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Radiation Grafting of Chloromethylstyrene on Polyethylene, Followed by Quaternization and Heparinization

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ABSTRACT

Several investigators have indicated an interest in binding heparin to polymers, because of the generally enhanced thrombo-resistance of such materials. This report describes such a technique for polyethylene (PE) film which involves radiation-induced grafting of chloromethylstyrene (CMS) on PE, followed by quaternization with pyridine, then reaction with sodium heparin to replace chloride ions in the film with heparin anions. Extraction of the heparinized final product with benzene, water, and 1.0 M aqueous sodium chloride solution produced no detectable removal of the heparin from the film samples. A plot of amount CMS grafted on PE film versus time was curved in shape rather than linear, showing an increase in grafting rate as the length of the run increased.

INTRODUCTION

It has been shown by several investigators [1, 2] that polymers containing bound heparin exhibit thromboresistant properties. This

report describes the radiation chemistry of the production of suitable graft copolymers and the technique for reacting the heparin with the copolymers, but does not go into the medical or physiological aspects of thromboresistance.

During recent years, much effort has been devoted to the development of polymeric materials that would not cause thrombosis (clotting) when placed in contact with the bloodstream [3-7]. Extensive studies of blood-polymer interactions suggest that surface characteristics such as wettability and zeta potential have no direct influence on their compatibility with blood, but rather that the chemical nature of the polymer surface is more important than other factors.

The synthetic technique described below results in a graft copolymer similar to that of Gott [1], but employs a method of preparation that is perhaps simpler and more controllable. Procedures not involving grafting have been devised by Grode [8], who treated polymers with a solution of tridodecylmethylammonium chloride (TDMAC). The hydrocarbon chain of the salt dissolves in the polymer and, on removing the solvent, quaternary groups are left on the surface which can be complexed with heparin (due to its negatively charged sulfate groups). In a modification of this process [8], heparin-TDMAC complex is preformed and then applied to the polymer. Eriksson [9] has described a process wherein a polymer is softened by heating in an aqueous solution of a quaternary salt. The hydrocarbon part of the salt penetrates into the softened polymer which, on cooling, is "fixed", leaving the quaternary part of the salt on the surface. Gradual leaching of the heparin from the surface by flowing blood is characteristic of such surface-coating techniques, but may be reduced by crosslinking the coating with glutaraldehyde [10].

One feature of the grafting approach described in this report is that the bound heparin is quite resistant to removal by washing with solvents or sodium chloride solutions as discussed below.

EXPERIMENTAL

The ^{60}Co source and the method of placing film samples in the same position in the source have been discussed in earlier reports [11]. Careful positioning of the film sample allows reproducible grafting rate measurements to be made. An exposure dose rate at the film capsule location of 360 R/hr was measured by ferrous sulfate dosimetry [12].

Polyethylene film samples of 0.02 to 0.03 g in weight were used as substrates in the grafting runs. The film samples were of 0.93 density and 0.012 in. thickness, and were supplied by Consolidated Thermoplastics (Woonsocket, R. I.).

The chloromethylstyrene (CMS) monomer from Polysciences,

Incorporated was washed three times with 10% aqueous sodium hydroxide, then three times with distilled water, dried with anhydrous calcium sulfate, distilled at reduced pressure, and stored over anhydrous calcium sulfate at refrigerator temperature prior to use. Just before making each run, the monomer sample containing the immersed film was deoxygenated by freeze-thaw cycling in a vacuum system. The capsule containing film and monomer was frozen down and sealed from the vacuum line, then placed in the ^{60}Co source in the predetermined position.

After each run the grafted film sample was extracted by stirring in warm benzene for approximately 24 hr. The purpose of the extraction was to remove CMS homopolymer formed within the film. All grafting runs were made at the temperature of the irradiation room, 23 ± 1°C. Percent grafting was computed from original film weight (P_0) before grafting, and the weight after grafting and drying to constant weight (P_g) by the use of Eq. (1):

$$\text{Percent grafting} = [(P_g - P_0)/P_0] \times 100 \quad (1)$$

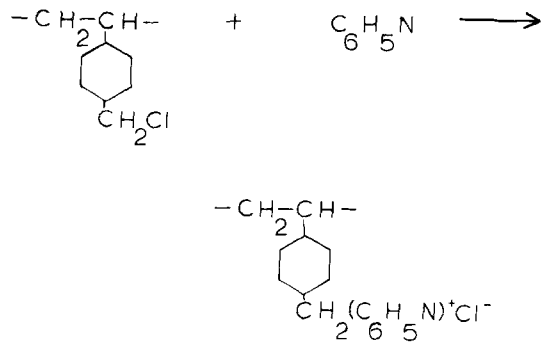
The weight of the monomer-swollen film (P_s) at the end of each run was measured by quickly blotting the swollen film between filter papers and then weighing it in a closed weighing bottle. The percent swelling (uncorrected for homopolymer content) was computed as in Eq. (2):

$$\text{Percent swelling} = [(P_s - P_0)/P_0 \times 100 \quad (2)$$

After incorporating chloromethyl groups into the film by radiation-induced grafting, these groups were quaternized by stirring the grafted film in pyridine for approximately 24 hr at 50°C. This is similar to the procedure of Stamm [13] for the quaternization of polypropylene grafted with CMS, but in that case the grafted samples were reacted with pyridine for 1 hr at 50-60°C. In the present work the quaternized samples were blotted with filter paper and allowed to dry until constant weight was reached. Separate experiments showed that the same final constant weight was attained whether the quaternized films were extracted with benzene prior to drying, or simply allowed to dry until all of the excess pyridine evaporated from the film.

The quaternization of the grafted CMS groups can be summarized by by Eq. (3).

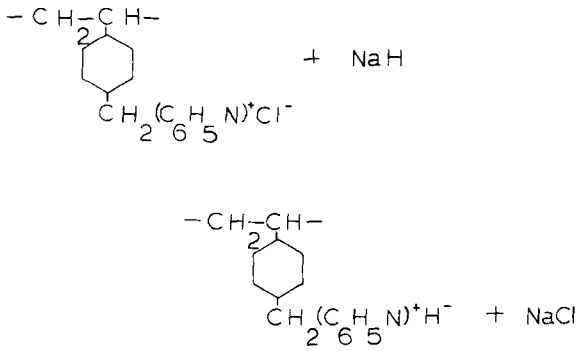
The quaternized, grafted films were next heparinized by placing in a 5.0% aqueous solution of sodium heparin (Sigma Chemicals) and holding at a temperature of about 70°C for 24 hr. In order to conserve



(3)

sodium heparin, each film sample was placed in about 2.0 ml of sodium heparin solution in a small screw-capped vial, the vial in turn being placed in a water bath at 70°C.

While questions remain about the structure of heparin, it is generally agreed that ionic compounds such as sodium heparin contain heparin anions which are negatively charged due to the presence of sulfate groups [2]. The reaction between quaternized graft copolymer and sodium heparin probably involves the exchange outlined in Eq. (4),



(4)

where the chloride in the copolymer is replaced by the heparin anion (H^-). In any case, the heparin is bound so tightly to the quaternized graft copolymer that it is not removed by the various extraction procedures described below.

RESULTS AND DISCUSSION

Grafting runs in the CMS/polyethylene system were carried out for various irradiation times, with the resulting plot of percent

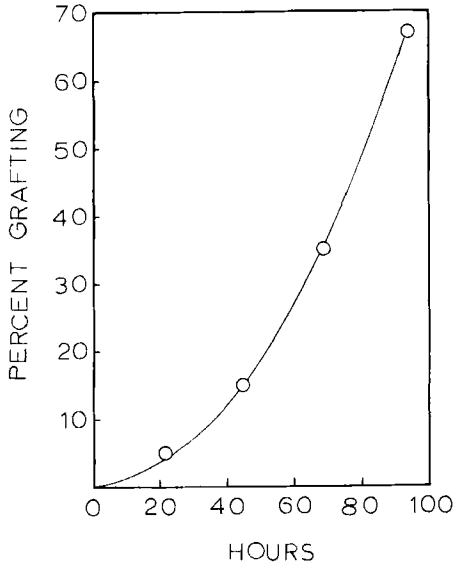


FIG. 1. Percent grafting of chloromethylstyrene on polyethylene film vs. irradiation time at room temperature; exposure dose rate of 360 R/hr.

grafting versus irradiation time shown in Fig. 1. Pertinent data for the individual runs are presented in Table 1. The most important conclusion to be drawn from Fig. 1 is that CMS grafting on polyethylene takes place readily. While many plastics are of interest in nonthrombogenic applications, polyethylene was selected for investigation as a "typical" plastic, whose behavior may simulate in some degree the behavior of other polymers of interest. A grafting versus time plot of the type shown in Fig. 1 is not unusual, although such plots are often linear [14, 15].

A plot of percent swelling versus percent grafting for the CMS/polyethylene system is presented in Fig. 2. Such plots are generally linear for most monomer/polymer grafting systems [16, 17], and the linearity observed in Fig. 2 tends to confirm the correctness of the grafting data.

Each CMS-grafted sample was subjected to quaternization with pyridine. The percent weight gain of each sample (based on original sample weight) is shown in Table 1. If it is assumed that every CMS grouping in the grafted polymer is quaternized, the ratio of weight gain on quaternization to grafting weight gain should equal the ratio of pyridine molecular weight to CMS molecular weight, namely,

TABLE 1. Summary of CMS/PE Grafting Runs

Run	Sample no.	Run length, (hr)	Original sample wt (g)	CMS add-on (%)	Pyridine add-on (%)	Pyr./CMS ratio $\times 10^2$	Heparin add-on (%)
7	23	22.3	0.0311	5.1	3.2	62.7	0.0
11	28	44.5	0.0244	15.2	12.3	80.9	3.5[a]
10	27	68.6	0.0265	35.1	26.0	74.0	7.9[a]
9	26	93.7	0.0303	67.3	45.5	67.6	24.1

[a]Two-step heparin addition.

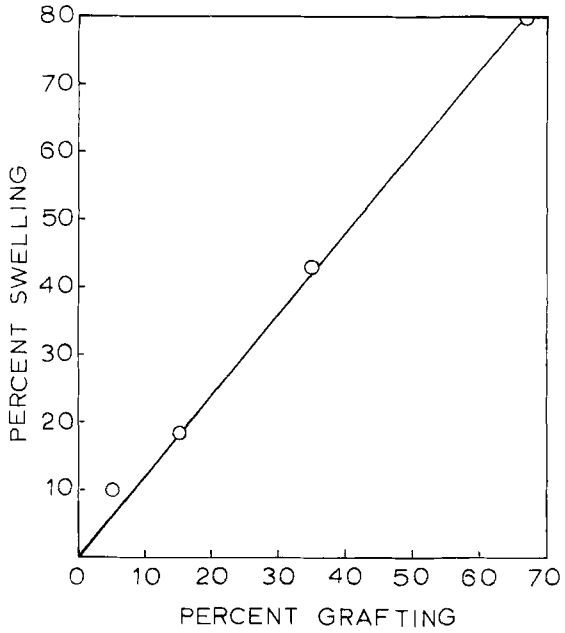


FIG. 2. Percent swelling (uncorrected for homopolymer formation) vs. percent grafting in the CMS/polyethylene system.

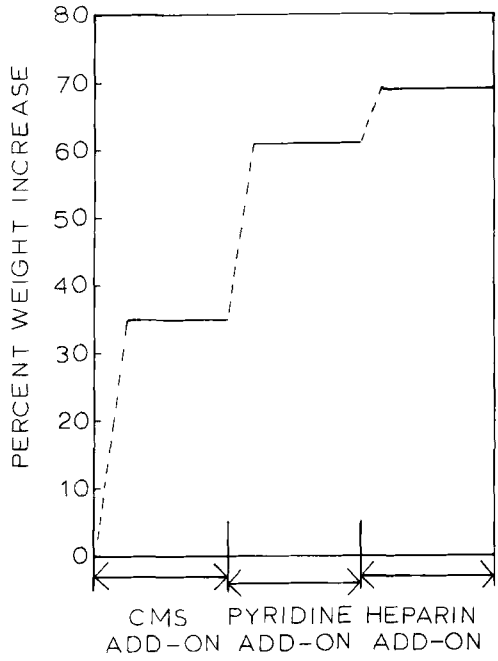


FIG. 3. Diagram showing relations between add-on of CMS, pyridine, and heparin for run 10 (abscissa not intended to be quantitative in time).

91.0/151.5 or 60.1×10^{-2}. The next to the last column of Table 1 shows the experimental value ranged from 62.7 to 80.9×10^{-2}. While experimental error may be involved at the lowest grafting level, another possible explanation for the generally high ratio is that the CMS may have contained some impurity bearing more than one chloromethyl group per molecule on the benzene ring. The weight gains for grafting and quaternization for one of the runs are given in graphical form in Fig. 3.

After drying to constant weight, the grafted, quaternized samples were reacted with sodium heparin by the procedure described above. The percent weight gain due to heparin combination for each sample is shown in the last column of Table 1. The amount of heparin added increases with the grafting and quaternization weight gain, but not in any simple proportion to the gain on grafting or quaternization. The amount of heparin combined is zero for the lowest quaternization weight gain (3.2%), and increases progressively to larger fractions of the quaternization weight gain as the latter increases. This may

TABLE 2. Percent Weight Loss in Extraction Tests on Heparinized Samples Using Solvents Indicated

Sample no.	Weight loss (%)			
	Benzene	Distilled water	0.012 M NaCl (aq.)	1.00 M NaCl (aq.)
26[a]	0.0	0.0	0.0	0.0
27[b]	–[c]	0.0	0.0	0.0

[a]24.1% heparin add-on.
[b]7.9% heparin add-on.
[c]Benzene extraction not run.

be due to the fact that sodium heparin was employed in water solution, and that the copolymer film becomes more readily penetrated by water as it becomes more highly quaternized.

One of the critical questions about any polymer containing bound heparin is whether the heparin can be removed from the polymer by washing or stirring in various solvents. Table 2 shows the results of solvent extraction tests on two of the grafted and heparinized samples discussed above (samples 26 and 27). For each solvent or solution, the film sample was stirred in the liquid for 24 hr at about 50°C, removed, allowed to dry to constant weight, and finally re-weighed. No weight loss was noted for benzene or water extraction tests carried out under these conditions. The next to last column of Table 2 shows no weight loss after extraction with 0.102 M sodium chloride in water solution, which duplicates the molar concentration of chloride in blood plasma [18]. The last column shows no heparin weight loss after extraction with 1.0 M aqueous sodium chloride.

Some investigators have employed "accelerated" extraction tests involving quite high concentrations of sodium chloride. For example, Grode [19] used 4.0 M aqueous sodium chloride extraction for 4 hr at 37°C. In considering the effect of such high chloride concentrations, it should be noted that the principles of chemical equilibria apply even for reactions within a polymeric phase, as discussed by Morawetz [20] and others. If Eq. (4) is abbreviated to read

$$SMP^+Cl^- + H^- \longrightarrow SMP^+H^- + Cl^- \quad (5)$$

The equilibrium constant for this reaction is

$$K = (SMP^+H^-)(Cl^-)/(SMP^+Cl^-)(H^-) \quad (6)$$

where the quantities in parentheses are the activities of the reactants and products. Since K is a constant, an increase in $[Cl^-]$ would tend to decrease $[SMP^+H^-]$ and increase $[SMP^+Cl^-]$. However, it would not be expected that $[Cl^-]$ in blood plasma would ever have the effect of 4.0 M sodium chloride on the equilibrium of Eq. (5). The apparent purpose of some investigators in using 4.0 M sodium chloride was to simulate an accelerated extraction by the much lower $[Cl^-]$ in blood plasma, but the actual effect would be to shift the equilibrium as indicated by Eq. (6). It is recognized that the chloride concentration within the polymer phase would probably always be smaller than that in the external aqueous phase in any extraction experiment, regardless of whether plasma or 4.0 M sodium chloride was used as the extractant.

While some retention of the heparin by the polymer is desirable, several investigators have found it advantageous for the heparin to leak off at a finite rate at the blood/polymer interface. For example, Tanzawa [21] found that the critical value for patency of heparin coated shunts was above 4×10^{-8} g/cm^2-min. Salzman [22], on the other hand, states that materials having heparin bonded to the surface were clot-inhibiting in vitro and free of fibrin in vivo even without desorption of heparin. Salzman [22] has also postulated that a microatmosphere of heparin in solution may exist in relation to a heparin-coated surface and foil any attempt to establish the effect of the surface-bound heparin, which could simply serve as a heparin reservoir. Sensitive clotting tests and studies with radioactive heparin indicate that the antithrombogenic effect does not depend on leaching of heparin into the blood [23, 24].

It is of interest to compare the grafting of CMS on polyethylene with the corresponding CMS/polypropylene grafting system studied by Stamm [13]. Stamm observed that the initial CMS add-on was linear with time. (Actually, percent CMS grafted was plotted versus dosage, but the latter was presumably proportional to irradiation time.) Above a dosage of 0.3 Mrads, the amount of CMS grafted leveled out at about 40%. The initial deviation from linearity in the grafting versus dosage plot took place at about 0.05 Mrads. The leveling out at higher dosages was not observed in the present work, probably because the dosage levels were much lower.

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