Carbohydrate Polymers 5 (1985) 181–199

Fluorescence Polarisation Studies on the Interaction of Cellulose Polycations with Polyanions

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(Received: 24 February 1984)

SUMMARY

The interaction of two commercially available cellulose-based polycations, Polymer JR-400 and Celquat L200, with polyacrylates (PAA-Na) and polyvinylsulphonates (PVS-Na) of various molecular weights was investigated by covalently labelling the polycations with dansyl hydrazine and then studying the fluorescence polarisation of the dansyl group. Celquat L200 was shown to form complexes that were more stoichiometric than the complexes formed by Polymer JR-400 at pH 6·1. This was attributed to the higher charge density and lower average molecular weight of the Celquat L200. At pH 3·5, no complex formation was observed with any of the samples of PAA-Na; PVS-Na samples did form complexes with the polycations and the ones with Polymer JR-400 were more stoichiometric than the complexes formed at pH 6·1.

The critical electrolyte concentration (C.E.C.) of each of the complexes was studied using sodium chloride as the electrolyte. The C.E.C. values for Polymer JR-400 complexes were in the order: PAA-Na (230 000) > PVS-Na (15 000) > PAA-Na (90 000) > PVS-Na (4300) > PAA-Na (5000). For complexes of Celquat L200, the order was: PAA-Na (230 000) > PVS-Na (15 000) = PVS-Na (4300) > PAA-Na (90 000) > PAA-Na (5000). The numerical values of the C.E.C. for complexes of Celquat L200 were found to be greater than the values for complexes of Polymer JR-400, thus implying that Celquat L200 binds more strongly to the polyanions. The order of binding indicated that, for a given molecular weight, the complexes formed by the polyvinylsulphonates are stronger than those formed by the polyacrylates.

Carbohydrate Polymers 0144-8617/85/$03.30 –

INTRODUCTION

Cellulosic polycations containing quaternary substituted nitrogen atoms have been widely used for some time in preparations for the treatment of human hair and other toiletries. Two that are available commercially are Polymer JR-400 (Union Carbide Corporation, 1969) and Celquat L200 (National Starch and Chemical Corporation, 1978). These are reported to be strongly attracted to the anionic surface of hair thereby conferring certain beneficial properties such as manageability and ease of combing.

The objective of the present work is the study of the nature of the complexes that are formed between the cellulosic polycations and polyanions. Two types of polyanions have been used as models: polyacrylates and polyvinylsulphonates. In each case a study has been made of the stoichiometry and the strength of the binding between the polyions. These measurements have been made possible as a result of our being able to label each polycation with a covalently linked dansyl hydrazone group. It was found that a study of the polarisation of the fluorescence of the labelled polycations allowed the interactions to be followed over a wide range of polyanion concentrations. The techniques used have also been employed by previous workers to study macromolecular interactions (Weber, 1952; Deranleau & Neurath, 1966; Kinoshita *et al.*, 1974; Cundall *et al.*, 1982).

MATERIALS AND METHODS

Polymer JR-400 was kindly donated by L'Oréal and is a cellulose derivative synthetically produced by Union Carbide Corporation (1969). Celquat L200, a graft copolymer based on cellulose, made by National Starch and Chemical Corporation (1978), was also donated by L'Oréal. Both polycations contain quaternary substituted nitrogens as the source of positive charges and are water-soluble. Their molecular weight per charge (Table 1) was calculated from their percentage nitrogen content (assuming one charge per nitrogen atom). Polyacrylic acids (PAA) of molecular weights 5000 and 90 000 were obtained from the Aldrich Chemical Company. PAA of average molecular weight 230 000 was obtained from BDH Chemicals Ltd. Both PAA of molecular weights 90 000 and 230 000 were dialysed using dialysis tubing obtained

TABLE 1
Base Molecular Weights of Polyelectrolytes

Polyelectrolyte	*Nitrogen (%)*	*Weight of polyelectrolyte per ionic site (air equilibrated)*
Polymer JR-400	1·55	900·9
Celquat L200	2·00	700·0
PAA-Na	–	94·0
PVS-Na	–	130·0

from Medicell International Ltd (pore size 24 Å, cut-off molecular weights 12 000–14 000) to eliminate low molecular weight molecules. All PAA were finally neutralised with sodium hydroxide to the sodium salt form (PAA-Na). Polyvinyl sulphonate-sodium salts of two different molecular weights (4300 and 15 000) were supplied by L'Oréal Laboratories, France (their average molecular weights were estimated using their intrinsic viscosities in aqueous solutions). Dansyl hydrazine (5-dimethylamino-1-naphthalene sulphonyl hydrazine) was purchased from Sigma Chemical Co. All other chemicals and reagents used were of Analar grade.

Dansylation of polycations

Dansyl hydrazine was used by Crabtree & Fujimori (1980) to react with the aldehyde groups in collagen. It is known that one end of the cellulose chain exists in the reduced state as an aldehyde group. Thus it is expected that dansylation will take place at that end of each cellulose chain. The dansylation procedures were similar for both polycations Polymer JR-400 and Celquat L200. The polycation (40 mg) was dissolved in carbonate/bicarbonate buffer, pH 10·0 (4 ml) and the dansyl hydrazine (20 mg in 2 ml of acetone) slowly added with stirring. The mixture was stirred at 57°C for 29 h. Sodium chloride (NaCl in crystalline form) was added to make the solution 1·0 M in NaCl, and 1·7 ml of this mixture was applied to a Sephadex G25 column (1·2 × 50 cm^2) and eluted with distilled water. The absence of extra NaCl in the eluted samples was checked by flame photometry

using appropriate standards. The elution of both dansylated polycations was monitored by their absorbance at 334 nm using a continuous flow cell set up in a Cecil CE272 spectrophotometer. The polycation concentration in these samples was determined by fluorescence spectroscopy using Eosin Y. The method was based on that of Jones (1980). The dansyl hydrazone group was estimated by its absorbance at 334 nm using $\epsilon_{334} = 3400$ litre mol^{-1} cm^{-1} (Tsuji *et al.*, 1974). Degrees of labelling were in the range 0·02 to 0·04 dansyl hydrazone groups per base mole of polycation (a base mole is the mass per positive charge (b.m.)). Base molecular weights are listed in Table 1. Absorption spectroscopy measurements were carried out on a Cecil CE 272 spectrophotometer.

Fluorescence intensities were recorded using a grating spectrofluorimeter constructed according to Cundall & Evans (1968).

Polarisation and total fluorescence intensity measurements were carried out on an instrument similar to that designed by Teichberg & Shinitzky (1973). Solutions were excited at 365 nm by means of a xenon lamp pulsed at 20 Hz, the excitation wavelength of which was selected by an Oriel interference filter. The emission was viewed by two EMI 9502B photomultiplier tubes which permitted the simultaneous recording of the horizontal (X) and vertical (Y) components of the fluorescence emission. The polarisation was estimated from

$$P = \frac{1 - X/Y}{1 + X/Y}$$

and the total fluorescence intensity (TFI) from

$$\text{TFI} = Y(1 + 2(X/Y))$$

Fluorescence lifetimes

These measurements were performed on a 199 fluorescence single photon counting spectrophotometer from Edinburgh Instruments. It has a similar basic function to that described by Lewis *et al.* (1973).

Polycation: polyanion ratios (PC : PA)

These were calculated as the ratio of positive sites on the polycation to negative sites on the polyanion. To facilitate the calculation of PC : PA ratios, all concentrations were expressed in base moles per litre.

RESULTS

(i) Estimation of the stoichiometry of polycation–polyanion complexes

The fact that the fluorescence excited lifetime of the covalently bound dansyl hydrazine approximately equals the rotational relaxation time of polycations dansyl-JR400 and dansyl-Celquat L200 allows the direct monitoring of the movement of these macromolecules when free or complexed to polyanions in solution. Thus, on addition of aliquots of polyanion solutions (3×10^{-3} b.m. litre^{-1}) to dansyl-Polymer JR-400 (6×10^{-5} b.m. litre^{-1}, pH ~ 6·0) the fluorescence polarisation (FP) increased linearly at first reaching a maximum value at a PC : PA ratio of approximately 1 : 1·2 and then curved off with a final slight decrease at higher polyanion concentrations (Fig. 1). The total fluorescence intensity (TFI) showed a consistent profile of a continuous decrease up to a PC : PA ratio of approximately 1·15 and finally levelled off at higher ratios. Within a given group of polyanions, e.g. PAA-Na salts, the greater increase in FP was obtained with the polyanion of higher

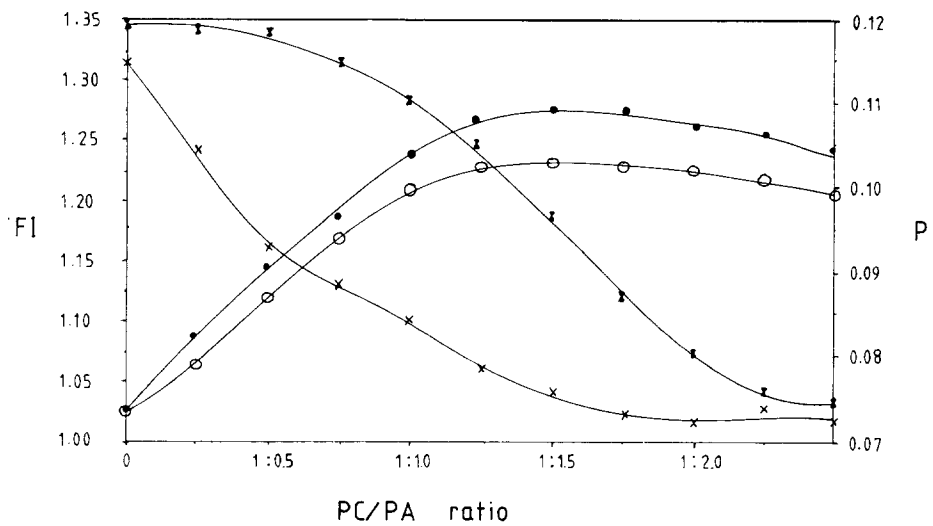

Fig. 1. Changes in the total fluorescence intensity (x, ⧗) and fluorescence polarisation (○, ●) of dansyl-Polymer JR-400 (6×10^{-5} b.m. litre^{-1}) on the addition of sodium polyacrylates (x, ○, mol. wt 5000; ⧗, ●, mol. wt 90 000) at pH 6·0.

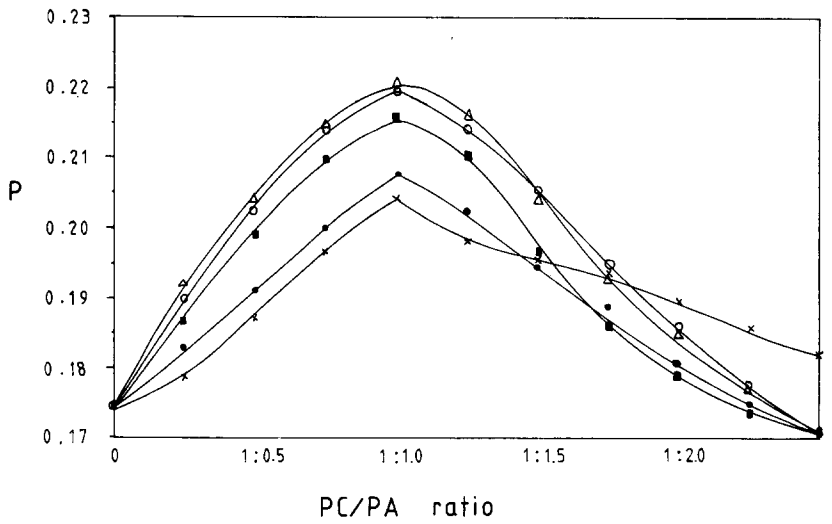

Fig. 2. Changes in the fluorescence polarisation of dansyl-Celquat L200 (10^{-4} b.m. litre^{-1}) on the addition of sodium polyvinylsulphonates (●, mol. wt 4300; ■, mol. wt 15 000) and sodium polyacrylates (x, mol. wt 5000; ○, mol. wt 90 000; △, mol. wt 230 000) at pH 6·2.

molecular weight, i.e. 230 000, followed by the 90 000 and 5000 polyanions respectively. The same trend was followed in the PVS-Na group where the polyanion of 15 000 molecular weight give a higher FP increase than that of 4300 molecular weight.

Similar PC–PA binding profiles (Fig. 2) were obtained when the same polyanions (5×10^{-3} b.m. litre^{-1}) were added to dansyl-Celquat L200 (1×10^{-4} b.m. litre^{-1}, pH ~ 6·2). A profound difference observed in these interactions was that the FP, after reaching a maximum value at PC : PA ratio approximately 1 : 1, decreased dramatically to a value lower than the initial one, i.e. in the absence of polyanion. As is illustrated in Fig. 2, PVS-Na of molecular weight 4300 gave a higher FP increase compared with PAA-Na salt of molecular weight 5000. In both Polymer JR-400 and Celquat L200 polyanion interactions PVS-Na seemed to give relatively higher FP increase compared with PAA-Na salts (Fig. 2). Two methods for estimating the stoichiometry values of PC–PA complexes in the case of dansyl-Polymers JR-400 were found to be necessary. The first is by extrapolating the initial and final parts of the curve to the height of the end point where their interception was

TABLE 2
Stoichiometry of Polycation-Polyanion (PC-PA) Complexes Determined by Fluorescence Polarisation

Polyanion and molecular weight	*Polymer JR-400, pH 6·0 (PC : PA)*		*Celquat L200, pH 6·2 (PC : PA)*
	Obtained by extrapolation	*Obtained by maximum polarisation*	
Polyacrylic acid-Na salt			
5 000	1 : 1·15	1 : 1·25	1 : 1
90 000	1 : 1·34	1 : 1·50	1 : 1
230 000	1 : 1·08	1 : 1·25	1 : 1
Polyvinyl sulphonate-Na salt			
4 300	1 : 1·17	1 : 1·25	1 : 1
15 000	1 : 1·15	1 : 1·25	1 : 1

taken as the stoichiometric value of the PC–PA interaction (Fig. 1). The other method, which gives values higher than those estimated by extrapolation, is by considering the amount of polyanion required to cause maximum FP increase. In the case of dansyl-Celquat L200 both methods resulted in similar stoichiometric values due to the abrupt fall of FP after maximum polarisation (Fig. 2). All stoichiometry values for both Polymer JR-400 and Celquat L200 polyanion complexes are listed in Table 2.

(ii) Effect of pH on the stoichiometry of polycation–polyanion complexes

The technique, methods and conditions that were used in this study were the same as those used for the estimation of the stoichiometries of the various PC–PA complexes, except that the pH values of all polycation and polyanion solutions used were reduced to pH 3·5 using 0·1 M HCl. When PAA-Na salts of all three molecular weights (i.e. 5000, 90 000, 230 000) were added to either dansyl-Polymer JR-400, or dansyl-Celquat L200, no significant change in the FP was obtained. Thus, there was no end point in the FP profile of these titrations (Fig.

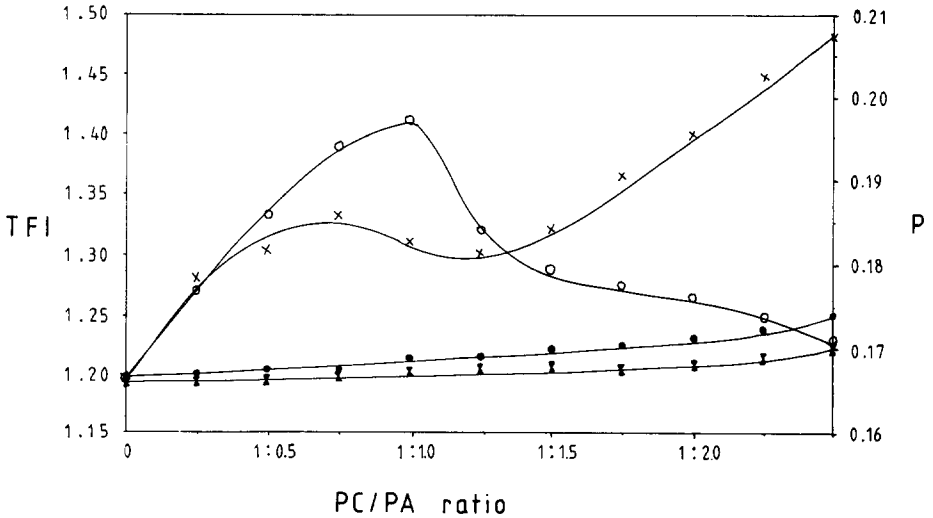

Fig. 3. Changes in the total fluorescence intensity (●, x) and fluorescence polarisation (⧗ o) of dansyl-Polymer JR-400 (6×10^{-5} b.m. litre^{-1}) on the addition of sodium polyvinylsulphonate (mol. wt 15 000, x, o) and sodium polacrylate (mol. wt 230 000, ●, ⧗) at pH 3·5.

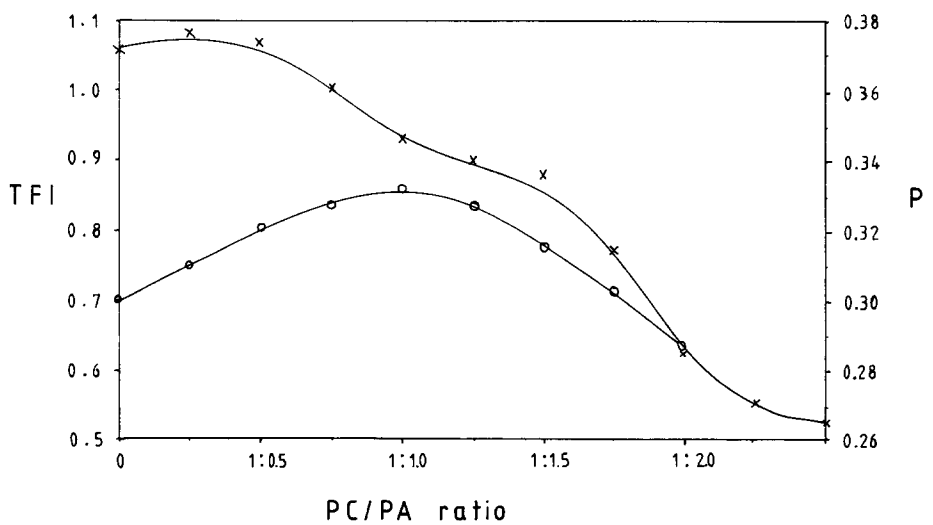

Fig. 4. Changes in the total fluorescence intensity (x) and fluorescence polarisation (o) of dansyl-Celquat L200 (10^{-4} b.m. litre^{-1}) on the addition of sodium polyvinylsulphonate (mol. wt 4300) at pH 3·5.

3). Also, the TFI of these titrations appeared to follow a similar pattern to the FP, i.e. there was no significant change. When PVS-Na salts (mol. wts. 4300 and 15 000) were added to either dansyl-Polymer JR-400 or dansyl-Celquat L200, the FP and TFI profiles obtained were found to be very similar to the corresponding ones at pH 6·01 (Figs 3 and 4). In the case of dansyl-Polymer JR-400 the FP drop after reaching a maximum value is much greater and the stoichiometry values obtained are nearer to PC : PA = 1 : 1. All stoichiometry values obtained at pH 3·5 were estimated to be very close to PC : PA = 1 : 1.

(iii) Effect of salt on polycation–polyanion interactions

The fact that the dansyl hydrazone group is covalently bound to polycations allows the study of the effect of NaCl on the various PC–PA complexes by the use of fluorescence polarisation methods. The effect of adding NaCl to pre-equilibrated solutions of dansylated polycation–polyanion complexes (PC : PA = 1 : 1, pH ~ 6·1, T° = 25°C) is illustrated in Figs 5 and 6. The end points of these titrations (i.e. when an extra-

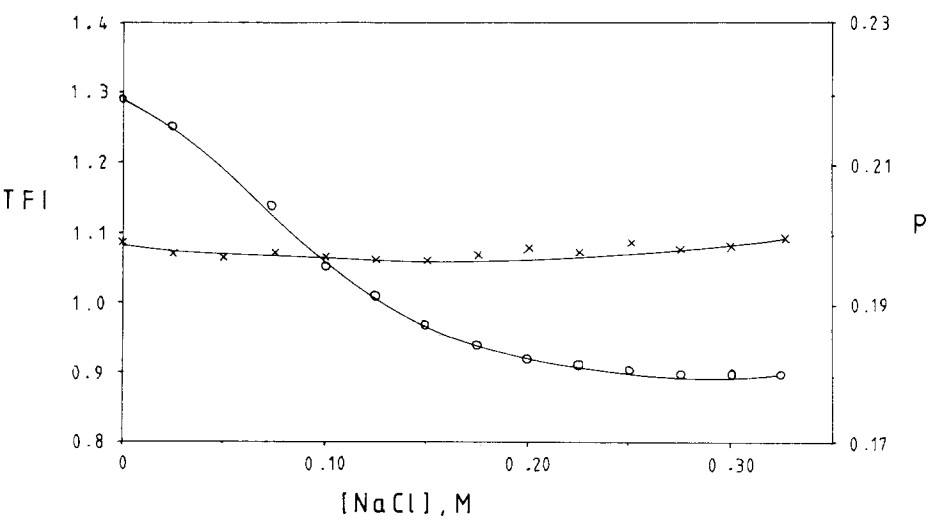

Fig. 5. The effect of the addition of sodium chloride on the total fluorescence intensity (x) and the fluorescence polarisation (o) of a stoichiometric (1 : 1) complex of dansyl-Celquat L200 and sodium polyacrylate (mol. wt 90 000) at pH 6·2. The concentration of each polyion was 10^{-4} b.m. litre^{-1}.

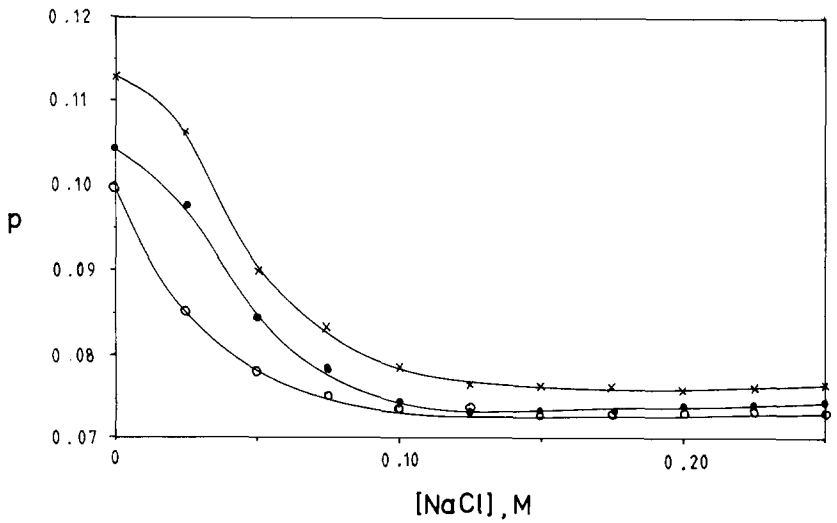

Fig. 6. The effect of the addition of sodium chloride on the fluorescence polarisation of stoichiometric complexes (1:1) of dansyl-Polymer JR-400 with sodium polyacrylates of various molecular weights (○, 5000; ●, 90 000; x, 230 000) at pH 6·0. The concentration of each polyion was 6×10^{-5} b.m. litre^{-1}.

polation of the decreasing polarisation intersects the extrapolation of the minimum polarisation plateau) are taken as the critical concentration (C.E.C.) values of the corresponding PC–PA complexes. The C.E.C. values for all PC–PA complexes studied are listed in Table 3 and are shown to vary throughout the different types of polycations or polyanions used.

(iv) Measurement of excitation lifetimes of dansylated polycations and their complexes

To eliminate any doubts about the origin of the changes in FP and TFI, and to show that these were not artifacts due to changes in the fluorescence excitation lifetime of the dansyl hydrazine group, but really were a direct indication of PC–PA formation or breakdown, the fluorescence lifetime of the dansyl group was measured at various stages of this study using a single photon counter. The lifetime of the dansylated PC was found to increase in the presence of polyanions and decrease in the presence of salt (Table 4).

TABLE 3
Critical Electrolyte Concentrations (C.E.C.) of PC : PA (1 : 1) Complexes Determined by Fluorescence Polarisation

Polyanion and molecular weight	*C.E.C. (M)*	
	Polymer JR-400 (6×10^{-5} *b.m. litre*$^{-1}$)	*Celquat L200* (1×10^{-4} *b.m. litre*$^{-1}$)
Polyacrylic acid-Na salt		
5 000	0·077	0·100
90 000	0·108	0·195
230 000	0·115	0·235
Polyvinyl sulphonate-Na salt		
4 300	0·085	0·200
15 000	0·110	0·200

TABLE 4
Fluorescence Lifetimes of Dansyl-Celquat L200 and Complexes of Dansyl-Celquat L200 and Sodium Polyvinylsulphonate (Mol. Wt 15 000) at pH 6·2. (The excitation wavelength was 356 nm and the emission wavelength was 510 nm)

Ratio of dansyl-Celquat L200 sites to PVS-Na salt sites	*Type of exponential decay considered*	*Fluorescence lifetimes (ns)*	*Standard deviation*	χ^2 *analysis*	*Mean lifetime (ns)*
Dansyl-Celquat L200 only (1×10^{-4} b.m. litre^{-1})	Single	11·998	0·141	14·28	–
	Double	$T_1 =$ 1·254 $T_2 =$ 13·43	0·069 0·064	1·757	11·24 ± 0·15
1:1	Single	12·073	0·135	13·161	–
	Double	$T_1 =$ 1·145 $T_2 =$ 13·543	0·074 0·061	1·708	11·39 ± 0·16
1:2·25	Single	12·40	0·109	10·423	–
	Double	$T_1 =$ 1·328 $T_2 =$ 13·724	0·118 0·058	1·666	11·88 ± 0·18
1:1 in 0·225 M NaCl	Single	11·612	0·142	15·989	–
	Double	$T_1 =$ 0·717 $T_2 =$ 13·061	0·062 0·060	1·626	10·37 ± 0·22

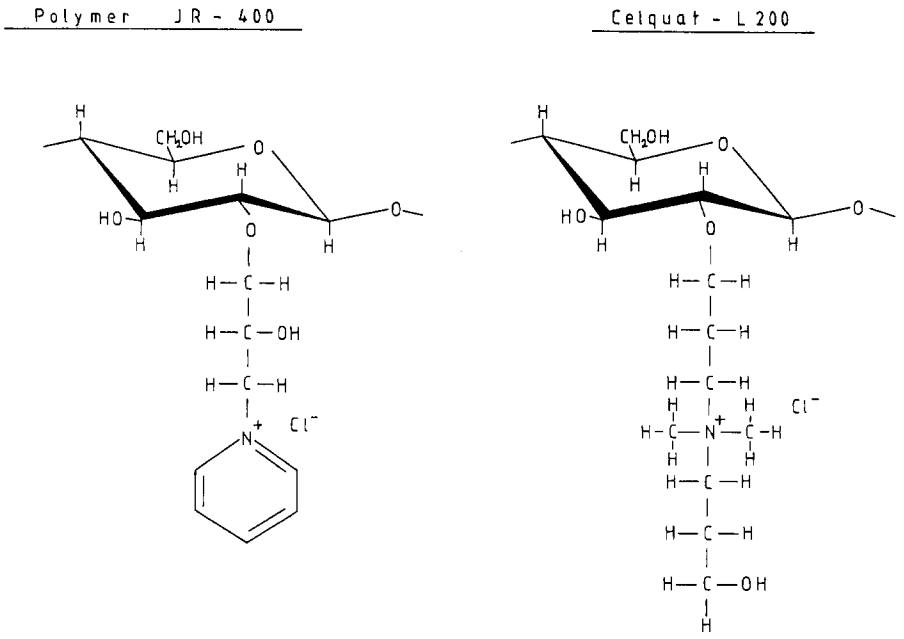

Fig. 7. Possible structures of charged glucopyranosyl units in Polymer JR-400 (National Starch and Chemical Corporation, 1978) and Celquat L200 (Union Carbide Corporation, 1969).

DISCUSSION

(i) Estimation of the stoichiometry of polycation–polyanion complexes

Polymer JR-400 and Celquat L200 are cellulose-based polycations and it would be expected that due to the positive charges repelling each other, each of the polycations would have an open conformation in aqueous solutions. The patents relating to the preparation of the two polymers (National Starch and Chemical Corporation, 1978; Union Carbide Corporation, 1969) suggest that the substituted glucopyranosyl residues have the structures given in Fig. 7. Thus, the main structural difference between them is that Celquat L200 has a higher charge density than Polymer JR-400 (approximately one positive charge per four glucopyranosyl units compared with one positive charge per five glucopyranosyl units, based on the nitrogen contents). Thus, it could

be argued that the former would have a more expanded conformation than the latter. The higher charge density of Celquat was confirmed by metachromatic studies using Eosin Y as the dye (Salveridis, 1982). From various preliminary studies on the two polycations including gel filtration through a Sephadex G-25 column and dialysis experiments using dialysis tubes of known pore size and cut-off ability, it was estimated that the average molecular weight of Polymer JR-400 was higher than that of Celquat L200 (Salverdis, 1982). Thus, Celquat L200 has a higher charge density with a lower average molecular weight when compared to Polymer JR-400. The difference in these two parameters was found to govern the behaviour profile with the various polymers in the present study.

The increase in polarisation of the dansylated polycations (DNS-Polymer JR-400 and DNS-Celquat L200), upon addition of various polyanions shown in Figs 1 and 2, indicated that either a PC–PA complex was formed, or that the lifetime of the dansyl group was decreased as a result of the addition of polyanion. Estimation of the lifetimes of DNS-Celquat L200–PVS-Na (molecular weight = 15 000) complexes at various PC : PA ratios indicated that the lifetime of the dansyl group increases as the PC : PA ratio increases (Table 4). Such an increase in the lifetime can only cause a decrease in the polarisation of the excited dye molecule and never an increase. This eliminates the possibility of the increase in polarisation being an artifact of a decrease in the fluorescence lifetime.

The lifetime results gave a better fit to a double exponential decay model curve, which suggests the possibility of the existence of two different lifetimes for the dansyl hydrazone group: one short lifetime (1·23 ns) and another longer lifetime (13·5 ns). Jones (1980) assigned two different lifetimes to dansyl protamine and attributed this to the possibility of the dansyl group being bound to two different amino acids in the protamine molecule or even more probably to the existence of the dansyl group in two different environments. Wahl & Wever (1967) amended their work on gamma-globulin conjugates by assigning two fluorescence lifetimes to dansylated 7S gamma-globulin. Also, Wahl & Lomi (1967) found several time constants for the fluorescent decay of dansylated lysozyme. To account for the presence of two lifetimes of the dansylated Celquat L200 it has been suggested that the fluorescence groups occupy different sites on the polycation. The existence of two excited lifetimes emitting simultaneously from the same dansyl

group is forbidden by 'Kasha's rule' (Kasha, 1950). The possibility that the longer lived fluorescence component was free dansyl hydrazine which had not been removed during the purification procedure was eliminated using thin layer chromatography (t.l.c.) with acetone as elution solvent. No free dansyl groups were found. The majority of the dansyl hydrazone groups might be in an aqueous environment as they are expected to be bound at the reducing end of the cellulose chain, but a small proportion may be found in the vicinity of hydrophobic environments. Such hydrophobic environments could be formed by the substituent groups on the quaternary nitrogen of Celquat L200 (Fig. 7). It is possible that two such groups might interact to create a hydrophobic region.

In Table 2, two methods are mentioned for the determination of complexes of Polymer JR-400. The value obtained from the maximum polarisation is higher due to the nature of the equilibrium that is present in the solution. This can be represented as:

$$PC.Cl + PA.Na = PC\text{–}PA + NaCl$$

Here, to form a complex from all the labelled polycation (PC.Cl) an excess of the polyanion will be required and thus the apparent stoichiometry will be greater when the point of maximum polarisation is used.

As Table 2 indicates, the stoichiometric values of the complexes formed between JR-400 and the various polyanions used differ from 1:1. Michaels *et al.* (1965), studying PC–PA interactions, stated that a stoichiometric reaction can only occur when the reacting polyelectrolytes are in a sufficiently open extended conformation. The same authors postulate that the quantitative pairing of ionic functions depends on the ability of long portions of the oppositely charged polymer chains to come into juxtaposition, on the rearrangements of rotational conformations to bring the resulting groups close enough to form stable links, and possibly on local re-alignment to correct short-range errors in the pattern of ionic pairing. All these requirements for a stoichiometric complex require high flexibility of the polymer chains in aqueous solution. The majority of the polyelectrolyte complexes described in the literature are products of the interaction of sterically irregular polymers. These complexes are shown to contain at least 20–30% of pairs of disconnected units, i.e. units incorporated in defects as a result of the interaction of sterically irregular macromolecules (Michaels *et al.*, 1964), Zezin & Kabanov, 1982). As far as Polymer

JR-400 is concerned such flexibility would be hindered by its high average molecular weight and the substituent groups on its quarternary nitrogens. Also, its low charge density would cause Polymer JR-400 to be in a relatively folded conformation in solution. In the case of the PAA-Na salt used as a polyanion in the JR-400–PAA-Na complexes, at pH 6·0 (Fig. 1) a certain proportion of the carboxyl groups (~ 9%) would be fully protonated and so unavailable for complex formation. In such a complex (i.e. JR-400–PAA-Na) there would be trapped unreacted sites on both polymer chains causing deviations from a stoichiometric value of 1:1. In contrast to Polymer JR-400, Celquat L200 was found to form PC–PA complexes with a stoichiometric value very near to 1:1. This difference may be due to the higher charge density on Celquat L200, which will cause it to have a more open conformation. Also, its lower average molecular weight would be expected to be associated with a higher flexibility. In both polyanion types used, i.e. PAA-Na and PVS-Na, the negative charges are uniformly spread along the polymer backbone with an intersite distance at 0·25 nm (Masamitsu *et al.*, 1977) and they bear quite an open spherical conformation at natural pH. When one considers the intersite distances in the two polycations (approximately 26 nm for Polymer JR-400 and 21 nm for Celquat L200) it is apparent that the complexes cannot be formed by the simple linear pairing of a polycation with a polyanion. To yield the observed stoichiometric values, several polycations must come into close association with a polyanion. Thus, a 1:1 stoichiometric complex is a remarkable occurrence since it implies a very complex entanglement of the polyions. It would appear that deviations from the stoichiometric point ought to be common in complexes where the intersite distances of the polyanions are not identical. Such deviations have been reported by others workers (Cundall *et al.*, 1982; Vij, 1981).

Another phenomenon that is apparent in Figs 1and 2 is that fluorescence polarisation (FP) drops after maximum polarisation has been reached. Cundall *et al.* (1982), working on the binding of heparin to dansylated protamine, found that FP, after reaching a maximum value, either stayed unchanged or decreased slightly. This unexpected drop in FP, which was higher for Celquat L200 than for Polymer JR-400, is thought to be an effect of both lifetime increases (Table 4) and also collapsing of the effective volume of the complex formed. The total fluorescence intensity (TFI) for all PC–PA complexes studied in this work kept a constant profile which is represented by an abrupt drop

as the PC : PA ratio reaches 1:1 after which it changes only slightly (Fig. 1). The TFI changes are a measure of the environment in which the dansyl hydrazine group exists (Li *et al.*, 1974). Thus the decrease in the TFI of the dansyl hydrazone suggests a change in its environment from a hydrophobic one (non-polar) to a more polar hydrophilic one (aqueous). Subsequently, this allows one to assume a change in the conformation at least of the terminal segment of the polycation chain (where dansyl hydrazine is expected to bind) during PC–PA complex formation.

(ii) Effect of pH on the stoichiometry of polycation–polyanion complexes

At pH 3·5 the carboxyl groups of the PAA-Na salts would be expected to be mostly protonated (Mandel, 1970). This renders them unavailable for PC–PA complex formation via electrostatic interactions, although other types of weaker interaction such as hydrophobic interactions or hydrogen bonding could take place. Both reacting polymers must come close together before any such secondary interaction occurs.

As Fig. 3 indicates there is no end point in the titration profiles of fluorescence polarisation when PAA-Na salt is used as a polyanion at pH 3·5, with either Polymer JR-400 or Celquat L200. The reason for this, already described above, lies in the fact that the carboxyl groups are unavailable for PC–PA complex formation. Any indications of secondary interactions taking place appear only at high PC : PA ratios when the FP increases slightly. This slight increase might be due to an increase in viscosity which is expected to occur at high PC : PA ratios. Finally this slight increase in the FP at high PC : PA ratios might be attributed to both secondary interactions and the increase in viscosity acting simultaneously.

In the case where PVS-Na is used as a polyanion, the pH change from 6·1 to 3·5 seemed to have no effect on the stoichiometry of the Celquat-L200-PVS-Na complexes (Fig. 4), whereas Polymer JR-400–PVS-Na complexes become more stoichiometric at pH 3·5.

(iii) Effect of salt on polycation–polyanion interactions

The initial abrupt fall in FP on the addition of NaCl to PC–PA complexes (PC : PA = 1:1) (Figs 5, 6) is an indication of the complex

breaking down. It is not an artifact due to an increase in the lifetime of the dansyl hydrazone group in the presence of salt. As Table 4 illustrates, the lifetime decreases in the presence of salt. Such a decrease could only cause an increase in the FP and never a decrease. In the present work the anionic groups on the polyanion chain are exclusively carboxylates (PAA-Na) and sulphonates (PVS-Na).

C.E.C. VALUES

The C.E.C. has been defined previously as the electrolyte concentration required to remove all turbidity from a PC–PA system. C.E.C. values of PC–PA complexes associated with sulphate groups have been reported to be much higher than those associated with carboxylates or phosphates (Scott, 1973; Vij, 1981).

A similar trend was followed in the present work where PVS-Na salts gave relatively high C.E.C. values compared with PAA-Na salts, considering the lower molecular weights of the former salts. C.E.C. values obtained from PVS-Na salt (4300) and PAA-Na (5000) with an approximately similar molecular weight are a good example of this. The C.E.C. values obtained for PVS-Na (4300) were found to be much higher to those of PAA-Na (5000) (Table 3) bearing in mind the fact that at pH 6·1 where the study took place the PAA-Na salts are not fully ionised. This in turn would reduce their C.E.C. values.

The selective binding of Na^+ in connective tissues by acidic polysaccharides has been discussed by Veis (1970) who suggested that Na^+ binds preferentially to phosphate ($-OPO_3^{2-}$) or carboxylate ($-COO^-$) groups compared with sulphate ($-OSO_3^-$) groups. This is explained by the fact that $-OPO_3^{2-}$ and $-COO^-$ groups are more polarisable than water so they can displace some of the solvation shell of the Na^+ ion and so can bind to it more strongly. The $-OSO_3^-$, being less polarisable than water, cannot displace the solvation shell of a Na^+ ion and so remains at a distance and is not as strongly held. Thus NaCl is expected to be more efficient in complex breaking of PC–PA systems where the ionisable sites on polyanions are either $-OPO_3^{2-}$ or $-COO^-$.

The C.E.C. values of various PC–PA complexes were reported (Scott, 1973; Cundall, 1982; Jones, 1980) to mainly depend on the type of binding site, the charge density, intersite distance and molecular weights of the interacting polymers. Scott (1973) postulated that there

is a maximum value of C.E.C. for a given polymer which is determined by its chemical structure and he suggested the identification of polymers using C.E.C. values. Moreover, he stated that the dependence of C.E.C. on the molecular weight is not very pronounced for high molecular weight polymers. This statement was supported from his studies on cetylpyridinium polyacrylate complexes when he noticed that the C.E.C. value decreased only by 25% for a 300-fold decrease of molecular weight. Jones (1980) has verified this trend working on fractionated heparin interactions with polycations using fluorescence polarisation methods.

In a given polyanion group (PAA-Na or PVS-Na) the higher molecular weight polyanions gave the higher C.E.C. values (Table 3). A comparison between the two polyanion groups proves that the sulphonate groups (PVS-Na) are associated with higher C.E.C. values when compared with carboxylate groups (PAA-Na).

Comparing the two polycations used, Polymer JR-400 and Celquat L200, the latter gave the higher C.E.C. values. This might be attributed to the higher charge density of Celquat L200 even though its average molecular weight is lower than that of Polymer JR-400.

CONCLUSIONS

The fluorescence polarisation method has proved to be a very useful method for studying the stoichiometry and strength of binding of PC–PA complexes, provided that a successful labelling of either the PC or PA with a covalent fluorescent probe is feasible. Also, a main feature throughout this work that could be generalised is that the charge density parameter in these complexes was found to be a more important influence on the stoichiometry and strength of binding than the molecular weight parameters. Thus Celquat L200, even though it has a lower molecular weight than Polymer JR-400, nevertheless, because of its higher charge density, forms stronger and more stoichiometric complexes.

ACKNOWLEDGEMENTS

We wish to thank M. G. Vanberbergh of L'Oréal for valuable advice and we are grateful to L'Oréal for financial support for C.S.

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