and about 80% of their initial extension. There can be noted a linear character of a durability decrease with an average rate of 1.13% per month. Whereas sharp decrease of extension was observed within half a year (40–50% with the rate of 8.3% per month) following by slow decrease with the rate of 0.8% per month. The weight loss of the samples being not visible during 2–3 years experiments *in vivo*, was finally found of 3.8%.

Molecular weight (M_η) which was measured of 80,000 using characteristic viscosity of PP solution in decaline decreased slowly against time of implantation. However, after 4 years the loss of molecular weight was of 17% (Tab. VII).

The surface of fibres was investigated by the methods of light and electronic microscopy in order to clarify the reasons for the loss of mechanical properties of fibres and increase of their brittleness. At earlier time of implantation fibres morphology remains basically unchanged. Cracks appear after 5 months. The development of cracks accelerates on the surface of fibres during the first year. During the second year this process is quantitatively stabilised whereas some quantitative changes can be noted. lead to the breakdown and the separation of the fibres fragments. The above destruction processes take place in relatively thin layer of the fibre surface (about 1 mm or 3% of the fibre diameter) and do not affect molecular weight of the polymer and material weight loss. The weight loss, which was measured of 3.5% after 4 years of implantation, was caused by the partial separation of the fibre surface and destruction of the surface layer. The curves representative of the surface effects are shown in Figure 2. To avoid the effect of temperature on the analysis of the process of PP ageing in the living body the fibres were studied at 37°C

TABLE VII Viscosity of PP solutions after material implantation in the living body

Period of implantation/ months	$[\eta]/(ml/g)$
original material	1.08
sterilised material	1.08
6	1.02
15	1.01
27	1.02
48	0.9

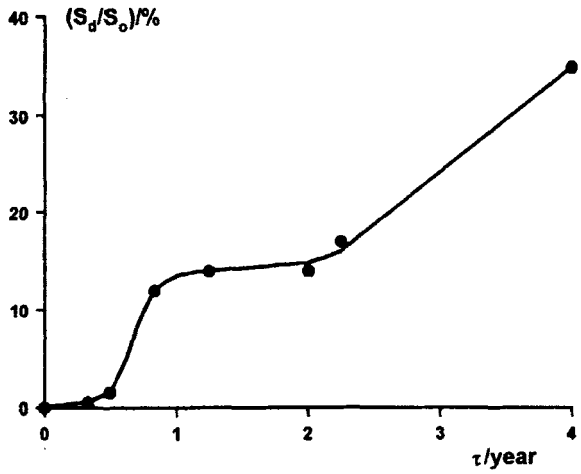


FIGURE 2 Alteration of the relative amount of defects on the surface of fibres by implantation.

light free in the air, in water and in Ringer solution. Results of investigation of mechanical properties (Fig. 3), and of measurements of weight and molecular weight of polymer showed that all these parameters remained unchanged during all period of exposure (4 years). No changes were also observed in fibres morphology. Therefore, the conclusion can be drawn that the ageing of PP fibres is affected by living body only.

To understand the role of structural changes, which occurred in PP fibres, in the process of fibres destruction, the stress relaxation was studied using tensile method. Figure 4 shows curves representative stress relaxation of PP fibres (original, and implanted for 2 and 4 years) through the dependence of ratio between remaining and initial load on time (τ).

There were found no visible changes in orientation of supermolecular structures of fibres in all time frame of experiment; all results were within the experimental error (6–10%).

To realise the changes occurred in orientation of fibres, X-ray analysis of PP fibres (either initial or implanted for 2 and 4 years) was performed. Some reorientation of structural elements of fibres was detected which was referred to the angle of crystallites reorientation

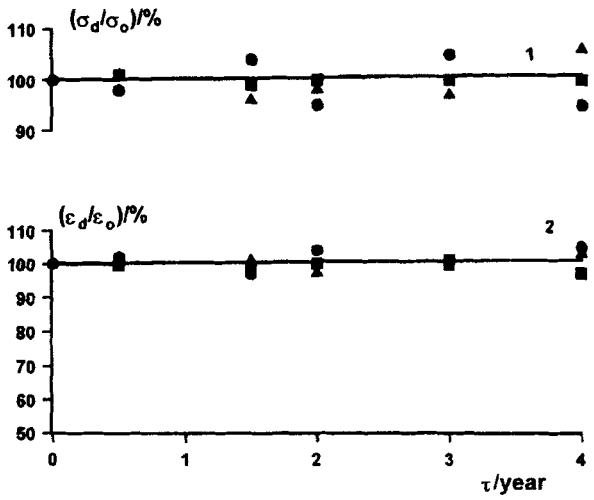


FIGURE 3 Stress (1) and relative extension (2) at rupture of PP fibres after exposure in model media. Symbols: circle = air, 37°C; square = water, 37°C; triangle up = Ringer solution, 37°C.

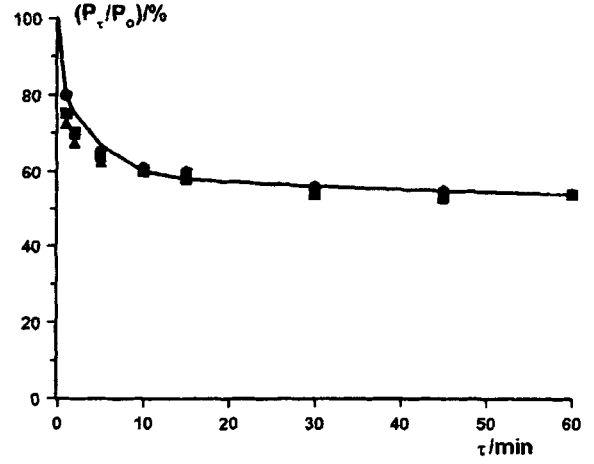


FIGURE 4 Alteration of stress relaxation of PP fibres by period of implantation (shown by symbols: circles = 0; squares = 2 years; triangle up = 4 years).

(ϕ). The angle ϕ varies from 8 (original fibres) to 13–15° for samples implanted for 4 years.

Therefore, resuming results of an analysis of PP fibres after their implantation in underskin cellular tissue of rabbits the following

conclusion can be drawn. The ageing process of fibres occurs which is justified by

- certain decrease of polymer molecular weight;
- some reorientation of the structural elements of fibres;
- loss of mechanical properties (*e.g.*, stress and relative extension at rupture);
- destruction of surface layers of fibres resulting in the development of cracks.

The most typical are changes of such properties as relative extension (first weeks) and surface quality (after 3 – 5 months of implantation).

1.3.2.2. Ageing of PP Fibres in Contact with Elements of Connective Tissue and Liquid of Tissue The experiment described in the previous paragraph was performed either with Russian or Italian (trade mark "Moplen") PP implants. The results of investigation of Moplen fibres, which were implanted in underskin cellular tissue of rabbits for 4 years, showed no changes in their properties. The weight of samples, molecular weight and mechanical characteristics remained unchanged in comparison with initial values.

Therefore, PP and Moplen fibres showed different behaviour in the same medium of implantation. The reason for such a difference is apparently the difference in polymer capsulation. In PP (Russian) case the sample was germinated by connective tissue following by capsulation of separated fibres and fibre groups. Whereas the Moplen sample was capsulated as a unit without germination of tissue between threads and fibres.

Since the growth of connective tissue is well known response of the body to inflammation process provoked by foreign bodies (*e.g.*, PP and Moplen implants), the absence of germination of Moplen samples by connective tissue can be explained by more inert character of this material than of polypropylene. Since Moplen properties remain unchanged even after 4 years of implantation, the slow interaction of this polymer with living tissue and be assumed. To test this assumption, more detailed analysis of the effect of connective tissue and tissual liquid on polymer was made.

The following method of the implantation of samples was developed. The first group of PP braid was implanted directly into the underskin tissue of the experimental animals (white rats) whereas the second group was placed inside silicone pipes with length of 40 mm and internal diameter of 5 mm. The germination of the samples of the first group began from the first week of implantation *in vivo*. The samples of the second group were basically in contact with tissual liquid. Although the germination of these samples was not completely prevented, the formation of capsules began with 3 month delay in comparison with the first group.

Mechanical tests, which were performed after 3 months of implantation, showed (Fig. 5) the decrease of relative extension at rupture of 40 and 25% for 1-st and 2-nd groups of samples, respectively. The highest rate of decrease of the relative extension was observed during first 2–3 months. For all periods of study, the difference between values of relative extensions remained within the range of 10–15%. The loss of durability was also observed more for the first group remaining for both groups of samples within the range of 10–20%.

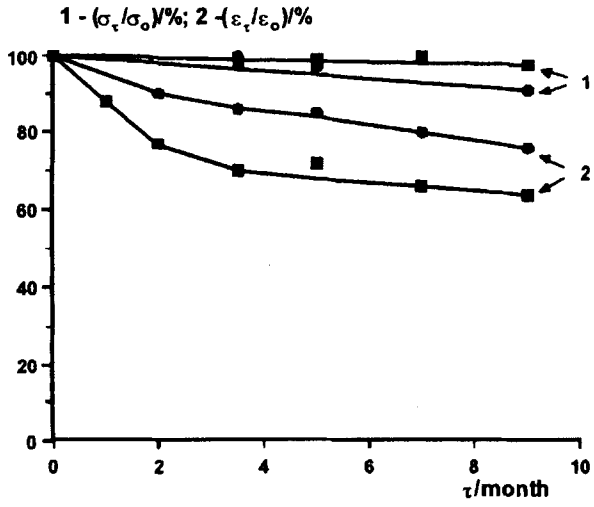


FIGURE 5 Stress (1) and relative extension (2) at rupture of polypropylene fibres implanted in living body. Symbols: circles = samples germinated by connective tissue; squares = samples in contact with tissual liquid.

The microscopic study showed the appearance of cracks perpendicular to the axis of the fibre. The character of cracks was typical for chemical degradation. Figure 6 shows that 3% of the surface were destroyed after 3 months for the first and of samples and after 9 months for the second group.

The difference in mechanical properties and surface quality between two groups of samples can be explained by the different mechanisms of the destruction of the surface layer of the material. For the first group aseptic inflammation and formation of the capsule of connective tissue occurred close to the surface of fibres whereas for the second group tissual liquid served as a barrier for inflammation process.

Therefore, degradation processes can be assumed in the surface layers of PP fibres which alter mechanical properties of the material leading to the formation of cracks.

The reasons for formation of cracks can be internal structure of fibres and anisotropic distribution of stresses in fibres. The elements of connective tissue contribute in the development of cracks thus provoking a difference in properties of PP fibres for two groups investigated.

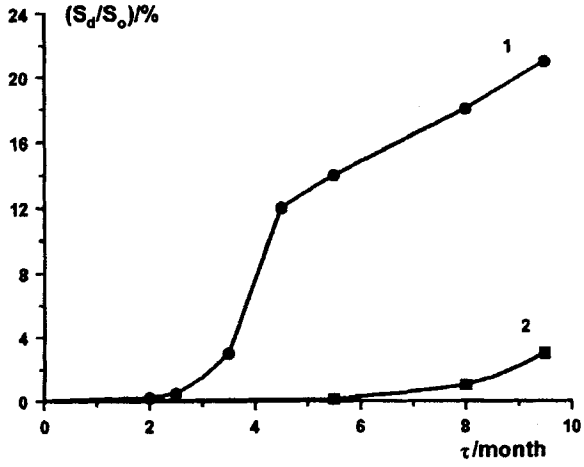


FIGURE 6 Destruction of the surface of PP fibres in conditions of their germination by connective tissue (1) and in tissual liquid of the body (2).

1.4. Ageing of Polypropylene Films

To understand the role of PP orientation, the samples of bi-axis oriented films were studied. Films were implanted under skin of white rats for the period of 1 year and 7 months. The thickness of films (0.03 – 0.035 mm) was relevant to the diameter of fibres earlier studied.

Either stabilised or non-stabilised film samples of polypropylene were studied. The most of PP stabilisers, which are applied to prevent polymer degradation during processing, are toxic although polypropylene itself is an inert material. Hence, for experiment with animals, stabilising system on the basis of Irganox 1010 was chosen which is sufficiently harmless [12].

Prior to experiments, the efficiency of stabilising systems on the basis of Irganox 1010 and of two similar Russian compounds, phenosan-23 and phenosan-28 was evaluated.

The efficiency of stabilising systems was evaluated estimating the induction period of oxidation of original PP films and analysing the changes in material properties after isothermal heating at 150°C [13].

Results showed the same induction period for all three systems (55 – 60 minutes) whereas isothermal heating for 6 hours at 150°C caused no changes of mechanical properties of the material. Therefore, the resistance of all three PP formulations to thermooxidation process is substantially the same. Therefore, the same stabilising effect of these additives during PP processing can be assumed.

For *in vivo* studies film stabilised with phenosan-23 and non-stabilised PP film was selected. Results of analysis of the effect of implantation on the mechanical properties of PP films are shown in Figure 7.

These properties remain unchanged *in vivo* for stabilised films. No changes in material structure nor chemical degradation were detected. Whereas for non-stabilised PP films, the stress at rupture reduced in 20% after 19 months of implantation. Probably this parameter is the best for a characterisation of PP ageing in the living body.

2. BIODETERIORATION OF POLYETHYLENE UNDER ACTION OF MICRO-ORGANISMS

Biodeterioration (biodamaging) of polyolefin materials proceeds at their contact with living organisms and may lead to the change of the

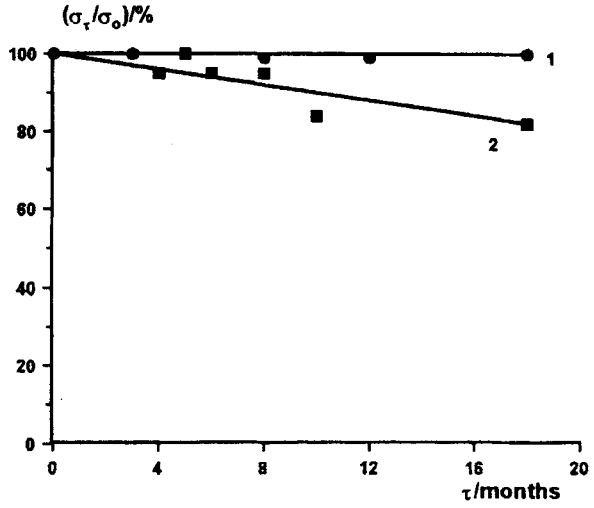


FIGURE 7 Stress at rupture of films of stabilised (1) and non-stabilised polypropylene (2) after exposure in the media of the living body.

exploitation properties. In general case the following processes may proceed at biodamaging:

- adsorption of micro-organisms or substances existing in tissues of the living organism on the material surface;
- decomposition of the material as a result of specific influence (living organisms apply polymeric material as a feeding source) or under the influence of metabolism products.

As the first process proceeds, the chemical structure of polyethylene does not change as a rule. The material plays the role of the support on which the adhesion and the growth of colonies of micro-organisms (bioovergrowth) or the formation of collagen-like capsule take place. Adhesion of micro-organisms is the initial stage of material bioovergrowth which defines all further picture of bioovergrowing and biodamaging of polymeric materials. At the stage of bioovergrowth the determination of the amount of biomass on the surface of polymeric material is of the great interest because it alters surface quality (optical, adhesive, *etc*).

The second process leads directly to the ageing of polymeric material under the influence of chemically active substances. In this

case the "volumetric" exploitation properties such as mechanical, dielectric and others will be affected.

2.1. Kinetics of the Biomass Growth. Methods and Results

The growth and development of microscopic fungi directly on solid surfaces of polymers is usually estimated by the growth of diameter of colonies of definite type or by selection of microscopic fungi. This method, which follows five-level scale Russian standard (GOST), is connected with difficulties of biomass recognition in amounts of μg/cm^2 at the initial stages of growth.

To obtain kinetic parameters of accumulation of biomass we have applied sensitive radio isotopic method [14, 15]. Polymeric films contaminated by suspension of microscopic fungi of 10^6 cells/ml in water or in nourishing medium [6, 7]. The amount of biomass was measured as the difference between changed and the test samples for each polymer. The intensity of irradiation was measured by liquid scintillation counter "Mark-388".

The growth of micro-organisms was estimated according to the change of amount of dry biomass per unit of the sample surface.

Figure 8 shows the kinetic curves of biomass accumulation on the surface of PE and hydrophilic cellulose. derivative (cellophane). In both cases the kinetics of accumulation follows an exponential trend

$$m/m_\infty = 1 - \exp(-kt) \tag{5}$$

where m is biomass accumulated at time t, m_∞ is biomass at equilibrium, k is an apparent constant of biomass growth.

Table VIII shows m_∞ values, initial rate of biomass growth (v_{init}), and k for two types of spore suspensions derived from logarithmic trend of Eq. (5), in water and in nourishing medium of Chapeck–Dox. The values of m_∞ and v_{init} are two times larger than those in water whereas k values are nearly the same for both media. Larger bioovergrowth of cells in the case of cellophane links to the polymer hydrophilicity.

2.2. Kinetics of Adhesion of Micro-organisms

Biodegradation follows the interaction of micro-organisms with polymeric surface and begins with adhesion of micro-organisms.

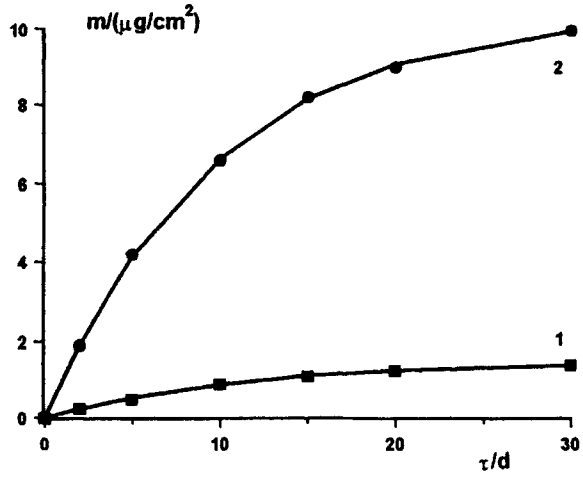


FIGURE 8 Kinetics of biomass accumulation of the microscopic fungi on PE (1) and cellophane (2) surface.

TABLE VIII Equilibrium biomass (m_∞) on the surface of various polymeric materials and the initial rate of biomass accumulation (v_{init})

Investigated material	*Treatment by mixture of spores in Chapeck–Dox medium*				*Treatment by mixture of spores in water*	
	m_∞/ ($\mu g/cm^2$)	v_{init}/ ($\mu g/cm^2 d$)	$k/(10^{-6}s^{-1})$	m_∞/	v_{init}/ ($\mu g/cm^2$)	$k/(10^{-6}s^{-1})$ ($\mu g/cm^2 d$)
Cellophane	10.5 ± 1	0.60 ± 0.05	1.0	5.80 ± 0.60	0.40 ± 0.080	1.5
Polyethylene	1.5 ± 0.20	0.27 ± 0.02	0.9	0.27 ± 0.02	0.01 ± 0.001	1.3

Adhered cells act as aggressive bioagents precipitating exoferments or other molecular substances. For this reason the quantitative parameters of adhesion are definitive for further stages which are bioovergrowing (biomass accumulation and Biodegradation [4, 16, 17].

Adhesion strength (F_{adh}) measured by the method of centrifugal detachment may serve as macroscopic parameter accessible for quantitative estimation. To estimate F_{adh} suspension of cells of micro-organisms (10^6 spores/ml) was attached to the surface of polymeric film and exposed during fixed time at different external conditions (temperature (T, °C) and humidity (ϕ, %)). Then films were fixed on metal plates and centrifuged in the field of intensities acting

perpendicular to the surface. The number of cells (γ), which was detached from the surface into the bulk of centrifugal glass filled with distilled water at the particular field of intensities, was calculated with the help of optical microscope. Adhesion was estimated according to the force of spores detachment from polymeric surface

$$F = (1/675)\pi^3 r^3 \omega^3 R(\rho_{\text{cell}} - \rho_1) \tag{6}$$

where r is the radius of spores, ω is the angular rate of rotation, R is the distance to rotor axis, ρ_{cell} is the density of spores (cells) and ρ_1 is the density of the liquid in which detachments is performed.

The effect of the material nature on adhesion of spores is shown in Figure 9 presenting kinetic curves of adhesion on different polymers at certain hygrothermal conditions. Adhesion equilibrium was reached within 24 hours. Table IX shows calculated values of adhesion intensities and of constants of formation of adhesion intensities γ_∞.

Figure 10 shows the dependence of the adhesion index of *Aspergillus niger* spores on time at constant humidity and different temperatures. Table X collects the values of parameters calculated from these kinetic curves for two substances representative of two extreme solubility in

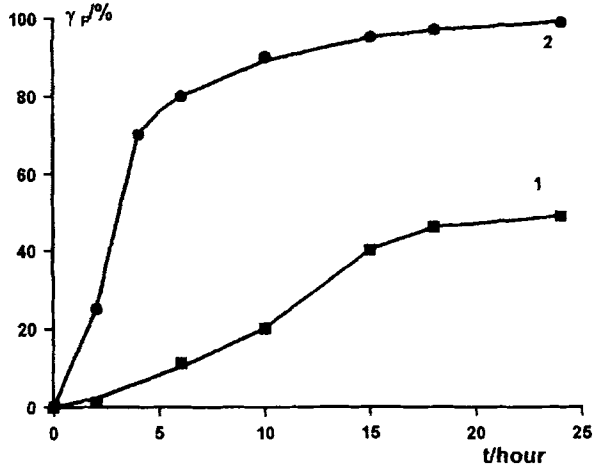


FIGURE 9 Kinetics of the *Aspergillus niger* spores adhesion to the polymer surface at $\phi = 98\%$ and $T = 22°C$.

TABLE IX Parameter of adhesion of *Aspergillus niger* conidia to the surface of different polymeric materials

Material	$k/(h^{-1})$	$F^{50}/(dyn/cell)$	$\gamma_\infty/\%$
polyethylene	0.06	3.3×10^{-4}	55
cellophane	0.36	1.6×10^{-3}	85

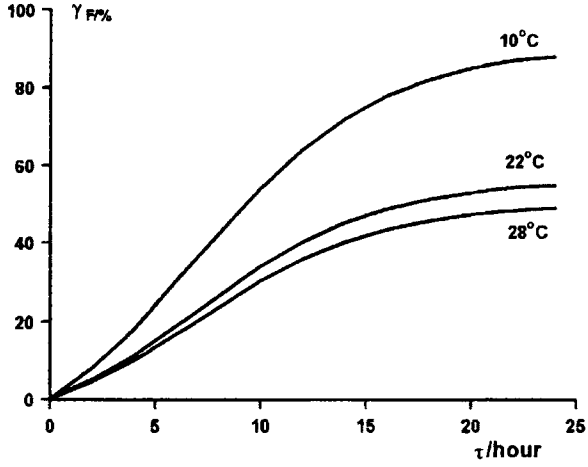


FIGURE 10 Kinetics of the *Aspergillus niger* spores adhesion to the polyethylene surface at $\phi = 98\%$ and different temperatures.

TABLE X Adhesive parameters of interaction for polyethylene and cellophane at different temperatures and relative humidity $\phi = 10\%$

$T/°C$	*polyethylene*			*cellophane*		
	$k/(h^{-1})$	$F^{50}(dyn/cell)$	$\gamma_\infty/\%$	$k/(h^{-1})$	$F^{50}(dyn/cell)$	$\gamma_\infty/\%$
10	0.06	5.2×10^{-4}	85	0.36	2.6×10^{-3}	90
22	0.06	3.3×10^{-4}	55	0.36	1.6×10^{-3}	85
38	0.06	1.3×10^{-4}	50	–	–	–

water (polyethylene and cellophane). Table X shows the same rate constant of the formation of adhesion intensities measured at different temperatures and the increase of adhesion intensity with the decrease of temperature. This tendency is valid for both polymers.

Figure 11 shows kinetic curves of adhesion of *Aspergillus niger* spores to the surface of polyethylene at constant temperature and variable environmental humidity. The constants of adhesion, which

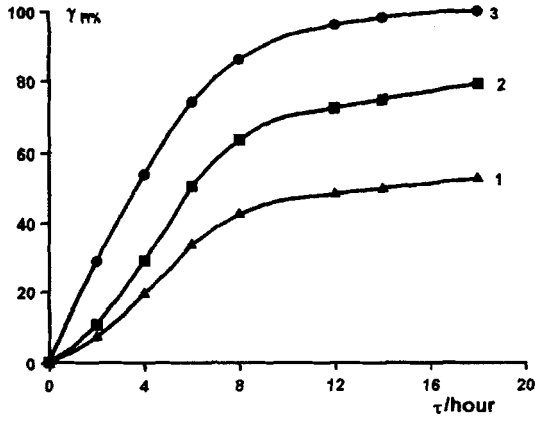


FIGURE 11 Kinetics of the *Aspergillus niger* spores adhesion to the polyethylene surface at $T = 22°C$ and different humidifies: $1 - \phi = 0\%$, $2 - \phi = 30\%$, $3 - \phi = 98\%$.

TABLE XI Adhesive parameters of conidia for polyethylene at different humidity and constant temperature 10°C

ϕ/%	$k/(h^{-1})$	$F^{50}/(dyn/cell)$	γ_∞/%
0	0.08	3.0×10^{-4}	70
30	0.66	5.2×10^{-4}	85
100	0.56	1.9×10^{-3}	100

were calculated at different humidifies and collected in Table XI, showed remarkable changes in k values as γ_∞ changes were sufficiently small.

The values of adhesion parameters which were calculated from the kinetic curves (Fig. 12) of adhesion of conidia of different microscopic fungi spores, are collected in Table XII.

The final scope of the investigation of the quantitative regularities of adhesion and bioovergrowth was an obtaining macroscopic kinetic parameters of biodamaging ability of polymers. The experimental data collected in Table XIII show the link between adhesion and the growth of biomass. Whereas additional study is required in model media to better understanding of the type of degradation.

Biostability of the material can be predicted with the help of experimental values of microscopic kinetic parameters of the interaction of micro-organisms cells (spores conidia) with polymeric surface. In general case biostability (B) depends on adhesion intensity, the

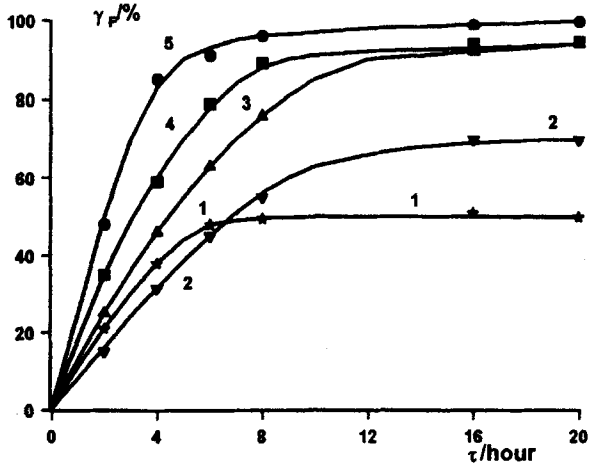


FIGURE 12 Kinetics of adhesion of different fungi to polyethylene surface. 1 – *Aspergillus niger*; 2 – *Penicillium cyclopium*; 3 – *Paec. varioti*; 4 – *Penicillium chrysogenium*; 5 – *Aspergillus terreus*.

TABLE XII Parameter of adhesion of microscopic fungi to the surface of polyethylene at $\phi = 10\%$ and $T = 22°C$

Fungus type	$k/(h^{-1})$	$F^{50}/(dyn/cell)$	$\gamma_\infty/\%$	$r^{1}/(\mu m)$
Aspergillus niger	0.23	0.26×10^{-1}	80	5.0 ± 0.5
Aspergillus terreus	1.96	0.31×10^{-3}	94	1.0 ± 0.05
Paec. varioti	0.30	0.76×10^{-1}	98	5.5 ± 0.7
Penicillium chrysogenium	0.40	0.45×10^{-3}	94	1.5 ± 0.06
Peinicillium cyclopium	0.50	0.10×10^{-1}	55	2.6 ± 0.4

[1] r radius of spores for different type of fungi.

TABLE XIII Parameters of biodamaging ability of PE and cellophane

Material	*Adhesion parameters*[1]			*Growth*	*Degradation*
	$k^{adh}/(h^{-1})$	$F^{50}/(dyn/cell)$	$\gamma_\infty/\%$	$m_\infty/(\mu m/cm^2)$	$k_{enzyme}/(s^{-1})$
Polyethylene (LDPE)	1.6×10^{-5}	3.3×10^{-4}	55 ± 5	$1.5 \times \pm 0.2$	1.2×10^{-9}
Cellophane	1.0×10^{-4}	1.6×10^{-3}	85 ± 5	10.5 ± 5	0.5×10^{-6}

[1] k^{adh} = constant of adhesion of *Aspergillus niger* microscopic fungus conidia; F = adhesion intensity of a single cell to polymer surface; γ_∞ = extreme adhesion index; m_∞ = biomass per surface unit; k_{enzyme} = rate constant of degradation affected by enzyme.

amount of biomass and apparent rate constants of degradation of accessible bonds:

$$B \sim (F_{adh} \Delta m_{\infty} k^{adh})^{-1}$$

For example, the values of biostability of cellulose calculated from this expression (humidity, $\phi = 98\%$) was equal 0.5×10^6 s cm^2/dyn μg whereas this parameter calculated for polyethylene was 5 orders of magnitude higher: 0.4×10^{11} s cm^2/dyn μg.

Therefore, it was shown that the investigation of kinetic regularities of microscopic processes, such as adhesion, bioovergrowth, biodegradation allows to make models of the mechanism of the complex process of biostability and biodecomposition of polymeric materials.

3. BIOMEDICAL, TECHNICAL AND ECOLOGICAL PROBLEMS OF BIODESTRUCTION OF POLYOLEFINS

3.1. Polypropylene

Polypropylene can undergo only oxidative degradation. Therefore, PP should be stable in the tissues of the living body decomposing only under the effect of oxidative enzymes and oxygen dissolved in the living medium.

The overall picture of the degradation of PP filaments and films in the body is characterised by the following phenomena: a worsening of mechanical properties (decrease in the breaking load and in the relative extension) [18]; formation of cracks in the initial stages of the implantation and fragmentation of the polymeric implant at longer times (the fragmentation to be observed quicker in PP samples without antioxidant) [18]; unchanged molecular weight during the implantation; appearance C = O absorption bands in the IR spectra of the PP samples [19]; an increased activity of oxyreductase and cytochrome oxidase in the capsule surrounding the implant [5].

The above experimental results on PP degradation in the living body show that this polymer undergoes the S-type of degradation under the effect of oxidising enzymes which cannot penetrate into the polymer matrix (Eqs. 3, 4). It is well known that oxidative degradation

accompanies the breakdown of the main chain thus leading to the decrease of molecular weight. However, since the catalysts are unable to penetrate into the bulk of the polymer, the breakdown of bonds occurs only on the surface provoking negligible decrease of the molecular weight of the whole sample. The accumulation of carbonyl compounds also takes place on the surface of cracks rather than over the whole volume of the sample.

Special experiments which were performed with PP exposed in a solution of cytochrome oxidase did not show the expected result because of the rapid deactivation of this enzyme in experiments *in vitro*. Therefore, experiments with bacteria, which produce oxidative enzymes able to open C —C bonds, are required to understand the role of these enzymes in PP oxidation.

3.2. Polyethylene

The biodegradation of PE was detected when the possibility of exploitation of polymeric by-products with the aid of micro-organisms and bacteria was studied. It was found that commercial polyethylenes (especially HPPE) undergo nearly no biodegradation by the radical mechanism [20, 21].

The following features of the biodegradation of PE have been detected:

- The main condition for the biodissociation is an increase of the polymer surface (*e.g.*, by using powder) [22].
- The dissociation process takes place with a decrease in weight accelerating during initial stages of exposure and then slowing down.
- Samples of low-pressure polyethylene (LPPE) exposed with micro-organisms are found to include carbonyl groups, especially in presence of fillers sensitive to oxidases and esterases.

PE labelled with ^{14}C was used to study the rate of degradation of slowly decomposing PE [24]. Samples incubated with micro-organisms for different times (up to 3 years) were irradiated with UV light in order to accelerate the degradation, and the weight loss caused by the action of the bacteria and micro-organisms was determined. Extra-

polation to zero time of irradiation yields the weight loss due to the biodegradation. The weight loss of the powders was found by this method of 0.5% in 3 years.

These data indicate S-type mechanism of PE degradation. The decomposing species can be oxidative enzymes generated by bacteria and micro-organisms, for which PE acts as a carbon feed medium.

Combining the data for PP and PE degradation we can conclude that although these polymers decompose slowly, the rearrangement of their structure during oxidation can lead to undesirable consequences for the living body.

The main problem, which limits further application of polyethylene in orthopaedics and other areas of implant surgery, is the uncertain effect of the living body on physico-mechanical characteristics of the material. Specific oxidative ferments make the process of biodegradation random.

The experimental finding of the existence of adhered cells and bioovergrowth on the surface of PE samples allows to assume the possible biodamage of this polymer.

Alternatively, ecological problem of polyolefin wasting provokes a need in rapid decomposition of polyolefins with an aid of micro-organisms or in alteration of polymer structure by irradiation or by other processes initiating structure the breakdown of chemical bond.

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