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Microscopy in the studies of polymer biodegradation

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SUMMARY: The goal of the paper is to overview different possibilities of microscopy, which can be helpful in estimating some of the changes undergoing in the materials exposed to the action of micro-organisms.
The types of microscopic techniques and methods discussed in the paper are: optical transmission microscopy (OTM), optical transmission microscopy with polarised light (OPTM), optical reflected microscopy (ORM), optical reflected microscopy with polarised light (OPRM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The above methods allow to follow the changes of the surface view, the changes inside the polymer and the changes in birefringence (in the case of semi-crystalline polymers). The results can be compared with other techniques used for monitoring of polymer biodegradation or biostability. The examples from author's studies on different types of polyurethanes and the blends of polyethylene with starch are also described in the paper.

Introduction

The investigation of polymer degradation including biodegradation is stimulated by two opposite goals. On the one hand, there are many applications where biologically resistant materials are needed. In such applications the polymer is exposed to the attack of various micro-organisms and must be resistant against them as much as possible. On the other hand, there is an increasing demand for biodegradable plastics. In both cases microscopy can be helpful in estimating some of the changes occurring in the materials exposed to micro-organisms. One can find mostly the results of surface observation by SEM.

But there are more types of microscopic techniques and methods which can be taken into account i. e. optical transmission microscopy (OTM), optical transmission microscopy with polarised light (OPTM), optical reflected microscopy (ORM), optical reflected microscopy

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with polarised light (OPRM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), electron diffraction.

The above methods allow to follow the changes of the surface view [1,2], the changes inside the polymer [3], the changes in birefringence in the case of semi-crystalline polymers [4,5], the biodegradation products and the results can be compared with others techniques used for monitoring of polymer biodegradation or biostability.

For the last 10 years microscopy has strongly developed again and some new techniques like atomic force microscopy [6] or confocal microscopy [7] have been introduced in scientific laboratories. These types of microscopy, however, will not be discussed in the paper.

Optical microscopy vs transmission electron microscopy

The main difference between optical and electron microscope is the wavelength of the light, which for optical microscopes is in the range of 400 - 800 nm and for electron microscopes of the order of 0.005 nm (when the electrons are for example accelerated by 60,000 V). Optical microscope magnifies images about 1000 times with a beam of light reflected off a mirror through condensing lenses and then through the specimen. From there the image undergoes magnification through additional sets of lenses, finally focusing at the eyepiece. Electron microscope is similar in principle to the optical microscope (Fig. 1) but its lenses are formed by electro-magnetic coils that affect an electron beam in much the same manner as glass lenses affect light. A filament generates a high-voltage electron beam which passes through a series of condensing lenses and then through the specimen. From there the electrons are scattered, creating an invisible image that is made visual by a system of electronic or magnetic lenses that focus the image onto a fluorescent screen, viewed through an eyepiece. The magnification of electron microscopes can be in the range of 100 - 100 000 times and more.

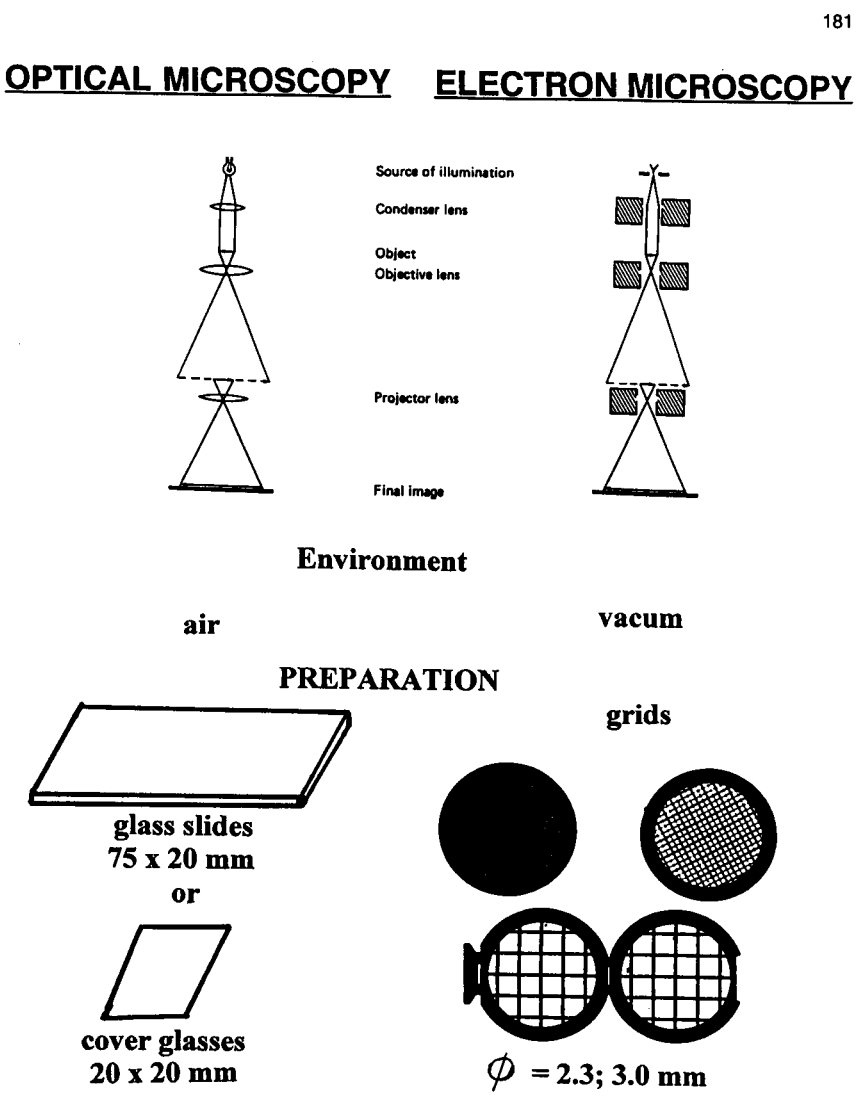


Fig. 1: Similarities and differences between optical and transmission electron microscopy

Apart from differences in wavelength and construction, optical and electronic microscopes differ in two other points: preparation scale and vacuum inside an electron microscope. Keeping in mind all these differences both types of microscopes give us the power to follow the changes undergoing during and /or after micro-organism attack on polymeric materials.

Techniques of microscopic observation.

Generally the mode of observation we can deal with is:

- optical microscopy: bright field, polarised light (Fig. 2), polarised light plus compensator
- electron microscopy: bright field, dark field, electron diffraction (Fig. 3,4)
- thermal microscopy together with DSC cell (the sample can be heated and analysed during observation with a new device of Mettler FP 900 Thermosystem - Fig. 5)

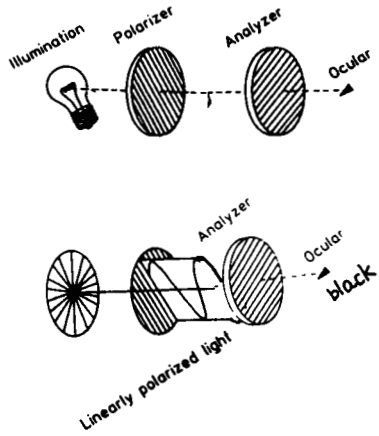
Preparation techniques.

Despite the various possibilities of mode observation we can prepare our material for studies in many different ways to follow the desired changes in the samples.

For optical microscopy we are mostly examining

- polymer surface of the film of any thickness (optical reflected microscopy)
- polymer sections of the sample of the thickness of 10 - 20 micrometers (bright and polarised mode of observation under transmission optical microscope)
- polymer film from solution of the thickness of 100 nm and more (like above)
- polymer film from the melt (like above)
- powder

THE IMAGE WITHOUT THE CRYSTAL



THE CRYSTAL IN POLARIZED LIGHT

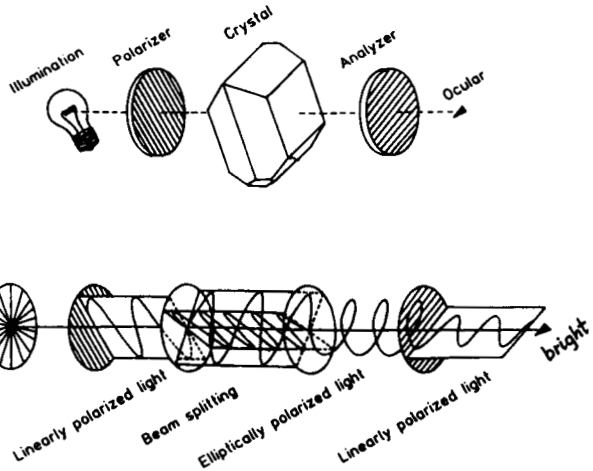


Fig. 2: The schemate of the idea of polarised microscopy for the studies of birefringent materials.

ELECTRON DIFFRACTION-MEASUREMENTS

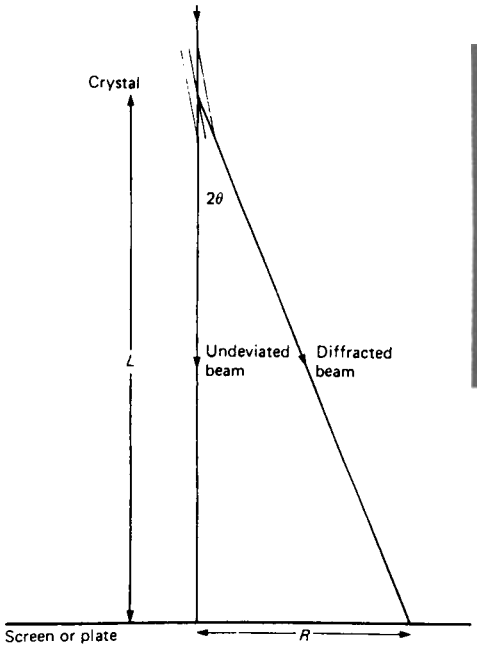
2 d SINΘ = n λ **BRAGG's LAW**

R= L tan2θ **GEOMETRY of DIFFRACTION CAMERA**

R/L=nλ/ d
d=λL/R

TEM DIFFRACTION **STANDARD SAMPLE**

$$K = \lambda L = d_s \times R_s$$



No. of ring	Au *hkl*	Au d_s *d* (nm)
1	111	0.2355
2	200	0.2039
3	220	0.1442
4	311	0.1230
5	222	0.1774
6	400	0.1020
7	331	0.0936

INVESTIGATED SAMPLE

$$d_i = K / R_i$$

R_i **from diffraction pattern**

computer ----> identification of the substance

Fig. 3: Electron diffraction mode of observation : background of calculations.

ELECTRON DIFFRACTION

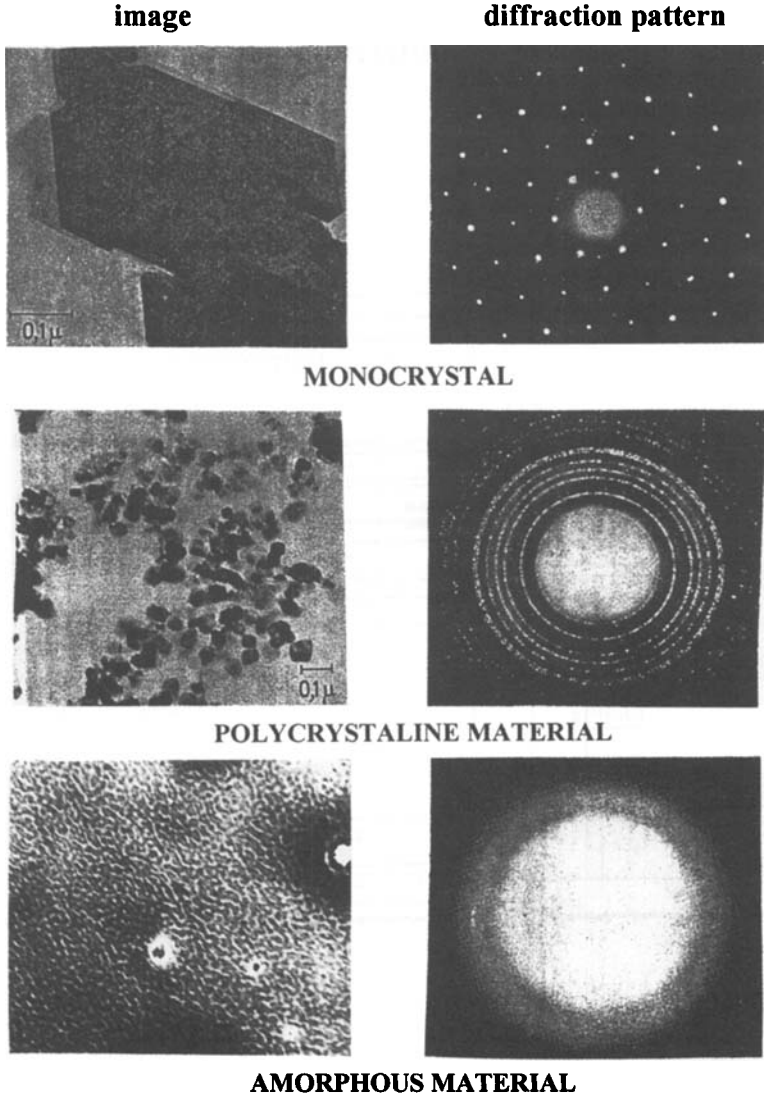


Fig. 4: Electron diffraction pattern for various materials.

The METTLER FP900 Thermosystem

METTLER FP84HT

thermal microscopy + differential scanning calorimetry

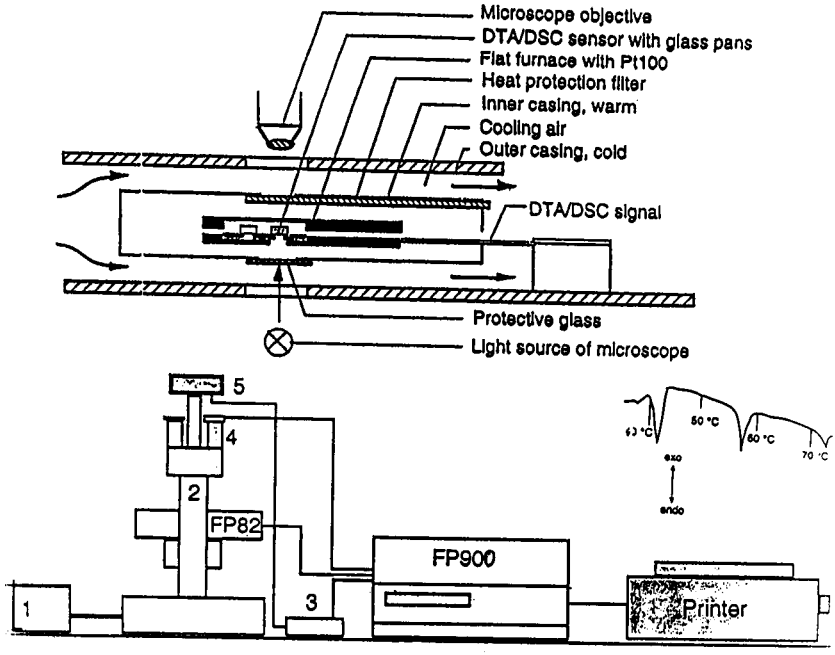


Fig. 5. The idea of combined thermal microscopy and differential scanning calorimetry in fully equipped thermo-optical Mettler FP 84 HT measuring station (1-voltage stabilizer, 2 - microscope, 3 - handset, 4 - photomonitor, 5 - camera).

In the case of electron microscopy the techniques are very specialised and we can prepare:

- polymer ultra thin sections (TEM, Fig. 6) of the thickness of 50 - 100 nm from the material as it is or after embedding in a hard resin
- polymer surface for SEM or TEM after replication (Fig. 7)
- polymer film from solution of the thickness of 20 -100 nm for TEM (bright , dark or electron mode of observation)
- powder (TEM-bright, dark or electron mode of observation, SEM)

Microscopic monitoring of biological stability

The following properties in polymeric material can be analysed after biological attack:

- surface view or fractured surface view (Fig. 8 b, 9)
- birefringence (Fig. 8a,10, 11)
- crystalline structure
- crystallinity (thermal microscopy with DSC cell)

These methods are very important both to follow the biological degradation and biological stability against microorganisms. In the first case we need them at the very biginning to check the first polymer's detoriation, and in the second case after a long period of time to make sure that our material resisted the attack (eg. application as polymer implants).

ULTRATHIN SECTIONING OF POLYMERIC MATERIALS:

**CUTTING POSSIBILITIES*

A: Material

- material as it is **- Material embedded in a hard resin**

B: Mode

WET CUTTING **ROOM TEMPERATURE CUTTING**

DRY CUTTING **CRYOCUTTING**

***SCHEMATE OF CUTTING*

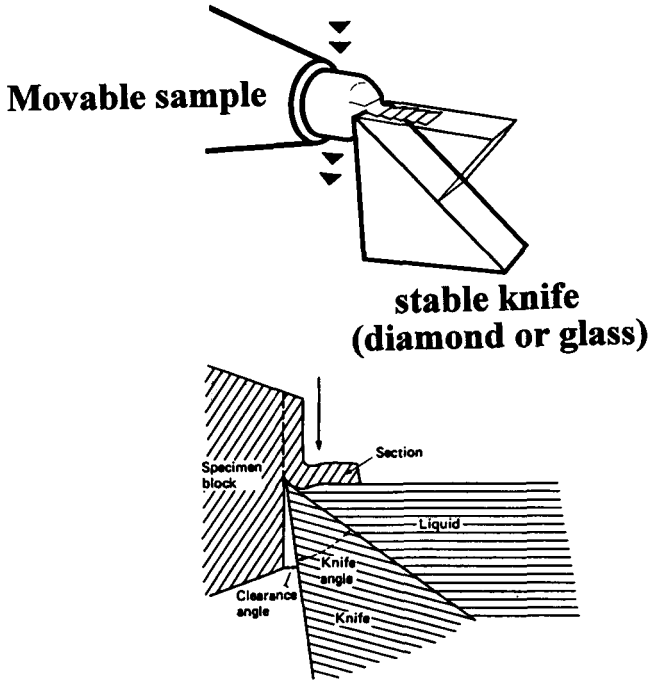


Fig. 6: Schemate of ultrathin sectioning in ultramicrotome station.

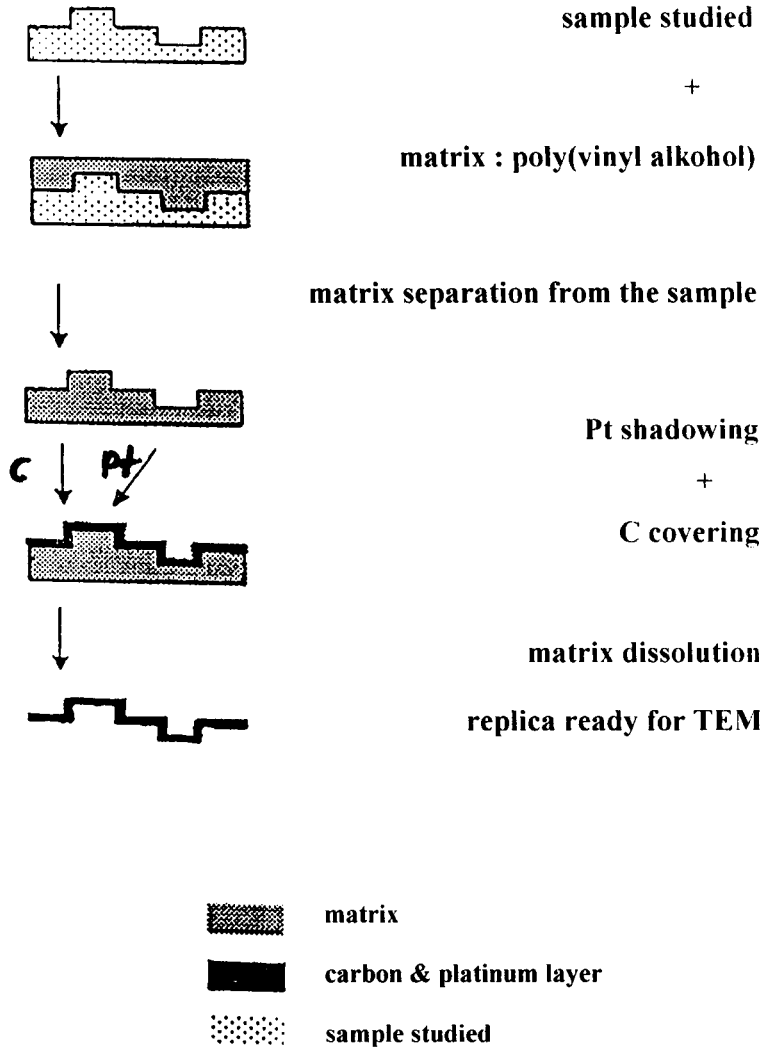


Fig. 7: Schemate of replica preparation for TEM

Polyurethanes

Segmented polyurethane block copolymers are very often used as a material for medical applications [2)] . Their morphology is very complicated [8-10] and their biological stability is controversial.

Figure 8 gives us examples of possible morphologies in some segmented polyesterurethanes. Similar morphologies have been found in our studies of polyesterureaurethanes. Studying their biostability against an attack of Aspergillus Niger [3,11] we have found strong correlation between initial morphology and biostability against the attack. The samples with the radial structure on the fracture surface were more stable than those with pseudoradial or non-radial feature of cryogenically fractured samples. Thus polyesterurethanes could be as much resistant as polyetherurethanes provided the former had a proper physical structure.

In the case of the presence of dispersed phase of micrometers scale and crystalline origin in segmented PU we have found the correlation that the more dispersed phase the less resistant the polyurethane.

Here microscopy can be used not only to follow the changes in microstructure but also to predict biostability of the samples.

Polycaprolactones

Polycaprolactones are materials in which birefrengent changes before and during the course of biological attack can be followed with the use of microscopic studies . An example of the studies is shown in fig. 10. The samples of different types of polycaprolactones were treated by sea water, buffered salt and the changes of different properties including birefrengence were followed before and after treatment [4] . Microscopic observation shows detoriation ofpolycaprolactone starting with the increase of birefringence and orientation during attack in sea water. Different course of changes was observed in plant treatment sludge.

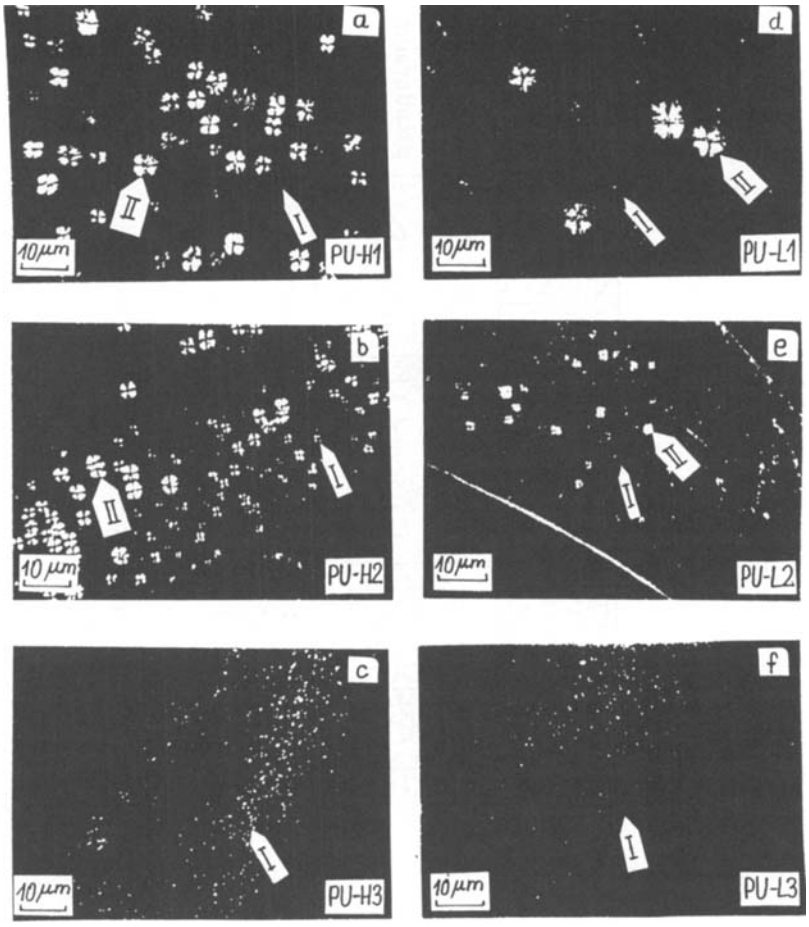


Fig. 8: The morphology of some segmented PU
a. observed under polarised light (seria L -low temp. synthesis, seria H - high temp. synthesis, 1, 2, 3 - temp. of the mould : 110, 90, 60 ° C respectively)

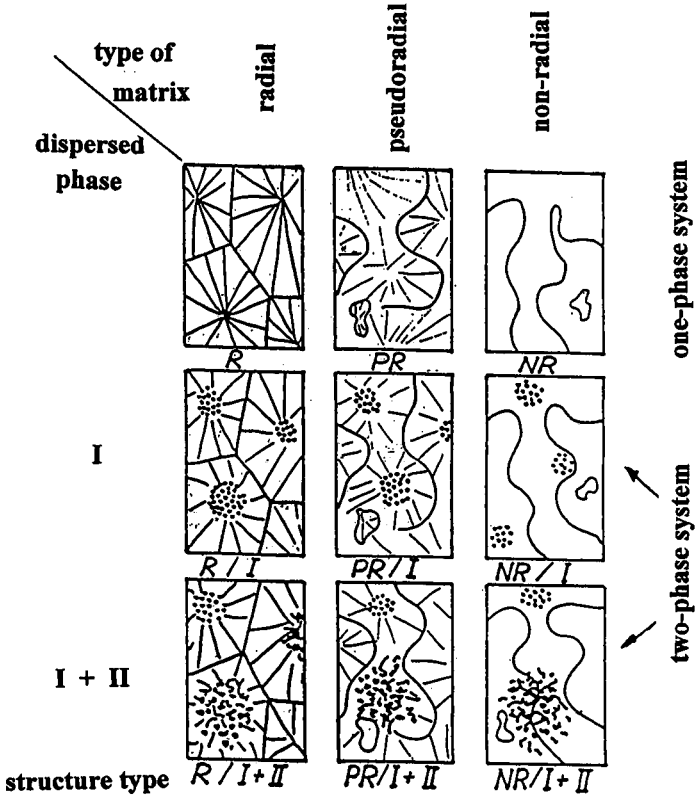


Fig. 8: The morphology of some segmented PU
b. schemes of the images observed under TEM by replica technique from cryogenically fractured samples

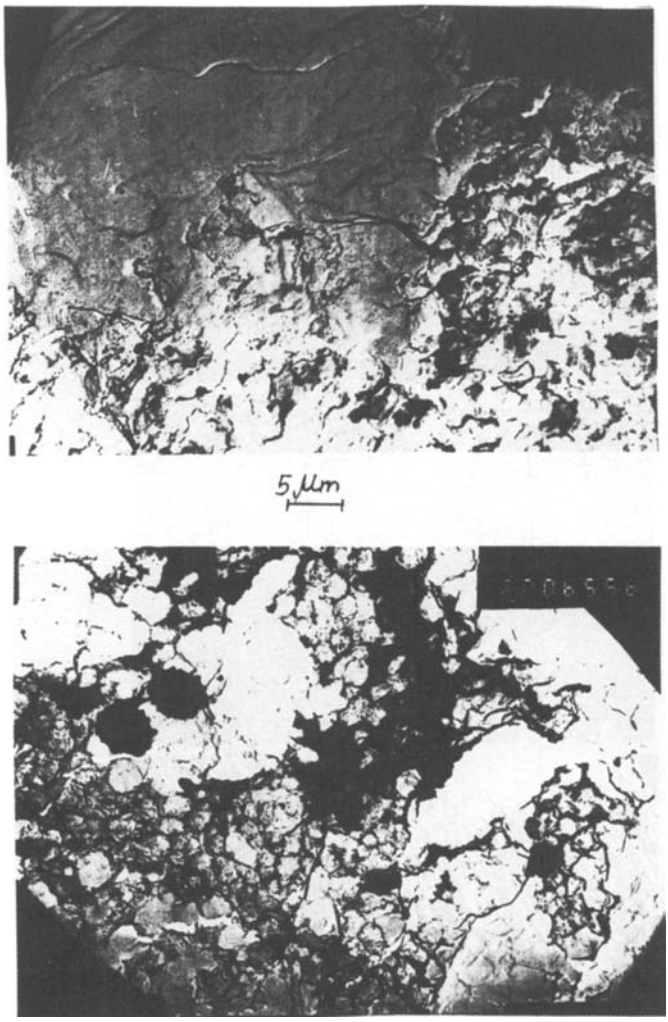


Fig. 9: The trace of biological attack viewed inside bulk polyurethanes after cryofracturing and replication.

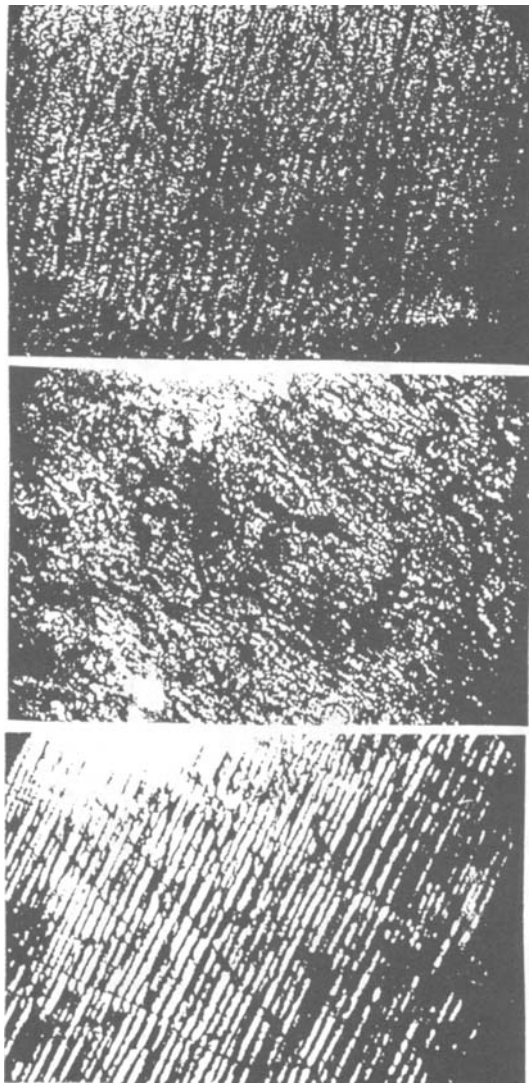


Fig. 10. The image of polycaprolactone films under reflected optical microscope [4] (polarised mode of observation)
a. before degradation
b. after 2 weeks of incubation in buffered salt solution
c. after 4 weeks of incubation in buffered salt solution

Low density polyethylene with additives

The films of low density polyethylene (LDPE) with some pro-degradant additives in form of batch were studied by reflected optical microscope in bright field and polarised light before and after treatment of sea water. The changes in birefringence of the samples both in the matrix and dispersed phase were observed. After six months of incubation in sea water a strong increase of birefringence of the matrix (LDPE) and decrease of birefrengence of dispersed phase (MB) have been found (fig. 11)

Conclusions

Microscopic techniques are very important both to follow the biological degradation and biological stability against microorganisms. In the first case we need them at the very biginning to check the first polymer's detoriation, and in the second case after a long period of time to make sure that our material resisted the attack (eg. application as polymer implants).

Depending on the polymer type and its morphology different level of changes is observed during bilogical attack especially in the case of birefringence.

Due to this the next useful advantage can be taken into account in the case of microscopy , the advantage to predict, at least partially, biological stability / biodegradibility of our samples and select them for the proper application.

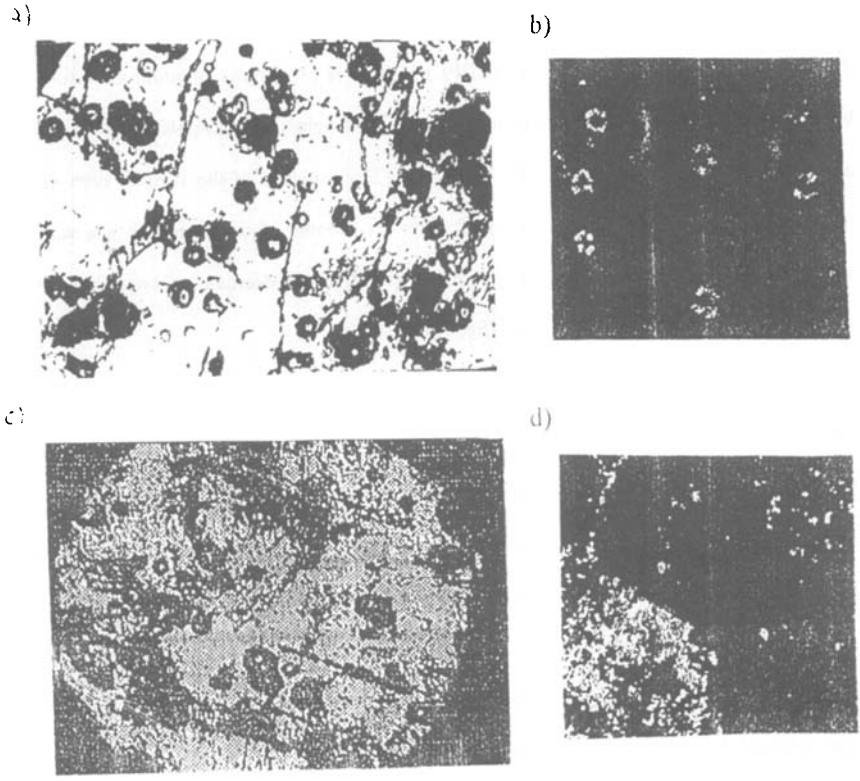


Fig. 11: The surface of LDPE - MB1 film observed under optical microscope [12]
a: before degradation (parallel polarisers)
b: before degradation (crossed polarisers)
c: after 6 months of incubation in sea water (parallel polarisers)
d: after 6 months of incubation in sea water, (crossed polarisers)
note: clearly visible birefringent disperesed phase in fig. 11b; the increase of birefringence of the matrix and decrease of birefringence of disperesed MB1 in fig.11d.

References

[1] B. Sedlacek, C. G. Overberger, H. F. Mark, *J. Polym. Sci. Polym. Symp. 66, „Medical Polymers: Chemical Problems"*, **66**, e. g. pp. 298, 299, 302, 303, 308 (1979).
[2] H. Planck, G. Egbers, I. Syre *"Progress in Biomedical Engineering"*, Elsevier, Amsterdam - Oxford - New York - Tokyo 1984, e. g. pp. 6-63.
[3] H. Janik, T. Zieniawa , *Proc. Conference: Polymers - Recycling - Environment*, Szczecin - Miedzyzdroje, PL, 1995, p.315.
[4] M. Rutkowska, M. Jastrzebska, H. Janik in *Reactive and Funcional Polymers*, Elsevier Publishers in press
[5] H. Janik, *Proc. IUPAC Symp. „ Molecular Architecture for Degradable Polymers"*, Stockholm, S 1997, p. 285
[6] S. N. Magonov, H. J. Cantow, *J. Poly. Sci. Appl. Polym. Symp.* **51,** 3 (1992).
[7] N. Petford, J. A. Miller, A. Rankin, *Microscopy and Analysis* **43**, 5 (1996).
[8] H. Janik, J. Foks, in: *Advances in Urethane Sience and Technology* **11**, Technomic Publishers, Lancaster - Basel, 1992, pp. 137-172
[9] H. Janik , J. Foks, *Progr. Colloid Polym. Sci.* **90**, 241 (1992)
[10] H. Janik, J. Foks, *Cellular Polym.* **11**, 298-318, (1992)
[11] H. Janik, *Proc. Polyurethane World Congress*, Amsterdam NL 1997, p. 739
[12] M. Rutkowska, M. Jastrzebska , H. Janik, *Proc. International Symposium on Composite Materials*, Osaka , Japonia 1997, pp. 483-486