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Polymeric carriers for covalent immobilization of amino groups containing compounds

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Summary

Graft copolymers of acryloylchloride (AC) with polyethylene (PE) and polyethyl eneterephtalate (PET) have been synthesized by grafting of AC from the vapor phase under mutual γ-irradiation. It has been shown that both the amount of grafted reactive chains and their structure was determined by the polymer: monomer ratio and the total irradiation dose. The high grafting degree of reactive groups and their ability to interact with free amino groups of biological molecules both in organic media and in water solutions make these functionalized polymers suitable as reactive carriers for covalent immobilization of various amino groups containing compounds.

Introduction

Saturated acylchlorides are known to be effective acylating agents for amino groups containing compounds under mild conditions (1). It might be of interest to graft unsaturated reagents having acylchloride groups onto polymeric surfaces for reactions with amino groups containing molecules to produce modified materials by a simple and effective technique. Various activation techniques have been developed for the introduction of reactive groups in polymers, including radiation grafting of functional reactive monomers with preformed polymers (2-4). We developed a new approach by creation of AC functionalized polymers by γ-irradiation of PE and PET in AC vapors using a direct mutual procedure and studied the properties of the modified polymers as reactive carriers.

Experimental

Materials

Low density PE and PET sheets (Talas-Ommen, The Netherlands) were cleaned and dried in vacuum at 60°C overnight. Acryloylchloride (Merck, Schuchardt), was distilled under low pressure. Monoethanolamine and L-arginine (Fluka Biochemica) with $[\alpha] = +26{,}5°$ (C = 5 in 5M HCl) were used without purification.

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Grafting procedure

PE and PET sheets were placed in the upper part of a glass ampule. Separated by a porous glass filter, AC was brought into the bottom of the ampule. The ampule was thoroughly degassed by a freezing-thawing cycle (5 times) and then sealed. The ampules were irradiated by ^{60}Co γ-rays with a dose rate of 180 KRad/h (Radiation Chemistry Dept., Technical University of Delft, The Netherlands) to various total doses. After irradiation the polymer films were washed and dried in vacuum.

Polymer characterization

The grafting degree (G) was determined using the equation: **G = $[(W_g-W_o)/W_o]$x100%**, where **W_o** and **W_g** are the weights of the films before and after grafting, respectively. The water uptake was established by the same weight-difference method. For immobilization of amino containing substances the modified films were placed in solutions with stirring at ambient temperature and after 4-6 h they were washed with water and ethanol and dried in vacuum at 60°C. The efficiency of immobilization (E) was estimated by the difference between the initial (**C_o**) and final (**C_t**) substance concentrations measured using the optical rotation value of L-arginine in solutions of Na_D-line (Perkin-Elmer 241 Polarimeter) and calculated as **E = $[(C_o-C_t)/C_o]$x100%**.

IR measurements were carried out using a Bio-Rad FTS-60 FTIR spectrophotometer. Contact angles of films were measured by the electrobalance technique (5) using an Electrobalance RM-2 apparatus. Surface images were obtained by SEM using a JEOL JSM-35 CF Scanning Microscope.

Results and Discussion

Irradiation of the polymers in AC vapor resulted in a weight increase and introduction of reactive centres onto their surfaces. The latter fact was proved by the appearance in the FT-IR spectra of a very intense band at 1720-1750 cm^{-1} (characteristic for the carbonyl C=O group).

Table 1

Influence of initial polymer:monomer ratio upon grafting of AC onto PE (2,5 MRad at 20°C and p = 1,5x10^{-2}mm Hg).

Polymer (g)	Monomer (g)	Polymer:monomer ratio	Grafting (G) %±0,5%
0,4114	0	1:0	0
0,3300	0,2228	1:0,67	44,5
0,2007	0,2288	1:1,10	89,7
0,2530	0,4456	1:1,76	126,3
0,2466	0,6684	1:2,71	152,5
0,2846	1,1114	1:3,90	149,9
0,2607	1,6684	1:9,15	150,0

Table 1 gives data about the effect of the polymer: monomer ratio on the degree of grafting **G**. It can be seen that a decrease of this ratio until about 1:2 resulted in an increase in **G** whereas a further decrease did not affect the maximal grafting. The use of polymer:monomer ratios lower than 1:2 did not lead to a higher grafting degree.

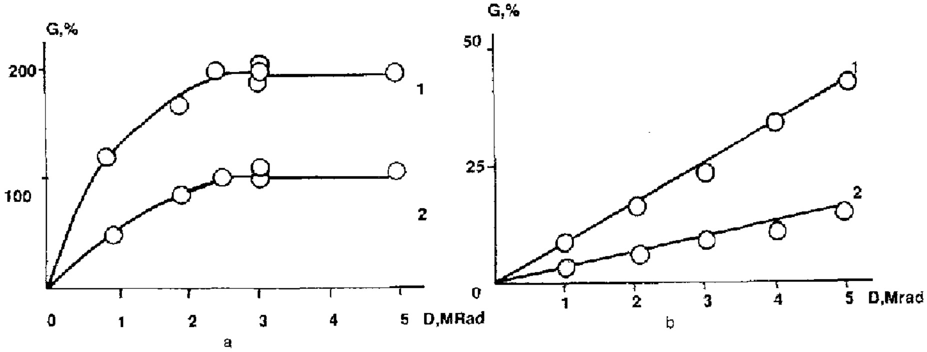
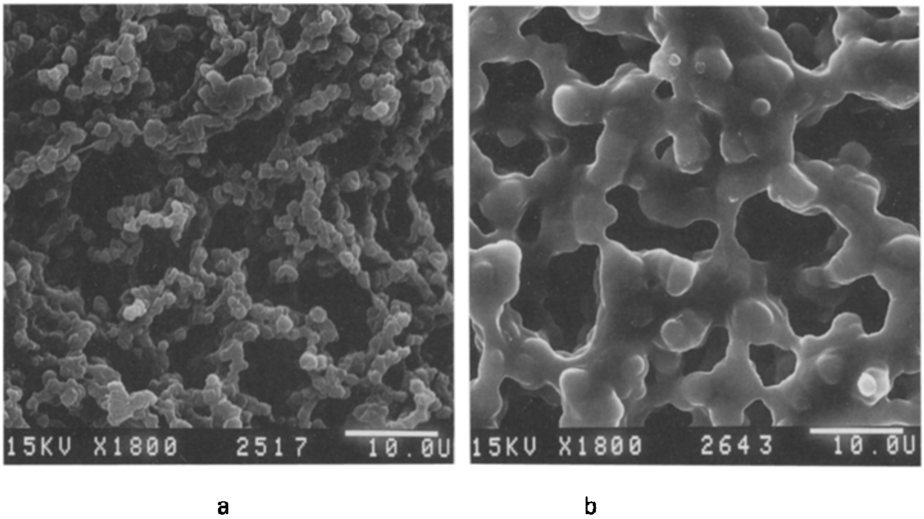


Figure 1.
Grafting degree vs total dose for (a) PE (1) and PET (2) under γ-irradiation in AC vapor and (b) for preliminary irradiated polymers in vacuum, followed by contact with AC vapor. ($p=4x10^{-2}$ mm Hg, 20°C, polymer:monomer ratio 1:2).

As can be seen from Figure 1-a, increasing the irradiation dose leads to increasing **G**-values for both polymers under doses below 2,5 MRad only. Increasing the dose to 5 MRad does not change the G-values neither for PE, nor for PET. The behaviour of AC in this grafting process is surprising because the grafting of AC from the vapor phase under irradiation onto polymers studied by the "post-polymerization" technique (6) increases continuously from 2,5-5 MRad (Figure 1-b). The reason for the grafting limitation might be the crosslinking of propagating chains.

Figure 2 shows SEM photographs of AC homopolymer and of PE grafted with AC by irradiation with 5 MRad. It has been observed (7) that in the absence of polymer, irradiation of freshly distilled AC with a dose of 2,5 MRad or more, leads to crosslinked products with structures as shown in Figure 2-a. Similar structures were found on the films after mutual irradiation of AC with PE or PET (Fig.2-b), possibly indicating that during the grafting also crosslinking of AC had taken place. Assuming the mechanism of bulk polymerization of AC and of grafting under γ-irradiation to be the same, the form of the curve in Figure 1-a can be explained, because without crosslinking a linear curve such as in Figure 1-b should be expected.



a

b

Figure 2.
SEM-photographs of samples of (a) AC homopolymer and (b) of PE grafted with AC by irradiation with 5 MRad.

The interaction of functionalized polymers with NH_2-containing substances in organic media (e.g. with liquid monoethanolamine) leads to a complete conversion in the reactive **-COCl**-groups into **-CONH**-groups according to the scheme:

P-COCl + H_2N-R ---> P-CONH-R + HCl, which was confirmed by the appearance in the IR spectra of the treated polymers by the absorbance at 1640 and 1560 cm^{-1} corresponding to amide-A and amide-B bands.

When the interaction of AC grafted polymers with amino group containing compounds occurs in aqueous solutions, AC may react in two different ways:

P-COCl + H_2N-R ---> P-CONH-R + HCl (k_1) and

P-COCl + H_2O ---> P-COOH + HCl (k_2).

The ratio of the two reaction rate constants will determine the immobilization efficiency (8).

Table 2 presents some properties of modified polymers after reaction with water (4h) and with aqueous solutions of L-arginine(4 h). After 4 h all **-COCl** groups had reacted and the concentration of L-arginine remained constant. In the IR spectra of the modified films bands of **COOH**-groups (1720 and 1150 cm^{-1}) were detected as well as amide bands. These data are evidence of the immobilization process onto carriers.

The efficiency of the immobilization of L-arginine and its ethyl ester onto AC grafted polymers from water solutions reached 50-60% and can be regulated by the molar ratio of AC-units to NH_2-groups. It was observed that the ratio of k_1/k_2 was the same for the two types of AC grafted polymers.

Table 2.
Some properties of initial and modified polymers.

Sample*	Water uptake, %	Contact angle, °	Nitrogen content, %
PE-1		89,3 ± 1,6	
PE-2	7,8 ± 0,3	16,0 ± 1,0	
PE-3	17,0 ± 0,5	< 10	3,19 ± 0,03
PET-1		93,5 ± 1,4	
PET-2	2,9 ± 0,4	20,2 ± 1,0	
PET-3	14,2 ± 0,5	14,0 ± 1,2	1,07 ± 0,03

* 1- initial polymers, 2 and 3 - grafted with AC and modified with water (pH = 8,9) and aqueous solutions of L-arginine at pH = 8,9, at 20°C, respectively.

Conclusions

Radiation induced copolymerization of AC onto PE and PET from the vapor phase results in grafted polymeric carriers and is determined by three parameters: the nature of polymer used, the polymer: monomer ratio before irradiation and the total irradiation dose. The proper choice of these parameters can be processed for materials with almost any desired properties. The polymers grafted with AC react with amino groups containing molecules resulting in the covalent coupling of these substances from both organic solutions and aqueous ones. This method can be used for the creation of modified polymeric materials and devices for biomedical and other applications.

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