Biodegradation of Chemically Modified Gelatin Films in Soil

P. G. DALEV,[1] R. D. PATIL,[2] J. E. MARK,[2] E. VASSILEVA,[3] S. FAKIROV[3]

[1] Department of Biology, University of Sofia, 1421 Sofia, Bulgaria

[2] Department of Chemistry and the Polymer Research Center, University of Cincinnati, Cincinnati, Ohio 45221-0172

[3] Laboratory on Structure and Properties of Polymers, University of Sofia, 1126 Sofia, Bulgaria

Received 23 August 1999; accepted 28 March 2000

ABSTRACT: Gelatin films that had been chemically modified (crosslinked with formaldehyde, glyoxal, glutaraldehyde, hexamethylene diisocyanate, butadiene diepoxide, or diepoxyoctane) were tested for their biodegradability by soil burial testing in a laboratory environment under temperature and humidity control. The relationship between weight loss and time of biodegradation showed a linear behavior for all the samples, but the rate of biodegradation showed a dependence on the type of crosslinking agent. The most stable films were those crosslinked with aldehydes, and these biodegraded by the 10th day. The samples crosslinked with hexamethylene diisocyanate and diepoxides completely biodegraded by the fourth and sixth days, respectively. It was shown that the rate of biodegradation depended on the density of crosslinking, which was calculated by a modified Flory–Rehner equation. The biodegraded samples showed considerable changes in the fingerprint region of FTIR spectra, and, thus, these spectra could be used for evaluation of the soil burial biodegradation of chemically modified gelatin samples. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 1341–1347, 2000

Key words: soil biodegradation; modified gelatins; weight loss; crosslink density; FTIR spectra

INTRODUCTION

The assessment of biodegradability of chemically modified biopolymers is a key issue in the development of environmentally friendly materials.[1–3] Earlier, we reported the biodegradative behavior of chemically modified gelatin with aldehydes, diepoxides, and hexamethylene diisocyanate employing a proteolytic enzyme (alkaline proteinase) and in sewage, lake, and river waters.[4–6] For the complete assessment of biodegradability of chemically modified gelatin, the study of its soil biodegradability is indispensable. Usually for such investigation, standard-sized samples of the material are buried either in protected outdoor locations or in a laboratory environment where better control of temperature and humidity can be maintained.[7]

Outdoor soil burial experiments involve real environmental conditions to test samples for rapidity of biodegradation. But due to uncontrollable factors (human or animal spoiling, climatic changes), it lacks reproducibility. Nevertheless, many authors conducted soil burial experiments in outdoor conditions for varying periods of time.[2,8]

Laboratory soil burial tests also suffer from the disadvantage of lack of control over the microbe population. To avoid this disadvantage, some authors inoculated soil with known test organisms,

Correspondence to: J. E. Mark (markje@email.uc.edu; World Wide Web: jemcom.crs.uc.edu).

Contract grant sponsor: National Science Foundation; contract grant number: INT-9514149.

Journal of Applied Polymer Science, Vol. 78, 1341–1347 (2000)

with or without soil sterilization.[9] However, both cases of microbiological treatments of soil have produced only partially satisfactory results, since, in both cases, symbiotic relationships between various native microorganisms strains are lost. The soil microbial society consists of large numbers of individual microorganism populations (bacteria, actinomycetes, fungi, yeasts, algae, and protozoa) usually with approximate counts ranging from 500×10^3 to 500×10^6 per gram of soil.[10] Normally, these microorganisms are heterotropic and require brothlike substances for carbon, nitrogen, and energy. Thus, microorganism distribution in soil depends on the presence and distribution of organic sources of carbon and nitrogen in the soil. A confirmation of this is the fact that the enzyme activities which are important for the turnover of organic substances in soil correspond closely with the number of soil microorganisms.[10] Biodegradation thus depends on the complex and balanced biosocieties in the soil in which study must be conducted, under conditions which do not disturb this balance. The present investigation dealt with the biodegradation behavior of chemically modified gelatin in soil burial experiments conducted in a laboratory, under conditions similar to those of the natural environment.

EXPERIMENTAL

Materials

Gelatin type A, Bloom value 300 (Sigma, St. Louis, MO), was used in the present investigation. Crosslinking agents formaldehyde (FA), glyoxal (GO), glutaraldehyde (GA), hexamethylene diisocyanate (HMDIC), 1,3-butadiene diepoxide (BDE), and 1,2,7,8-diepoxyoctane (DEO) were purchased from Aldrich (Milwaukee, WI) and used as received. 2,2,2-Trifluoroethanol (TFE), potassium thiocyanate (KCSN), and all other chemicals were of AR grade and used as obtained.

Soil was taken from the Burnet Woods park in an urban area of Cincinnati, Ohio, from the surface layer of the ground. All inert materials were carefully removed to obtain a relatively homogeneous mass.

Preparation of Crosslinked Gelatin Samples

Two grams of gelatin was soaked overnight in distilled water at 5°C and then dispersed at 45°C in 20 mL 0.02*M* phosphate buffer (pH 5.8). Solutions of FA, GO, or GA in the same buffer were added to the gelatin solution with thorough mixing, in an appropriate quantity to obtain a 0.012*M* concentration of the crosslinking agent. Crosslinking was performed at the same temperature (45°C) for 120 min. Crosslinking with BDE and DEO was performed in the same way as in the case of the aldehydes, specifically under identical conditions (concentrations of crosslinking agent and gelatin, temperature, solvent, and reaction time). The crosslinking with HMDIC was performed by adding appropriate amounts of HMDIC and triethylamine (1/3 part of the HMDIC quantity) to a 5.5 wt % solution of gelatin in TFE at room temperature (25°C). In all cases, the resulting solutions were poured into aluminum dishes having a diameter of 10 cm and were then dried at room temperature for 24 h to obtain isotropic films having a thickness of approximately 0.25 mm. Uncrosslinked gelatin films were cast as above from a 5.5 wt % gelatin in 0.02*M* phosphate buffer (pH 5.8). The crosslinked gelatin samples were designated by the crosslinking agent designation followed by "G" for gelatin, for example, FAG, GOG, etc., with NG specifying no crosslinking agent at all (the uncrosslinked gelatin). The sample designations and conditions of preparation are given in Table I.

Crosslink Density Determination

The total crosslink density expressed inversely as the molecular weight between crosslinks was calculated by the Flory–Rehner equation[11] as reported elsewere.[12] The samples were swelled in distilled water at 20°C for 24 h to reach swelling equilibrium.

Biodegradation Experiments

Soil was poured into a plastic tray ($10 \times 20 \times 5$ cm) up to a thickness of about 4 cm. The samples ($10 \times 20 \times 0.3$ mm) were weighed and then buried in the soil to a depth of 1 cm. The tray was covered with a iron net and then with a thick paper moistened with tap water. Water was sprayed twice a day to sustain the moisture. During the fixed periods (first, second, fourth, sixth, and tenth day), samples were carefully taken out, washed with distilled water, and dried under ambient temperature (22–25°C) and humidity (70–80%) conditions for 24 h and then weighed.

Controls were prepared in sterile soil. To sterilize the soil, 500 g of soil was heated in an oven at

Table I Sample Designations, Crosslinking Agents, Solvents, and Molecular Weight (Da) Between Crosslinks

Sample Designation	Crosslinking Agent	Solvent	Molecular Weight Between Crosslinks (Da)
FAG	Formaldehyde	Phosphate buffer, pH 5.8	480
GOG	Glyoxal	Phosphate buffer, pH 5.8	650
GAG	Glutaraldehyde	Phosphate buffer, pH 5.8	840
HMDICG	Hexamethylene diisocyanate	2,2,2-Trifluoroethanol	3200
BDEG	Butadiene diepoxide	Phosphate buffer, pH 5.8	4150
DEOG	Diepoxyoctane	Phosphate buffer, pH 5.8	3700
NG	—	Distilled water	11,250

[a] All crosslinked samples were obtained from a 5.5 wt % solution of gelatin at 25°C and the time of crosslinking was 120 min.

125°C for 6 h. Then, it was cooled at ambient temperature and weighed to determine the water content. This soil was placed into a plastic tray (10 × 20 × 5 cm) and thoroughly mixed with a 0.02 wt % aqueous solution of sodium azide (NaN_3) to obtain a homogeneous mass. The amount of the NaN_3 solution mixed was equal to the amount of water lost during the sterilization process.

Fourier Transform Infrared (FTIR) Analysis

Gelatin samples for FTIR analysis were taken on the fourth day of biodegradation (for HMDICG, on the second day). Control samples on the 10th day of biodegradation were also used. Spectra were obtained using a Perkin–Elmer 1600 Series spectrometer.

RESULTS AND DISCUSSION

As in our previous work, where biodegradability in lake and river waters was studied,[6] crosslinking agents with various functional groups (aldehydes, epoxides, and isocyanates) and various lengths of the —CH_2— chain (1–8 —CH_2— units) were used. The appearances of the gelatin films on various days of soil biodegradation are shown in Figure 1. Even on the first day, very strong changes in the samples were seen. During the first 3 days, the films were swollen remarkably, after which they began to lose their outline shapes. On the 10th day, the biodegradation was complete for all the films. In contrast to the experiments with natural soil, the samples buried in sterilized soil soaked with a NaN_3 solution kept their outlines even to the 10th day, and their weight losses (WL) were negligible (2–4%). All samples changed their colors to brown.

The time dependence of the WL of the crosslinked and uncrosslinked gelatin are shown in Figure 2. The considerable differences between the samples are seen even on the first day. The highest WL was for HMDICG, which completely degraded between the third and the fourth days. (Complete degradation was determined when the sample disappeared from the soil mass or it turned into thin slimy mucous which was ab-

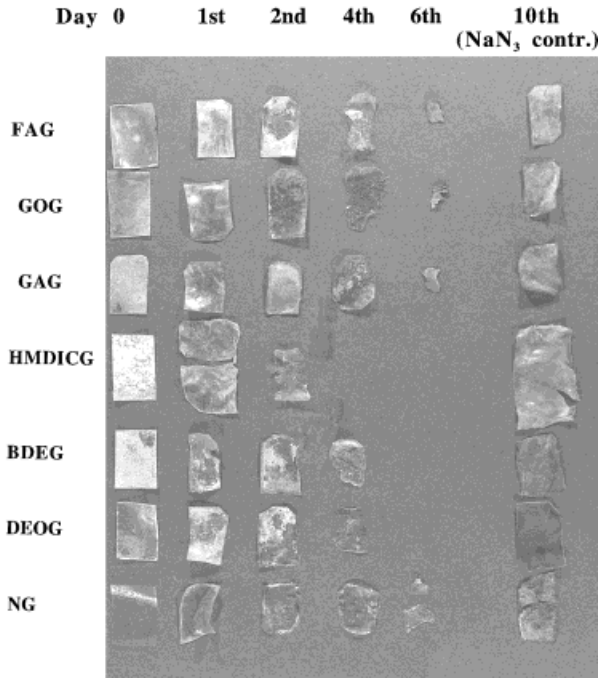

Figure 1 Appearances of the gelatin films after the specified days of soil biodegradation. The last column shows the NaN_3 controls.

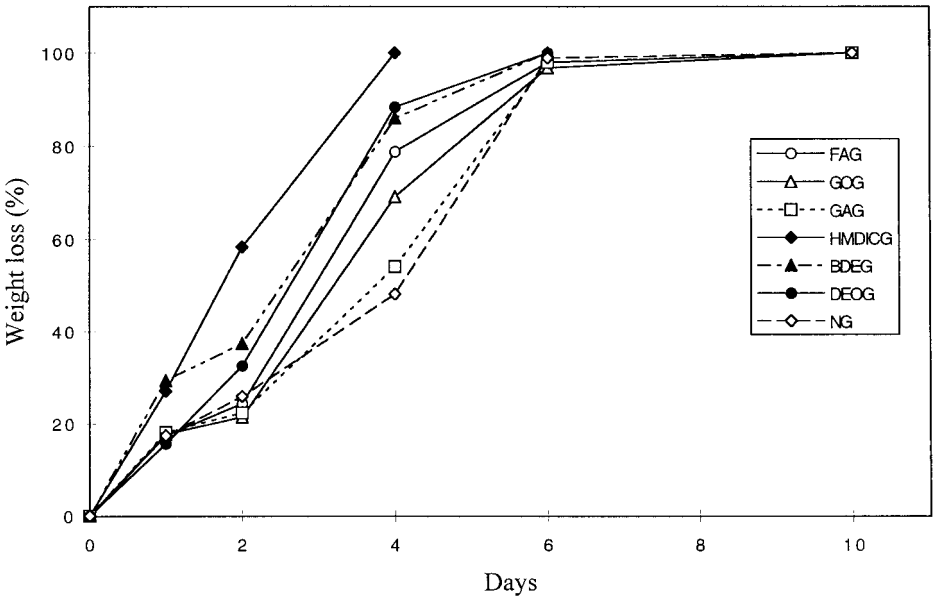

Figure 2 Weight losses of the films.

sorbed into the soil.) This kind of biodegradation occurred with NG, HMDICG, BDEG, and DEOG. The films of FAG, GOG, and GAG preserved their comparative stiffness and it was possible to remove the residues of the films from the soil even after the sixth day.

The curves of WL against time for the other samples were almost parallel to each other. Complete degradation occurred for NG, BDEG, and DEOG by the sixth day. Remainders from FAG, GOG, and GAG could not be recovered on the 10th day, and for all practical purposes, they could be considered as completely degraded.

A close relationship between the crosslink densities (shown in Table I) and values of the WL (Fig. 2) was established. The samples with higher crosslink densities (FAG, GOG, and GAG) were found to be the most stable. For these samples, complete degradation occurred by the 10th day. Complete biodegradation of BDEG and DEOG was twice as fast as for those crosslinked with aldehydes. Biodegradation of HMDICG was twice as fast as for the diepoxide crosslinked samples, although their crosslink densities were similar. The explanation of this difference is the fact that the crosslinking of HMDICG was carried out in TFE, a nonaqueous medium, where the formation of physical (hydrogen) bonds was hindered because of its polarity.

The absorption of water into the crosslinked gelatin samples depended strongly on the density of crosslinking. It was hindered by the network structure, and the higher the crosslink density, the lower the adsorption. This is important because the penetration of microorganisms and their enzymes is impossible without water adsorption and swelling of the crosslinked gelatin network.

The very low estimated network density for NG (11,250 Da between crosslinks) did not correspond to the comparatively high stability of NG in soil. It could be assumed that in soil NG underwent additional changes in its network structure during incubation.

The soil microorganisms are involved in the synthesis of simple and complex organic molecules, which may belong to the cell biomass or to different extracellular products.[13] For example, fungi form phenolic compounds from a simple aliphatic molecule, which may polymerize both abiotically or become linked with amino acids, peptides, amino sugars, and other organic compounds to finally form dark humic acid-type polymers.[13] These processes are catalyzed by microbial phenoloxidases, but analogical processes could be catalyzed by other microbial enzymes such as hydrolyases, transferases, and ligases. These processes may take place and form additional crosslinks in NG. In crosslinked samples, such processes could not take place, since active functional groups in gelatin are already engaged.

FTIR spectra of FAG, HMDICG, and NG are shown in Figure 3. The spectra of undegraded

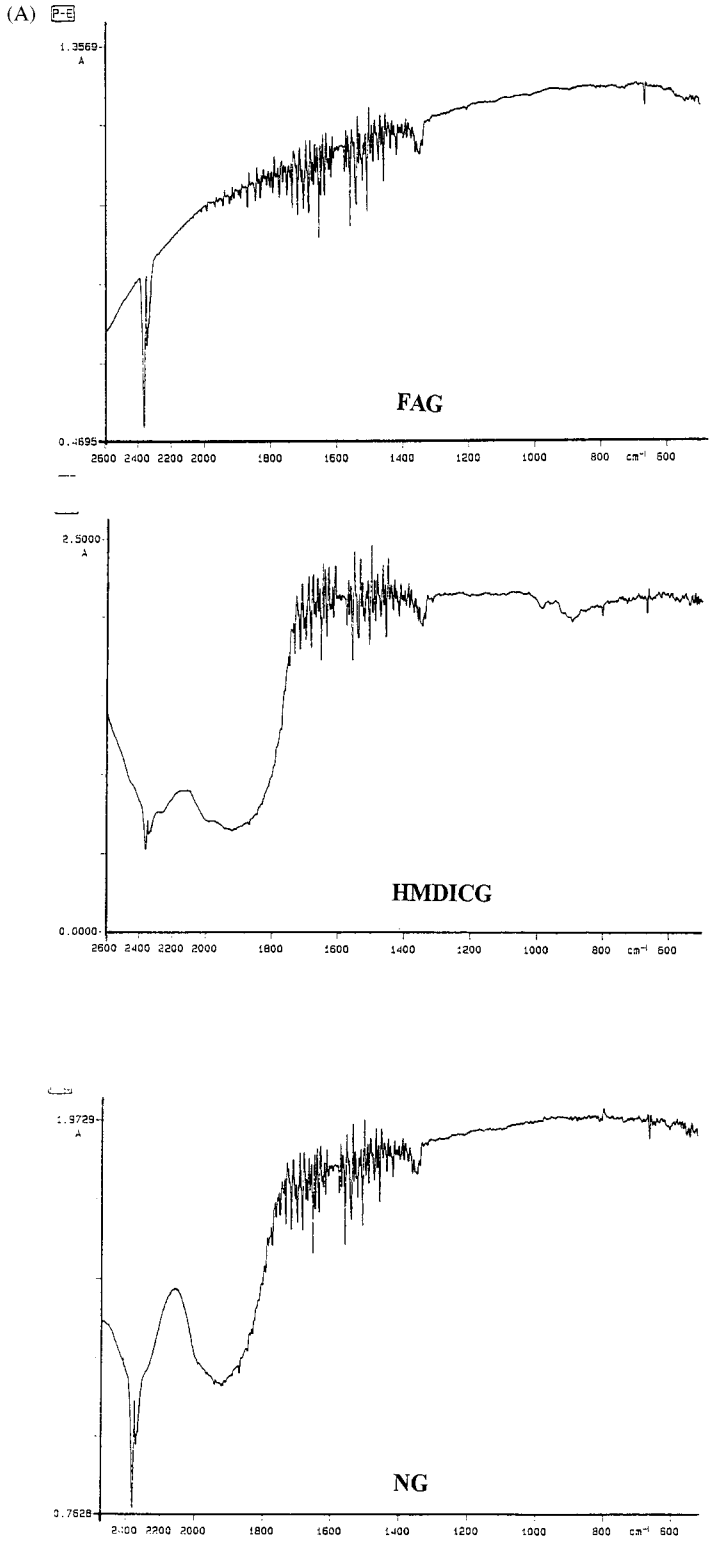

Figure 3 FTIR spectra of (A) unbiodegraded samples and (B) those biodegraded in soil for 3 days.

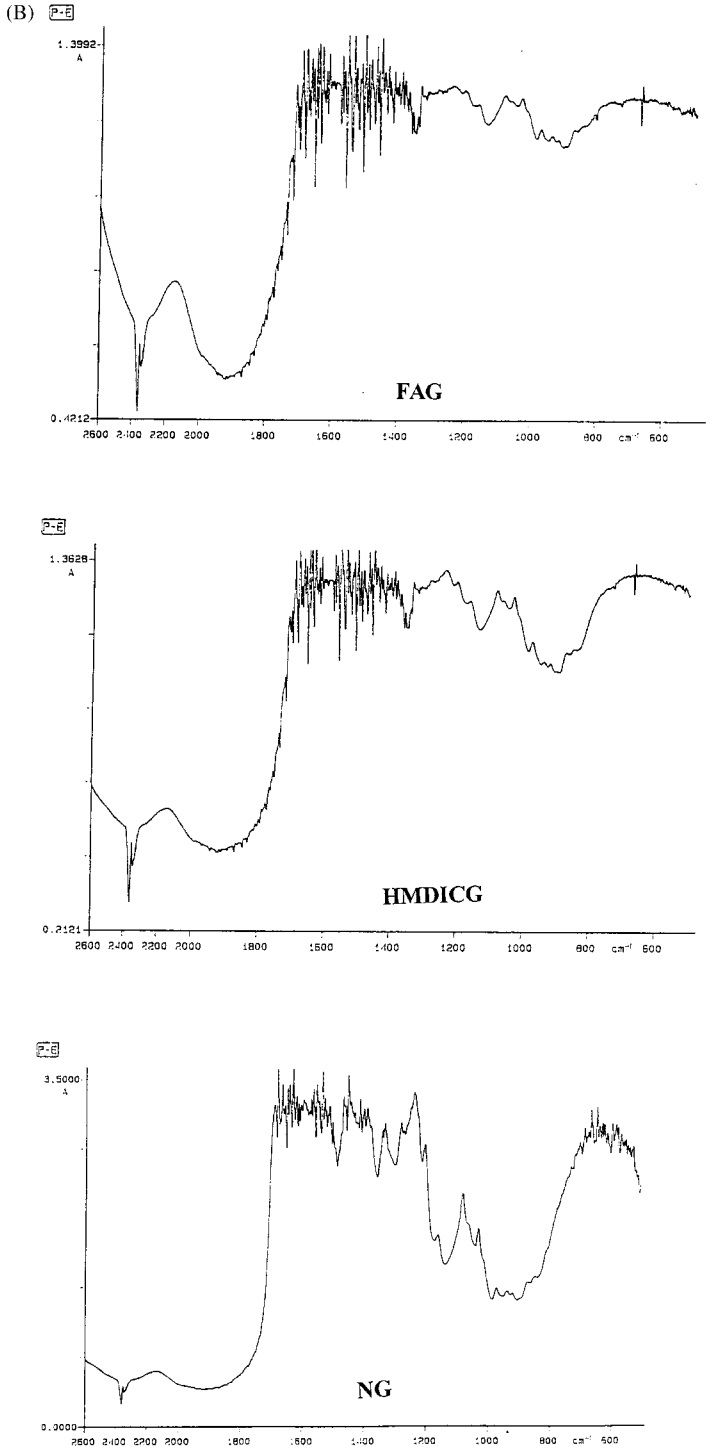

Figure 3 (*Continued*)

samples are comparatively simple and without any characteristic bands in the fingerprint region. In contrast, all biodegraded crosslinked gelatin samples exhibited characteristic bands in the fingerprint region (1250–600 cm^{-1}). Particularly strong and characteristic bands were found in the regions 1200–1100 cm^{-1} and 800–1020 cm^{-1}. The complexity of the spectra could be due to either the unfolding of the modified protein structure and exposure of crosslinked sites or to for-

mation of new compounds as by-products of the metabolism of the microorganisms.

CONCLUSIONS

The chemically modified gelatin with either FA, GO, GA, HMDIC, BDE, or ODE showed rapid biodegradability in soil burial experiments in a laboratory environment. The biodegradability was measured by means of weight loss of the films or with the aid of FTIR spectroscopy. The rate of biodegradability showed dependencies on the type of the crosslinking agent and on the crosslink density.

It is a pleasure for the authors to acknowledge the financial support provided by the National Science Foundation through Grant INT-9514149. One of the authors (P. G. D.) appreciates the hospitality of the Department of Chemistry at the University of Cincinnati.

REFERENCES

1. Potts, J. E. In Aspects of Degradation and Stabilization of Polymers; Jellinek, H. H. J., Ed.; Elsevier: Amsterdam, 1978; p 617.
2. Kumar, G. S.; Kalpagam, V.; Nandi, U. S. J Appl Polym Sci 1985, 30, 915.
3. Lo, F.; Petchonka, J.; Hanly, J. Chem Eng Prog 1993, 89(7), 55.
4. Patil, R. D.; Mark, J. E.; Dalev, P.; Vassileva, E.; Fakirov, S. Polym Prepr 1998, 39(2), 717.
5. Dalev, P.; Staromanova, E.; Dalev, D.; Patil R. D.; Mark, J. E; Vassileva, E.; Fakirov, S., submitted for publication in Biodegradation.
6. Patil, R. D.; Dalev, P. G.; Mark, J. E.; Vassileva, E.; Fakirov, S. J Appl Polym Sci 2000, 76, 29.
7. Osmon, J. L.; Klausmeier, R. E. In Biodeterioration Investigation Techniques; Walters, A. H., Ed.; Applied Science: London, 1977; p 77.
8. Otake, Y.; Kobayashi, T.; Asabe, H.; Murakami, N.; Ono K. J Appl Polym Sci 1995, 56, 1789.
9. Tsuchii, A.; Takeda, K.; Suzuki, T.; Tokiwa, Y. Biodegradation 1969, 7, 41.
10. Filip, Z. In Biodegradable Polymers and Plastics; Vert, M.; Feijen, J.; Albertsson, A.; Scott, G.; Chiellini, E., Eds.; RSC, Thomas Graham House: Cambridge, 1992; p 45.
11. Flory, P. J.; Rehner, J. J Chem Phys 1943, 11, 521.
12. Oikawa, H.; Nakanishi, H. Polymer 1993, 34(16), 126.
13. Haider, K.; Martin, J. P.; Filip, Z. In Soil Biochemistry; Paul, E. A.; McLaren, A. D., Eds.; Marcel Dekker: New York, 1975; Vol. 4, p 195.