PII: S0032-3861(97)00601-0

Polymer Vol. 39 No. 13, pp. 2751–2757, 1998
© 1998 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0032-3861/98/$19.00+0.00

Poly (vinyl alcohol) functionalized by monosuccinate groups. Coupling of bioactive amino compounds

Manuel Sánchez-Chaves*, Felix Arranz and Monica Cortazar
Instituto de Ciencia y Tecnologia de Polimeros (CSIC), Juan de la Cierva 3, Madrid 28006, Spain
(Received 4 June 1997; accepted 14 July 1997)

Poly (vinyl alcohol) (PVAL) partially functionalized with monosuccinate groups was obtained by reaction of PVAL with succinic anhydride using triethylamine as catalyst and N-Methyl-2-pyrrolidone as solvent. The structure of the resulting polymers was determined by ^{1}H and ^{13}C nuclear magnetic resonance (n.m.r.) spectroscopy. The influence of the type of catalyst, solvent composition and temperature was evaluated. The activation energy was found to be 31.8 kJ/mol. ^{13}C n.m.r. spectroscopy was used for the determination of the sequence distribution of partially modified PVAL [considered as a vinyl alcohol (VAL)-vinyl succinate (VSU) copolymer]. The results obtained show that VSU units have a random distribution with a slight alternating tendency in the copolymer. High degrees of modification were obtained in the coupling of model bioactive amino compounds (benzocaine, phenethylamine and 2-amino-2-deoxy-D-glucose (glucosamine)) to monosuccinylated PVAL activated with ethyl chloroformate. Interaction experiments between glucosamine-carrying PVAL (PVAL-G) and Concanavalin A (Con A) in a phosphate buffer (pH 7.2) at room temperature showed that PVAL-G interacts with Con A. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: poly(vinyl alcohol) functionalization; monosuccinate groups; sequence distribution)

INTRODUCTION

Much attention has been paid to controlled release polymeric systems in which bioactive compounds are bound as pendant groups to a polymeric backbone via covalent bonds of limited stability in biological environments[1,2]. The macromolecular nature of the delivery system allows control of the period of effectiveness of the bioactive agent. A gradual release of the bioactive agent, whether pharmaceutical or agrochemical, can be achieved by hydrolytic or enzymatic cleavage of the linking bond.

Similar attention has been focused on many kinds of polymers having pendant sugar groups because of their interest as cell-specific biomedical materials[3], as pharmacological substances[4] and also as tools for investigating biomedical recognition phenomena[5].

In this respect, poly(vinyl alcohol) (PVAL) possesses several properties which make it a feasible carrier molecule for the preparation of macromolecular derivatives of bioactive agents[6,7]. However, sometimes this polymer has the disadvantage of low chemical reactivity with the bioactive compound at low temperature. A route to solve this problem may be the creation of a new more reactive functional group in its structure that enables the coupling of the bioactive agent.

In this paper we report the applicability of pendant monosuccinate groups, previously linked to PVAL, in the coupling of model bioactive amino compounds, such as benzocaine, phenethylamine and 2-amino-2-deoxy-D-glucose (glucosamine).

Preliminary experiments on the interaction of glucosamine-carrying PVAL (PVAL-G) with a lectin, Concavalin A, were also carried out.

EXPERIMENTAL

Materials

The PVAL was a commercial product (Gohsenol NLO5, 98.5–100 mol% hydrolysed). Its molecular weight, as measured by osmometry, was 22 000 g/mol, and purification was performed by conventional precipitation methods using a water/methanol mixture as the solvent/precipitant system. The purified polymer was dried to constant weight *in vacuo* in the presence of phosphorous pentoxide. N,N-Dimethylformamide (DMF) (from Panreac), pyridine (from Panreac) and triethylamine (from Scharlau) were purified following one of the conventional methods[8–10]. N-Methyl-2-pyrrolidone (NM2P) (from Fluka), dimethylsulfoxide (DMSO) (from Ferosa) and ethylchloroformate (from Fluka) were purified by distillation and then dried for a few days with a Merck 4 Å molecular sieve. Succinic anhydride (from Fluka) was dried to constant weight *in vacuo* in the presence of phosphorous pentoxide. 4-Dimethylaminopyridine (from Merck), 1-methylimidazole (from Fluka) benzocaine (from Fluka), phenethylamine (from Fluka), D (+) glucosamine hydrochloride (from Fluka), Concanavalin A (Con A) (from Fluka) and methyl orange (from Fluka) were used without further purification.

Reaction of PVAL with succinic anhydride

The PVAL was dissolved in NM2P at 90°C using a Pyrex double-walled reactor through which thermostatted water

* To whom correspondence should be addressed

was circulated. The solution obtained was maintained at the reaction temperature and the calculated amounts of tertiary amine and succinic anhydride were added whilst stirring. The polymer remained soluble throughout the process. After 22 h, the modified polymer was isolated by precipitation. Different precipitants were used to isolate the polymer, depending on the extent of modification. All polymers were purified by precipitation using methanol as solvent and distilled water or propan-2-ol/diethyl ether mixtures as precipitants, and then dried *in vacuo* in the presence of phosphorous pentoxide. In the kinetic study, the extent of modification was followed by sampling the reactant solution, extracting aliquots at specific time periods. The isolation and purification of the modified polymers was carried out as indicated above.

Characterization of succinylated PVAL

The ^{1}H n.m.r. spectra were registered either in deuterated pyridine at 50°C or in DMSO-d_6 at 70°C using a 200 MHz Bruker AM-200 spectrometer. ^{13}C n.m.r. spectra were obtained from a Bruker AM200 spectrometer operating at 50.4 MHz in deuterated pyridine at 50°C or deuterated water at 70°C. Values of the extent of modification were determined by means of titrimetric analysis of the carboxylic content, using a standard aqueous solution of sodium hydroxyde in the presence of phenolphthalein. The extent of modification was also determined by ^{1}H n.m.r. The results by both methods proved to be in good agreement.

Reaction of amino compounds with succinylated PVAL activated with ethyl chloroformate

The succinylated PVAL was dissolved in DMF at 90°C and the solution cooled to −8°C. Calculated amounts of triethylamine and ethyl chloroformate were added whilst stirring. After 1 h of stirring at −8°C, a cooled solution of the corresponding amino compund in DMF was then added dropwise. Stirring was continued at −8°C for another hour and then at room temperature for 18 h. Triethylamine hydrochloride was removed by filtration and the filtrate containing the modified polymer was precipitated into propan-2-ol or distilled water and filtered off. All polymers were purified by reprecipitation using DMF or THF as solvents and propan-2-ol/diethyl ether mixtures or distilled water as precipitants, then dried *in vacuo* in the presence of phosphorous pentoxide.

Characterization of amino compounds-carrying PVAL

The i.r. spectra were obtained on a Perkin-Elmer spectrometer using KBr discs. The ^{1}H n.m.r. spectra were registered either in deuterated water at 70°C for glucosamine-carrying PVAL or in deuterated pyridine at 50°C for benzocaine- or phenethylamine-PVAL adducts, using a 200 MHz Bruker AM-200 spectrometer. ^{13}C n.m.r. spectra were obtained from a Bruker AM-200 spectrometer operating at 50.4 MHz either in deuterated water at 70°C for glucosamine-carrying PVAL (PVAL-G) or in DMSO-d_6 at 80°C for the modified PVAL polymers with benzocaine or phenethylamine groups. The content of amino compound was quantitatively determined from ^{1}H n.m.r. For PVAL-G polymers by comparing the integrated intensities of the signals that appear in the interval 5.4–4.9 ppm (corresponding to the α and β anomeric protons as well as to the methine protons in the main chain) with the peak at 2.1 ppm (ascribed to the methylene protons in the main chain). For PVAL-benzocaine and PVAL-phenethylamine adducts, the composition was determined from the ratio of amido proton absorption to the methylene protons in the main chain absorption.

Interaction between PVAL-G and methyl orange

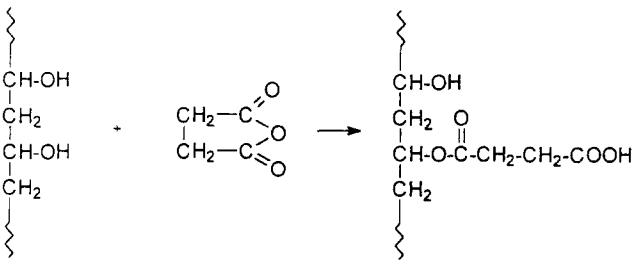
For this study we used a u.v. Perkin-Elmer 554 double-beam spectrophotometer. Phosphate buffer at pH 6.88 was used as solvent at room temperature. The methyl orange concentration was $1.0\ 10^{-4}$ M and the PVAL-G (97.2 mol% of glucosamine groups) concentrations were 2.5×10^{-3} M expressed on the basis of the glucosamine groups.

Interaction between PVAL-G and Con A

Buffer solutions (0.01 M phosphate, pH 7.2, containing 0.5 M NaCl, 0.1 m M $MnCl_2$, and 0.1 m M $CaCl_2$) of PVAL-G (97.2 mol% of glucosamine groups) and Con A were mixed and shaken at room temperature. At regular intervals, the turbidity of the solution was measured at 360 nm with a u.v. Perkin-Elmer 554 double-beam spectrophotometer.

RESULTS AND DISCUSSION

According to the literature, succinates of polysaccharides have been previously synthesized for various purposes by reaction of the polysaccharide with succinic anhydride in the presence of a tertiary amine as acylation catalyst[11–13]. A similar reaction with PVAL would be expected, according to the following scheme:



The structure of the resulting polymers was confirmed by ^{1}H and ^{13}C n.m.r. spectroscopy. The ^{1}H n.m.r. spectra of partially monosuccinylated PVAL show a signal at 1.9–2.1 ppm, which corresponds to methylene protons in the main chain. The peak at 2.9 ppm is due to the methylene protons in the grafted monosuccinate groups. The signal at 5.4 ppm can be assigned to the methine protons of modified units. The ^{13}C n.m.r. spectra of the same polymers (*Figure 1*) show the presence of a signal at 30.2 ppm and two bands centered at 172.8–173.8 ppm and 175.0 ppm indicative of the presence of methylene carbon atoms and carbonyl ester and carboxylic acid groups, respectively, corresponding to the grafted monosuccinate groups. The bands at 40.3, 43.6 and 45.9 ppm correspond to the methylene carbon atoms in the main chain. These bands arise from the different neighbouring units of the methylene carbons. The bands that appear in the interval 64.1–66.0 ppm are associated with methine carbons of unmodified units. The multiplet centered at 70.2 ppm corresponds to methine carbons of modified units.

Table 1 shows the effect of the type of catalyst and the solvent composition on the reaction of PVAL with succinic anhydride. Data presented in *Table 1* indicate clearly that no acylation reactions take place in the absence of pyridine. *Table 1* also shows that 4-dimethylaminopyridine (4DAP) is more efficient than the other catalysts used. This fact may be due to the well known excellent properties of 4DAP as

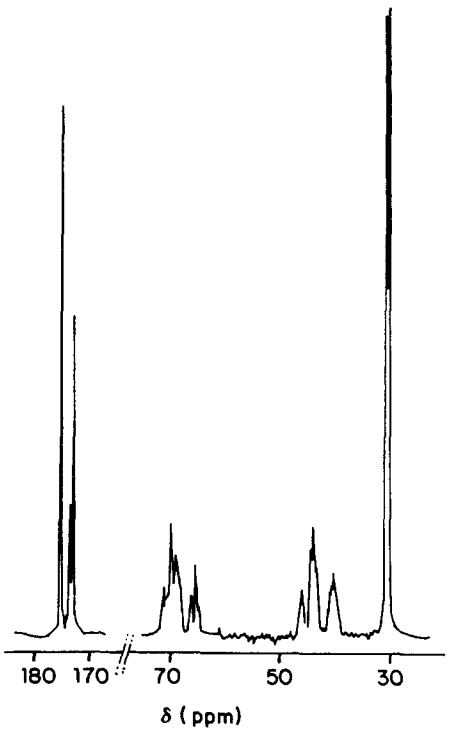


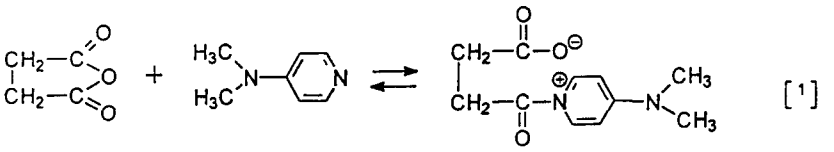
Figure 1 50.4 MHz ^{13}C n.m.r. signals of a partially monosuccinylated PVAL (58.7 mol% of monosuccinate groups) measured in deuterated pyridine at 50°C

Table 1 Reaction of PVAL ([OH] = 0.45 mol.l^{-1}) with succinic anhydride (0.45 mol.l^{-1}) using a tertiary amine (0.45 mol.l^{-1}) as catalyst at 60°C[a]

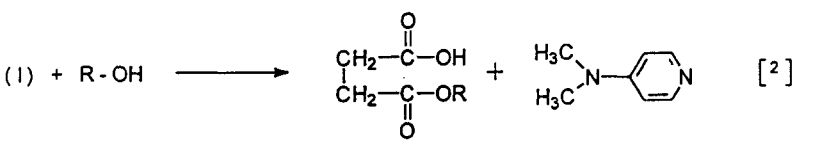
Tertiary amine	Solvent	Extent of modification (mol %)
—	NM2P	0
Pyridine	NM2P	19.6
1-Methylimidazole	NM2P	69.2
4DAP	NM2P	96.0
Triethylamine	NM2P	81.4
Pyridine	DMSO	9.5

[a]Time = 22 h.

acylation catalyst[14]. The nucleophilic catalysis of 4DAP in the esterification reaction can be attributed to the intermediate formation of the corresponding succinyl pyridinium salt (I):



(I)



With 4DAP the equilibrium (step 1) is shifted to the right to a much greater extent than for the other tertiary amines, due to the mesomeric stabilization[15] of the intermediate (I).

In addition to the nucleophilic catalysis of the acylation reaction by tertiary amines, the succinylation of alcohol is

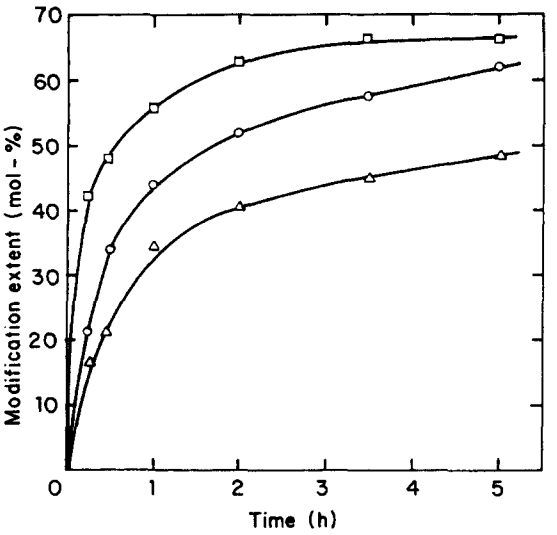
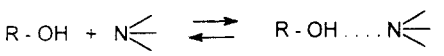


Figure 2 Kinetics of the reaction between PVAL ([OH] = 0.45 mol.l^{-1}) and succinic anhydride (0.34 mol.l^{-1}) using triethylamine (0.34 mol.$^{-1}$) as catalyst and NM2P as solvent at various temperatures: (□): 80°C (○): 60°C (△): 40°C

also sensitive to general base catalysis, as was demonstrated in the literature[16,17]. A pre-equilibrium between the amine and the alcohol to form a hydrogen-bonded complex was assumed:



This general base catalysis effect will increase with increasing basicity of the catalyst. The pK_A values of the acylation catalysts presently used decreased in the order: triethylamine (pK_A = 10.6) > 4DAP (pK_A = 9.7) > 1-methylimidazole (pK_A = 6.9) > pyridine (pK_A = 5.3). As may be observed from *Table 1*, triethylamine shows a lower catalytic activity than that expected from its pK_A value as compared with 4DAP. A tentative explanation of the higher catalytic activity of 4DAP might be that with the latter the extent of nucleophilic catalysis is predominant with respect to the general base catalysis.

As shown in *Table 1*, the extent of modification is also affected by the nature of the solvent. The lower extent of modification in DMSO as compared with the other solvent used may be due to a more pronounced solvatation of the intermediate (I) (step 1) by DMSO. It has been demonstrated[18] that the reactivity of N-acylpyridinium salts decreases with increasing solvatation.

Figure 2 shows the kinetic results for the reaction between PVAL and succinic anhydride at various temperatures using triethylamine as catalyst and NM2P as solvent. It

Table 2 Variation of the initial reaction rate (v) with temperature in the reaction of PVAL ([OH] = 0.45 mol.l^{-1}) with succinic anhydride (0.34 mol.l^{-1}) using triethylamine (0.34 mol.l^{-1}) as catalyst and NM2P as solvent

Temperature (°C)	v × 10^4 (mol l^{-1}.s^{-1})
40	0.76
60	1.15
80	3.01

can be seen that the esterification reaction is faster in the first stages of the reaction, and it was observed that for each one of the three reactions carried out total completion of the reaction was not attained. These results may be explained by assuming that the reactivity of the alcoholic groups is sterically affected due to the succinate groups incorporated into the polymeric chain in the first stages of the reaction. *Table 2* depicts the dependence of the initial reaction rate on the temperature in the reaction of PVAL with succinic anhydride. The activation energy of this process, calculated by the Arrhenius plot, is 31.8 kJ/mol.

On the other hand, the ^{13}C n.m.r. spectra of monosuccinylated PVAL (*Figure 1*) (which can be considered as a vinyl alcohol (VAL)-vinyl succinate (VSU) copolymer) exhibit three peaks at 40.3, 43.6 and 45.9 ppm in the region the methylene carbon resonance of the main chain. The presence of these peaks is due to the influence of the structure of the neighbouring units. The assignments of the three signals was done by comparison with the ^{13}C n.m.r. spectra of vinyl alcohol-vinyl acetate copolymers[19]. Therefore, the peaks at 40.3, 43.6 and 45.9 ppm were assigned to VSU–VSU, VAL–VSU and VAL–VAL diads, respectively.

Assuming an essentially equal degree of enhancement due to the nuclear Overhauser effect between the three methylene carbon peaks[20], the mol fractions of the three diads can be accurately determined from the intensities of the lines. The results obtained are shown in *Table 3*, where the η value is also shown. The block character η is an adequate way to characterize the sequence distribution in binary copolymers. It is given by the expression[21]:

$$\eta = \frac{[\text{VAL} - \text{VSU}]}{2[\text{VAL}][\text{VSU}]}$$

where [VAL–VSU] is the mol fraction of VAL–VSU diads, [VAL] and [VSU] being the mol fractions of VAL and VSU units, respectively. $0 \leq \eta < 1$ reflects a more block character, and $1 < \eta \leq 2$ means a more alternating tendency in the copolymer, than that expected from a random distribution; $\eta = 1$ means a completely random distribution.

As can be seen, the η values obtained for the VAL–VSU copolymers are between 0.9 and 1.2. These results show that the VSU units have a random distribution with a slight alternating tendency in the copolymer.

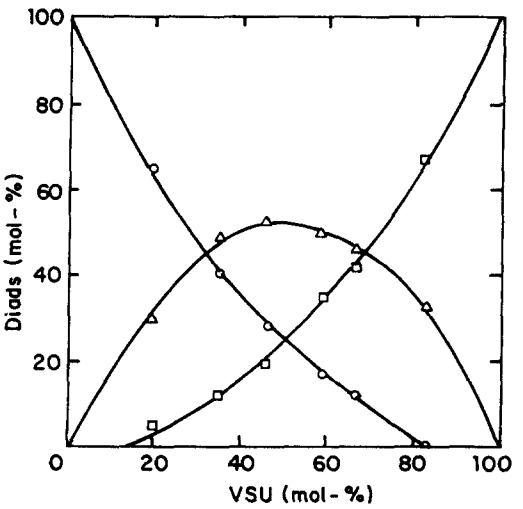
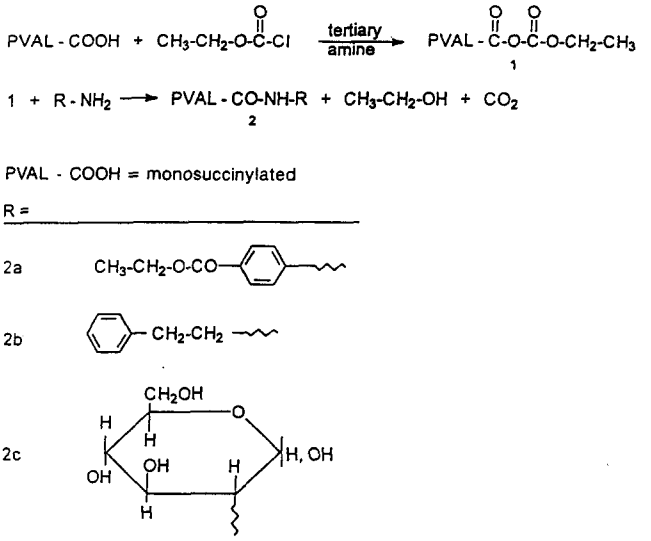


Figure 3 Plot of mole fraction of VAL-VAL (○) VAL-VSU (△) and VSU-VSU (□) diads vs. mole fraction of VSU in VAL-VSU copolymers

Figure 3 shows the mol fraction of diads as a function of the mol fraction of VSU in VAL–VSU copolymers.

It is known that carboxylic acids can react with chloroformates in the presence of a basic catalyst to give carbonic carboxylic anhydride. The aminolysis of the anhydride groups formed gives the expected amides with the formation of carbonic anhydride[22–24]. The synthesis of amide derivatives of monosuccinylated PVAL was accomplished as shown below:



In one approach, succinylated PVAL was allowed to react with ethyl chloroformate followed by aminolysis of the

Table 3 Composition, mole fractions of diads and parameter η of VAL-VSU copolymers

	Composition (mol-%)		Diad content (mol-%)			
Sample	VAL	VSU	VAL-VAL	VAL-VSU	VSU-VSU	η
1	80.2	19.8	65.2	29.8	4.9	0.9
2	64.4	35.6	40.2	48.3	11.5	1.1
3	53.9	46. 1	27.3	53.1	19.6	1.1
4	41.3	58.7	16.4	49.8	33.8	1.0
5	33.5	66.5	13.0	40.9	46.1	0.9
6	16.9	83.3	0.0	33.6	66.3	1.2

Table 4 Characteristic data of the ^1H and ^{13}C n.m.r. spectra of monosuccinylated PVAL containing pendant amido groups

Pendant group from	Group	δ (ppm) ^1H n.m.r.	δ (ppm) ^{13}C n.m.r.
2a	$-CH_3$	1.3	13.6
	$-CH_2-O-$	4.3	59.7
	$-C_6H_4-$	8.0-8.1	118.7, 124.0, 129.5, 142.9
	-NH-CO-	10.6	165.0-170.0
2b	C_6H_5-	7.3	127 9-139.0
	-NH-CO-	8.2	170.0-171.1
	$-CH_2-NH-$	2.8	43.2
	$C_6H_5-CH_2-$	3.7	34.7

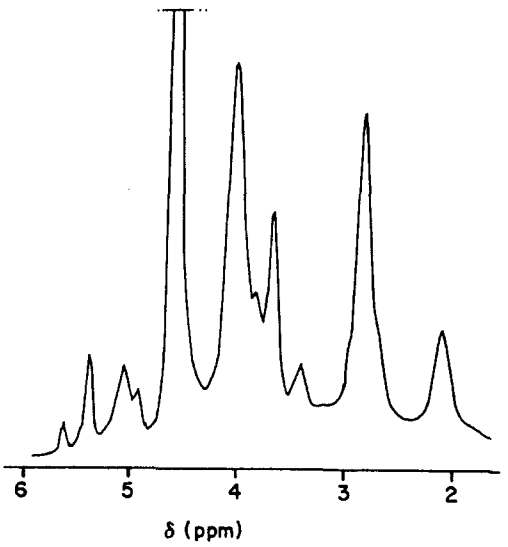


Figure 4 200 MHz ^1H n.m.r. signals of a PVAL-G polymer (97.2 mol% of glucosamine groups) measured in deuterated water at 70°C

formed anhydride groups with benzocaine (2a), phenethylamine (2b) or glucosamine (2c). The structure of the resulting polymers was confirmed by i.r. ^1H and ^{13}C n.m.r. spectroscopy. All i.r. spectra showed marked changes in the range of the carboxyl frequency compared to the spectrum of the succinylated PVAL. The resulting modified polymers show bands at 1665–1650 cm^{-1} due to the carbonyl in amido groups as well as the characteristic –NH-deformation vibration (amide II band) at 1550–1520 cm^{-1}. The ^1H and ^{13}C n.m.r. spectra of succinylated PVAL modified by pendant benzocaine (2a) or phenethylamine (2b) moieties show the characteristic bands of these groups, as can be seen in *Table 4*.

The ^1H n.m.r. spectrum of a PVAL-G (97.2 mol% of glucosamine groups) (2c), *Figure 4*, shows the signals corresponding to the grafted glucosamine groups. The

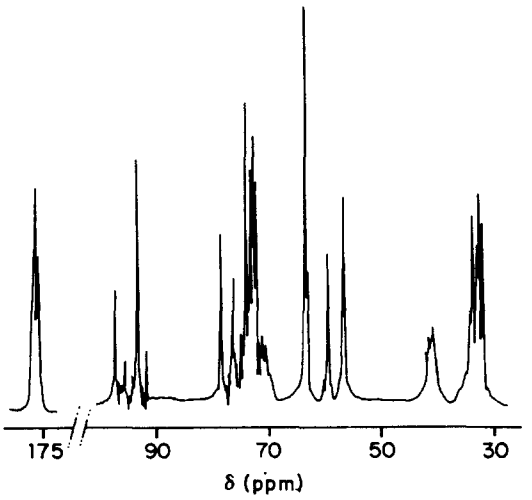


Figure 5 50.4 MHz ^{13}C n.m.r. signals of a PVAL-G polymer (97.2 mol% of glucosamine groups) measured in deuterated water at 70°C

multiplet centered at 3.6–4.0 ppm is due to the sugar protons[25]. The bands at 5.4 and 4.9 ppm can be assigned to the anomeric protons of the α and β isomers[25] respectively. The ^{13}C n.m.r. spectrum of the same polymer, *Figure 5*, shows bands between 97.0 and 55.0 ppm, which can be assigned to the sugar carbon atoms of the α and β isomers[25,26]. The multiplet at 175.9 ppm was attributed to carbonyl carbon atoms. The anomer ratio (α/β) of PVAL-G polymers was evaluated from ^{13}C n.m.r. spectra by comparing the peak at 92.6 ppm (ascribed to the α-anomeric carbon atoms) with the signal at 96.7 ppm (corresponding to the β-anomeric carbon atoms). The anomer ratio value was found to be 1.49, which is very similar to that previously obtained for the starting glucosamine hydrochloride. This means that the reaction of succinylated PVAL with glucosamine proceeds without anomeric change.

Table 5 shows satisfactory couplings in the reaction of succinylated PVAL with benzocaine (2a), phenethylamine (2b) or glucosamine (2c). It was found that the extent of modification in the reaction with benzocaine was notably lower than that of the reaction with phenethylamine. The difference probably arises from the different basicity of the two amines, being higher in the case of the aliphatic amine. It is noticeable that in the case of the reaction with glucosamine, the aminolysis reaction was practically quantitative.

It is important to point out that succinylated PVAL modified by pendant benzocaine (2a) or phenethylamine (2b) moieties, which are water-insoluble polymers, can find potential application as drug delivery systems. The release of the active compound from PVAL matrix can be achieved by hydrolytic or enzimatic cleavage of the linking bond.

Table 5 Reaction of monosuccinylated PVAL[a] (99.8 mol% of carboxylic acid groups), activated by ethyl chloroformate, with amino compounds in NNDF

Amino compound	[Triethylamine] (mol l^{-1})	[Ethyl chloroformate] (mol l^{-1})	[Amino compound] (mol l^{-1})	Extent of modification (mol-%)
Benzocaine (2a)	0.07	0.07	0.07	24.1
	0.15	0.15	0.15	53.6
Phenethylamine (2b)	0.07	0.07	0.07	45.4
	0.15	0.15	0.15	89.1
Glucosamine (2c)	0.30	0.15	0.15	97.2

[a][Carboxylic acid groups] = 0.14 mol.l^{-1}.

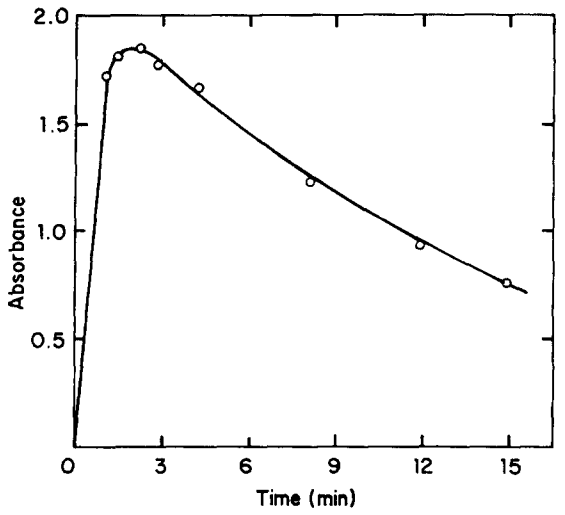


Figure 6 Turbidity as a function of time in the interaction of a PVAL-G polymer (97.2 mol% of glucosamine groups [PVAL-G] = 0.6 mg/ml) with Con A (0.6 mg/ml) in an aqueous buffer solution (pH 7.2) at room temperature

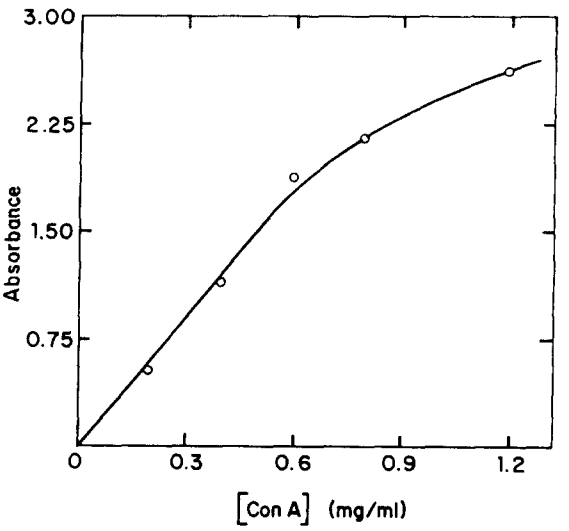


Figure 8 Turbidity as a function of Con A concentration in the interaction of a PVAL-G polymer (97.2 mol% of glucosamine groups [PVAL-G] = 0.4 mg/ml) with Con A in an aqueous buffer solution (pH 7.2) at room temperature

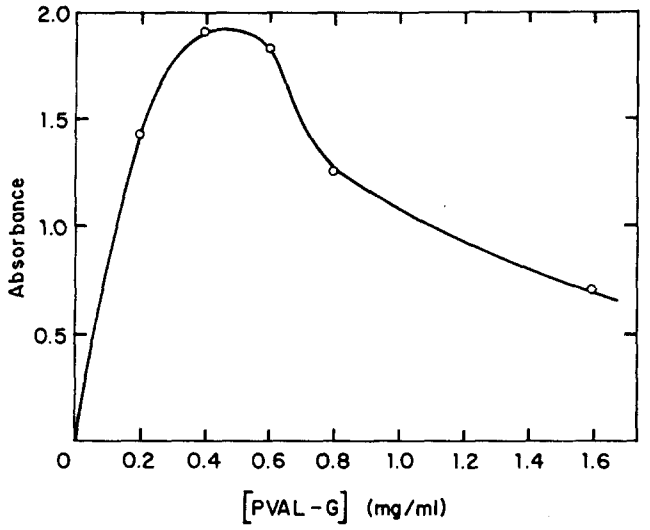


Figure 7 Turbidity as a function of PVAL-G concentration in the interaction of a PVAL-G polymer (97.2 mol% of glucosamine groups) with Con A (0.6 mg/ml) in an aqueous buffer solution (pH 7.2) at room temperature

On the other hand, the water-soluble sugar polymer (2c) is an amphiphile consisting of hydrophilic and hydrophobic moieties. These amphiphilic polymers are expected to show some specific interaction with organic solutes, such as methyl orange, in aqueous solutions[27]. However, under the experimental conditions used in this work, no interaction between PVAL-G (97.2 mol% of glucosamine groups) and methyl orange has been observed. Considering that the hydrophobic regions of the polymer play an important role in this interaction, it would be suggested that the lower hydrophobic character of the used PVAL-G in comparison with other polymers described in other papers[28,29], could explain the obtained results.

It is known that lectins are highly specific carbohydrate-binding proteins that agglutinate cells and/or precipitate glycoconjugates[28,29]. Concanavalin A (Con A), the most extensively investigated member of the lectin family, has been widely used in protein-carbohydrate interaction studies[30–33]. Con A is a tetramer with four carbohydrate binding sites and it recognises and binds, specifically α-D-glucopyranosyl, α-D-manopyranosyl or β-D-Fructofuranosyl residues. All these carbohydrate residues have the same configuration of the hydroxyl groups at the C-3, C-4, and C-6 positions of pyranosyl or furanosyl rings. The interaction of PVAL-G (97.2 mol% of glucosamine groups) with Con A at room temperature is reflected in *Figure 6*, where the time dependence of the absorbance, when an amount of Con A is added to an aqueous buffer-solution (pH 7.2) of the polymer, is shown. The curve obtained shows a precipitin type reaction with Con A. The turbidity of the solution increases rapidly, reaching a maximum after about 2 min, then gradually decreases and a precipitate is deposited. The precipitation can be interpreted on the basis of crosslinks between the tetrameric Con A molecules and multivalent polymer molecule carrying glucose residues.

Figure 7 shows the influence of PVAL-G concentration on Con A binding. The curve obtained allows the determination of the optimum PVAL-G/Con A ratio for maximum turbidity. The influence of the Con A concentration on the maximum absorbance value at a constant amount of PVAL-G (0.4 mg/ml) was also examined (*Figure 8*). This curve shows that, at relatively low concentrations of Con A, the turbidity increases with increasing amounts of protein. In addition, when D-mannose was added to the coagulated suspension mixture of PVAL-G and Con A, the precipitate was dissolved and a clear solution was restored. This phenomenon may be attributed to the fact that the mannose expels the glucose residues from the binding sites, resulting in dissociation of the multivalent interaction, according to the known fact that the ability of Con A to form a complex with mannose is greater than that with glucose[31]. This behaviour supports the attribution that the turbidity change in *Figure 8* is due to the specific recognition of sugars by the tetrameric protein Con A. Further investigation on the interaction of PVAL-G with Con A is now in progress.

ACKNOWLEDGEMENTS

We are grateful to the Dirección General de Investigación Cientifica y Técnica (DGICYT) for financial support. (PB 93–1250).

REFERENCES

1. Dumitriu, S., Popa, M. and Dumitriu, M., *J. Bioact. Compat. Polym.*, 1989, **4**, 151.
2. Kenawy, E. R., Sherrington, D. C. and Akelah, A., *Eur. Polym. J.*, 1992, **28**, 841.
3. Nakaya, T., Nishio, K., Memita, M. and Imoto, M., *Makromol. Chem., Rapid Commun.*, 1993, **14**, 77.
4. Rozalski, A., Brade, L., Kunh, H. M., Brade, J., Kosma, P., Appelmk, B. J., Kusumoto, S. and Paulsen, H., *Carbohydr. Res.*, 1989, **193**, 257.
5. Nishimura, S., Matsuoka, K., Furuike, T., Ishii, S., Kurita, K. and Nishimura, K., *Macromolecules*, 1991, **24**, 4236.
6. Schacht, E. H., Desmarets, G., Goethals, E. and Pierre, T. St., *Macromolecules*, 1983, **16**, 291.
7. Arranz, F., Bejarano, E. M. and Sanchez-Chaves, M., *Macromol. Chem. Phys.*, 1994, **195**, 3789.
8. Vogel, A. I., Furniss, B. S., Hannaford, A. J. Rogers, V., Smith, P. W. G. and Tatchell, A. R., Textbook of Practical Organic Chemistry, 5th edn. Longman, New York, 1989, p. 409.
9. Riddick, J. A. and Bunger, W. B., Techniques in Chemistry, Vol 2, 3rd edn. Wiley-Interscience, New York, 1970, p. 838.
10. Korshak, V. V., Vinogradova, S. V. and Vasnev, V. A., *Polym. Sci. USSR (English Translation)*, 1968, **10**, 1543.
11. Ferruti, P., Tanzi, M. C. and Vaccaroni, F., *Makromol. Chem.*, 1979, **180**, 375.
12. Vermeersch, J. and Schacht, E., *Makromol. Chem.*, 1986, **187**, 125.
13. Bruneel, D. and Schacht, E., *Polymer*, 1994, **35**, 2656.
14. Hofle, G., Steglich, W. and Vorbruggen, H., *Angew. Chem., Int. Ed. Engl.*, 1978, **17**, 569.
15. Wakselman, M. and Guibe-Jampel, E., *Tetrahedron Lett.* 1970, 1521.
16. Butler, A. R. and Gold, V., *J. Chem. Soc.* 1961, 4362.
17. Battye, P. J., Ihsan, E. M. and Moodie, R. B., *J. Chem. Soc., Perkin Trans*, 1980, **2**(2), 68.
18. Hofle, G., Steglich, W. and Vorbruggen, H., *Angew. Makromol. Chem.*, 1978, **90**, 602.
19. Moritani, T. and Fujiwara, Y., *Macromolecules*, 1977, **10**, 532.
20. Schaefer, J. and Natusch, D. F. S., *Macromolecules*, 1972, **5**, 416.
21. Ito, K. and Yamashita, Y., *J. Polym. Sci.*, 1965, **3**(Part A), 2165.
22. Boissonnas, R. A., *Helv. Chim. Acta*, 1951, **34**, 874.
23. Hartmann, M., Bauer, H. J. and Wermann, K., *Polym. Bull. (Berlin)*, 1985, **13**, 195.
24. Hartmann, M., Wermann, K., Clausen, F., Schwachula, G., Feistel, L. and Kraus, D., *Angew. Makromol. Chem.*, 1988, **161**, 185.
25. Kadokawa, J., Kato, M., Kobayashi, N., Karasu, M., Togaya, H. and Chiba, K., *Macromol. Rapid Commun.*, 1994, **15**, 971.
26. Klein, J. and Herzog, D., *Makromol. Chem.*, 1987, **88**, 1217.
27. Kobayashi, K. and Sumitomo, H., *Macromolecules*, 1980, **13**, 234.
28. Kobayashi, K., Sumitomo, H. and Ina, Y., *Polym. J.*, 1985, **17**, 567.
29. Goldstein, I. J. and Hayes, C. E., *Adv. Carbohydr. Chem. Biochem.*, 1978, **35**, 127.
30. Kobayashi, K., Sumitomo, H., Kobayashi, A. and Akaike, T., *J. Macromol. Sci., Chem.*, 1988, **25**, 655.
31. Nakamae, K., Miyata, T., Jikihara, A.and Hoffman, A. S. Biomater, J., *Sci., Polym. Edn.*, 1994, **6**, 79.
32. Ostafe, V., Petrescu, A. D., Szedlacsek, S. E. and Schell, H. D., *Rev. Roum. Biochim.*, 1988, **25**, 47.
33. Miyata, T., Jikihara, A., Nakamae, K. and Hoffman, A. S., *Macromol. Chem. Phys.*, 1996, **197**, 1135.