Physical and Biological Properties of Water Soluble Polyelectrolyte Complexes

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ABSTRACT: Water soluble non-stoichiometric polyelectrolyte complexes have been investigated as potential drug carrier systems for parenteral administration. Complexes between the polycationic quaternized poly(vinyl imidazole) (QPVI) and an excess of a higher molecular weight partially sulfonated dextran (pDS) were designed to inherit the biocompatible properties of dextran. Polyelectrolyte complexes with an overall anionic nature prepared from excess poly(methacrylic acid) (PMAA) and low molecular weight quaternary polyamines were also studied. Sedimentation velocity techniques and size exclusion chromatography showed that complexation was present in all of the systems studied under the appropriate conditions. These studies also indicated that an increasing [polycation]/[polyanion] ratio resulted in a smaller, more compact complex conformation. Platelet aggregation studies showed that toxic aggregatory effects normally induced by the polycations, and to a lesser extent the pDS, were eliminated *in vitro* when they formed part of a soluble polyelectrolyte complex. *In vivo* distribution studies in mice using ^{125}I-labelled polycation complexed with pDS or PMAA showed accumulation of 40–50% of the administered dose in the liver after 2 hours. The polycation present in these complexes appears to have been prevented, to some extent, from interacting with negatively-charged biological surfaces, such as platelets *in vitro*. However this complexation was not sufficient, at least for the macromolecules we have examined, to prevent the extensive incidence of unwanted interactions *in vivo*, leading to removal of the polyelectrolyte complexes from the circulation. In conclusion it has been shown that the ionic bonds which hold this type of complex together, are not sufficiently strong *in vivo* to preserve a stable complex structure.

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INTRODUCTION

A wide range of carrier systems for the delivery of drugs have been investigated. These have been the subject of a number of recent reviews [1–3]. Those systems following the intravenous route of administration include both soluble macromolecular conjugates and solid microparticles. Steric factors exercised by macromolecular carriers can in certain cases be responsible for protecting the drug molecules from enzymatic attack, or from unwanted interactions with non-target tissue. Additionally a decrease in antigenicity may be observed. This steric barrier, which can also be introduced around particulates by surface modification, is believed to be responsible in many cases for a substantial increase in circulation time. Increased tolerance within the vasculature would appear to be due to the reduction of any interaction between the carrier, and biological macromolecules and cells. This leads, directly or indirectly, to an increased avoidance of the cells of the mononuclear phagocytic system (MPS). It is the MPS which is responsible for the clearance of foreign materials from the circulation. The phagocytosis of blood-borne foreign material by the MPS is understood to be mediated by the interaction of serum factors called opsonins. The opsonized material adheres to the phagocytic cell membrane and is subsequently engulfed by the phagocytosing cell. It is postulated that phagocytosis can be reduced if the interaction of carrier systems with proteins and cells of the vascular compartment can be largely or completely prevented.

The aim of this work was to investigate the potential of a class of interpolymer complexes termed non-stoichiometric polyelectrolyte complexes (N-PEC) as drug carrier systems. These structures have been reviewed by Kabanov [5], Philipp [6], Tsuchida [7] and Petrak [8]. Certain conditions of stoichiometry, relative molecular mass, pH and ionic strength have been described [5,9] under which mixtures of oppositely charged polyelectrolytes can form discrete water-soluble N-PEC structures. A self-assembly principle based on structural complementarity is responsible for their formation. This is perhaps similar to the interactions due to non-covalent forces found in many biochemical processes. N-PEC differ from the more commonly observed polyelectrolyte complexes in which phase separation occurs and stoichiometric polyelectrolyte complexes are precipitated. The rules governing the formation of N-PEC require that one of the polyelectrolyte species be in excess, and that this component must also have a higher degree of polymerization than the other. Fully ionized polyelectrolytes are normally required making the solution pH a factor in complex formation. Addi-

tionally the ionic strength of the media is also an influence. In many systems both a minimum and maximum ionic strength is indicated for N-PEC formation. It has been proposed from light scattering and sedimentation velocity data [5], that the composition of an individual N-PEC "particle" consists of a single high molecular "lyophilizing" chain, complexed with a number of the smaller "blocking" polyelectrolyte molecules. A conformation might be adopted by the N-PEC in which the "hydrophobic microdomains" formed by the short stretches of complexed polymer chain reorient themselves to form a "core." The uncomplexed, lyophilizing, high molecular weight chains, which provide N-PEC solvency, could also provide a protective effect when used as a drug carrier. To be feasible such a system must be able to provide protection for the drug by avoiding both degradation (until required by release) and interaction with opsonins and consequent take-up by the cells of the MPS. A drug molecule might be linked to the blocking polyelectrolyte backbone or in the case of a peptidic (pro)drug might form that species. In the complexes studied the blocking polyelectrolyte is always polycationic and the "outer" lyophilizing polyelectrolyte is either a polyanion, or an anionic-nonionic copolymer.

The biological behavior of polyelectrolytes and their use in drug delivery has received attention. Polyanions, whether natural or synthetic, are known to elicit a range of biological responses [10]. Poly(carboxylic acids) in particular are known to act as macrophage activators [11]. This effect could well be related to factors such as the degree of polymerization and the charge density as the moderately charged polysaccharides found on cell surfaces do not elicit this response. Polyanionic carriers have been the subject of investigation with regard to their potential as a carrier system [12]. Polycations such as poly(L-lysine) have been investigated as carriers [13] but exhibit undesirable effects. This arises from the universal property of polycations to interact strongly and non-specifically with cell surfaces thereby precluding their use for specific targeting. McCormick et al. [14] have investigated the use of a cationic N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer as a potential drug carrier. They postulated that a biocompatible polycation might be useful for delivery to cell surfaces. The cationic HPMA proved less toxic than other polycations *in vitro* but rapidly accumulated in the liver following intravenous administration. A number of non-ionic polymers such as dextran show an ability to avoid uptake by the MPS, and have been proposed as a carrier system [15–17].

N-PEC are proposed to have different macromolecular properties than polyelectrolytes [5]. Their potential for drug delivery therefore

warrants investigation. We first examined polyelectrolyte complexes formed from poly(methacrylic acid) (PMAA) with either quaternized poly(vinylimidazole) (QPVI) or quaternized poly(vinyl pyridine) (QPVP). These would be expected to have an outer polyanionic coat. Additionally complexes have been examined which were designed to have an outer shell of non-ionic, biocompatible dextran. A small number of ionic groups were introduced into the dextran by partial sulfonation, and this was complexed with the polycation QPVI. It was hoped that the complex would adopt a conformation in which the ionic groups and bonds would be inaccessible in the core, and that the carrier would inherit the desirable properties of dextran. The choice of dextran follows from its extensive use as a plasma expander in human patients.

Sedimentation velocity experiments, in both preparative and analytical ultracentrifuges, were conducted to determine whether complexation had occurred. Size exclusion chromatography also gave indications of the changing conformation of the complex with different polyelectrolyte ratios. Platelet aggregometry was used to examine the interaction between the complexes and platelets *in vitro*. The importance of looking at platelet interaction lies in the involvement of platelets with blood coagulation and thrombogenicity. This is thought to be a major factor in the determination of thrombogenicity when any foreign particle is put into blood. Whilst platelet aggregation studies cannot be linked directly to the processes involved in uptake by the MPS, it does provide a sensitive, and reliable measure of the interaction between platelets and carrier. Some indication of the likely incidence of unwanted interactions *in vivo* could be gained from these results. In particular undesirable carriers can be rejected from further investigation. Finally, animal distribution studies in mice were conducted to determine the circulating half-life of these potential N-PEC carriers *in vivo*.

EXPERIMENTAL

Preparation and Characterization of Polyelectrolytes

Preparation of Poly(methacrylic acid) (PMAA)

Poly(methacrylic acid) was prepared by free radical polymerization. Methacrylic acid monomer (BDH) was distilled prior to use. The initiator 4,4-Azo-bisisobutyronitrile (Fluka) and NaCl (BDH) were both recrystallized. A 10% solution of NaCl in distilled water (920 ml) was prepared with pH adjusted to 10.5 (by addition of NaOH). This was purged with N_2 and equilibrated at 60°C. Monomer was added to the solution (155 ml, 1.8 mole) followed by the initiator (1.2 g). This mix-

ture was left stirring overnight. Filtration and extensive dialysis against distilled water was followed by freeze drying. Fractional precipitation of the PMAA was achieved by sequential addition of a miscible non-solvent (acetone) to a PMAA solution ($MeOH/H_2O$) in a round bottomed 5 l flask thermostatted in a water bath. The polyelectrolyte was characterized by size exclusion chromatography.

Preparation of Poly(vinylimidazole) (QPVI)

QPVI was synthesized according to the method of Henrichs et al. [18] from N-vinylimidazole (86 ml), distilled water (125 ml) and 4,4′-azobis(4-cyanovaleric acid) (0.2 g). Fractionation of the sample was achieved by a series of ultrafiltrations in aqueous solution using Amicon membranes (YM10 and YM30). This gave a sample in the molecular weight range of 10,000–30,000. Quaternization of the sample with excess bromoethane was carried out in dry DMF at 70°C overnight.

Preparation of Quaternized Poly(vinyl pyridine) (QPVP)

Poly(4-vinyl pyridine) (P4VP) (Polyscience, M_w 73,000) was purified by ultrafiltration (Amicon, XM50) followed by freeze drying. The P4VP (2.0 g) was dissolved in a solution of nitromethane (300 ml) and MeOH (20 ml). 4-Bromoanisole was added and the mixture left refluxing for 30 hrs at 60°C. Excess bromoethane (Aldrich) (5 ml) was added and treated a further 30 h under the same conditions. The reaction mixture was precipitated into ether, washed, filtered, dried and redissolved into distilled water. The product, poly(4-vinyl-1-ethyl pyridinium bromide)-co-(4-vinyl-1-anisoylpyridinium bromide), contained approximately 10% of the quaternized groups which had an activated aromatic ring suitable for ^{125}I labelling.

Preparation of Ionene 3X (I3X)

Ionenes (ionic amines) are polycations which can be formed by reaction between diamines and dihalogen compounds. The polymerization is a type of Menschutkin reaction and proceeds at a fairly fast rate when both compounds are present in equimolar amounts. Ionene 3X was prepared according to the method of Tsuchida et al. [19] with modifications to give a polycation with end groups suitable for radiolabelling. Equimolar amounts of N,N,N′,N′-tetramethylenediamine (TED) (1.72 g) and p-xylene dichloride (XDC) (2.0 g) were dissolved in dry DMF in a 3-neck flask fitted with a condenser. The reaction mixture was maintained at 40°C. After 7 minutes more TED (1 ml) was added to ensure amino-terminated ends. Then, 4-methoxybenzoyl

chloride (MBC) (2.0 ml) was added to end-cap the polycation. The product was precipitated into MeOH, filtered, washed and dried.

Vapour Pressure Osmometry

The number-average molecular weight of I3X was measured by vapour pressure osmometry (Knauer, model No. 731.1100000, fitted with a 70–130°C universal thermister probe). An aqueous solvent system was used, equilibrated at 37°C for 2 h and NaCl was used as a calibrating solution.

^{125}I Labelling of Polycations

Each of the three polycations was radiolabelled with ^{125}I using the Chloramine T method [19]. The polycation was dissolved in phosphate buffered saline at pH 7.4 (10 μl, 1 mg ml^{-1}) (adjusted to 8.25 for the QPVI) to which 100 μCi of Na^{125}I and Chloramine T solution (10 μl, 5 mg ml^{-1}) was added. The reaction was stopped after 1 h by the addition of sodium metabisulfate (10 μl, 300 mg ml^{-1}). Extensive dialysis against NaI solution followed by phosphate buffered saline was used to remove all the free label and other impurities.

Preparation of Partially Sulfonated Dextran (pDS)

A modified method by Ricketts et al. [21] was used to produce partially sulfonated dextran (pDS). Clinical-grade dextran (Sigma M_w 82,000, 12 g) was added to chlorosulfonic acid (10 ml) in pyridine (130 ml) at 65°C. This was reacted for 30 min, cooled, and purified by separation into a water phase. This was followed by repeated precipitations into ethanol and extended dialysis. The product was characterized on a microbondagel High A column with a 1% 1-heptane sulfonic acid (Na^+-salt) in phosphate buffer. Flow rate was 0.3 ml min^{-1}. Elemental analysis showed a 7.4% degree of substitution.

Polyelectrolyte Complexes

Complexes

These were prepared by mixing solutions of the polyelectrolytes (0.1% w/v) in phosphate buffered saline (pH 7.4) in the molar ratio of [polycation]/[polyanion] required.

Size Exclusion Chromatography

Size exclusion chromatography was used to determine the M_{peak} of the PMAA. These are reported relative to poly(acrylic acid) standards (Polysciences). A Waters Associates system was used which was com-

prised of a model 600 pump, 490 UV/vis detector (operating at 254 nm), 410 differential refractometer and 840 datastation. Microbondagel columns (E500, E1000 and High A) (Waters Associates) were used with a mobile phase of 13.6 g l^{-1} sodium acetate and 13.6 g l^{-1} monobasic sodium (pH adjusted to 7.4 by NaOH).

Sedimentation Velocity Analysis

Analytical ultracentrifigation: "Analytical" sedimentation velocity experiments were performed on the QPVP-PMAA polyelectrolyte complexes. Additionally the interaction of the complex with Bovine Serum Albumin (BSA) (Sigma) was examined. These studies were performed on an MSE Centriscan-75 analytical ultracentrifuge fitted with both scanning absorption and Schlieren optics, and with a monochromator. For the Schlieren work the monochromator was set at 546 nm; and for absorption, at 256 nm (corresponding to the absorption maximum for QPVP). Experiments were normally run, with suitable multiplexing, in multiples of three to ensure that experimental conditions (rotor speed and temperature) were similar for both complexes and the corresponding controls. All measurements were performed at 25 °C and at rotor speeds of 45,000–51,000 rev/min. Total sample loading concentrations were typically 1.5 mg ml^{-1}. Possible concentration dependence effects on the interpretation of sedimentation data were minimized, by ensuring that polyelectrolyte concentrations were identical in both the complex solutions, and in the single polyelectrolyte solution controls.

Preparative ultracentrifigation: A series of "preparative" type velocity analysis experiments were performed to determine the interactions between the pDS and QPVI and the degree of complex formation. These experiments used a preparative ultracentrifuge (MSE 65M) fitted with a 6 × 4.2 swing-out rotor. The centrifuge tube contained a homogeneous solution of either complex, polyanion or polycation. After centrifugation (at 58K rev/min) the rotor tube was placed in a piercing unit and the bottom pierced; the sample pumped out by displacement with liquid paraffin. Successive fractions were collected using a fraction collector. These were analyzed by UV absorption at 210 nm.

Platelet Aggregometry

Blood from human volunteers was collected into acid-citrated dextrose (0.11 ml ACD/1 ml blood). Platelet-rich plasma (PRP) was obtained by centrifugation of the blood at 100 g for 20 min at 25 °C (Mistral 2000), and platelet-poor plasma (PPP) at 800 g for 20 min at 25 °C. The upper and lower limits of the aggregometer (Payton Dual Channel) were set with reference to PRP and PPP, respectively. The response was

calibrated with reference to the addition of 50 μl of 100 μg/ml collagen fibrils (Hormon-Chemie, Munich) to 500 μl PRP at a stirring speed of 700 rpm and maintained at a temperature of 37 °C. Aliquots of QPVI, pDS, and QPVI-pDS complexes were tested over a concentration range of 0.075–0.3% w/v. In addition, a commercial sample of fully sulfonated dextran (Sigma, M_w 500) was tested for comparison with the pDS.

In Vivo Distribution Studies

Adult male NIH/OLA mice (28–30 g) were injected via the tail vein with ^{125}I-labelled polyelectrolyte complex (50 μl, 0.3% w/v). After a period of 2 h the animals were rapidly decapitated and the trunk blood was collected. The skin from the right side of the abdomen was sampled and the liver, spleen, gut, kidneys and lung were dissected out. Abdominal fat was sampled and both vastus lateralis muscles taken. The radioactivity content of each tissue sample was measured and expressed as a percentage of the total injected dose after a correction for the blood content.

RESULTS AND DISCUSSION

Characterization of the polyelectrolytes gave a molecular weight of 117,500 for the PMAA by size exclusion chromatography, which corresponds to a degree of polymerization (*DP*) of 1365. Vapour pressure osmometry yielded a M_n of 1835 ± 300 for the I3X polycation which gives a $DP = 6.5$. DP values for the QPVI, QPVP and pDS were estimated as approximately 270, 700 and 460, respectively.

Qualitative confirmation of complexation of the QPVP-PMAA system, as examined by (analytical) sedimentation velocity analysis, came from the observation that the center of a given sedimenting boundary (the point of inflexion) recorded using absorption optics at 256 nm coincided with the center of the boundary recorded using Schlieren optics: the latter optical system being sensitive to both the QPVP and the PMAA in the mixture (Figure 1).

Quantitative proof of complexation comes from a consideration of the sedimentation coefficients. This sedimenting single "Schlieren" boundary for the complex mixture (which sedimented at the same rate as the absorption boundary) was found to sediment at approximately double that of the individual QPVP and PMAA components. Coefficients of sedimentation of approximately 1.8 S and 2.2 S were found for the complex (with [QPVP]/[PMAA] ratios of 0.2 and 0.5, respectively), compared to 1.0 S for the QPVP, and 1.1 S for the PMAA. These results in-

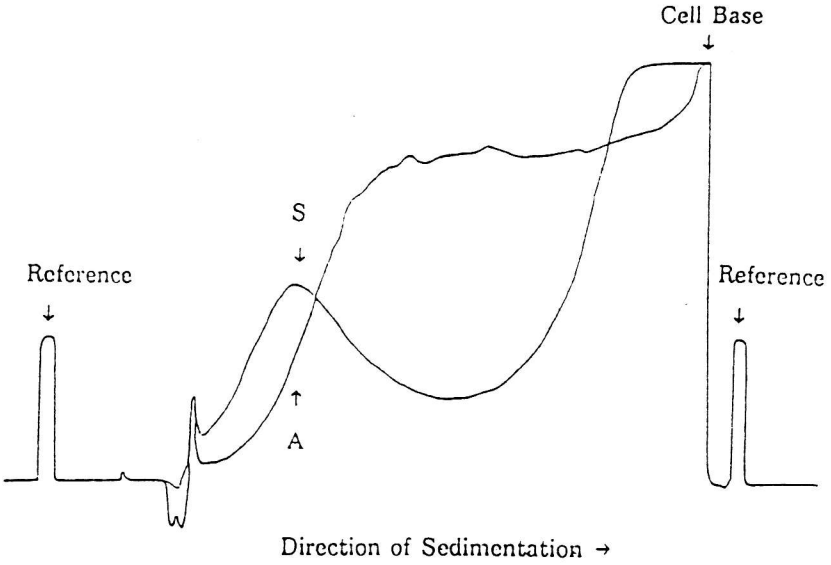

Figure 1. "Analytical" sedimentation velocity diagram for a QPVP-PMAA complex— S: center of Schlieren sedimenting boundary; A: center of absorption sedimenting boundary. Ratio of loading concentrations, [QPVP]/[PMAA] = 0.2. Rotor speed = 45,000 rev min^{-1}; temp. = 25.0°; Schlieren knife edge angle = 84°.

dicate little evidence of significant amounts of unbound QPVP or PMAA (Table 1).

It is also interesting to note that the complex formed with a [QPVP]/[PMAA] ratio of 1:2 had a higher S_{25} value than the complex formed with a 1:5 ratio; both solutions having the same total loading concentration. This suggests that the net mass of the complex is higher and/or that the complex has a tighter, more compact structure with increasing proportions of the QPVP polycation. The precise stoichiome-

Table 1. Sedimentation velocity: QPVP, PMAA and QPVP-PMAA.

	S_{25}
QPVP	1.00 ± 0.10S
PMAA	1.10 ± 0.15S
[QPVP]/[PMAA] = 0.2	1.80 ± 0.10S
[QPVP]/[PMAA] = 0.5	2.20 ± 0.10S
{[QPVP]/[PMAA] = 0.2} + BSA	3.99 ± 0.03S

S_{25}: apparent sedimentation coefficients at 25°C in Svedberg units, S (10^{-13} sec).

try of these interactions can not be found from these measurements alone, due to the complicating factors of polydispersity and diffusion. Studies with mixtures of the QPVP-PMAA complex and albumin showed an interaction with further complexes being formed between the QPVP-PMAA and BSA. These sediment with a higher sedimentation coefficient than was found for the polyelectrolyte complexes alone, but with a lower value than for BSA by itself.

Results from the sedimentation studies using the preparative ultracentrifuge on the QPVI-pDS system are shown in Figure 2. Upon application of a centrifugal force, the macromolecules move at a rate dependent on their size and shape. A moving boundary is formed between the buffer and the polymer solution. The position of this boundary can be found from the UV analysis of the fractions, the QPVI showing a far greater absorbance than the pDS. This information was used to indicate whether complex formation has occurred. The smaller extended polycation molecules showed no sedimentation under the conditions used. The larger and denser pDS molecules, however, sedimented to the bottom of the tube and appeared in the first fractions. The QPVI-pDS complex solution also showed a strong sedimentation. Interpretation of these results qualitatively indicates that complexation of the QPVI and pDS had taken place. Experimental difficulties, such as disturbances caused during tube piercing and adherence of material to the needle, preclude a more quantitative analysis. In particular the question as to whether complexation is complete remains undetermined.

The size exclusion chromatography results given in Table 2 show that an increasing [polycation]/[polyanion] ratio yields an increasingly smaller size for both the QPVI-PMAA and the QPVP-PMAA complexes. This correlates well with the sedimentation velocity results from the QPVP-PMAA system which showed higher S_{2s} values with increasing polycation content.

The results of the platelet aggregation studies are listed in Table 3. The cationic polyelectrolyte QPVI induced a dose dependent aggregation of platelets that was marked by an aggregatory phase followed by a recovery phase. Addition of collagen to the incompletely aggregated platelets led to a gross aggregation of the suspension. The QPVP behaved similarly. A markedly different effect was observed with polycation I3X. A dose-dependent aggregation was observed, with a threshold concentration required to "trigger" the aggregation, and visual observation of the PRP showed diffuse platelet aggregates. In contrast, the polyanion, PMAA, showed no aggregatory effect, and provided some protection against collagen. This latter observation depended on there being a relatively high concentration of PMAA present. The pDS sam-

ple was found unable to aggregate platelets, in contrast to the aggregatory effects found with fully sulfonated, commercially available dextran sulfate. Significantly, the interpolymer complexes of both QPVP-PMAA and of QPVI-PMAA showed no aggregation of platelets. They did not, however, protect against collagen. The polyelectrolyte complex of QPVI-pDS similarly induced no platelet aggregation. However, the polyelectrolyte complex of I3X-pDS behaved differently from the other complexes and gave a dose dependent aggregation. The I3X polycation was of a significantly lower molecular weight than the others studied. This factor may be the reason why it failed to show non-toxic behavior when complexed.

The *in vivo* distribution data shown in Table 4 represent the percentage of injected dose (% dose) found in a range of tissues, corrected for blood content. Results for the QPVP-PMAA complex show a high recovery. This may be due to the low specific activity of the QPVP. The results show clearly an accumulation of ^{125}I-label in the liver for all three systems studied, indicating a rapid clearance of the material by the MPS.

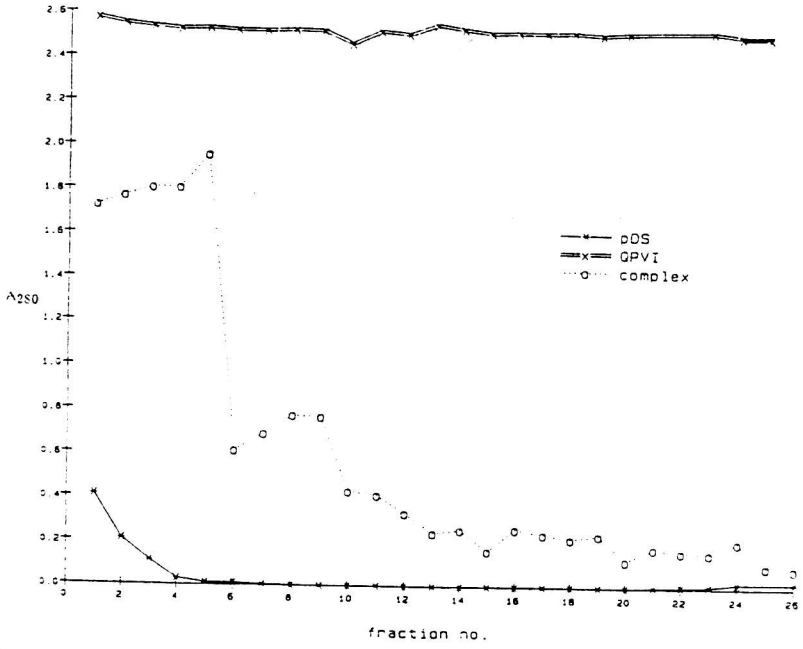

Figure 2. Sedimentation velocity data for a QPVI-pDS complex system – A_{280}: absorbance at 280 nm; -x-: pDS control; =x=: QPVI control; o: complex of [QPVP]/[PMAA] = 0.2; Temperature = 25 °C; rotor speed = 60,000 rev min^{-1}.

Table 2. Size exclusion chromatography: QPVI-PMAA and QPVP-PMAA.

	Retention Time (mins.)	Equiv. M_w
(a) QPVI-PMAA:		
PMAA	12.33	117,500
[QPVI]/[PMAA] = 0.10	12.32	107,000
[QPVI]/[PMAA] = 0.20	12.47	102,000
[QPVI]/[PMAA] = 0.30	13.20	73,000
[QPVI]/[PMAA] = 0.40	14.90	38,000
[QPVI]/[PMAA] = 0.50	15.20	34,000
(b) QPVP-PMAA:		
PMAA	12.33	117,500
[QPVP]/[PMAA] = 0.10	12.13	117,500
[QPVP]/[PMAA] = 0.20	12.43	101,000
[QPVP]/[PMAA] = 0.25	12.43	77,600
[QPVP]/[PMAA] = 0.30	13.17	77,600
[QPVP]/[PMAA] = 0.35	15.37	32,500
[QPVP]/[PMAA] = 0.40	15.50	31,400
[QPVP]/[PMAA] = 0.50	15.40	31,600

Table 3. Platelet aggregometry.

Polyanion		Polycation		Polyelectrolyte complex	
PMAA	-	QPVP	+	QPVP-PMAA	-
PMAA	-	QPVI	+	QPVI-PMAA	-
pDS	-	I3X	+	I3X-pDS	+
pDS	-	QPVI	+	QPVI-pDS	-

+ aggregation observed; - aggregation not observed.

Table 4. Tissue distribution results.

(a) QPVI-PMAA: $n = 4$; $t = 2$ h; [QPVI]/[PMAA] = 0.33					
	% dose	se		% dose	se
Blood	1.91	0.12	Skin	2.96	0.19
Liver	43.07	3.73	Spleen	1.23	0.11
Gut	6.75	1.43	Kidney	4.39	0.17
Lung	0.51	0.02	Fat	0.68	0.05
Muscle	3.34	0.23	Bone	2.30	0.08
Tail	0.53	0.08			
Total	67.222	3.79			
(b) QPVP-PMAA: $n = 3$; $t = 2$ h; [QPVP]/[PMAA] = 0.2					
	% dose	se		% dose	se
Blood	13.96	1.05	Skin	6.03	0.20
Liver	51.20	1.94	Spleen	5.80	1.34
Gut	9.34	0.60	Kidney	13.04	0.47
Lung	4.91	0.37	Fat	3.48	0.26
Muscle	10.12	0.42	Bone	9.95	1.09
Tail	2.05	0.14			
Total	129.86	5.26			
(c) QPVI-pDS: $n = 3$; $t = 2$ h; [QPVI]/[sDS] = 0.5					
	% dose	se		% dose	se
Blood	2.37	0.35	Skin	4.33	0.49
Liver	37.75	4.14	Spleen	0.90	0.13
Gut	5.72	0.47	Kidney	3.28	0.40
Lung	1.31	0.92	Fat	1.64	0.21
Muscle	7.12	2.12	Bone	0.70	0.08
Tail	2.86	0.12			
Total	67.97	7.98			

CONCLUSIONS

Water soluble non-stoichiometric polyelectrolyte complexes have been investigated as potential drug carrier systems for parenteral administration. Complexes were prepared between the polycation quaternized poly(vinyl imidazole) (QPVI), and an excess of partially sulfonated dextran. The resulting structure was designed to inherit the biocompatible properties of dextran. Additionally polyelectrolyte complexes between the polycation quaternized poly(vinyl pyridine) and the polyanion, poly(methacrylic acid) (PMAA), and between QPVI and PMAA, were studied. These both had excess PMAA and an overall anionic nature.

Sedimentation velocity experiments using a preparative ultracentrifuge indicated a degree of complexation between the partially sulfonated dextran (pDS) and quaternized poly(vinylimidazole) (QPVI) under the appropriate conditions. Also, data from "analytical" sedimentation velocity analysis showed that the QPVP-PMAA system was complexed. Additionally size exclusion chromatography results indicated that complexation was occurring in both the QPVP-PMAA and the QPVI-PMAA systems. These studies also showed that increasing [polycation]/[polyanion] ratios result in a smaller more compact complex conformation.

Platelet aggregation studies were carried out on both complexes and their individual polyelectrolyte components. All of the polycations (QPVP, QPVI and I3X) alone, and to a lesser extent the pDS, caused the aggregation of platelets. However, the polyelectrolyte complex of the two macromolecules QPVI and pDS did not exhibit this property *in vitro* when the pDS was present in excess in the complex. Likewise, complexes formed between QPVP and PMAA, or QPVI and PMAA eliminated the aggregatory effect exhibited by the polycations alone. A polyelectrolyte complex between the polycation I3X and pDS however showed toxic aggregatory effects. It would appear that in most cases the effects normally found with cationic polymers were eliminated *in vitro* when they formed part of a soluble polyelectrolyte complex.

In vivo distribution studies in mice using ^{125}I-labelled QPVI or QPVP complexed with pDS or PMAA showed a 40–50% accumulation of the administered dose in the liver after 2 hours.

It would, therefore, appear that platelet aggregation studies are not a reliable indicator for predicting *in vivo* distribution results; however favourable the indications may be. In these studies the polycation present in the complexes appears to have been prevented, to some extent, from interacting with negatively-charged biological surfaces, such as

platelets *in vitro*. Complexes using the low molecular weight polycation I3X were the exception to this rule.

In conclusion it has been shown that the ionic bonds which hold this type of complex together, are not sufficiently strong *in vivo* to preserve a stable complex structure. These types of polymer complexes may readily undergo exchange reactions, for example, with other charged macromolecules present. This extensive incidence of unwanted interactions *in vivo* leads to the rapid removal of polyelectrolyte complexes from the circulation; at least for those complexes examined.

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