Vinyl polymers containing amido and carboxylic groups as side substituents: I. Synthesis of *N*-acryloyl-glycine and *N*-acryloyl-6-caproic acid and their grafting on cellulose membranes

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Two vinyl monomers containing amido and carboxylic groups, namely *N*-acryloyl glycine and *N*-acryloyl-6-caproic acid, have been synthesized and polymerized by radical initiators. The polymers of high molecular weight were prepared in a dioxane solution by radical polymerization of the corresponding monomers. Graft copolymers of both monomers on cellulose films have been obtained by use of ceric salts. The surface modifications of the grafted cellulose films have been checked by attenuated total reflection/*FT* i.r. spectra. The difference spectra of the surface graft polymers were found to be very similar to those of the homopolymers.

(Keywords: polyelectrolytes; graft copolymerization; cellulose porous membranes; ceric salts; ATR/*FT* i.r.)

INTRODUCTION

Cellulosic material is widely used as supports because it is the most abundant renewable material. Regenerated cellulose is currently the prevalent material used for haemodialysis membranes. Graft copolymerization of vinyl monomers onto cellulose has been the subject of extensive investigations[1]. Recently interest has been devoted to their potential surface wettability for ion exchange, control release of bioactive molecules and a variety of other uses. The modification of the cellulose with vinyl monomers carrying a carboxylic group is studied with difficulty because homopolymers are always formed as an undesirable by-product.

Our main aim is to develop a novel membrane system in which the rate of permeation of biological molecules through porous cellulose film can be controlled by pH-sensitive graft polyelectrolytes[2–4]. In this respect we have grafted on cellulose membranes two acidic monomers using Ce(IV) as initiator. We reported previously a novel membrane system to control delivery of insulin at rates dependent on the external glucose concentration. This was made of poly(acrylic acid) grafted on porous cellulose membrane containing glucose oxidase immobilized on the graft chain. The enzyme catalyses the conversion of glucose to gluconic acid which, in turn, can protonate carboxylate groups on the side chains of the graft vinyl polymers. The reduced electrostatic repulsion of the protonated carboxylic groups causes coiling of the graft chains which open the pores of the membrane and thus enhances faster delivery of insulin in the presence of glucose[4].

Hydrophobicity of polyelectrolytes is believed to be important especially in conformational changes or other property changes[5]. The more hydrophobic the character, the greater the pH-induced conformational transition[6]. Following these premises, we purposely synthesized two vinyl monomers having the structure of *N*-acryloyl amino acids, in which a different number of non-polar methylene groups were inserted between amido and carboxylic groups. This was done in order to study the effects of side-chain length and flexibility on backbone chain conformation. The solution properties of both polymers as well as the permeation of insulin through grafted porous cellulose membranes controlled by pH changes will be reported in forthcoming papers[7].

In this paper we report the preliminary results of the synthesis and characterization of free and graft vinyl

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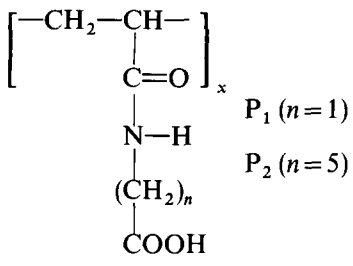
polymers of the following structure:



P_1 ($n=1$)

P_2 ($n=5$)

These multifunctional synthetic polymers, in their linear form, are also of interest in relation to macromolecular drugs[8] or as macromolecular catalysts[9]. Further, many of them can act as complexing agents with heavy metal ions in aqueous solution[10].

EXPERIMENTAL

Viscometric measurements

Viscometric data were obtained in DMF at 30°C with a Cannon Ubbelohde 50 E 998 viscometer having a flow time of 170 s for the DMF solvent. Solutions were freshly prepared by dissolving *c.* 40 mg of each of P_1 and P_2 in 15 ml of DMF solvent, and were immediately used after filtration.

^1H n.m.r. spectroscopic measurements

Samples were prepared by dissolving *c.* 10 mg of each of M_1 and M_2, and the corresponding polymers in 0.5 ml DMSO-d6. Proton n.m.r. spectra were recorded on a Varian XL 200 spectrometer. All spectra were obtained at 25°C and a delay of 2 s was used after each $\pi/2$ pulse in order to observe full intensities of the proton signals. Chemical shifts were measured by taking the residual DMSO peak as internal reference signal at 2.48 ppm.

In the proton n.m.r. spectra of the polymers, broad lines were found for both backbone and side chain resonances. The characteristic signals of the vinyl double bond disappear, while a high-field shift of the N–H proton and the presence of intense broad signals at 3.3 and 3.7 ppm, respectively, for the two polymers were observed. These findings are consistent with the presence in solution of a slowly tumbling molecular species such as the proposed polymers.

FT *i.r. spectroscopic measurements*

FT i.r. spectra on both monomers and corresponding polymers were recorded with KBr pellets using a Perkin-Elmer *FT* i.r. spectrophotometer M1800 equipped with a Data Station 7500 professional computer. A.t.r./*FT* i.r. spectra were recorded to check the surface modification using a KRS5 crystal with an incident angle of 45°.

The apparatus was purged with nitrogen. Typically 300 scans at a resolution of 2 cm^{-1} were averaged and the spectra were stored on a floppy disk.

The infra-red spectra of polymers did not show the absorption at 990 and 1615 cm^{-1} due to the double bond of monomers, an indication of the formation of a polymer. The latter also reveals the broadening of the infra-red bands, due to the polymeric nature.

SYNTHESIS OF MONOMERS

N-*acryloylglycine* (M_1)

To a well-stirred aqueous solution of glycine (10.5 g, 0.14 mol), sodium hydroxide (4.4 g, 0.11 mol) and 2,6-di-*t*-butyl-*p*-cresol (0.02 g) in water (25 ml), acryloyl chloride (7.93 g, 0.09 mol) and sodium hydroxide solution (4.0 g, 0.1 mol in 25 ml) was contemporaneously added dropwise over a 30 min period. The reaction mixture was kept at −18°C by external cooling with a dry ice–acetone bath. The reaction mixture was stirred for a further 1 h while rising to room temperature. Then it was acidified to pH 2 with concentrated hydrochloric acid, and filtered. The filtrate was evaporated to dryness *in vacuo*. The temperature was never allowed to rise above 40°C during evaporation. The residue was then extracted with preheated boiling ethyl acetate (100 ml). The product crystallized by cooling. The yield was 3 g (30%) with a m.p. of 127–128°C. Elemental analysis: calculated for $C_5H_7NO_3$: C, 46.5%; H, 5.5%; N, 10.8%. Found: C, 46.2%; H, 5.5%; N, 10.6%.

The carboxyl group in M_1 was assayed by titration with standard sodium hydroxide solution. This showed it to be 100% pure.

These results, taken in conjunction with spectroscopic analysis (^1H n.m.r. and *FT* i.r.), identified a product of analytical grade.

N-*acryloyl-6-caproic acid* (M_2)

To a well-stirred aqueous solution of 6-aminocaproic acid (18.4 g, 0.14 mol), sodium hydroxide (5.5 g, 0.14 mol) and 2,6-di-*t*-butyl-*p*-cresol (0.02 g) in water (25 ml) acryloyl chloride (7.93 g, 0.09 mol) and sodium hydroxide solution (4.0 g, 0.1 mol in 25 ml) was contemporaneously added dropwise over a 30 min period. The reaction mixture was kept at −18°C by external cooling with a dry ice–acetone bath. The reaction mixture was allowed to rise at room temperature over a 1 h period under stirring. Then it was acidified to pH 2 with concentrated hydrochloric acid, extracted with chloroform, dried over Na_2SO_4, and evaporated to dryness *in vacuo* after adding a small amount of 2,6-di-*t*-butyl-*p*-cresol. The temperature was never allowed to rise above 40°C during evaporation. The residue crystallized in cold n-heptane, and it was recrystallized from benzene. The yield was 2 g (11%), with a m.p. of 89–90°C. Elemental analysis: calculated for $C_9H_{15}NO_3$: C, 58.4%; H, 8.2%; N, 7.6%. Found: C, 58.3%; H, 8.1%; N, 7.5%.

The analytical grade of the M_2 monomer was tested by potentiometric titration with standard sodium hydroxide solution (purity 100%) and by spectroscopic analysis (^1H n.m.r. and *FT* i.r.).

POLYMERIZATION

Poly(N-*acryloylglycine*) (P_1)

To a solution of 2.0 g of M_1 in 20 ml of 1,4-dioxane, 15 mg of azobisisobutyronitrile (AIBN) was added. After purging with nitrogen the mixture was allowed to stand in a thermostatted oil bath at 60°C for 24 h under a nitrogen atmosphere. The polymer precipitated out, and the solvent was evaporated *in vacuo*. The product was purified by dissolving in ethanol (5 ml), reprecipitating with an excess of acetone, and drying at 60°C and

0.1 mmHg. It was a white powder, having an intrinsic viscosity $[\eta]=0.66\,dl\,g^{-1}$ (in DMF at 30°C). Its elemental analysis was quite satisfactory and consistent with a structure comprising 0.5 water molecules for each monomeric unit. Elemental analysis: calculated for $C_5H_7NO_3 \times 0.5H_2O$: C, 43.5%; H, 5.8%; N, 10.1%. Found: C, 43.2%; H, 5.3%; N, 9.5%.

Poly(N-acryloyl-6-caproic acid) (P_2)

A similar polymerization procedure was used to synthesize P_2 starting from 1 g of M_2 in 1,4-dioxane (15 ml), to which 10 mg of azobisisobutyronitrile (AIBN) was added. The polymer obtained showed an inherent viscosity $\eta_{inh}=0.58\,dl\,g^{-1}$ (0.2 wt%, in DMF at 30°C). The elemental analysis is consistent with a structure comprising 0.5 water molecules for each monomeric unit. Elemental analysis: calculated for $C_9H_{15}NO_3 \times 0.5H_2O$: C, 55.6%; H, 8.3%; N, 7.2%. Found: C, 55.0%; H, 7.8%; N, 6.9%.

Graft polymerization

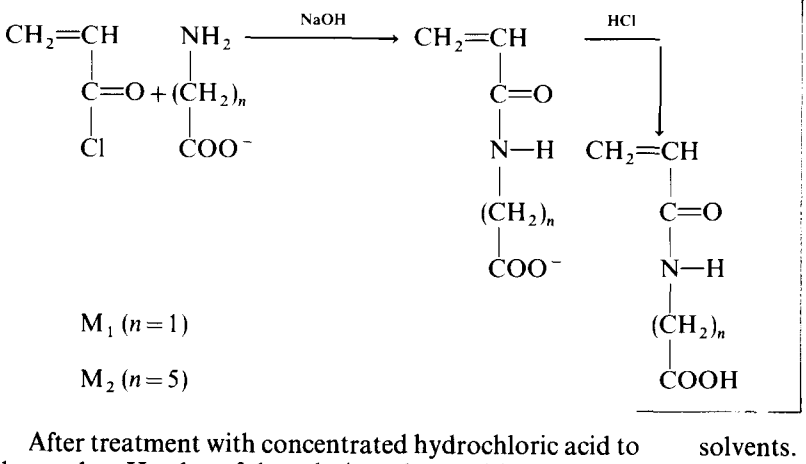
The polymerization procedure was based on the method described by Mino and Kaizerman[11]. Graft copolymerization was carried out at 20°C under constant bubbling of nitrogen in the reaction tube containing the porous cellulose filter of pore size 0.2 μm (regenerated cellulose, from Sartorius Co. Ltd or nitrocellulose, from Sigma Chemical Co.), ceric ammonium nitrate (from Sigma Chemical Co.) and monomer dissolved in a known amount of water.

A solution of ceric ammonium nitrate (0.09 g, 0.16 mmol) in 1M nitric acid (1 ml) was added to a solution of the appropriate monomer (M_1: 0.25–0.84 g, 1.94–6.50 mmol; M_2: 0.30 g, 1.62 mmol) in presence of the cellulose filter (regenerated cellulose 0.05 g; nitrocellulose 0.36 g) in 25 ml water. Polymerization was allowed to proceed for 12 h, then the grafted cellulosic material was washed several times with distilled water under magnetic stirring and finally air-dried.

RESULTS AND DISCUSSION

Synthesis and characterization of monomers and related polymers

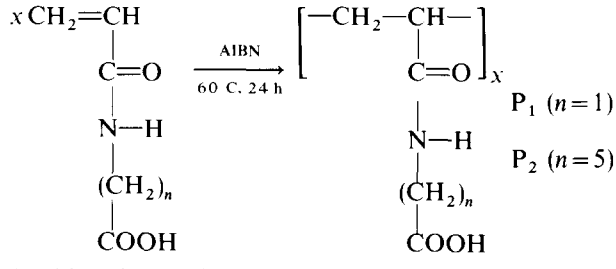
The two acidic vinyl monomers were obtained by a condensation reaction using acryloyl chloride and the appropriate aminoacid in alkaline solution:



After treatment with concentrated hydrochloric acid to lower the pH value of the solution, the resulting products were purified by recrystallization from hot ethyl acetate and benzene solutions, respectively, for M_1 and M_2 monomers. They are crystalline and freely soluble in many solvents such as dimethylsulphoxide, dioxane and water, and do not appear to be very sensitive to atmospheric moisture.

A complete assignment of proton signals of M_1 and M_2 monomers in DMSO-d6 was achieved by homonuclear decouplings and inspection of chemical shifts. The ^1H n.m.r. spectra are shown in *Figure 1*. *FT* i.r. spectra of the two monomers are very similar and the main i.r. frequencies are summarized in *Table 1* together with their identification. Only the N—H and the C=O stretching of the COOH group in M_2 showed lower frequencies with respect to M_1. This is due to the inductive effect, as also occurs in the simple amino acids[12] and poly(amidoamino)acids previously studied[13]. The lengthening of the aliphatic chain between the NH and COOH groups also reveals an increased number of characteristic bands in the 1300–1470 cm^{-1} region because of the greater number of CH_2 vibrational modes.

The polymerization of the monomers can be readily accomplished with radical initiators in dioxane solution, using azobisisobutyronitrile:



In this solvent, the compounds precipitate during the reaction to give high molecular weight polymers, as shown by viscosity under measurements. They are white powders, amorphous under X-ray examination, and do not show a definite melting point. Elemental analysis performed on both polymers gave quite satisfactory results, if the presence of 0.5 residual bound water molecules per monomeric unit was taken into account. This was already reported for other polymers carrying amido and carboxylic functional groups[14,15].

Polymer P_1 is soluble in aqueous media over a wide range of pHs, while polymer P_2 dissolves only at pH > 5. This is obviously due to a higher hydrophobicity of the latter. Both polymers are soluble in most organic solvents. Some solubility data on both polymers are reported in *Table 2*. It may be observed that only highly polar solvents such as dimethylformamide or dimethyl-

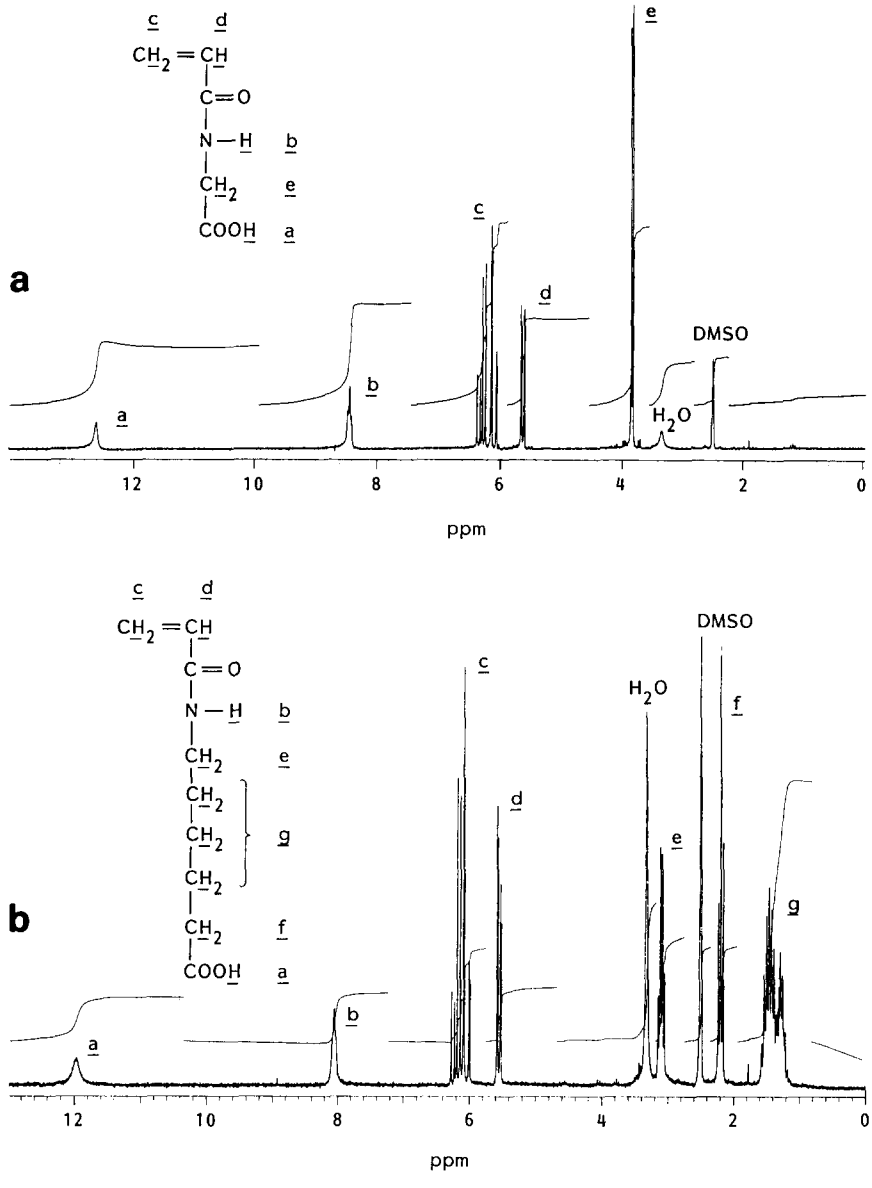


Figure 1 ^{1}H n.m.r. spectra of (a) *N*-acryloylglycine (M_1) and (b) *N*-acryloyl-6-caproic acid (M_2) in DMSO-d6 with the corresponding assignments of proton signals

Table 1 Main frequencies (cm^{-1}) observed for M_1 and M_2 monomers, for P_1 and P_2 polymers and their assignments

M_1	P_1	M_2	P_2	Assignments
3500 (sh)	3500 (sh)	3450 (sh)	3450 (sh)	OH (free) stretch
3320 (vs)	3320 (vs)	3300 (vs)	3300 (vs)	NH (H bonded) stretch
2800–3000	2800–3000	2800–3000	2800–3000	CH_2 stretch
1725 (vs)	1730 (vs)	1705 (vs)	1710 (vs)	C=O stretch of COOH group
1650 (vs)	1650 (vs)	1655 (vs)	1650 (vs)	Amide I band
1615 (vs)		1615 (vs)		C=C stretch
1555 (vs)	1555 (s)	1550 (vs)	1550 (s)	Amide II band
1300–1470	1300–1470	1300–1470	1300–1470	CH_2 def
1230 (vs)	1230 (vs)	1240 (s)	1240 (w)	C—O stretch + OH bend of COOH group
990 (s)		990 (s)		CH_2 bend of CH_2=CH group

vs = very strong, s = strong, w = weak, sh = shoulder

sulphoxide and ethanol are able to dissolve both polymers.

Viscometric measurements of the two polymers show that while polymer P_1, in DMF, shows a linear relationship between the reduced viscosity with concentration (η_{sp}/C), thus allowing an intrinsic viscosity value to be obtained, polymer P_2, in the same solvent, shows a typical polyelectrolyte behaviour[16]; its reduced viscosity increasing with decreasing concentration (*Figure 2*). The inherent viscosity has been calculated at

0.2 wt% polymer concentration. It is not easy at present to interpret this difference between the two polymers. However, it is probably related to the presence, in P_2, of a hydrophobic polymethylenic side chain. This is currently under investigation.

Graft copolymerization onto cellulose films

Along with many other synthetic methods, free-radical polymerization has been used for surface grafting by using Ce(IV) to generate free radicals on the surface[1].

Graft copolymerization of the two monomers M_1 and M_2 onto cellulose films was conducted at room temperature and at different monomer/substrate ratios, keeping the catalyst concentration constant. The nitrocellulose was partially nitrate, as purchased from Sigma Chemical Co. Two nitro groups for each glucosidic residue ensured the presence of at least one alcoholic reducing group in the initiation reaction mechanism. The increase in weight, which is usually determined in conventional graft copolymerization, was dependent on the monomer concentration[17]. The greater the concentration of monomer, the greater the number of polymer grafts. This suggests that the radical graft polymerization can be easily controlled by changing monomer concentration. *Table 3* summarizes the experimental conditions used to graft cellulosic materials. The percentage of grafting has been expressed as the increase in weight after drying/the initial weight × 100.

As reported by many authors[1], the chemical bonding of acidic monomers, such as acrylic or methacrylic acid, onto cellulosic materials leads to the formation of a large amount of homopolymer[1]. Homopolymerization also occurred with both synthetic monomers by using cellulose membranes as supports. The cellulose materials retain the polymers after being repeatedly washed with water. Thus the increase in weight and the i.r. spectra of the surface supports the covalent attachment of polymers to the cellulose membranes.

Properties of the grafted cellulose films

Graft copolymerization of M_1 gives rise to a noticeable change in wettability of the cellulose film, especially at high degrees of grafting. The film is rigid in the dry state and swells in water solution because the introduction of the polymer on the surface forms a more hydrophilic material. Moreover, the graft polyelectrolyte is able to form complexes with heavy metal ions. The copper(II) ions, in fact, are captured from the aqueous solution leading to a green coloured film at neutral pH; its release occurs only in acidic regions[18].

Measurements of a.t.r./*FT* i.r. were undertaken to examine whether graft copolymerization onto the film surface actually took place, even at low degrees of grafting.

Figure 3 shows the difference spectrum of the nitrocellulose membrane with the M_2 monomer. The striking similarity with the spectrum of the free polymer is diagnostic of graft reaction even at low degrees of grafting (9 wt%). Similar results were also obtained at low degrees of grafting with the monomers M_1 and M_2 onto regenerated cellulose membranes.

The increase of grafting leads to a better comparison

Table 2 Solubility data on poly(*N*-acryloylglycine) (P_1) and poly(*N*-acryloyl-6-caproic acid) (P_2)

Solvents	P_1	P_2
n-Hexane	I	I
Benzene	I	I
Toluene	I	I
Diethyl ether	I	I
Dioxane	I	I
Methylene chloride	I	SH
Chloroform	I	I
Carbon tetrachloride	I	I
Methanol	sw (SH)	S
Ethanol	S	S
iso-propyl alcohol	I	sw (SH)
Acetone	I	I
Dimethylformamide	S	S
Ethylacetate	I	I
Dimethylsulphoxide	S	S
Acetic acid (glacial)	SH	S
Water	S	I

S = soluble; SH = soluble at the boiling point; I = insoluble; sw = swells

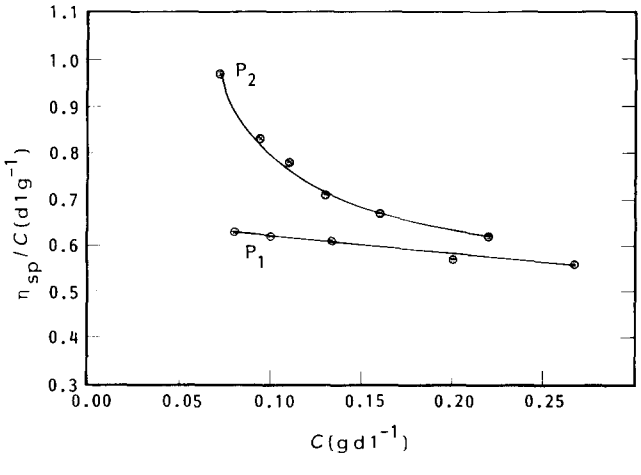


Figure 2 Reduced viscosity (η_{sp}/C) *versus* polymer concentration plots for P_1 and P_2, in DMF at 30°C

Table 3 Experimental conditions for the graft copolymerization of the cellulosic materials

Monomer compound	Amount of monomer (g)	Regenerated cellulose (g)	Cellulose nitrate (g)	Ce(IV) catalyst[b] (g)	Volume (ml)	Reaction time (h)	Wt% increase[a]
M_1	0.2514	0.0500		0.0868	25	12	9
	0.8400	0.0494		0.0877	20	12	305
M_2	0.3012	0.508		0.0901	25	12	11
	0.3012		0.3568	0.0901	25	12	9

[a] Percentage of grafting = $(W - W_0)/W_0$ (W: weight of grafted cellulose; W_0: weight of free cellulose)
[b] Each time the amount of ceric salt was dissolved in 1M HNO_3 (1 ml)

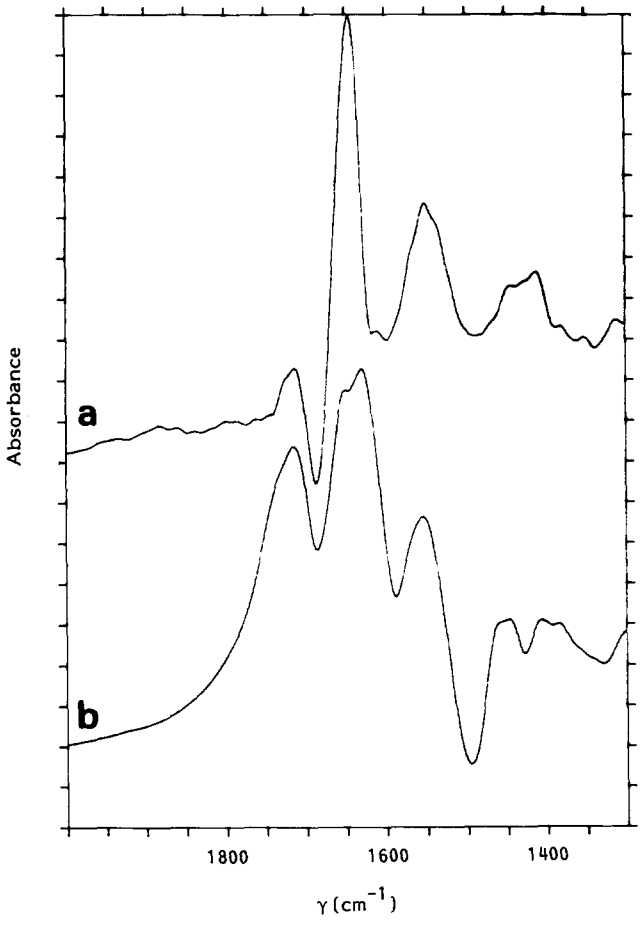


Figure 3 (a) A.t.r./*FT*i.r. difference spectrum of nitrocellulose grafted with M_2 (b) Spectrum of the free polymer P_2 in a KBr pellet

with the free polymer. *Figure 4* shows the a.t.r./*FT* i.r. spectra of the regenerated cellulose (a) and the grafted cellulose (b) with the M_1 monomer at high degrees of grafting (305 wt%). The comparison is clear even if the difference spectrum (c = b − a) show the same assignments as the free polymer P_1 in a KBr pellet. In particular, the characteristic bands due to the C=O stretching of the COOH group (1715 cm^{-1}), to the C=O stretching of the amidic group (1650 cm^{-1}), and to the NH stretching of the amidic group (1555 cm^{-1}) are well resolved.

Moreover, the loss of the band at 1615 cm^{-1} as well as that at 990 cm^{-1}, due to the C=C stretching, confirms that the polymerization of the monomers took place on the surface of the cellulose membranes.

CONCLUSIONS

The synthesis of the two monomers and their polymerization and graft copolymerization on regenerated cellulose and nitrocellulose porous films apparently provides a convenient way to obtain pH-sensitive membranes of potential utility in controlling the permeation rate of biological molecules because the grafted chains of polyelectrolytes work as 'chemical gates' in the pores of the film. Physico-chemical studies on the products with a view towards their application in the biomedical field are presently in progress and will be published in forthcoming papers.

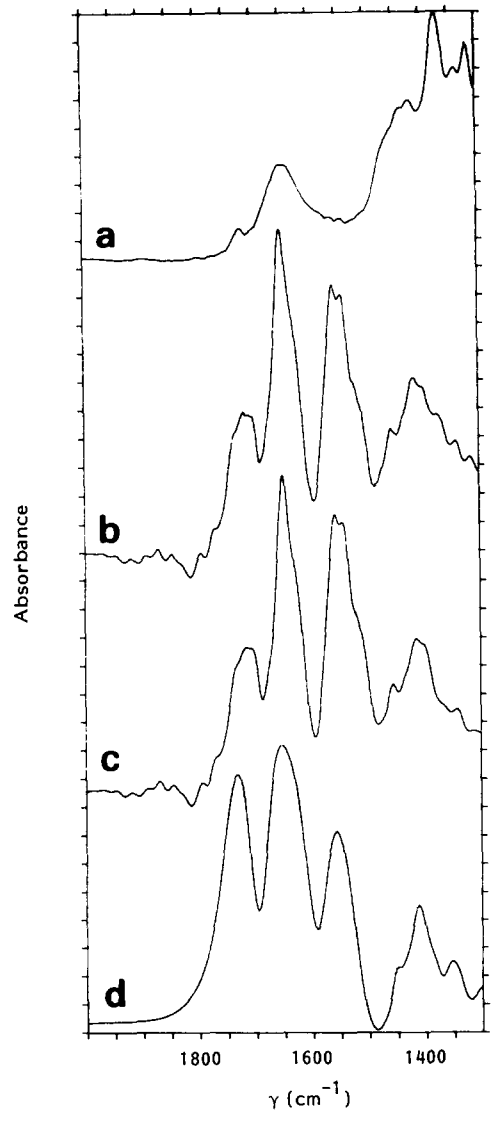


Figure 4 (a) The a.t.r./*FT*i.r. spectra of regenerated cellulose and (b) the spectra of regenerated cellulose with M_1 monomer. (c) Difference spectrum (b) − (a); (d) spectrum of the free polymer P_1 in a KBr pellet

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