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GEL PERMEATION CHROMATOGRAPHY OF A POLYAMIDE–EPICHLOROHYDRIN RESIN AND SOME OTHER CATIONIC POLYMERS

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SUMMARY

The gel permeation chromatography of cationic polymers, a polyamide–epichlorohydrin resin (Hercosett 125), poly(2-vinylpyridine) and polyethyleneimine, was compared on a selection of silica gel columns with neutral and cationic surface modifications.

The elution volumes of the polymer peaks were very dependent on the pH, ionic strength and the column, but were also influenced by the type of salts in the eluent and the polymer concentration. In some cases it was possible to elute the cationic polymer anywhere between the high-molecular-weight exclusion limit and the low-molecular-weight total permeation limit.

The polymer peak in Hercosett 125 split into two under certain conditions. This appears to be related to the presence in Hercosett 125 of polymers of different structures.

Universal calibration methods and viscosity measurements have been used to indicate conditions where interaction between the cationic polymers and the columns and/or solvent was minimal and these conditions have been used to estimate the molecular weight of Hercosett 125.

INTRODUCTION

Cationic polyamide–epichlorohydrin (PAE) resins prepared by reaction of epichlorohydrin with polyamide derived from adipic acid and diethylenetriamine (Fig. 1) are widely used as paper wet-strength additives[1] and to shrink-resist wool[2,3]. Several chemical studies[4,5] have investigated the cross-linking reactions and reactive groups in PAE resins but little is known about the molecular weight or molecular weight distribution of PAE resins, apart from a recent gel filtration study[6].

This paper reports an investigation of the wool shrink-resist resin Hercosett 125 (Hercules, Australia) by gel permeation chromatography (GPC). The objective of this work was to develop analytical methods to follow chemical changes during the application of Hercosett 125 to wool.

Most GPC studies have been on neutral polymers rather than polyelectrolytes. One reason for this is that suitable columns for high-pressure aqueous GPC have only

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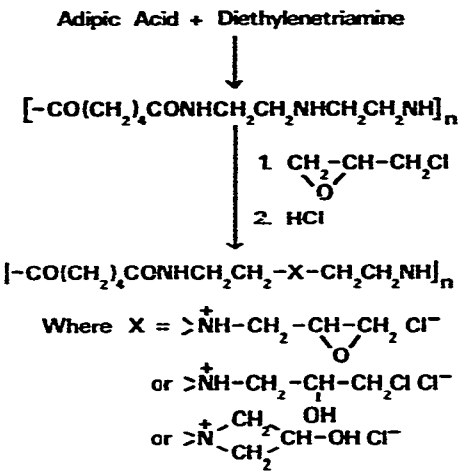

Fig. 1. Preparation and structure of the PAE resins.

become available recently, but even with these columns there are difficulties in molecular weight calibration with polyelectrolytes[7–12]. There are a number of GPC effects peculiar to polyelectrolytes due to ionic interactions between the polymer and column (*e.g.*, ion exclusion, Donnan equilibrium ion inclusion) and due to changes (*e.g.*, with pH or ionic strength) of the apparent dimensions of polyelectrolyte molecules in solution. These effects and ways to minimise them (*e.g.*, by choice of columns and eluents) have been discussed in detail elsewhere[7–12].

In the present study Hercosett 125 and some cationic polymers of known structure, poly(2-vinylpyridine) (PVP) and polyethyleneimine (PEI), were examined on two classes of surface-modified silica gel columns; A, with a neutral surface modification and B, with a cationic surface modification. Surface modification is necessary to prevent the strong adsorption of polyelectrolytes onto unmodified silica[7–9]. The second class was examined in the hope of preventing adsorption effects caused by the slight negative surface charge in the first class. Such cationic columns have recently been used by Talley and Bowman[13,14] for the GPC of certain cationic polymers.

EXPERIMENTAL

Materials

The Hercosett 125 sample was provided by Hercules. All the chromatograms of Hercosett 125 in this paper were run within a week approximately 3 months after the date of manufacture. This approach avoided the changes that occur due to cross-linking during storage.

The other polymers were commercial samples, used without further purification, and their sources and molecular weights are indicated in Table I. Other chemicals were analytical grade.

Quaternary ammonium salt solutions were prepared by diluting 25% solutions of the quaternary ammonium hydroxides (BDH) with water and then adjusting to the desired pH with either nitric or phosphoric acid.

TABLE I

POLYMERS

Name	*Source*	$\bar{M}_n$*
Dextran T10	Pharmacia	10,000
T40		40,000
T500		500,000
Poly(2-vinylpyridine) Batch 2	Pressure Chemical	30,000
Batch 3		90,000
Batch 5		230,000
Batch 7		600,000
Poly(ethyleneimine) PEI 6	Dow Chemical	600
PEI 12		1200
PEI 18		1800

* Manufacturer's data.

Chromatography

A Varian 4100 syringe pumping system, a Rheodyne loop injector using a 100-μl loop, a Varian refractive index detector and a Varian Vari-chrom variable-wavelength ultraviolet detector set at 230 nm were used. The supports prepared in this laboratory were packed into 250 × 4.6 mm I.D. stainless-steel columns. All chromatograms were run at room temperature (about 22°C) with a flow-rate of 1.3 ml/min for column II and 1 ml/min for the other columns. Table II gives details of the columns used in this study.

Unless otherwise stated, the GPC results have been presented as apparent molecular weights in order to make comparisons between columns. The molecular weights quoted in Table III are for peak maxima and were determined by direct comparison with dextran standards. They are hence referred to as apparent molecular weights.

Viscometry

Viscosities were determined with a Ubbelohde viscometer at 25°C.

RESULTS AND DISCUSSION

The most pronounced features (Table III) in the present aqueous GPC study were the shifts of the cationic polymer peaks, on all five columns, to longer elution volumes (*i.e.*, reduced apparent molecular weight) when the pH and/or the ionic strength was increased. Such shifts have been noted before in polyelectrolyte GPC[7–10], but the magnitude of the shifts on columns I and II appeared to be greater with the cationic polymers than reported previously[10,11] with anionic polymers. The reasons for these shifts have been discussed previously[7–11] and involve ion exclusion and conformational changes. However, adsorption (which appeared to increase as both the pH and the ionic strength was raised) must be significant. At very high ionic strengths (*e.g.*, Fig. 2) adsorption became so pronounced that the intensity of the cationic peaks decreased markedly and the peak tailed considerably.

TABLE II

COLUMNS

No.	*Type/Source*	*Source*	*Modification*	*Silica*	*Pore size* (nm)*
I	μBondagel E-Linear	Waters Assoc.	Hydrophilic ether*		12.5–100
II	TSK-G-3000SW	Toyo Soda	Glyceryl ether*		Unknown
III	Zorbax SAX	DuPont	Quaternary ammonium* ions		6
IV	Cationic modified**	Method from ref. 13**	$-(CH_2)_3NH-CH_2CH(OH)-CH_2NMe_3^+$	LiChrosorb Si 500 (E. Merck)	50
V	Cationic modified	Method from ref. 13**	$-(CH_2)_3NH-CH_2CH(OH)-CH_2NMe_3^+$	LiChrosorb Si 100 (E. Merck)	10

* Manufacturers' literature.

** Packed in these laboratories.

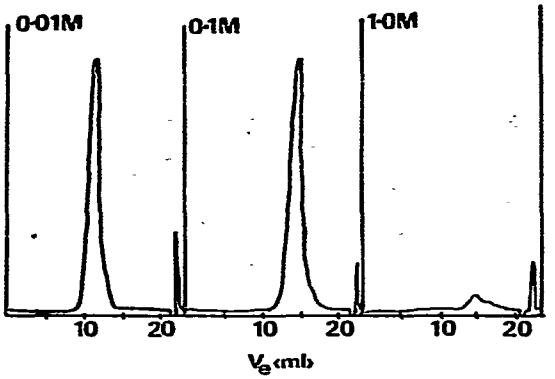

Fig. 2. GPC of mol.wt. 30,000 poly(2-vinylpyridine) in $NaNO_3$ solutions of various ionic strengths (pH 3) on column II (refractive index detector). V_e = Elution volume.

In addition to the above effects, Hercosett 125 (but not the other cationic polymers) showed two polymer peaks under certain conditions. This is illustrated in Figs. 3 and 4 (which show series on columns II and I at different pH values) and in Fig. 5 (which shows a series on column II of increasing ionic strength at constant pH).

There were also minor effects (which have not been illustrated) related to the polymer concentration (distortion and fusion of peaks which was avoided by the use of dilute solutions) and to the nature of the salts in the eluent. Only solutions of sodium or alkylammonium nitrate and phosphate were examined. Alkylammonium salts gave slightly sharper peaks than when sodium salts were used. Nitrates avoided the possibility of ion binding of HPO_4^{2-} to cationic molecules but phosphate had a greater buffering capacity.

In view of these various effects, before Hercosett 125 was studied, experiments

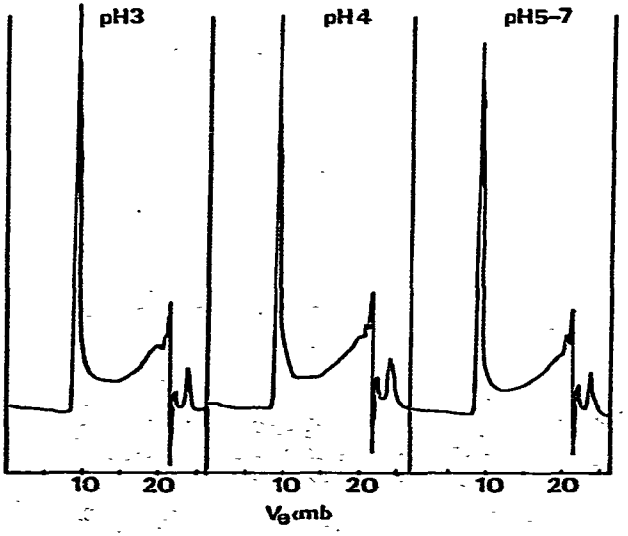

Fig. 3. GPC of Hercosett 125 in 0.1 *M* $NaNO_3$ solutions at various pH values on column II (refractive index detector).

TABLE III

APPARENT MOLECULAR WEIGHTS OF CATIONIC POLYELECTROLYTES AS DETERMINED BY GPC ON VARIOUS COLUMNS

Values are based on direct comparison with dextran standards. NE = not eluted; NT = not tested.

Column	*Salt*	*Conc. (M)*	*pH*	*PVP*				*PEI*			*Hercosett 125*
				30,000	*90,000*	*230,000*	*600,000*	*6*	*12*	*18*	
I	$NaNO_3$	0.05	3	NE	NE	NE	NE	6000	10,000	14,000	>500,000 tailing to TPL*
		0.1	3	NE	NE	NE	NE	3500	5000	6000	TPL peak only
	$NEt_4H_2PO_4$	0.2	3	NT	NT	NT	NT	900	2000	500	>500,000 + 5000
		0.2	6	NT	NT	NT	NT	NT	NT	NT	TPL only
		0.1									
	$NEt_4H_2PO_4$ + NaH_2PO_4	0.1 + 0.2	3	NE				2000	Insol.	Insol.	>500,000 + 4000
II	$NaNO_3$	0.01	3	55,000	200,000	>300,000	>300,000	700	1400	2200	>300,000 + 7000
		0.1	3	35,000	110,000	>300,000	>300,000	600	1200	2200	>300,000 + 3000
		1.0	3	6000	25,000	NT	NT	600	1200	2200	>300,000 + 3000
	$NEt_4H_2PO_4$ + NaH_2PO_4	0.1 + 0.2	3	30,000	90,000	NT	NT	600	Insol.	Insol.	>300,000 + 3000
III	$NaNO_3$	0.1	3	>25,000	>25,000			8000	9000	11,000	>25,000 + 14,000
IV	HNO_3	0.001	3	>800,000	>800,000			NT	NT	NT	800,000 + 80,000
	NaH_2PO_4	0.001	4.8	NE	NE	>800,000	>800,000	NT	NT	NT	800,000 + 80,000
	$NaNO_3$	0.1	3	60,000	300,000	>800,000	>800,000	NT	NT	NT	500,000 + 20,000
	NEt_4NO_3	0.1	3	30,000	120,000	500,000	>800,000	NT	NT	NT	350,000 + 10,000
V	$NaNO_3$	0.1	2	>50,000		NT	NT	10,500	15,000	19,000	50,000 + 20,000
		0.1	3	>50,000		NT	NT	7500	10,500	12,500	50,000 + 20,000
		0.2	3	40,000	>50,000	NT	NT	4800	7800	10,000	50,000 + 20,000

* TPL = Total permeation limit.

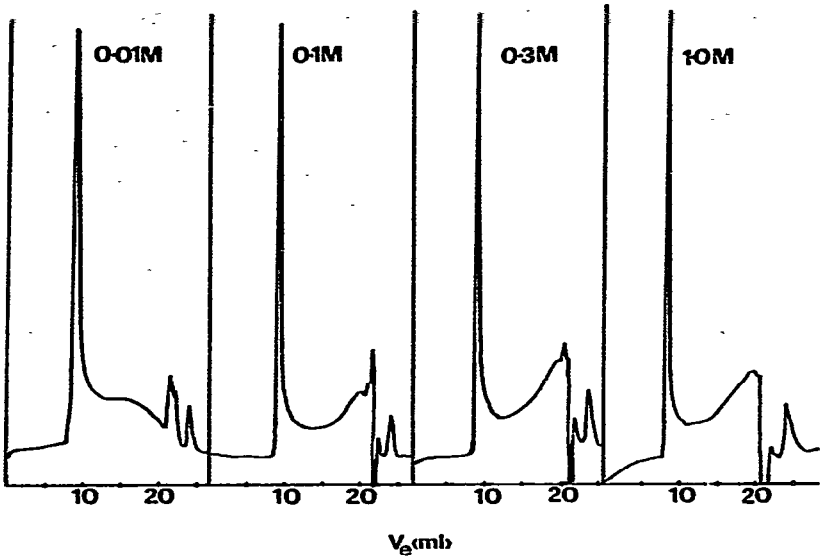

Fig. 4. GPC of Hercosett 125 in $NaNO_3$ solutions of various ionic strengths (pH 3) on column II (refractive index detector).

were performed with poly(2-vinylpyridine) and polyethyleneimine samples of known molecular weight.

Comparison of columns

If the two neutral modified columns I and II were compared, column II gave slightly better resolution, was less sensitive to changes in pH or ionic strength, but took a longer time for each analysis. PVP adsorbed strongly on column I at all ionic strengths examined, and the PEI peaks on this column tailed indicating some adsorption. With column I, the changes caused by increased ionic strength were more pro-

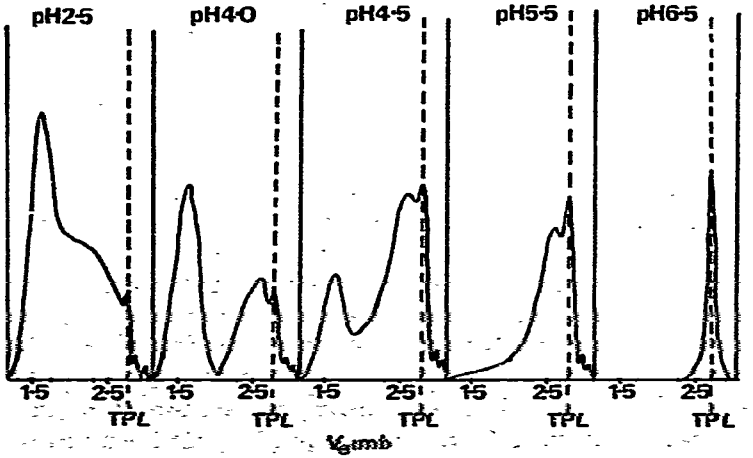

Fig. 5. GPC of Hercosett 125 in 0.05 *M* $NEt_4H_2PO_4$ on column I at various pH (refractive index detector). TPL = Total permeation limit.

nounced with Na^+ and Me_4N^+ than with Et_3NH^+, Et_4N^+ or Bu_4N^+ (where Me = methyl, Et = ethyl and Bu = butyl). In contrast, with column II, the various cations behaved similarly.

Column III which was designed for ion exchange chromatography was limited to molecular weights below about 25,000. Column IV prepared by Talley and Bowman's method[13] with silica (pore size 50 nm) deteriorated during use but column V (pore size 10 nm) was stable. With column IV the back pressure increased gradually until it was unusable, and this was faster at pH 2–3 than at pH 6 and replicate preparations deteriorated at different rates. Consequently, the cationic modified columns were limited to molecular weights around 40,000, although some results of higher molecular weights were obtained from column IV before deterioration became excessive. All the cationic columns showed significantly higher molecular weights (except at very high ionic strength) than expected for the PVP and PEI standards when calibrated directly against dextran standards, indicating ion exclusion effects.

If the cationic and neutral modified columns were compared for a given ionic strength, the cationic columns gave higher apparent molecular weights, presumably due to ion exclusion. As far as sensitivity to pH and ionic strength and adsorption effects were concerned, the cationic columns were generally similar in behaviour to column II and less sensitive than column I.

Determination of molecular weight

The approach used in the present study to obtain molecular weights for Hercosett 125 from the GPC data involved the use of established "universal" calibration methods[15,16] with dextran standards to find conditions where the correct molecular weights were obtained for PVP and PEI samples of known molecular weight. Hercosett 125 was then run under the same conditions and also the intrinsic viscosity was measured with the GPC eluent as solvent. The universal calibration method then gave a molecular weight for Hercosett 125.

The "universal" calibration line was drawn from the GPC data of a series of dextran standards which were eluted from a particular column at essentially the same position, independent of pH or ionic strength. The solvent composition was then varied until the points for the cationic polymer standards fell on the dextran calibration line. When this happens, it is an indication that interactions between the polymer and the column and/or solvent are minimal, but it may not apply to other cationic polymers, *e.g.*, Hercosett 125. This coincidence may be a fortuitous balance of opposing interactions, but viscosity measurements can be used to give an indication whether polyelectrolyte conformational changes are likely to be significant.

This universal calibration approach could only be used with column II since (i) on column IV the PVP calibration line did not coincide with the dextran line (Fig. 7) as observed before[13], and (ii) on the other columns the PVP standards were either strongly adsorbed or totally excluded. Universal calibration was performed, firstly by Benoit's method[15] (Figs. 6 and 7) and secondly by the "Southern" method[16] (Fig. 8), using published Mark–Houwink constants[17] for dextran and intrinsic viscosities, determined in this work, for PVP and PEI. With both methods the calibration lines for dextran, PVP and PEI coincided in 0.1 *M* $NaNO_3$ (adjusted to pH 3 with nitric acid) or 0.1 *M* $NEt_4H_2PO_4$ + 0.2 *M* NaH_2PO_4 (pH 3). From intrinsic viscosity measurements at different ionic strengths (Fig. 9) it can be seen that the eluent where

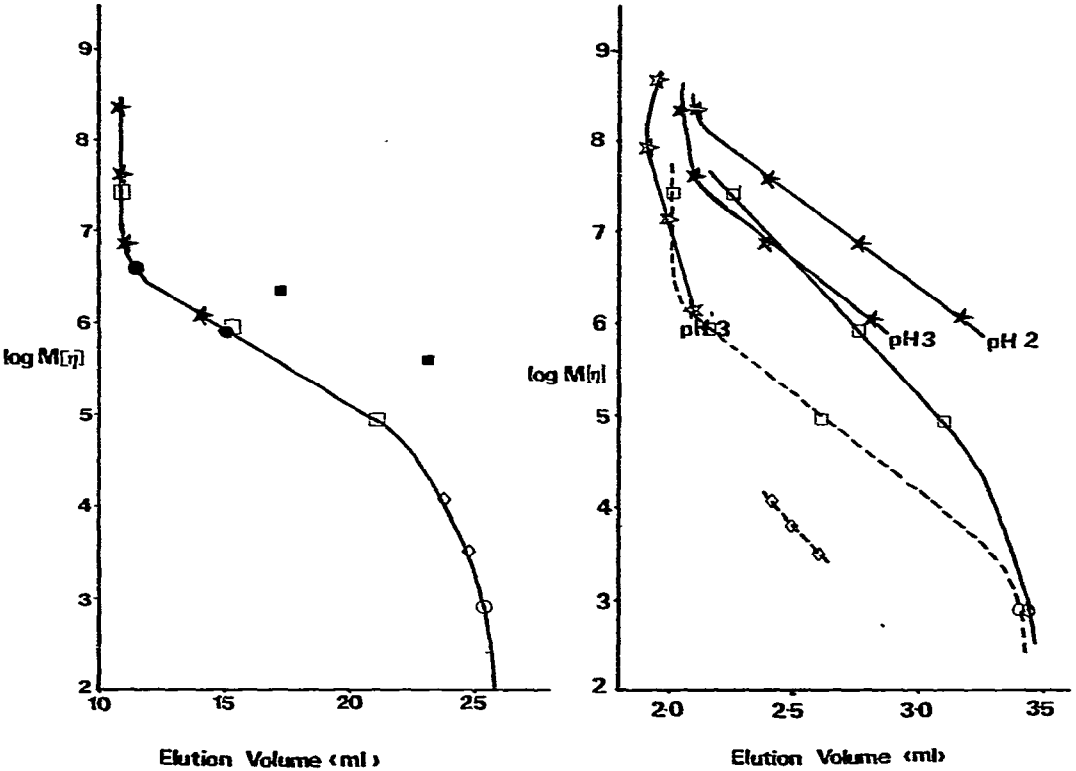

Fig. 6. Universal calibration {log ([η] × mol.wt.) *vs.* elution volume} of column II with dextran in water and PVP in aqueous salt solutions. □ = Dextran in water; ○ = sucrose in water; ✳ = PVP in 0.1 *M* $NaNO_3$ (pH 3); ■ = PVP in 1.0 *M* $NaNO_3$ (pH 3); ● = PVP in 0.1 *M* $NEt_4H_2PO_4$ + 0.2 *M* NaH_2PO_4 (pH 3); ◇ = PEI in 0.1 *M* $NaNO_3$.

Fig. 7. Universal calibration of column IV with dextran in water and PVP in aqueous salt solutions. ◇ = PVP in 0.01 *M* $NaNO_3$; other symbols as in Fig. 6.

the universal calibration lines coincided corresponded (for most polymer samples) to where the intrinsic viscosity had dropped nearly to its limiting value. Barth[7] has recommended that in polyelectrolyte GPC the ionic strength should be chosen such that the intrinsic viscosity has reached the limiting value in order to minimize effects from rod–coil conformational changes. In the present case Barth's recommendation could not be strictly followed as, in the region of the minimum intrinsic viscosity, adsorption started to occur.

Hercosett 125, under the "optimum" GPC conditions noted above (0.1 *M* $NEt_4H_2PO_4$ + 0.2 *M* NaH_2PO_4, pH 3 on column II) appeared as a broad peak with $\bar{M}_n = 2100$ and $\bar{M}_w = 11{,}000$ which merged into a peak of smaller area with molecular weight >300,000. The value assigned to the broad peak agrees closely with that obtained from gel filtration[6]. In addition to the errors inherent in the calibration method used, it should be noted that branching is possible and this would make the actual molecular weight slightly higher.

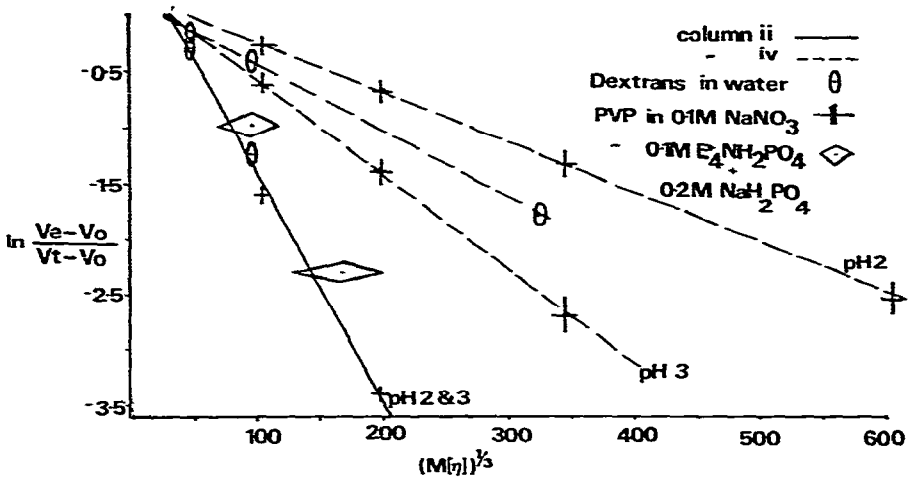

Fig. 8. "Southern" calibration method {$\ln (V_e - V_0)/(V_t - V_0)$ *vs.* (mol.wt. × $[\eta]$)$^{\frac{1}{3}}$} of columns II and IV with dextran in water and PVP in aqueous salt solutions. V_e = Elution volume; V_t = total permeation volume; V_0 = total interstitial volume; —— = Column II; – – – = column IV; ○ = dextrans in water; ÷ = PVP in 0.1 *M* $NaNO_3$; ◇ = PVP in 0.1 *M* $NEt_4H_2PO_4$ + 0.2 *M* NaH_2PO_4.

GPC of Hercosett 125

In the GPC of Hercosett 125 there were two peaks eluted after the total permeation limit which appeared to be low-molecular-weight species, since they were not present after dialysis or acetone precipitation. One of the peaks had a strong UV absorption maximum at 315 nm and adsorbed strongly onto columns above pH 4. This peak was also observed in the GPC on Sephadex G-20[6].

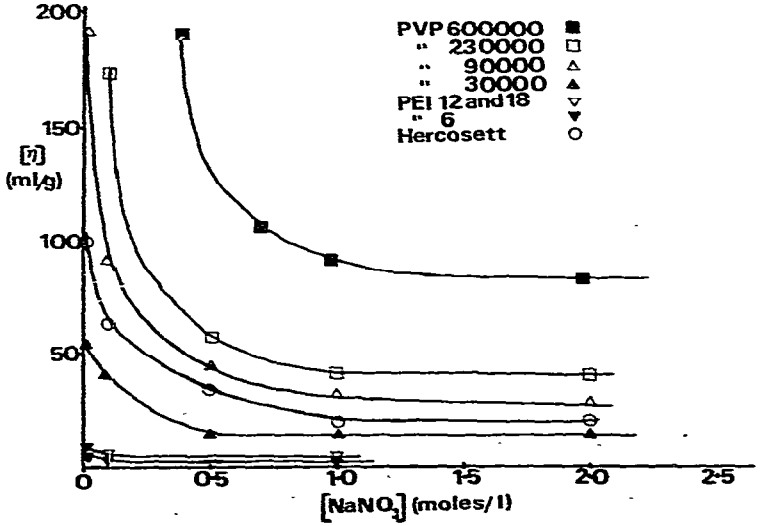

Fig. 9. Intrinsic viscosity, $[\eta]$, *vs.* ionic strength, ($NaNO_3$ solutions, pH 3) for cationic polymers used in this study.

The polymeric material in Hercosett 125 showed as two peaks which shifted position and/or intensity when the pH, ionic strength or column was changed (*cf.*, Figs. 3–5). As the pH and/or ionic strength increased, the intensity of the high-molecular-weight peak decreased, the low-molecular-weight peak shifted to lower apparent molecular weight (as observed before with PVP and PEI), and eventually there was only a peak at the total permeation limit. Peak area measurements indicated that when the high-molecular-weight peak decreased the low-molecular-weight peak increased. The high-molecular-weight limit of column I was higher than column II, and the high-molecular-weight peak on column II tailed from the exclusion limit. On column I the high-molecular-weight peak was eluted after the exclusion limit of the column, but did not shift its position with changes in pH or ionic strength.

It was demonstrated that the changes with increasing pH were reversible and were not due to cross-linking, by collecting fractions from chromatograms using one pH and rechromatographing at other pH values.

The explanation we advance for the peak splitting in Hercosett 125 involves changes in the extent of adsorption onto the column and the well known conformational changes[18] found in polyelectrolytes whereby extended rod-like molecules are converted to much more compact coiled conformations by reduction of the charge or increase in ionic strength.

The structure of Hercosett 125 in Fig. 1 is an oversimplification. In the first reaction, the excess of amino groups over carbonyl groups and the different reactivity of the amino groups result in a low-molecular-weight polyamide containing diethylenetriamine units, in which various combinations of from one to three of the amino groups have reacted. In the second reaction with epichlorohydrin, each amino group can be converted into several possible structures. Thus, in Hercosett 125, molecules of any molecular weight will have a number of different structures. Titration curves of Hercosett 125 with dilute NaOH and HCl indicate that there is a range of base strengths including some weakly basic groups which are not protonated at pH 4. This distribution of base strengths is consistent with the mixtures of different structures.

Our explanation for the two peaks is that some of the species present occur as an apparently high-molecular-weight peak due to either (i) ion exclusion from the column or (ii) by adopting a rod-like conformation with a large hydrodynamic volume. This peak is reduced when the ionic strength increases or the pH is raised (which reduces the number of positive charges), due to one or more of the following: (a) elimination of ion exclusion, (b) increased adsorption onto the column, and (c) collapse of a rod conformation to a more compact coiled conformation. The changes of viscosity with ionic strength (Fig. 9) are consistent with conformational changes; however, this could occur in both peaks.

CONCLUSIONS

The present study suggests that the commercial neutral modified silica columns I and II are more useful for the aqueous GPC of cationic polymers than the three cationic modified columns examined. Column II also can be used for a greater range of cationic polymers than column I. The commercial aqueous GPC columns I and II appear to be generally less suitable for cationic than for neutral or anionic polymers. Cationic polymers show greater changes than anionic ones in the elution position

when the pH or ionic strength is changed. This is probably a reflection that silica (even at pH 2–3) has a negative surface charge which increases as the pH becomes more alkaline. This problem was not overcome by modification of the silica surface with cationic groups, as this resulted in anomalously high apparent molecular weights due to ion exclusion effects (*i.e.*, electrostatic repulsion). In addition, satisfactory cationic modified columns for molecular weights above about 40,000 could not be prepared.

Universal calibration methods have been used to indicate the conditions with column II where polymer–column interactions appear to be minimal, and these conditions have been used to assign to Hercosett 125 a $\bar{M}_n = 2100$ and $\bar{M}_w = 11{,}000$. The errors in this operation are probably greater than in the molecular weights assigned by GPC for neutral and anionic polymers.

In summary, of the GPC systems examined, the one which was applicable to a wider range of cationic polymers used column II, pH 3–4, and 0.1–0.5 *M* ionic strength. These eluents are not unexpected as this pH range is where the negative surface charge on the silica is least, and the ionic strength is sufficient to suppress ion exclusion. The use of higher ionic strengths (*e.g.*, in order to overcome conformational changes) resulted in increased adsorption onto the column, which does not appear to have been noted before in polyelectrolyte GPC.

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