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Use of Additives to Enhance Radiation Grafting of Monomers to Poly(vinyl Chloride) and Application of These PVC Copolymers to Immobilization of Enzymes and Heterogenization of Homogeneous Metal Complexes

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ABSTRACT

The effect of solvent structure, particularly the application of the low molecular weight aliphatic alcohols, on the radiation grafting of monomers to PVC with the use of cobalt-60 and the simultaneous procedure is discussed. The addition of sulfuric acid to a typical system such as styrene/methanol/PVC enhances both the grafting yield and the size of the Trommsdorff peak. The results are shown to be consistent with a mechanism involving charge-transfer complex formation between monomer and grafting site in PVC. The role of hydrogen atoms in these reactions, particularly in the presence of sulfuric acid, is emphasized, and the importance of the magnitude of G(H) on the grafting yields and the Trommsdorff effect is discussed. These principles, developed for radiation grafting styrene, are shown to be of value for the radiation copolymerization of p-nitrostyrene and vinyl phosphorus monomers to PVC. The use of these PVC copolymers for immobilizing enzymes such as trypsin and heterogenizing typical catalytically active homogeneous metal complexes is described.

INTRODUCTION

The use of radiation to crosslink and initiate grafting to PVC has been extensively investigated because of the wide use of PVC as a relatively cheap, flame-resistant plastic in a number of commercial fields, i.e., textiles, wire insulation, and cable jacketing. For radiation grafting, both preirradiation [1] and the simultaneous method [2-5] have been used. With radiation-grafted PVC, there is the problem of thermal stability, since radiation may lead to additional sites which are capable of initiating dehydrochlorination. Recent work [4] has shown that PVC-g-styrene prepared by a radiation technique exhibits improved thermal stability when compared with unmodified PVC. Because irradiation is known to enhance thermal dehydrochlorination, in this trunk polymer, the lowest possible radiation dose for grafting to PVC is beneficial.

The current paper describes the use of novel additives which are found to accelerate the radiation grafting of monomers to PVC, thus permitting the use of lower doses of radiation than previously required to achieve grafting. The present authors have already utilized similar additives to enhance radiation copolymerization of monomers to other trunk polymers, particularly the polyolefins [6, 7], cellulose [8-10], and wool [11, 12]. In the present work, PVC is shown to exhibit unique properties in these accelerated radiation grafting reactions. The data are compared with corresponding results obtained with the other trunk polymers. Because of the high grafting efficiencies observed, particularly in the presence of the additives using the simultaneous method, the mutual irradiation grafting technique only has been studied in this PVC work. The project has been extended to include the effect of solvent structure on the copolymerization reaction, specifically a systematic investigation of the role of chain length of alcohol in the PVC grafting process.

For most of the studies described in the present work, styrene has been used as a representative monomer; however, less detailed experiments for the radiation grafting of certain other monomers for specific purposes are also reported. Thus studies have been made of the use of radiation copolymers of PVC as supports for the immobilization of enzymes and for the heterogenization of homogeneous metal complexes of potential catalytic value. For these latter two reactions, specific monomers such as p-nitrostyrene and phosphorus-containing monomers have been grafted. For the radiation grafting of such monomers, the accelerated technique is important, since p-nitrostyrene, particularly, is relatively difficult to copolymerize under conventional radiation conditions. PVC is shown to be of unique value as a trunk support in such processes when compared with analogous materials such as the polyolefins and crosslinked polystyrene. Finally, the advantages of the radiation grafting technique compared with conventional chemical methods for performing the above processes will be evaluated for the PVC copolymer system.

EXPERIMENTAL

The procedures used in the present ionizing radiation work are similar to those previously described [10, 13]. Some modifications to these techniques were necessary, especially since powders, as distinct from films, were being grafted; most of the preceding publications [6, 7, 10, 13] involved film copolymerization. Styrene (Monsanto Chemicals Ltd.) and p-nitrostyrene (Polysciences) were purified as before [10, 13]. The phosphorus-containing monomers (Strem) were used as supplied after being checked for purity by gas chromatography.

In a typical experiment, 5. g samples of PVC powder [ICI Ltd. (Australia) fine particle size] or polypropylene powder (Shell, isotactic, doubly oriented) were irradiated in stoppered vessels in air as suspensions in a solution of monomer in solvent (6 ml). Irradiations were performed in a cobalt-60 source at the Australian Atomic Energy Commission. To minimize post-irradiation effects [10, 13-16] extraction of the grafted material was commenced as soon as practical after irradiation, the samples being kept at 2°C until extraction was begun (no longer than 8 hr). For removal of homopolymer, the crude copolymer was extracted in a preweighed thimble for 30 hr at 65°C in a Soxhlet extractor. The solvent used for this process was a 3:1 mixture of benzene and chloroform as previously recommended [13]. After washing, the thimbles were dried to constant weight at the same relative humidity (65%) as the empty thimble. Increase in weight was used as graft, this procedure previously being shown [13, 16] to be a valid estimate of degree of copolymerization.

For the immobilization of a typical enzyme, trypsin, p-nitrostyrene was radiation-grafted to PVC or polypropylene under the conditions mentioned in the relevant tables. After homopolymer had been removed, the nitro group was reduced and converted to the isothiocyanate derivative [17, 18]. Covalent attachment of the trypsin to the poly(p-isothiocyanatostyrene-g-PVC) or poly(p-isothiocyanatostyrene-g-propylene) was carried out by suspending the copolymer in a solution of trypsin in a bicarbonate buffer (pH = 9.6, 25 ml). The weight of protein bound to the polymer was determined by acid hydrolysis of the polymer-enzyme conjugate. The esterase activity of trypsin in the conjugate was determined by using N-α-benzoyl-L-arginine ethyl ester, while the proteolytic activity was obtained with N,N-dimethylhemoglobin [19].

For the insolubilization of the homogeneous metal complexes, procedures analogous to those previously reported [20] were utilized. Thus p-nitrostyrene was radiation-grafted to polypropylene as above, then the nitro group reduced to the amino derivative. For the synthesis of the p-chlorostyrene and vinylphosphine copolymers with PVC and polypropylene, the monomers were radiation-grafted to the relevant trunk polymers [20]. The copolymers were then directly reacted with the homogeneous complexes dissolved in appropriate solvents. The heterogenized complexes were washed, dried, and hydrogenations carried out by using these complexes by the same technique as before [20].

RESULTS AND DISCUSSION

Effect of Solvent on Radiation Grafting to PVC

The data in Table 1 show that, as the molecular weight of the alcohols is increased, there is a decrease in PVC grafting efficiency from methanol to n-octanol. Branched chain alcohols (tert-butanol) are less effective than the corresponding straight-chain compounds in the promotion of grafting to PVC. Benzene and even polar dimethylformamide are less satisfactory as grafting solvents than tert-butanol under the radiolysis conditions used in Table 1. A marked Trommsdorff effect is observed with all four alcohols studied, the position of the peak in grafting varying from 30 to 40% styrene concentration for a particular alcohol. The radiation grafting characteristics of PVC are thus similar to the polyolefins [6, 7] and cellulose [8, 10, 13, 14] where analogous gel effects have previously been observed. However, the pattern of radiation grafting of styrene in the alcohols to PVC is intermediate between the polyolefins and cellulose, since, for the latter trunk polymer, grafting of styrene virtually cuts out at n-butanol, whereas with polypropylene, copolymerization remains high even with the longer chain alcohol, n-octanol [7].

As the radiation dose is increased from 0.1 to 0.5 Mrad at constant dose rate (Table 2), there is a corresponding increase both in grafting yield and the intensity of the Trommsdorff peak for the grafting of styrene in methanol to PVC. The concentration of monomer in solvent at which this peak is observed also varies with dose but appears to occur between 30 and 40% monomer over the total radiation dose range studied.

The mechanism of the present PVC grafting reaction appears to be analogous to that proposed previously for other trunk polymers such as the polyolefins [6, 7, 15], cellulose [10, 13, 21] and wool [12, 15]. Because PVC has a relatively high G_R value [2], radical

TABLE 1. Effect of Various Alcohols, DMF, and Benzene on Radiation Grafting of Styrene to PVC[a]

	Graft (% in additive to styrene)					
Styrene (%)	Methanol	Butanol	tert-Butanol	Octanol	DMF	Benzene
15	26	3	0	0	-	-
20	85	7	26	0	19	-
25	230	32	-	1	18	-
27	-	50	-	8	-	-
30	444	53	65	21	-	29
35	562	270	45	51	14	29
40	101	203	27	133	28	26
50	31	123	-	-	25	-
60	19	63	11	92	15	33
80	8	-	-	-	-	24

[a] Radiation dose, 0.5 Mrad at 0.1 Mrad/hr.

sites for grafting of monomers are readily formed with reasonable doses of ionizing radiation. Charge-transfer intermediates with monomers can thus be readily formed at these sites [21], provided that the monomer solution used for the simultaneous grafting technique swells the PVC sufficiently. Either monomer or solvent needs to possess this swelling property to lead to efficient radiation copolymerization. In the present work, the additive alcohols also have a property which enhances radiation grafting, i.e., these compounds, especially the low molecular weight homologs, show relatively high G(H) values in radiolysis [22-24]. It has already been demonstrated that an increase in hydrogen atom yields can be an advantage in radiation grafting certain monomers to polyolefins [6], cellulose [8] and wool [11, 12]. A similar hydrogen atom effect also appears to be applicable to the present styrene/solvent/PVC radiation grafting system. Thus, after radical sites are formed in the trunk PVC polymer, charge-transfer complex formation occurs between these sites and the monomer. After formation of this intermediate complex, it is proposed that grafting results via mechanisms analogous to those already described for radiation copolymerization to other trunk polymers [6, 10, 12, 21].

TABLE 2. Effect of Dose on Radiation Grafting of Styrene in Methanol to PVC

Styrene in methanol (%)	Graft (%) at various doses		
	0.1 Mrad[a]	0.25 Mrad[a]	0.5 Mrad[a]
15	0	-	26
20	0	44	85
25	0	73	230
27	2	130	-
30	13	269	444
33	56	250	-
35	58	148	562
40	66	-	101
50	6	-	31
60	3	-	19
80	-	-	8

[a] Total dose at 0.1 Mrad/hr in air.

Effect of Acid on Radiation Grafting to PVC

The addition of mineral acid (H_2SO_4) increases the grafting efficiency of styrene in methanol to PVC at all total radiation doses studied in Table 3. An analogous acid enhancement effect is also observed when dimethylformamide is used as solvent (Table 3). The presence of this acid also increases the intensity of the Trommsdorff effect and may alter the concentration of monomer in solvent at which the peak is observed. This acid effect with PVC is thus similar to that observed with radiation grafting to the polyolefins [6], cellulose [8] and wool [11, 12]. It is proposed that a similar mechanism [21] to that suggested for the three latter trunk polymers is also relevant for the present acid-enhanced radiation copolymerization of styrene to PVC. Thus it is suggested that the effect is due to an increase in radiolytic hydrogen atom yields [Eq. (1)] from electron scavenging, leading to an increase in grafting sites in the trunk polymer by hydrogen abstraction.

$$CH_3OH_2^+ + e \longrightarrow CH_3OH + H \qquad (1)$$

TABLE 3. Effect of Sulfuric Acid on Radiation Grafting of Styrene in Methanol and DMF to PVC[a]

Styrene (% v/v)	Graft (%)			
	0.1 Mrad[b]	0.1 Mrad + H^{+b}	0.5 Mrad[b]	0.5 Mrad + H^{+b}
15	0	0	-	-
20	0	10	19	121
25	0	15	18	114
27	2	18	-	323
30	13	34	-	140
33	56	65	14	178
35	58	68	28	-
40	66	70	25	-
50	6	25	15	-
60	3	10	-	-

[a] Sulfuric acid, 7.5×10^{-2} M; dose rate, 0.1 Mrad/hr in air.
[b] Methanol solvent, remainder DMF.

Radiation Grafting of p-Nitrostyrene for Enzyme Immobilization

Additive effects, such as the presence of appropriate solvents and the acid phenomenon discussed for the radiation grafting of styrene in previous sections of this paper, are particularly important when monomers such as p-nitrostyrene are to be grafted, since the nitro group can be strongly deactivating in such reactions [20]. The presence of additives which might accelerate the grafting of p-nitrostyrene is thus valuable. In addition, for the radiation grafting of p-nitrostyrene, PVC possesses a unique advantage when compared with most common trunk polymers such as the polyolefins and cellulose. PVC is soluble in appreciable concentration in dimethyl formamide which is one of the most effective solvents for radiation copolymerization. It is therefore now possible to graft p-nitrostyrene to PVC under both homogeneous and heterogeneous conditions, and the resulting copolymers may well have different properties. After grafting in homogeneous solution, the PVC copolymer can be readily precipitated from the grafting medium by the addition of compounds such as methanol. Both homogeneous and heterogeneous PVC

TABLE 4. Effect of Dose on Radiation Grafting of p-Nitrostyrene to Soluble and Insoluble Poly(vinyl Chloride)

Total dose (Mrad)	Graft (%)[a]	
	Soluble PVC[b]	Insoluble PVC[c]
1.0	6.0	8.3
2.0	8.2	11.7
3.0	8.9	11.9
4.0	9.1	18.8

[a] Calculated from elemental composition.

[b] Poly(vinyl chloride) (1 g) dissolved in 30% p-nitrostyrene in DMF (5 ml), irradiated at 200 krad/hr in air (cobalt-60).

[c] Poly(vinyl chloride) (1 g) suspended in 33% p-nitrostyrene in methanol (5 ml), irradiated at 200 krad/hr in air.

copolymers of p-nitrostyrene can then be compared for immobilization and heterogenization reactions.

The data in Table 4 show that heterogeneous grafting of p-nitrostyrene to PVC in methanol is slightly more efficient than the corresponding homogeneous copolymerization in DMF under the radiation conditions used. However, both grafting techniques yield copolymers which are potentially useful for both immobilization and heterogenization. The heterogeneous grafting procedure may, however, be preferable for these two processes, since a higher percentage of surface graft would be expected under heterogeneous conditions than when the corresponding homogeneous procedure is used, thus larger percentages of enzyme and/or complex will be capable of being attached to the surface of the copolymer prepared by the heterogeneous method (Table 5). The reactivity of the heterogeneous copolymer should also be better, due again to the higher surface graft, since the average diffusion time of both substrate and product molecules to reach the surface of this heterogeneous copolymer for reaction would be reduced to a minimum. Lastly, it is also possible to graft styrene to PVC, then nitrate the copolymer. The present authors believe that grafting p-nitrostyrene to PVC directly is the preferred procedure for reasons similar to those discussed elsewhere for the copolymerization of p-nitrostyrene to polypropylene [17].

Immobilization of Enzymes

For the immobilization of a representative enzyme, trypsin, the nitro group in the graft copolymer (after removal of homopolymer)

TABLE 5. Preparation by Radiation Grafting of p-Nitrostyrene Copolymers with PVC and Polyolefins for Enzyme Immobilization

Reference	Trunk polymer[a]	Graft (%)[b]
PVC 1	Poly(vinyl chloride)	8
PVC 2	Poly(vinyl chloride)[c]	13
PVC 3	Poly(vinyl chloride)[d]	15
PPI	Polypropylene[e]	30[f]
LDPE	Low-density polyethylene	4

[a] Trunk polymer (5 g) suspended in monomer solution (30%) in DMF (6 ml) and irradiated in air at 200 krad/hr to a total dose of 3 Mrad.

[b] Calculated from elemental analysis.

[c] 50% p-nitrostyrene in DMF (PVC-soluble).

[d] 30% p-nitrostyrene in methanol (PVC-insoluble).

[e] Polypropylene without stabilizer.

[f] Data from Garnett et al. [17].

was reduced to the amino derivative. With the use of carbon disulfide and ethyl chloroformate in the presence of triethylamine [25] this amino compound was then converted to the corresponding isothiocyanate derivative through which the trypsin was immobilized. By amino acid analysis, it has been shown that the isothiocyanate functional group reacts with trypsin via lysine residues, presumably through the ϵ-amino group in the side chain. To date, lysine has not been implicated in the active site of trypsin [26].

The data in Table 6 show that PVC, when compared with polypropylene and low-density polyethylene, is valuable as a support for the immobilization of trypsin. Both homogeneous and heterogeneous grafted PVC are useful, although as predicted, the latter copolymer is slightly better in performance. In Table 6, quantitative data were obtained only for one of the polymer-enzyme conjugates, namely the polypropylene-trypsin system, where the proteolytic activity of trypsin in the conjugate is lower than the esterolytic activity. This difference in reactivities has been attributed to steric effects [27], and similar differences in reactivity patterns with the PVC conjugates may be expected. This aspect is presently being clarified.

TABLE 6. Reactivity of Radiation PVC Copolymer-Enzyme Conjugates[a]

Conjugate	Protein bound (mg/g)	Esterase activity
PVC 1-trypsin	b	c
PVC 2-trypsin	20	a
PVC 3-trypsin	25	c
PPI-trypsin	6.9	31[d]
LDPE-trypsin	b	c

[a] Copolymer prepared as in Table 5.

[b] Protein definitely bound but quantitative reproducibility of determination low.

[c] Finite esterase activity; being determined quantitatively at present.

[d] Relative to an equal weight of soluble trypsin; protease activity 13%.

Heterogenizing of Homogeneous Metal Catalysts

The data in Table 7 show a principle, analogous to the preceding enzyme immobilization process, for the heterogenizing of homogeneous metal complexes. Most of the results listed are with copolymers of polypropylene; however, preliminary data for PVC copolymers are also reported. For this work, vinyldiphenylphosphine has been radiation-grafted to PVC and the resulting copolymer used to heterogenize the iridium hydride-carbonyl complex shown and also rhodium trichloride. Both PVC insolubilized complexes exhibited appreciable conversions in the hydrogenation of vinylcyclohexene to ethylcyclohexene. Quantitative data for kinetic studies of these reactions are still being carried out, however preliminary work [28] shows that the hydrogenation rates of the heterogenized complexes are at least comparable to those found when the same homogeneous complex is used in solution under homogeneous conditions. Analogous hydrogenation reactions with a much wider range of PVC copolymers similar to the polypropylene copolymers listed in Table 7 are at present being carried out. Thus p-aminostyrene, p-chlorostyrene, and cis-bis(1,2-diphenylphosphino)ethylene (DPE) have been radiation-grafted to PVC, and the resulting copolymers are currently being evaluated for heterogenization. These preliminary studies suggest

TABLE 7. Hydrogenation of Cyclohexene with Heterogenized Complexes Prepared by Radiation Grafting with Polypropylene and PVC

Heterogenized complex[a]	Metal on surface (%)	Temp (°C)	Hydrogenation (%)
pAST/PP/$RhCl_3$	0.43	50[b]	100
pAST/PP/$RhH(CO)(Ph_3P)_3$	0.12	50[b]	100
pClST/PP/$RhCl(CO)(Ph_3P)_2$		50[b]	36
VDP/PP/$RhCl(Ph_3P)_3$	0.26	130[c]	31
VDP/PP/$RhCl_3$	0.26	130[c]	79
DPE/PP/$RhH(CO)(Ph_3P)_3$	0.03	130[c]	75
VDP/PVC/Ir $H(CO)(Ph_3P)_3$		80[d]	e
VDP/PVC/$RhCl_3$		80[d]	e

[a] Complexes: pAST = p-aminostyrene; PP = polypropylene powder; pClST = p-chlorostyrene; VDP = vinyldiphenylphosphine; DPE = cis-bis(1,2-diphenylphosphino)ethylene.

[b] Hydrogenation time 96 hr [20].

[c] Hydrogenation time 140 hr [20].

[d] Hydrogenation time 72 hr [20].

[e] Appreciable hydrogenation of vinylcyclohexene to ethylcyclohexene after 72 hr. Quantitative kinetic studies at present being performed. Rates of hydrogenation of insolubilized complex are at least comparable to rates when nonheterogenized complex is used in homogeneous solution.

that radiation-grafted copolymers of PVC will possess certain advantages when compared with other radiation-grafted copolymers for the heterogenization reaction. Radiation-grafted copolymers, in turn, appear to be potentially more useful for these processes [20] than chemically prepared styrene-divinylbenzene beads [29]. A detailed evaluation of these advantages will be discussed elsewhere [28].

CONCLUSIONS

Radiation grafting of styrene in methanol to PVC is enhanced in the presence of sulfuric acid; thus the radiation dose required to achieve a certain percentage graft to PVC can be significantly reduced in the presence of such acid additives. This development may be of value in the preparation of PVC-g-styrene for various applications, particularly those requiring improved thermal stability. The acid radiation technique may also be useful for the synthesis of PVC copolymers for unique purposes. Two such examples are described involving the immobilization of enzymes and the heterogenization of homogeneous catalytically active metal complexes. γ-Radiation has been used exclusively in the present paper to prepare the PVC copolymers; however the method is also amenable to rapid-cure ultraviolet light [28] and electron-beam [28] initiation. The radiation-grafting technique is thus potentially the most versatile method for both enzyme immobilization and the heterogenizing of homogeneous catalytically active metal complexes.

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