Grafting onto wool: 7. Determination of end groups of grafted polymer and homopolymer formed in methanol–benzoyl initiating system

Kozo Arai
Department of Chemistry, Faculty of Technology, Gunma University, Kiryu, Gunma, Japan
(Received 4 October 1976)

A previous assessment of the end group incorporated in poly(methyl methacrylate) and polystyrene chains, based on the synthesis of the 2,4-dinitrophenyl (DNP) derivatives of hydroxyl and amino groups, has been extended. It has been found that in methanol–benzoyl peroxide initiating systems, hydroxyl, phenyl and benzoyloxy radicals initiate homopolymerization. The total number of end groups has been determined. From the results of the number of DNP-amino-acid end groups in the isolated polymer chain from the grafted fibres and from chemically modified fibres before and after grafting, it is postulated that two DNP-end groups are linked to the polymer chain, and one DNP–amino-acid is situated at each end of the polymer chain. No initiating mechanism visualized with respect to the homopolymerization is involved in the graft copolymerization.

INTRODUCTION

It has been attempted by the present author to determine quantitatively the number of truly grafted poly(methyl methacrylate) (PMMA) and polystyrene (PS) chains, and to determine the mechanisms of the initiation and termination reactions in the viscous media of wool fibres[1–5]. Quantitative determinations of the amino-acid end groups incorporated in separated graft copolymer chains have been made using 1-fluoro-2,4-dinitrobenzene. Information about the end group contents of the isolated polymer and the homopolymer not only makes it possible to identify and differentiate the initiating species of the two different macromolecules but also throws some lights on the nature of the termination steps. The aim of this investigation is to demonstrate the grafting mechanism from the knowledge of the end group of the grafted polymer and homopolymer formed in the methanol–benzoyl peroxide initiating system including methyl methacrylate (MMA) or styrene (S).

EXPERIMENTAL

Materials

The tops of fine Australian Merino wool fibres were purified by Soxhlet extraction with acetone for about 24 h, followed by washing with cold water and air-drying. Purified MMA and S were used as the monomers. Purified acrylonitrile (AN) and 2-vinylpyridine (2VP) were used as blocking agents. Commercially available methanol was dehydrated by refluxing for 5 h with calcium oxide and then distilling. Benzoyl peroxide (BPO), thioglycollic acid (TGA), tri-n-butyl phosphine (TBP), 1-fluoro-2,4-dinitrobenzene (FDNB), triethylamine (TEA), and dimethyl sulphoxide (DMSO) were special reagent grade and used without further purification.

Ethyl benzoate and β-phenethyl n-butyrate as standard materials for spectrophotometric analysis were special reagent grade from Wako Chemical Industries, Ltd.

The 2,4-dinitrophenyl derivatives of the amino-acids DL-methionine, L-leucine, and L-tyrosine were obtained by the method of Rao and Sober[6]. DNP-DL-methionine (Theoretical for $C_{11}H_{13}N_3O_6S$: C, 41.9; H, 4.1; N, 13.3. Observed: C, 41.9; H, 4.2; N, 13.3), DNP-L-leucine (Theoretical for $C_{12}H_{15}N_3O_6$: C, 48.5; H, 5.1; N, 14.1. Observed: C, 49.1; H, 5.1; N, 14.2), and *O*, *N*-di-DNP-tyrosine (Theoretical for $C_{21}H_{15}N_5O_{11}$: C, 49.1; H, 2.9; N, 13.7. Observed: C, 49.2; H, 3.0; N, 13.4) were used for calibration.

O-mono-DNP-L-tyrosine was of analytical reagent grade from Mann Research Laboratories, USA. (Theoretical for $C_{15}H_{13}N_3O_7$: C, 51.9; H, 3.8; N, 12.1. Observed: C, 50.4; H, 3.7; N, 12.0.)

Stearyl 2,4-dinitrophenyl ether was prepared by the method of Whalley[7]. A solution of stearyl alcohol (1.0 g), FDNB (5 g), and TEA (3 drops) in acetone (20 ml) were kept in the dark at 30°C for 24 h. After the reaction, 1% sodium carbonate was added to the solution. The solid residue that separated was washed with water, dried, and then crystallized from ethyl acetate. The product was recrystallized and stearyl 2,4-dinitrophenyl ether was obtained as colourless needles, m.p. 70°–70.3°C. (Theoretical for $C_{24}H_{40}N_2O_5$: C, 66.0; H, 9.2; N, 6.4. Observed C, 66.1; H, 9.3; N, 6.6.)

Chemical modification of fibres

In the reduction of wool fibres, samples were treated for 3 h at 30°C with 0.2 N sodium thioglycollate solution adjusted to pH 4.7 (50 ml); they were then washed with water. washed with ethanol again washed with water, pressed out with filter paper, and then subjected to *S*-carboxymethylation and to grafting.

In another method[8], samples (1 g) were treated for 24 h at 30°C with a solution containing TBP (1 g), n-propanol (25 ml), and a borate–phosphate buffer adjusted to pH 8.0 (25 ml); they were then washed with water, pressed out

with filter paper, and then subjected to *S*-cyanoethylation, to *S*-2-pyridylethylation, and to grafting.

In the *S*-cyanoethylation[9], both reduced wool and the reduced fibres which had been grafted with MMA were treated for 24 h at 30°C with a solution containing AN (1 g), n-propanol (25 ml) and the same buffer (25 ml); they were then washed with water, with acetone, washed again with water and then air-dried.

In the *S*-2-pyridylethylation[10], the reduced fibres which had been grafted with MMA were treated with 2VP under the same conditions as the *S*-cyanoethylation.

In the 2,4-dinitrophenylation[11] the grafted fibres (0.5 g of wool portion) were treated for 24 h at 30°C with a solution containing FDNB (0.5 g), sodium bicarbonate (0.5 g), ethanol (25 ml), and water (25 ml); they were then washed with water, Soxhlet extracted with acetone for 5 h, followed by washing with water and air-drying.

Synthesis of PMMA by Fenton's reagent

Polymerization was carried out for 48 h at 30°C in a mixed solution of methanol (25 ml) and water (25 ml) containing MMA (5 g), H_2O_2 (0.05 g), and $FeSO_4$ (0.005 g). The materials collected were washed with 0.2 N HCl with water several times with warm water dissolved in acetone and precipitated from methanol.

Graft copolymerization

The native and chemically modified wool fibres (1 g) were treated with a solution containing 0.4 g (or 1.0 g) BPO, 8 g MMA and 91.6 g (or 91.0 g) MeOH. The dried wool fibres were immersed for 1 h in methanol which was then replaced by fresh solvent and the fibres immersed for a further 30 min. The samples were placed in the bottom of a three-necked flask equipped with a reflux condenser. The reaction liquor was poured into the flask and flushed with nitrogen for 20 min at room temperature, and then kept at 60°C for 2, 3, and 5 h with the fibres well immersed in the solution.

Grafted fibres obtained were Soxhlet extracted with acetone for 12 h to remove a large amount of homopolymer, washed with water and then air-dried.

The same procedure as above was used for the grafting of styrene. The composition of the reaction liquor was 1.0 g BPO, 30 g S, and 69 g MeOH. The grafted fibres were Soxhlet extracted with benzene for 12 h, followed by washing with MeOH, with water, and then air drying.

Isolation and purification of grafted polymer

Separation of grafted polymer from the grafted fibres was carried out by the two-step HCl digestion technique[1]. The grafted wool fibres (0.5 g of wool portion) were digested with 4–6 N HCl (35 ml) for 30 min at 80°–110°C. The partly digested residues were filtered off from the solution using a G3 glass filter and washed with the HCl. Again, the digestion was allowed to proceed for 24 h at a definite temperature of 80°–110°C in a 100 ml flask equipped with a reflux condenser, and the residue was washed with boiling water and then dried. Grafted PMMA and PS were obtained as white and insoluble brittle fibres.

The separated polymers obtained in the fibre state were extracted with a 10% v/v diethyl ether solution of methanol for 48 h. Further purification of the materials was carried out by using the two following different solvent–precipitant systems.

Ethyl acetate system. The materials (0.1 g) were dissolved in ethyl acetate (3 ml) and were precipitated by cold methanol, and dried after the same procedure as above was repeated two to three times.

DMSO system. The materials (0.1 g) were dissolved in hot DMSO (5 ml), precipitated from a mixed solution (5 ml) containing the same volume of diethyl ether and methanol, washed with diethyl ether, redissolved again in a mixed solution containing the same volume of DMSO and ethyl acetate, and followed by precipitation with methanol. The precipitates were swollen in a solution mixture (4 ml) containing DMSO (3 ml) and methanol (1 ml) at room temperature, washed three times with the solution, and then washed with methanol and dried.

Dinitrophenylation of the isolated polymer

Dinitrophenylation of the amino-acid incorporated in the separated graft polymer was carried out for 24 h at 30°C in a mixed solution containing 6 ml of a ~2 to 5% benzene solution of the isolated polymer, 0.3 g FDNB, and a few drops of TEA[1,7].

After the reaction was complete, the materials from PMMA were precipitated by adding an excess volume of diethyl ether and the other materials from PS were precipitated by adding an excess volume of cold methanol. The materials were collected, washed with methanol and then dissolved in ethyl acetate, followed by filtration through a G3 glass filter. Reprecipitation was repeated three times using ethyl acetate–methanol as a solvent–precipitant pair. Thus, bright yellow dinitrophenylated polymer (DNP-polymer) was obtained and subsequently dissolved in ethyl acetate.

Absorption spectra of polymers in the range 250–470 nm were obtained in ethyl acetate using a Hitachi spectrophotometer (Model 124) and 1 cm quartz cells. For the estimation of DNP-amino-acid end groups, the intensity of the absorption was corrected by subtracting the absorption in the same concentration of the isolated polymer.

Purification and 2,4-dinitrophenylation of the homopolymer

The materials obtained by Soxhlet extraction with acetone were purified thoroughly by repeating the procedure of dissolution and precipitation, three times using ethyl acetate–methanol, twice using benzene–methanol, and finally once using benzene–petroleum ether as a solvent–precipitant pair.

Dinitrophenylation of the homopolymer and subsequent purification were carried out using the same procedure as that applied to the isolated polymer. Thus, purified 2,4-dinitrophenylated homopolymer (DNP-polymer) was obtained.

Molecular weight and DNP-end group determination

The number-average molecular weight was obtained from osmotic pressure measurements in toluene at 37°C and ethyl acetate at 30°C using a high speed membrane osmometer (Hewlett Packard, Model 502). From the u.v. spectroscopic analysis of the number of DNP-end groups and the measurement of the average molecular weight of the DNP-polymer, the number of DNP-end groups linked to the grafted polymers was calculated.

RESULTS AND DISCUSSION

Characterization of the homopolymer

For the characterization of the radicals trapped as poly-

Table 1 Number-average molecular weight of isolated polymer and homopolymer formed in various grafting systems after 5 h

Wool sample obtained from different grafting system	Treatment	Thiol and disulphide contents of wool (μmol/g) SH	SS	2SS + SH	Concentration of BPO (%)	Graft-on (%)	Number-average molecular weight: Isolated polymer, $\bar{M}_{n,p} \times 10^{-4}$	Homopolymer, $\bar{M}_{n,h} \times 10^{-4}$
A	Untreated	35.9	416	868	1.0	62.8	8.85	3.00
B	Untreated	35.9	416	868	0.4	54.8	13.7	5.38
C	*S*-cyanoethylated	4.9	16.4	37.7	0.4	207.6	58.4	6.75

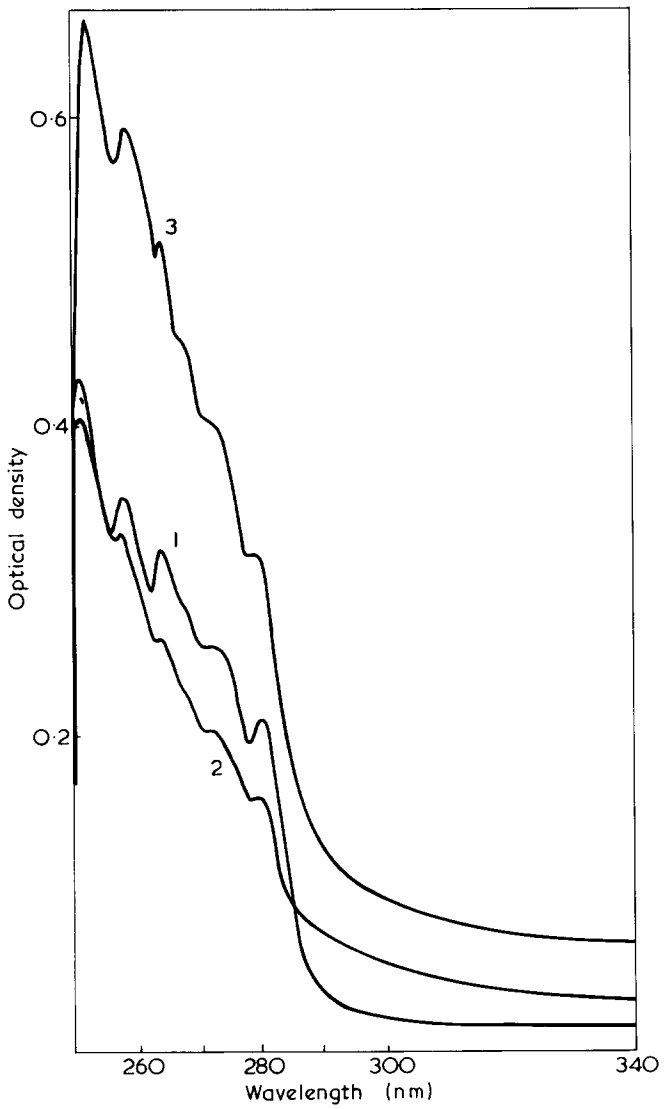

Figure 1 Absorption spectra of homopolymers formed in the grafting systems A, B, and C: 1, system A, 32.56 g/l; 2, system C, 35.18 g/l; 3, system B, 53.22 g/l solution of ethyl acetate

mer end groups in the homopolymer, experiments were carried out for three typical cases listed in *Table 1*. With grafting to the untreated wool fibres, on increasing the concentration of BPO the extent of grafting is slightly increased while the number-average molecular weights of both the isolated polymer, $\bar{M}_{n,p}$ and homopolymer, $\bar{M}_{n,h}$ are considerably decreased.

Disulphide bonds in wool are reduced with TBP under very mild conditions and produce thiol groups available for the selective alkylation with AN used for subsequent blocking of the cystine residues[8,12,13]. A marked promotion of grafting to the *S*-cyanoethylated wool is observed, which is probably a result of the increase of the rate of diffusion of monomer into the fibres with small amounts of disulphide crosslinks. The $\bar{M}_{n,p}$ value of the polymer reaches about four times that of the isolated polymer from the untreated wool, which corresponds to the same ratio as observed for the extent of grafting. However, no such remarkable change is observed for the homopolymer.

The u.v. absorption spectra of homopolymer in ethyl acetate have five maxima and two shoulders in the range 250 to 340 nm as shown in *Figure 1*. The five maxima are ~252, 257, 264, 272 and 280 nm, and the two shoulders ~260 and 267 nm, respectively.

It has generally been accepted that benzoyl peroxide is thermally decomposed in organic solvents to generate benzoyloxy and phenyl radicals which interact with the solvent used and also various radicals result from the chain dissociation of BPO in the system[14–16].

As a model, compounds of the end groups incorporated in the polymer chain initiated by phenyl and benzoyloxy radicals, β-phenethyl n-butyrate and ethyl benzoate could be used for the determination of both end groups. We can observe four maxima on the absorption curve of the ethyl acetate solution of β-phenethyl n-butyrate at 252, 257 264 and 267 nm, and two maxima for ethyl benzoate at 272 and 280 nm as shown in *Figure 2*. All these characteristic absorption bands appear to give discrete absorption maxima in the spectra of the homopolymer. An estimation of the absorption intensity of the polymer itself, which is independent of the fragments in the polymer can be made using the absorption spectra of polymer synthesised by the redox initiating system, Fe^{2+}–H_2O_2, since the characteristic absorption band of hydroxyl end groups is not present in the wavelength range in question. The absorption spectrum of the polymer in an ethyl acetate solution (1 mol/l) based on the monomer unit as molecular weight, $m_0 = 100$ is shown in *Figure 2*. Absorption maxima at 260 and 268 nm correspond to the bands in the spectra of homopolymer present as a weak shoulder near 260 and a shoulder at 267 nm.

Lambert–Beer's law is obeyed fairly well by the polymer solution up to a concentration of 60 g/l or more, at each wavelength in the range 250 to 340 nm. The clear separation of absorption maxima, the comparable magnitude of the molar extinction coefficients, and the validity of Lambert–Beer's law allow the simultaneous determination of the molar concentration of end groups resulting from the different initiating radicals, assuming that no interaction arises between chromophores in the comparatively high concentration of polymer. With the ε-values from *Table 2*, the following analytical equations are obtained:

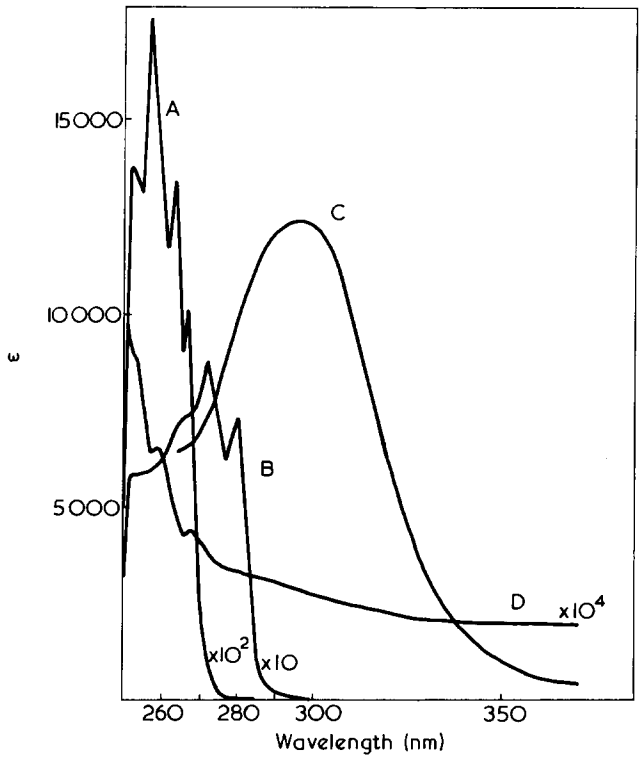

Figure 2 Molar extinction coefficients of model compounds of u.v. spectra: A, β-phenethyl n-butyrate; B, ethyl benzoate; C, stearyl 2,4-dinitrophenyl ether; D, polymer synthesized by Fenton's reagent (based on monomer unit)

Table 2 Molar extinction coefficient, ε of polymer (based on monomer unit), β-phenethyl n-butyrate and ethyl benzoate at 257 and 280 nm

Compound	ϵ_{257} (1000 cm²/mol)	ϵ_{280} (1000 cm²/mol)
$+CH_2C(CH_3)(CO_2CH_3)+$	0.628	0.335
$C_6H_5(CH_2)_2CO_2C_3H_9$	176	0
$C_6H_5CO_2C_2H_5$	571	725

$$c_2 = (5682D_{257} - 4472D_{280} - 20.6c_1) \times 10^{-6} \text{ mol/l} \quad (1)$$

$$c_3 = (1379D_{280} - 4.62c_1) \times 10^{-6} \text{ mol/l} \quad (2)$$

where c_1 is the concentration of polymer (g/l), c_2 is the concentration of phenyl end groups in the polymer solution, c_3 is the concentration of benzoyloxy end groups in the polymer solution, D_{257} is the optical density at 257 nm, and D_{280} is the optical density at 280 nm.

Then, we can evaluate the number of phenyl and benzoyloxy end groups per polymer chain, Z from equations (3) and (4), respectively:

$$Z_{\text{phenyl}} = \bar{M}_{n,h}\{[(5682D_{257} - 4472D_{280})/c_1] - 20.6\} \times 10^{-6} \quad (3)$$

$$Z_{\text{benzoyloxy}} = \bar{M}_{n,h}[(1379D_{280}/c_1) - 4.62] \times 10^{-6} \quad (4)$$

Although the simultaneous analysis of phenyl end groups is less accurate, especially for a higher molecular weight polymer, the number of phenyl end groups per polymer chain appears to be greater than the benzoyloxy end groups as calculated and listed in *Table 3*.

The u.v. absorption spectra for DNP-polymers are shown in *Figure 3*. The spectral features are similar to those of the characteristic spectrum of stearyl 2,4-dinitrophenyl ether (see *Figure 2*) showing an absorption maximum near 295 nm which might be expected to be taken as the reference standard curve for estimation of *O*-DNP end groups incorporated in the homopolymer. The number of *O*-DNP end groups per polymer chain can be represented by the following equation:

$$Z_{O\text{-DNP}} = \bar{M}_{n,h}(OD_{0.1} - OD_{\text{blank}})/\epsilon_m = \bar{M}_{n,h}OD_a/\epsilon_m \quad (5)$$

where $OD_{0.1}$ is the optical density observed at 293 nm for a 0.1% w/v DNP-polymer solution of ethyl acetate, OD_{blank} is the optical density for the homopolymer solution at the same concentration and wavelength as the DNP-polymer, OD_a is the optical density for *O*-DNP groups in a 0.1% w/v DNP-polymer solution, and ϵ_m (= 1.24×10^4) is the molar extinction coefficient of stearyl 2,4-dinitrophenyl ether.

Results of the calculated average number of hydroxyl end groups estimated as *O*-DNP groups are given in *Table 3*. It has been reported that FDNB reacts readily with primary alcohol[7]. In experiments of the prolonged reaction with FDNB, the values of the number of *O*-DNP end groups in the polymer formed in the system B are not changed sufficiently to confuse the interpretation of the number of end groups. The values of the number of hydroxyl end groups of polymer prepared by Fenton's reagent is estimated to be 0.84. It is noted that for the polymers tested, the total number of end groups per chain is approximately unity. These results agree well with the conclusion reached by Bevington, Melville, and Taylor. By using the method of ^{14}C labelled end groups the PMMA radical is disproportionation-terminated at 60°C[17]. It has been reported that from the identification of the dissociation products includ-

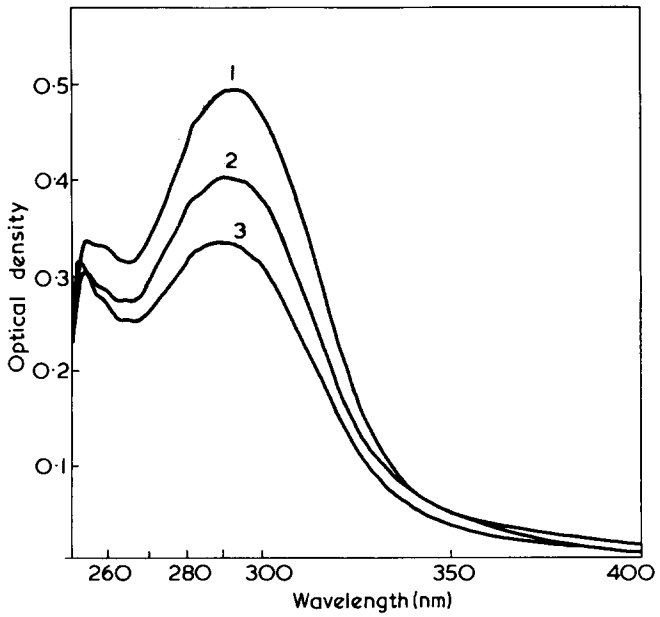

Figure 3 Absorption spectra of DNP-homopolymers formed in grafting systems A, B, and C: 1, system A, 2.89 g/l; 2, system B, 5.77 g/l; 3, system C, 7.94 g/l solution of ethyl acetate

Table 3 Average total number of end groups of homopolymer

Polymer obtained from different grafting system	Test for hydroxyl end groups at 293 nm			Number-average molecular weight of DNP-homopolymer	Average number of hydroxyl, phenyl and benzoyloxy end groups per polymer chain			
	$OD^{a}_{0.1}$ X 10^2	OD^{b}_{blank} X 10^2	OD^{c}_{a} X 10^2	$\bar{M}_{n,h}$ X 10^{-4}	HO–	C_6H_5–	$C_6H_5CO_2$–	Total
A	16.97	0.07	16.90	3.09	0.42	0.38	0.12	0.92
B	6.98	0.35	6.63	5.08	0.27	0.76	0.20	1.23
B[d]	7.02	0.35	6.67	5.38	0.29			
B[e]	6.81	0.35	6.45	5.33	0.28			
C	4.16	0.21	3.95	6.68	0.21	0.80	0.13	1.14
Polymer prepared by Fenton's reagent	1.41	0.31	1.10	94.3	0.84	–	–	0.84

[a]Optical density of 0.1% w/v DNP-polymer solution; [b]optical density of 0.1% w/v homopolymer solution; [c]optical density of *O*-DNP end groups in 0.1% w/v ethyl acetate solution of DNP-homopolymer; [d]treated with FDNB for 48 h; [e]treated with FDNB for 72 h

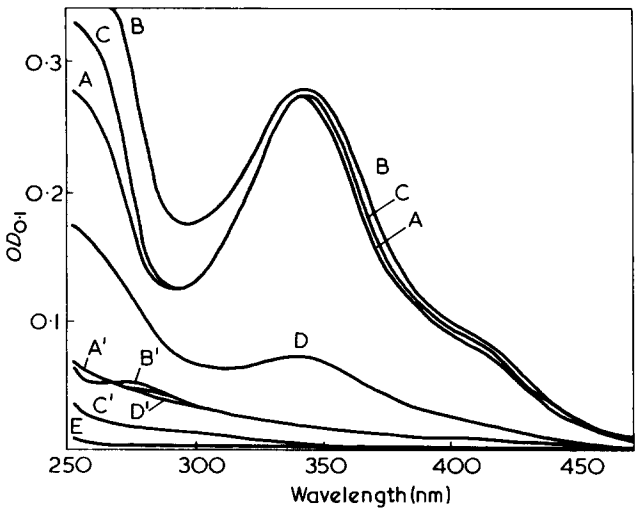

Figure 4 Optical density *versus* wavelength relationships of 0.1% w/v ethyl acetate solution of DNP-polymers synthesised from grafted fibres: A, 48.7% grafted wool, 0.4% BPO; B, 61.6% grafted wool, 1% BPO; C, 112.8% grafted wool after reduction, 1% BPO; D, 207.8% grafted wool after S-cyanoethylation, 0.4% BPO. From isolated PMMAs: A', B', C' and D' corresponding to the grafted samples, A, B, C and D, respectively, and E, unmodified homopolymer formed by Fenton's reagent

ing various fragments of the solvent moecule, a remarkable chain dissociation of BPO occurs in alcohol[14].

The initiation step of the polymerization is thus presumed to be due to reactions (6)–(11):

$$BPO \longrightarrow C_6H_5CO_2\cdot \quad (6)$$

$$C_6H_5CO_2\cdot \longrightarrow C_6H_5\cdot + CO_2 \quad (7)$$

$$R\cdot + CH_3OH \longrightarrow HOH_2C\cdot + RH \quad (8)$$

$$(R\cdot = C_6H_5CO_2\cdot \text{ and } C_6H_5\cdot)$$

$$HOH_2C\cdot + BPO \longrightarrow R\cdot \quad (9)$$

$$R\cdot + M \longrightarrow RM\cdot \quad (10)$$

$$HOH_2C\cdot + M \longrightarrow HOH_2CM\cdot \quad (11)$$

The end group data suggest: (a) mutual termination of reaction chains occurred in these reactions, (b) direct thermal polymerization was negligible, and (c) transfer of polymer radical to solvent and monomer could be neglected.

DNP-amino-acid end group in isolated polymer

It is expected that if the deposited polymers are truly grafted polymers, spectral changes between the isolated polymer and the homopolymer can be detected by the difference between the end groups incorporated in the two polymers. The spectral absorption curves from 250 to 470 nm for DNP-polymer are shown in *Figure 4*. The spectral shapes for DNP-polymers prepared from unreduced wool, reduced wool, and *S*-cyanoethylated wool are almost the same. An absorption maximum appears at 340 nm and a shoulder near 410 nm, which are similar to the characteristic absorptions of the common DNP-amino-acids, e.g. methionine, valine, leucine, etc. as illustrated in *Figure 5*. These spectra are totally different from the spectral curves of the DNP-polymer compared to the homopolymer. It appears that no characteristic absorption of *O*-DNP groups is involved in the system.

The number of *N*-DNP-amino acid end groups can be calculated using equation (12).

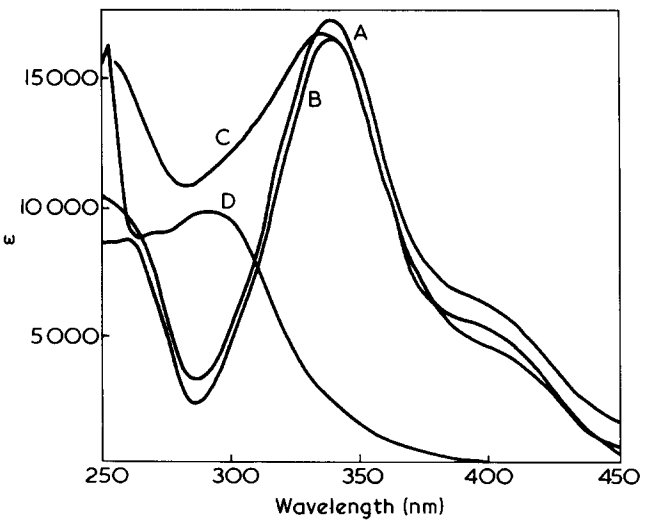

Figure 5 Molar extinction coefficient of various DNP-amino-acids: A, DNP-DL-methionine; B, DNP-L-leucine; C, *O,N*-di-DNP-L-tyrosine; D, *O*-mono-DNP-L-tyrosine

Table 4 Results of the number of DNP-amino-acid end groups incorporated in isolated PMMA

Treatment	Concentration of BPO (%)	Time of reaction (h)	Grafton (%)	Conditions of acid digestion: Concentration of HCl (N)	Conditions of acid digestion: Temperature (°C)	$OD^{d}_{0.1}$	OD^{e}_{blank}	OD^{f}_{a}	$\bar{M}_{n,p} \times 10^{-5}$	Z^{g}	s^{h} (μmol/g)
Unreduced	1.0	2	35.9	6[c]	100	0.336	0.011	0.325	0.933	1.79	6.9
	1.0	3	56.2	6	100	0.324	0.013	0.311	1.02	1.87	10.3
	1.0	5	62.8	6	100	0.417	0.015	0.402	0.885	2.09	14.9
	1.0	3	55.5	6	110	0.264	0.015	0.249	0.935	1.57	8.1
	1.0	3	55.9	6	110	0.283	0.026	0.259	1.05	1.59	8.5
	1.0	3	66.7[b]	6	110	0.226	0.011	0.215	1.15	1.45	8.5
	1.0	3	50.4	6	110	0.279	0.016	0.263	1.08	1.67	7.8
	1.0	3	69.4[b]	6	80	0.287	0.012	0.276	1.26	1.99	11.2
	1.0	3	56.7	5	100	0.298	0.011	0.287	1.25	2.11	9.6
	1.0	3	53.6	4	100	0.286	0.011	0.275	1.04	1.75	8.1
	0.4	3	49.0	6	110	0.302	0.015	0.287	1.03	1.74	8.3
	0.4	3	48.7	6	100	0.276	0.020	0.256	1.23	1.86	7.3
	0.4	3	52.3	6	100	0.272	0.010	0.262	1.33	2.05	8.1
	0.4	3	53.6	6[c]	100	0.356	0.023	0.333	0.980	1.92	10.5
	0.4	3	61.6[b]	6	100	0.280	0.020	0.260	1.48	2.28	9.5
	0.4	5	54.8	6	100	0.263	0.015	0.248	1.36	1.98	8.0
Reduced[a]	1.0	3	112.8	6	100	0.274	0.007	0.267	1.20	1.88	17.7
S-cyanoethylated	0.4	5	207.6	6	100	0.0686	0.0050	0.0636	5.36	2.00	7.7

[a]Thiol content of reduced wool: 128 μmol/g wool; [b]graft copolymerization was carried out in a sealed tube filled with N_2 gas; [c]*in vacuo*; [d]optical density of 0.1% w/v DNP-polymer solution of ethyl acetate at 340 nm; [e]optical density of 0.1% w/v isolated polymer solution; [f]optical density of *N*-DNP-amino-acid end groups in 0.1% w/v DNP-polymer solution; [g]average number of DNP-end groups per chain; [h]number of grafting sites in wool fibres

$$Z_{N\text{-DNP}} = \bar{M}_{n,p} OD_a / \epsilon_m \quad (12)$$

where OD_a is the optical density for *N*-DNP groups in a 0.1% w/v DNP-polymer solution, and ϵ_m (= 1.70×10^4) is the molar extinction coefficient of DNP-DL-methionine. The values of the molar extinction coefficient, ϵ_{max} at λ = 340 nm, of DNP-DL-methionine, DNP-L-alanine, DNP-L-serine, DNP-L-aspartic acid, and DNP-L-leucine are 1.70×10^4, 1.70×10^4, 1.68×10^4, 1.76×10^4, and 1.65×10^4, respectively. As an average of the values of the common DNP-amino-acids, DNP-DL-methionine was selected for calibration. The results are summarized in *Table 4*. It is found that the quantity of end groups in the polymer is about 2 per polymer chain except in the case for the polymers digested with 6 N HCl for 24 h at 110°C. It is not certain whether the decomposition of end groups incorporated in polymer chain ends or the extensive degradation of grafted polymer occurred during the strong hydrolysis. This situation is complicated by the fractionation during the purification of dinitrophenylated polymer.

Interpretation of the number of end groups

In order to clarify the two sites of DNP-amino-acid end groups in the polymer, spectral changes were investigated for the isolated polymer and the DNP-polymers from the grafted fibres before and after the chemical modification of wool peptides.

2VP[10] and AN[12,13] are useful reagents for selective modification of thiol groups in reduced wool. Quantitative conversion of thiol groups in reduced wool into *S*-β-(2-pyridylethyl)cysteine which is stable to acid hydrolysis, permits quantitative determination of cysteine or of cystine via cysteine by u.v. spectrophotometry[18,19]. No remarkable difference is observed in the spectral curves among the isolated polymers from the *S*-pyridylethylated wool (SPRG), the *S*-cyanoethylated wool (SCRG), and dinitrophenylated fibres after *S*-cyanoethylation (DSCRG), as shown in

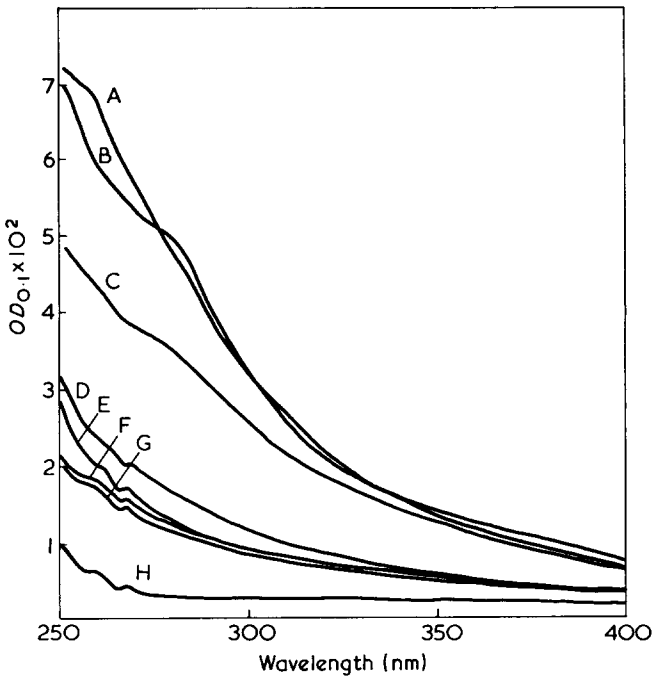

Figure 6 Optical density *versus* wavelength relationships of 0.1% w/v ethyl acetate solution of isolated polymers from the grafted fibres: A, SPRG wool; B, SCRG wool; C, G wool; D, SCRG wool; E, SPRG wool; F, DSCRG wool; G, G wool; H, homopolymer synthesised by Fenton's reagent. A–C were purified by the ethyl acetate method and D–G were purified by the DMSO method

Figure 6. It should be noted that the purification method for the isolated polymers affects the broad absorption spectra observed in the range 250 to 400 nm, showing much higher absorbance for the polymers purified using ethyl acetate rather than DMSO. The yields of the residues digested with 6 N HCl for 24 h at 100°C, were nearly 99.8% of the calculated polymer yields. Very good yield balance indi-

Table 5 Number of DNP-amino-acid end groups incorporated in isolated polymer from chemically modified fibres of wool-graft copolymers with 54.8% MMA

Treatment	Thiol and disulphide contents (μmol/g) SS	SH	2SS + SH	Purification method for isolated polymer	$OD_{0.1}$	OD_{blank}	OD_a	$\bar{M}_{n,p} \times 10^{-5}$	Z
Unmodified (G wool)	415	26.4	856	Ethyl acetate	0.263	0.015	0.248	1.36	1.98
S-Pyridylethylated (SPRG wool)	19.7	40.2	79.6	Ethyl acetate	0.200	0.014	0.186	1.51	1.65
	19.7	40.2	79.6	DMSO	0.181	0.005	0.176	1.41	1.46
S-Cyanoethylated (SCRG wool)	19.2	14.0	52.4	Ethyl acetate	0.284	0.018	0.266	1.23	1.92
	32.4	13.4	78.2	Ethyl acetate	0.223	0.029	0.194	1.43	1.63
	32.4	13.4	78.2	DMSO	0.235	0.006	0.229	1.38	1.86
Dinitrophenylated after *S*-cyanoethylation[a] (DSCRG wool)				Ethyl acetate	0.398	0.116	0.282	1.17	1.94
				DMSO	0.211	0.004	0.207	1.64	2.00

[a]Thiol and disulphide contents of *S*-cyanoethylated wool: SH = 14.0 and SS = 19.2 in μmol/g wool

cates that the wool polypeptide could be well digested to an individual amino-acid which might diffuse into the outward solution from the fibrous, insoluble residues. However, the isolated polymers have been spectrophotometrically stained with insoluble materials produced during the acid digestion of the grafted fibres. The unidentified materials intensifying the absorption could be removed by thorough washing with a solvent such as DMSO. These spectra are substantially the same as the spectrum for the polymer obtained by the redox system, $Fe^{2+} - H_2O_2$, which has two weak peaks at 260 and 268 nm. A definite absence of the bands observed for the homopolymer is also indicated. From the fact that no absorption band of *S*-β-(2-pyridylethyl)cysteine is present near 262 nm in ethyl acetate (ϵ_m is $\sim 10^4$)[20], the possibility of existence of cysteine or of cystine residues can be ruled out. The absence of ε-DNP-lysine, di-DNP-cystine, *O*-DNP-tyrosine and imidazol-mono-DNP-histidine is also confirmed by the fact that the spectrum of isolated polymer from DSCRG wool is very similar to that of the polymer from SCRG wool. Although the values of DNP-end groups are somewhat scattered, in any treated sample, approximately two DNP-end groups per polymer chain is obtained as shown in *Table 5*, this figure is the same value as that for grafted copolymer without these treatments. When a polypeptide with several amino-acid residues is linked at one end of an isolated polymer chain, only one DNP-end group is possible if the all amino-acids are common-type amino-acid residues, and the possibility of two or more amino-acid residues with DNP-groups on one end of a long polymer chain is also ruled out. It can, therefore, be concluded that one DNP-amino-acid end group is situated at each end of the polymer chain.

The spectral curves of the isolated polymers from the grafted fibres of *S*-cyanoethylated wool with 207.6% MMA (GSC wool) and from the grafted fibres followed by dinitrophenylation (DGSC wool) are shown in *Figure 7*. The spectrum of the former is similar to that of the isolated polymer from the graft copolymers of untreated wool. However, the latter spectrum has a weak shoulder near 290 to 300 nm. There is one absorption maximum near

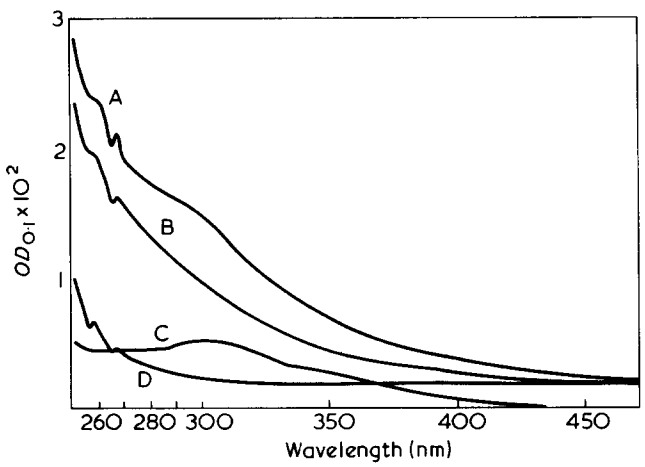

Figure 7 Optical density *versus* wavelength relationships of 0.1% w/v ethyl acetate solution of isolated and DNP-polymers: A, DNP-polymer from DGSC wool; B, isolated polymer purified by DMSO method; C, difference spectrum of A – B; D, homopolymer synthesised by Fenton's reagent

300 nm in the difference spectral curve in *Figure 7*. It remains, however, uncertain whether the characteristic absorption band belongs to *O*-DNP-tyrosine incorporated in the grafted polymer or to *O*-DNP group linked to the polymer chain initiated by oxymethylene radical, $HOCH_2 \cdot$ according to the reaction (11). The calculated number of DNP-end groups of the grafted polymer is reliable even if tyrosyl residues in the polypeptides are responsible for the initiating sites of grafting, since the ϵ_m-value of *O*,*N*-di-DNP-tyrosine is almost the same as that obtained in the common-types of *N*-DNP-amino-acid (see *Figure 5*). If the tyrosyl residues are concerned with grafting, the amounts of end groups can be determined as $Z_{O\text{-DNP-tyr}} = 0.27$. Assuming that the grafted polymres are composed of bicomponent systems, true grafted copolymer and unextractable homopolymer, the value of $Z_{O\text{-DNP}}$ (= 0.21) corresponds to approximately 10% of the average total number of end groups.

The results of the end group analyses are summarized in

Table 6 Number of DNP-amino-acid end groups incorporated in the isolated polymers from grafted fibres of *S*-cyanoethylated wool[a] with 207.6% MMA and from the grafted fibres followed by dinitrophenylation

Treatment	Optical density of 0.1% w/v polymer solution at 340 nm		Purification method for isolated polymer			
	$OD_{0.1}$ × 10^2	OD_{blank} × 10^2		OD_a × 10^2	$\bar{M}_{n,p}$ × 10^{-5}	Z
Unmodified (GSC wool)	7.16	1.98	Ethyl acetate	5.18	5.24	1.60
Unmodified (GSC wool)	6.86	0.50	DMSO	6.36	5.36	2.00
Dinitrophenylated (DGSC wool)	8.00	1.97	Ethyl acetate	6.03	5.84	2.07
Dinitrophenylated (DGSC wool)	7.50	0.80	DMSO	6.70	5.33	2.10

[a]Thiol and disulphide contents of *S*-cyanoethylated wool: SH = 9.9 and SS = 16.4 in μmol/g wool

Table 6. Approximately 2 end groups per chain come from the graft copolymers polymerized with no disulphide bonds. The end group data indicate that in the wool fibres: (a) wool radicals are formed in the polypeptide chains by the interaction with primary radicals, (b) wool radicals initiate grafting, (c) deactivation of polymer radicals occurs by recombination-termination, and (d) transfer reactions of polymer radicals to substrate, solvent and monomer molecules can be neglected.

As reported previously[2–5], the mode of termination would certainly be controlled by the mobility of growing polymer–radical end which would interact with the substrate chain. Disproportionation-terminated polymer–radical interaction is favoured by free movement of the copolymer chain while recombination-termination, which requires a much lower activation energy, takes place if there is only limited movement, as permitted for a gel structure. It has been hypothesised that in an aqueous medium, the mobility of the growing chain end is dependent on the mobility of the wool chain, which increases with increase in the thiol content catalysing the thiol and disulphide interchange reaction, and with decrease in the disulphide crosslinks[3,4].

It has been observed that in methanol, fibre swelling is less than in water, and the thiol and disulphide interchange reaction is inhibited in the strained fibres. Segmental motion of the peptides would naturally be lowered in a methanol environment. Under such circumstances, the interaction between the growing graft polymer radicals would be overwhelmed by recombination-termination even in a low crosslinked network or in a highly reduced structure.

The spectra of the isolated polymer and of the DNP-polymer from the grafted fibres with styrene are shown in *Figure 8*. Broad and high absorption spectra are observed in the range 270 to 450 nm for the grafted polymer purified using the ethyl acetate system. Applying the DMSO method for the purification, the insoluble hydrolysates in the isolated polymer can be removed, since the absorption spectra obtained for the polymers are virtually the same as that for styrene homopolymer. No significant variation is observed between the shapes of the absorption curves of DNP-polymers prepared from the polymers which had been purified with DMSO and with ethyl acetate (see *Figure 8*). It is indicated, therefore, that a portion of the insoluble hydrolysates reacts with FDNB to convert soluble materials in ethyl acetate and methanol, which could be removed during the purification of DNP-polymer. Accordingly, it seems unlikely that the value of the number of end groups per chain would be affected by the insoluble hydrolysates in isolated polymer.

It has been reported that the polystyryl radicals interactions are terminated by recombination in solution[17]. As shown in *Table 6*, the number of DNP-end groups per chain lies between 1.5 and 1.8 which is somewhat less than the value expected as end group. Two reasons for this are considered: (a) monomolecular termination reactions were included in predominant recombination reactions of the polymer radicals within the structure stabilized by cystine crosslinks and (b) the decomposition of end group in polymer during the acid hydrolysis. From the values of Z, proportions of recombination reaction occurrences can be calculated to be approximately between the values of 70 and 90% for the systems examined. Recombination is preceded by the other termination reactions of polystyryl radicals in the wool fibres. This also supports the result obtained with MMA that one end group of about two DNP-amino-acid end groups analysed is situated at each end of the polymer chain.

Number of grafting sites

The number of grafting sites produced in fibres in μmol/g of wool, s, can be calculated from the following equation:

$$s = 10^4 ZG/\bar{M}_{n,p} \qquad (13)$$

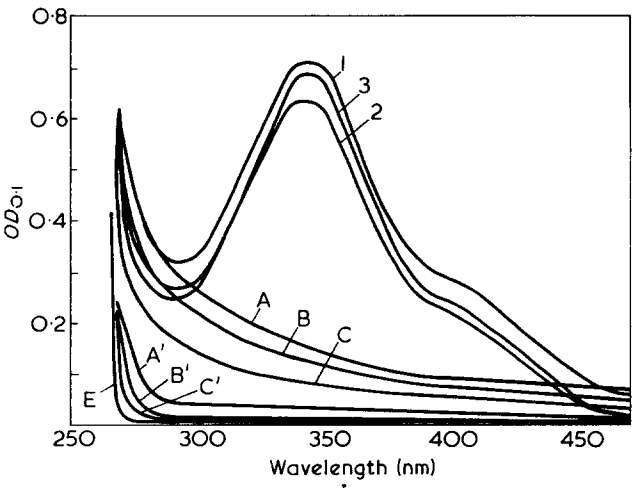

Figure 8 Optical density *versus* wavelength relationships of 0.1% w/v ethyl acetate solution of DNP-polymers and isolated polymers obtained from the styrene grafted fibres. Isolated polymers purified by ethyl acetate method: A, 21.5% grafted wool; B, 24.6% grafted wool and 72.8% grafted wool after reduction. Isolated polymers purified by DMSO method A', B', and C' correspond to the polymers A, B, and C, respectively. DNP-polymers 1 (system A); 2 (system B); 3 (system C) correspond to the isolated polymer A', B, and C', respectively

Table 7 Number of DNP-amino-acid end groups incorporated in isolated PS molecule

Sample	Graft-on[a] (%)	$OD^{b}_{0.1}$	OD^{c}_{blank}	OD_a	$\bar{M}_{n,p} \times 10^{-4}$	Z^{d}	s^{e} (μmol/g)
Unreduced	21.5	0.715	0.032	0.683	4.04	1.62	8.6
Unreduced	24.6	0.63	0.013	0.617	4.24	1.54	8.9
Reduced	72.8	0.694	0.008	0.689	4.41	1.78	29.5

[a]Reaction: 1% BPO, 30% S, and 69% MeOH (by wt); 60°C, 3 h. Hydrolysis: 6 N HCl, 100°C; [b]optical density at 343 nm; [c]isolated polymer purified by using DMSO method; [d]number of *N*-DNP end groups per polymer chain; [e]number of grafting sites produced in fibres

where G is the degree of grafting (%).

From equations (12) and (13), we obtain equation (14):

$$s = 10^4 G.OD_a/\epsilon_m \quad (14)$$

With the grafting system containing 1% BPO, the number of grafting sites produced in the untreated wool fibres reaches ~15 μmol/g of wool after 5 h. With the system involving different concentrations of initiator and different monomers, great similarity was observed in the number of grafting sites generated after 3 h reactions as shown in *Tables 4* and *7*. A somewhat lower level in the number of grafting centres can be seen for the *S*-cyanoethylated wool rather than the unmodified wool fibres. Consequently, production of the grafting centres is independent of the cystine content (see *Table 1*) and of the swelling of fibres which is closely related to the diffusion of monomer.

It should be noted, however, that the production of radicals available for grafting is considerably enhanced within the reduced wool fibres (see *Table 4*). This clearly indicates that the free thiol groups can initiate grafting through the interaction with the primary radicals. It appears that two different initiating mechanisms are involved in the reduced wool fibres.

ACKNOWLEDGEMENT

This research was supported financially in part by the International Wool Secretariat.

REFERENCES

1 Arai, K., Komine, S. and Negishi, M. *J. Polym. Sci. (A-1)* 1970, **8**, 917
2 Arai, K., Negishi, M., Komine, S. and Takeda, K. *J. Appl. Polym. Symp.* 1971, **18**, 545; *Int. Wool Text. Res. Conf. 4th Berkeley* 1970
3 Arai, K., Shimizu, M. and Shimada, M. *J. Polym. Sci. (Polym. Chem. Edn)* 1973, **11**, 3271
4 Arai, K., Shimizu, M. and Shimada, M. *J. Polym. Sci. (Polym. Chem. Edn)* 1973, **11**, 3283
5 Arai, K. in 'Block and Graft Copolymerization', (Ed. R. J. Ceresa), Wiley–Interscience, New York–London, 1973, Vol 1, p 193
6 Rao, K. R. and Sober, H. A. *J. Am. Chem. Soc.* 1954, **176**, 1320
7 Whalley, W. B. *J. Chem. Soc.* 1950, p 2241
8 Maclaren, J. A. and Sweetman, B. J. *Aust. J. Chem.* 1966, **19**, 2355
9 Robson, A., Williams, M. J. and Woodhouse, J. M. *J. Text. Inst.* 1969, **60**, 140
10 Friedman, M. and Noma, A. *Text. Res. J.* 1970, **40**, 1073
11 Fraenkel-Conrat, H. *Biochem. Biophys. Acta* 1952, **9**, 551
12 Maclaren, J. A. *Aust. J. Chem.* 1962, **15**, 824
13 Asquith, R. S., Otterburn, M. S., Buchanan, J. H., Cole, M., Fletcher, J. C. and Gardner, K. L. *Biochim. Biophys. Acta* 1970, **207**, 342
14 Burrell, C., Majury, T. and Melville, H. W. *Proc. Roy. Soc.* 1951, **205**, 309
15 Bengough, W. and Melville, H. W. *Proc. Roy. Soc.* 1954, **225**, 330
16 Bengough, W. *Nature* 1957, **180**, 1120
17 Bevington, J. C., Melville, H. W. and Taylor, R. P. *J. Polym. Sci.* 1954, **14**, 463
18 Friedman, M. and Tillin, S. *Text. Res. J.* 1974, **44**, 578
19 Maclaren, J. A. *Text. Res. J.* 1971, **41**, 713
20 Arai, K. unpublished results