Thermodynamics of Noncovalent Interactions in Hydrophobically-Substituted Water-Soluble Polymers from Intrinsic Viscosity Measurements: Application to Nucleobase-Substituted Pullulans

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ABSTRACT: Hydrophobically substituted water-soluble polymers (HSWSP) act as associative thickeners through the reversible crosslinking from noncovalent interactions between the various groups on the polymer chains in aqueous solution. This article shows how the intrinsic viscosity (IV) of nonionic HSWSP can be used to define the thermodynamics of these interactions. Literature data on the IV of pullulans substituted by nucleobase ester groups (thyminylbutyryl and adeninylbutyryl) (Mocanu et al., Can J Chem, 1995, 73, 1933) are used as an exemplar of these procedures. The intramolecular crosslinking in these substituted pullulans is deduced to be "unimolecular" (association constant $K_1 = 1\ M^{-1}$), as contrasted with the "bimolecular" behavior expected from the stacking of the free nucleobases; evidently the crosslinking results from hydrophobic interactions between the butyryl linking groups and the main chain. The results are compared with those from other HSWSP, and from cosolute binding systems. The use of the water–octanol partition coefficients of model systems to elucidate hydrophobic interactions in HSWSP, and of denaturant cosolutes (especially urea) to diagnose the presence and strength of these interactions, are also discussed. Emphasis is placed on the need for further such studies to identify the interactions underlying the rheological behavior of the nonionic HSWSP, and of the more common ionic types. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 657–671, 2012

Key words: crosslinking, reversible; intrinsic viscosity; hydrophobic bonds; noncovalent interactions; nucleobases, adenine and thymine; partition coefficients, water-octanol; urea, denaturing effect of; water-soluble polymers, hydrophobically substituted

INTRODUCTION*

Interactions in hydrophobically substituted water-soluble polymers

Water-soluble polymers (WSP) are a group whose small total value belies their importance in both the technical and the biological areas. A particularly important subgroup is that of their hydrophobically-substituted derivatives (HSWSP), where small amounts of alkyl or other nonpolar substituent groups have profound effects on the solution behavior of the polymer. Such HSWSP have found applications as associative thickeners (for non-drip/thixotropic paints and other coatings), flocculants, and so forth. These systems have been studied for more than 20 years;[1–3] they continue to attract attention both from their rheological behavior and for their ability to produce nanospheres.[4–17]

Most of these studies have involved polyelectrolytes, where the ionic charges further improve their performance in applications, and (as discussed below) prevent the precipitation that occurs with many nonionic forms, as well as bringing them closer in structure and behavior to biopolymers. However, from the viewpoint of trying to quantify the effects involved, the presence of the ionic groups complicates the situation.

This applies particularly in considering their hydrodynamic volume, a property given by such techniques as intrinsic viscosity (IV). Even the addition of small-molecule electrolyte (e.g., NaCl) to minimize or mask the ionic effects with the polyelectrolytes does little to simplify the situation. For example, in the studies of Zhou et al.[17] on samples of poly(acrylic acid) and poly(methacrylic acid) that had been substituted by fluorocarbon-containing ester groups, the viscosity measurements at increasing concentrations of NaCl showed that that the IV

*Three general points: (a) the abbreviations and symbols used in the article are listed in the Nomenclature section at the end; (b) all solutes and interactions are in aqueous solution unless otherwise specified; (c) numerical values are shown in the form 1.23(4) where 1.23 is the mean value and 0.04 is the standard deviation as the number of units in the last decimal place of the mean.

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was still decreasing even at 0.32 M concentration. Furthermore, extrapolation of IV versus $1/\sqrt{[NaCl]}$ to infinite salt concentration in the standard manner for polyelectrolytes[18] gives in all cases an apparently *negative* value of the IV.[19]

From this viewpoint, the use of wholly neutral polymers provides a simpler situation. Indeed, studies on nonionic systems, and at low concentrations, should be a necessary preliminary to understanding the behavior of the ionic types, particularly that around the critical concentration c^* at which the thickening effect becomes marked. One drawback of these nonionic systems is with higher amounts of the nonpolar substituent groups the polymer may become insoluble in water, which is evidently a drawback for any applications; however, viscosity studies may also be used to give information on the factors leading to this precipitation.

The presence of hydrophobic groups gives rise to intramolecular and intermolecular reversible crosslinks from noncovalent interactions such as hydrophobic bonds. This leads to shrinkage in the encompassed (hydrodynamic) volume, which it is important to take into account when interpreting data from viscometry and light scattering on such polymers. These effects are also significant in size-exclusion chromatography (gel filtration) of such polymers, where hydrodynamic volume is a controlling factor in the retention time/volume, while the hydrophobic groups may interact with the surface of the column packing leading to such unwanted effects as anomalous retention times and tailing.[20]

In the case of natural polymers, hydrophobic effects are one group of interactions that determine biological activity, insofar as they affect molecular conformation and biopolymer/cosolute interactions. Simple model systems therefore may provide data to interpret the behavior of the generally more complex biochemical systems. For example, studies of the interactions between hydrophobic groups and polysaccharides, as dealt with in this article, should be useful for interpreting the behavior of lipid/polysaccharide and glycolipid systems. Similar considerations apply to the nucleobases (adenine and thymine) also involved here.

Despite the considerations outlined above, there is little data in the literature on the solution behavior of nonionic HSWSP, and indeed there is an almost complete absence of any *quantitative* interpretation of the equilibria governing the behavior of the more common ionic HSWSP in any of the cited references.[1–17] One aim of this article is therefore to show how the simple measurement of IV for the nonionic HSWSP may be used to give estimates of the association constants for the noncovalent interactions involved, and hence to interpret these constants to show the nature of these interactions.

Dilute solution viscometry: Intrinsic viscosity and the Mark-Houwink-Sakurada equation

In advance of the specific discussion of the viscosity behavior of the HSWSP, it is useful to have a reminder of the basic quantities to be discussed.[21–23] The value of the intrinsic viscosity [η] for nonionic polymers is defined by the standard Huggins' equation

$$\eta_{sp}/c = [\eta] + k_H[\eta^2]c \tag{1}$$

where η_{sp} is the specific viscosity, [η] is the intrinsic viscosity (IV), c is the polymer concentration, and k_H is the dimensionless Huggins' slope parameter. Because the value of the IV refers to extreme dilution, and hence to isolated polymer molecules, any changes in the IV relate only to intramolecular effects. On the other hand, the value of the Huggins' parameter k_H relates to intermolecular effects, which may be expected to parallel the intramolecular effects.

The dependence of IV on molecular weight is given in general by the Mark-Houwink-Sakurada (MHS) equation

$$[\eta] = K_\eta M^\alpha \tag{2}$$

The value of the exponent α in eq. (2) is a useful diagnostic tool for the conformation of the polymer in solution. Its value shows, for example, that the flexible-chain random coil conformation is that taken up by the parent (unsubstituted) polymers discussed later in the article—poly(vinylpyrrolidone) (PVP), poly(vinyl alcohol), hydroxyethylcellulose, and pullulan.[24]

Quantitation of noncovalent interactions: poly(vinylpyrrolidone) with phenolic cosolutes

One point of entry into the quantitation of these noncovalent interactions is to use a theory of reversible crosslinking that was developed to deal with the effects of reversibly-bound phenols and related compounds on the solution conformation and solubility of poly(vinylpyrrolidone) (PVP), a nonionic water-soluble synthetic polymer.[25,26] Here the "substituent groups" are the molecules of cosolute (small-molecule solute in solution with the polymer) that are reversibly bound by the PVP chain.

In this interpretation, the reductions in IV that are observed when the cosolute is added are taken to be due to the bound cosolute molecules then forming reversible intramolecular crosslinks in the polymer coil by noncovalent interactions. If $[\eta]_0$ is the IV for the polymer alone and $[\eta]_r$ is that with degree of binding (cosolute molecules per PVP monomer unit), r, then the viscosity ratio V is defined as

$$V \equiv [\eta]_r/[\eta]_0 \tag{3}$$

In the case of the PVP/phenol interactions, two forms of behavior were observed for the dependence of the IV on the degree of binding r.[25,26]

First, with certain cosolutes the reduction in the ratio V was linear with the degree of binding:

$$V = 1 - S_1 r \tag{4}$$

This is referred to as unimolecular shrinkage behavior, since it is interpreted as due to reversible crosslinking between one bound cosolute molecule and another distantly connected part of the same polymer chain:

$$> S \sim A + S < \;\rightleftharpoons\; > S \sim A \sim S < \tag{5}$$

where A is the (bound) cosolute molecule, S is the binding site on the chain, and the symbol "$\sim$ "; represents the particular combination of noncovalent interactions involved in each case; the equilibrium constant for this process is denoted K_1. This behavior was seen with the cosolutes having hydroxethyl groups in place of phenolic hydroxyls, as well as with 4-nitrophenol ($HOPhNO_2$).[25,26]

Other cosolutes gave the contrasting bimolecular shrinkage behavior

$$V = 1 - S_2 r^2 \tag{6}$$

which is interpreted as the crosslinking takes place between distant *pairs* of bound cosolute molecules on the same chain:

$$> S \sim A + A \sim S < \;\rightleftharpoons\; > S \sim A \sim A \sim S < \tag{7}$$

This behavior was seen with most of the phenolic cosolutes (PhOH, HOPhOH, etc.).[25,26]

This interpretation was supported by a theoretical treatment of the known shrinkage effect of tetravalent crosslinks on the IV of a polymer,[27] using the persistence-length and statistical-element model of Kuhn and Majer for flexible chain polymers.[28] This model had been applied successfully by Kuhn and Balmer to the irreversible crosslinking of poly(vinyl alcohol) by terephthaldehyde (1,4-$Ph(CHO)_2$),[29] and by Ochiai et al. to the reversible crosslinking of the same polymer by borax (sodium tetraborate.)[30] In the PVP/phenols case, for the unimolecular shrinkage case this was shown to correspond to the expected equilibrium of eq. (5), and the association constant K_1 was related to the experimental shrinkage coefficient S_1 of eq. (4) by

$$K_1 = QS_1 \tag{8}$$

where Q is a numerical factor given for these viscosity data by

$$Q = 2^{1/2} b\, K_\eta^{(1/3a)} M_0 [\eta]_0^{[(2a-1)/3a]} \Phi^{1/3} \tag{9}$$

Here, $[\eta]_0$ is again the IV of the (cosolute-free) polymer, while K_η and α are the parameters from the Mark-Houwink-Sakurada relation of eq. (2), b is the length of the monomer unit along the polymer chain, M_0 is the molecular weight of this unit, and Φ is the Flory-Fox universal viscosity constant.

The similar application to the bimolecular case confirms the form of eq. (6) for the crosslinking equilibrium of eq. (7), with the bimolecular shrinkage coefficient S_2 given by

$$K_2 = QS_2 \tag{10}$$

where Q is again given by eq. (9).

The data were applied to calculate the association constants for the crosslinking processes of eqs. (5) and (7), as discussed in the cited references.[25,26]

The reductions in $[\eta]$ were accompanied by parallel increases in the Huggins' constant k_H, representing the reversibly crosslinking between different polymer chains, which in the case of four cosolutes (PhOH, HOPhOH, HOPhOMe, $HOPhNO_2$) can lead to the precipitation of the polymer.

Quantitation of noncovalent interactions for HSWSP

This above treatment may be extended to the case of HSWSP, involving now covalently attached substituent groups, and again with the two simplest cases—unimolecular crosslinking, or bimolecular crosslinking.

For the unimolecular picture already discussed, the group R is now covalently attached to the polymer chain so that the reversible crosslinking process takes the form of interactions between distantly connected parts of the same polymer chain:

$$> M - R + P < \;\rightleftharpoons\; > M - R \sim P < \tag{11}$$

Here "$P<$"; represents the distantly connected section of the chain to which the group R is attracted, and may therefore consist of several monomer units rather than just one.

The alternative bimolecular shrinkage process would involve noncovalent interactions between two substituent groups R on distantly connected parts of the same chain

$$> M - R + R - M < \;\rightleftharpoons\; > M - R \sim R - M < \tag{12}$$

In a subsequent application of the above specifically to HSWSP,[31] the IV behavior from the literature was considered for two such systems: (a) poly(vinyl acetate-*co*-vinyl alcohol) (PVAC-VAL) with low

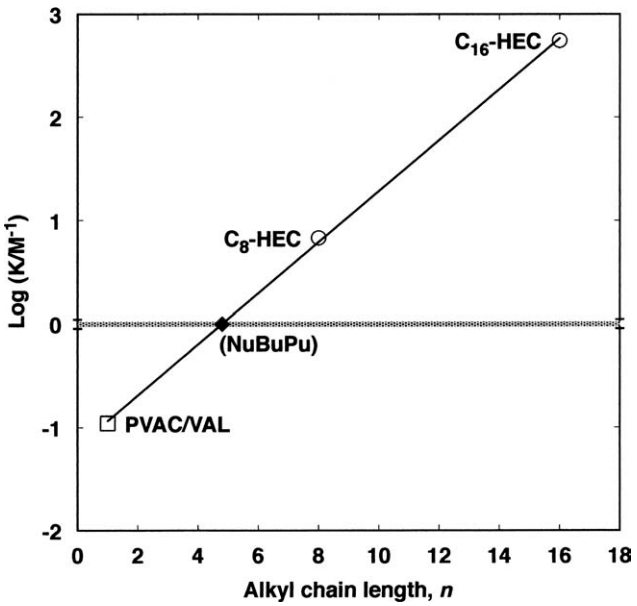

Figure 1 Association constants K (25°) for labile crosslinks in hydrophobically substituted water soluble polymers, as derived from intrinsic viscosity measurements; log K plotted against chain length of the alkyl group, n. Key: □ poly (vinyl acetate-*co*-vinyl alcohol)[31,32] and ○ alkyl-substituted hydroxyethylcelluloses[31,33,34] ["bimolecular" self-association K_2 values for eq. (12) in each case]; the horizontal shaded line gives the value of log K_1 ["unimolecular" association—eq. (11)] obtained in the present article for the nucleobase-substituted pullulans NuBuPu (where Nu is 1-thyminyl or 9-adenenyl), with the interpolated effective value (filled diamond) of n for the crosslinks in this case.

content of vinyl acetate (VAC),[32] and (b) samples of hydrophobically-substituted hydroxyethylcellulose (HSHEC) with octyl and hexadecyl groups.[33,34] In each case, there was a reduction in IV with increasing degree of alkyl group substitution, which points to a corresponding reduction in the hydrodynamic volume of the isolated polymer molecule. Also, in each case the IV reduction behavior was bimolecular, the reduction in IV being a linear function of the square of the alkyl group content according to eq. (6), where r is now replaced by x, the molar degree of covalent hydrophobic group substitution. This is again taken to indicate interactions between pairs of substituent groups (i.e., self-association) according to eq. (12). The values of the bimolecular constant K_2 calculated as detailed earlier are plotted against alkyl chain length in Figure 1, where the hydrophobic effect of the acetate group on PVAC-VAL is taken as that of one methyl group. This shows that there is a consistent effect of the alkyl chain length on the IV reduction, with the association constant increasing by a factor of 1.77(2) for each additional methylene group. This indicates in turn that for interaction between a pair of methylene groups

$$> CH_2 + H_2C < \rightleftharpoons > CH_2 \sim H_2C < \qquad (13)$$

the strength of the hydrophobic effect has a value for the standard free energy change $\Delta G(CH_2 \sim H_2C)$ of -1.4 kJ mol^{-1} This is equivalent, for a single methylene group entering into hydrophobic interaction, to a free energy contribution of -0.7 kJ mol^{-1}, which is comparable to the values estimated for similar systems.[31]

As with the PVP/phenols systems discussed earlier, the reductions in IV are accompanied by increases in the Huggins' constant k_H, ascribable again to interactions between different polymer molecules; with still greater degrees of substitution the polymer (PVAC-VAL, HSHEC) becomes insoluble from the same effect.[32–34]

This treatment therefore indicates how IV measurements may be used to quantify these noncovalent interactions. Here, IV may be replaced by other methods that give measures of coil size, such as light scattering (LS) or gel permeation chromatography. Light scattering has the advantage over IV that it also gives the parameter second virial coefficient, which is a more defined measure of coil-coil interactions than the Huggins viscosity parameter k_H.

Less generally, if the substituent groups are spectroscopically active (UV-absorbing, fluorescent, etc.), then the change in their environment when they enter into noncovalent interactions may give corresponding changes in their spectra, as discussed below in connection with pullulan.

HYDROPHOBICALLY SUBSTITUTED PULLULANS

Pullulan

The above theory is here applied to literature data[11] on the IV behavior of nonionic hydrophobically substituted derivatives of the water-soluble polysaccharide, pullulan (Pu). The data when treated as already outlined showed some unusual features that are through worth reporting, particularly in view of the scarcity of such data. This article[11] is therefore treated here as a further exemplar of the way in which such measurements may be treated quantitatively. Such quantitative interpretation leads to a number of unexpected conclusions, including the apparent inability of the nucleobase parts (thymine, adenine) of the substituent groups to show the stacking association known to occur with the free groups in aqueous solution, and with the observed crosslinking showing up an amphiphilic character to the pullulan chain.

Pullulan is a water-soluble fungal exopolysaccharide. Structurally, it is an α-D-glucose polymer (α-glucan), with α-1,4-linked maltotriose units that are then joined together by α-1,6-links (Fig. 2).[35] The polymer has been characterized extensively by

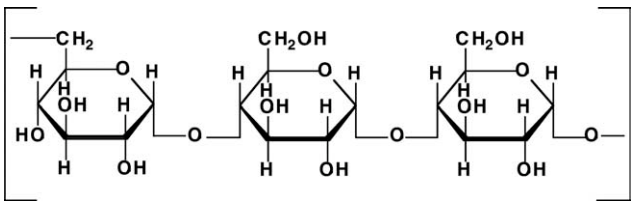

Figure 2 Chemical structure of the pullulan chain—maltotriose units linked α-1,6. In the substituted pullulans from the MCM paper[11] the substituent groups R—thyminylbutyryl (Fig. 3) in samples G3, G4, G5, and adenylbutryl (Fig. 4) in samples G6 and G7—are attached randomly to the glucose hydroxyl groups. The maximum degree of substitution (sample G5) is one R group per 14 glucose units.

standard methods (light scattering, IV, etc.) and shown to form essentially random coils in aqueous solution, indicating a freely linked chain.[36–39] Pullulan has applications as a water-soluble coating in the food industry, its films having a low permeability to oxygen.[35] It is also used as a standard for calibrating size-exclusion chromatography columns with water-soluble polymers.[40] It is also interesting for molecular modeling investigations of the relation between the configurations and linking of the component glucose rings, and the conformation of the polymer in solution.[41] Indeed, pullulan is a useful glucan because its behavior in aqueous solution does not show such complications as crystallization (cellulose) or helix formation (amylose) seen with other simple glucans.

Hydrophobically modified pullulans: Data of Mocanu et al. (MCM)

In paper under discussion by Mocanu et al.,[11] hereafter MCM, the starting polymer was a commonly used grade of pullulan designated as PI-20, as

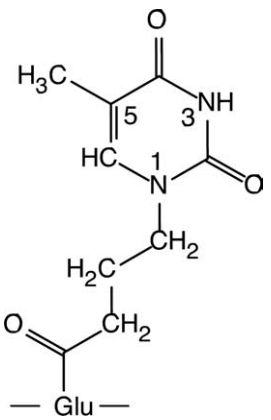

Figure 3 Structure of 3(1-thyminylbutyryl) (ThyBu) substituent group attached to the anhydroglucose unit (Glu) on the pullulan chain.[11]

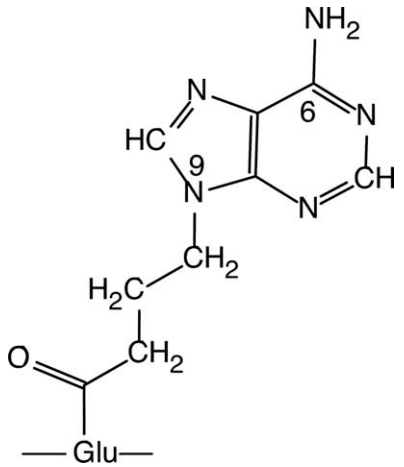

Figure 4 Structure of 3(9-adeninylbutyryl) (AdeBu) substituent group attached to anhydroglucose unit (Glu) on the pullulan chain.[11]

supplied by the major manufacturer, Hayashibari Biochemical. Here, the designation PI-20 indicates that the polymer is deionized, and that it has a nominal molecular weight of 200,000 g mol^{-1}. The pullulan was then substituted either by 3-(1-thyminyl)butyryl (TheBu) groups (Fig. 3) or by 3(9-adeninyl)butyryl (AdeBu) groups (Fig. 4) to low percentage molar content x, where the quoted values presumed to be the number of groups per glucose monomer unit, as measured by UV spectrophotometry. These two nucleobase (nucleic acid base) substituents, thymine and adenine, were presumably chosen in part to cast light on the interactions in the nucleic acids and related systems. The starting Pu and the derivatives were then studied by dilute solution viscosity in aqueous 0.1 M NaCl. The temperature of measurements was not specified, but it may be presumed to be 25°C from the parallel work by this joint group.[12] The plots of η_{sp}/c versus polymer concentration c were all linear, in accordance with eq. (1); the published data as reported[11] are plotted as [η] versus mole % substitution x in Figure 5.[†]

[†]There is some confusion in the MCM paper[11]over the data for the polymer mixture. In the first place, this is referred to evidently correctly as "G4+G7" both in their Fig. 2 and in the text, but incorrectly as "G6+G7" in their Table III. Also, the molar content of substituent groups is given incorrectly in Table III as the *sum* of those of the constituent polymers (5.8 + 2.99 = 7.99), rather than the *average* of these (7.99/2 = 4.0); the latter is the x-value plotted for this mixture in the present Fig. 5. The data for the samples G9(pyr) and G10(ad) in their Table III[11] are not of course relevant to the present treatment, since they refer to *carboxymethyl*pullulan derivatives where the ionic groups introduce complicating polyelectrolyte effects as discussed earlier.

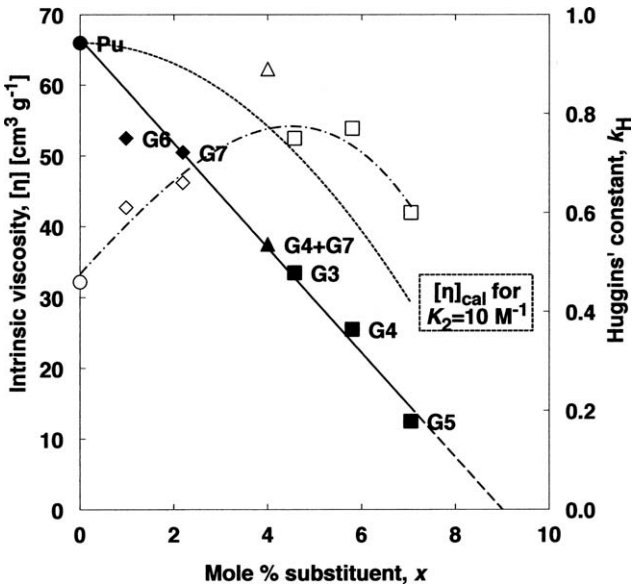

Figure 5 Plots of intrinsic viscosity [η] (left hand ordinate and filled symbols) and Huggins' constant k_H (right hand scale and open symbols) for substituted pullulan samples (0.1 M NaCl, 25°C) against the mole % substituent x; data of Mocanu et al.[11] Key: ●, ○ unsubstituted pullulan, Pu; ■, □ samples G3, G4, G5 (R = AdeBu—Fig. 4); ◊, ♦ samples G6 and G7 (R = ThyBu—Fig. 3); ▲, Δ 1 : 1 mixture of G4 and G7. The straight line is best fit to the intrinsic viscosity data for polymers Pu, G3, G4, and G5; chain dotted curve is cubic fit to all data for the Huggins' constant k_H omitting those for the mixture. The dotted parabolic curve represents the expected intrinsic viscosity dependence for bimolecular association using $K_2 = 10$ M^{-1} corresponding to alkyladenine association—see text at eqs. (21) and (22).

INTERPRETATION OF THE MCM PULLULAN DATA—ASSOCIATION EQUILIBRIA

Interpreting the data shown in Figure 5, for the parent pullulan Pu, and the three adeninylbutyryl-substituted sample G3, G4 and G5, there is a close to linear reduction in IV with the degree of substitution of the polymer chain. The average deviation of the points from the straight line is 6%; addition of a squared term to the fitting equation only reduces this deviation to 5%.

The situation with the thyminyl samples G6 and G7 is less clear-cut. In the original paper, the authors comment (Ref. [11] p 1935) that the viscosity change "is more pronounced for the adenine group [G3, G4, G5] than for the thymine group [G6, G7]." However, Figure 5 shows that this is simply due to the lower degree of substitution for the thymine group, and in fact the two types of groups show similar degrees of effect, since the sample G7 lies close to the line for the other samples (Pu, G3, G4, G5) and the sample G6 only somewhat off it. Moreover, the point for the 1 : 1 mixture G4+G7 also lies close to the line, and this would be expected to be the average of the values for the individual polymers, so that this confirms the point for G7, that is, this corresponds to this being a double point. It is evident that if the authors had used a plot such as Figure 5 in their interpretation of their data, they would have noted and corrected the discrepancy between the samples G6 and G7. In addition, as noted later, from the molecular viewpoint the latter samples would be expected to show if anything lesser effects (higher [η]) than the samples G3-G5 where the substituent group is larger (compare Fig. 3 for G6 and G7, with Fig. 4 for G3–G5). For these reasons, in the present article it is taken that the behavior of thyminylbutyryl-substituted samples G6 and G7 closely parallels that of the adeninylbutyryl ones G3-G5.

The straight line behavior in Figure 5 conforms to the unimolecular crosslinking picture previously discussed. The reversible crosslinking process show in eq. (11) now takes the more specific form

$$> \mathrm{Glu} - \mathrm{R} + \mathrm{Pu} < \;\rightleftharpoons\; > \mathrm{Glu} - \mathrm{R} \sim \mathrm{Pu} < \qquad (14)$$

Here Glu represents the local glucose unit of the chain to which the group R (here, either ThBu or AdBu—Figs. 3 and 4) is attached, while Pu is that distantly connected section of the chain to which the group R is attracted, and which may consist of one or of several such glucose units.

The alternative bimolecular process would involve noncovalent interactions between two substituent groups R on distantly-connected parts of the same chain

$$> \mathrm{Glu} - \mathrm{R} + \mathrm{R} - \mathrm{Glu} < \;\rightleftharpoons\; > \mathrm{Glu} - \mathrm{R} \sim \mathrm{R\ Glu} < \qquad (15)$$

However, this is apparently not important in the present case, since otherwise there would be a contribution from the square of the R group content, which as noted above does not seem to be the case from the experimental results (Fig. 5). The dotted curve in Figure 5 corresponds to such a contribution with bimolecular association constant $K_2 = 10$ M^{-1} for nucleobase association deduced later in the article.

The data for the Huggins' constants k_H also fit in with same crosslinking picture, since the value rises with increase in the degree of substitution, although there seems to be a final falloff. The scatter is greater here than with the IV data, since as eq. (1) shows, the value of k_H results from dividing the slope of the Huggins' plot by the square of the intercept, with a consequent propagation of errors. This increase is again a reflection of increasing interaction between different polymer molecules, that is, with the species in eq. (14) now on different

polymer chains. The 1 : 1 mixture G4+G7 shows a k_H value of 0.89, which is somewhat higher than the average value for the mixture of 0.72, showing some possible cross interaction between the two different bases (Ade and Thy), which would be in line with the favorable hydrogen-bonding seen in particular in the nucleic acids. However, this effect would not be expected if the intermolecular crosslinking that determines the value of k_H were the same unimolecular process of eq. (14) as for the intramolecular effects that determine the IV; in any case, hydrogen-bonding between the nucleobases is much weaker in aqueous solution because of the competition from the water itself.

There is also a mention in the MCM paper[11] of precipitation occurring with these samples at higher degrees of substitution "more than 5–6%." This is again in accord with this same crosslinking picture, and with the extrapolated trend of [η] values seen in Figure 5, as well with the behavior in the other systems already discussed that is, PVP/phenolic cosolutes, and the PVAC-VAL and HSHEC copolymers.

The IV data as plotted in Figure 5 may be used to obtain the value for the equilibrium constant K_1 for the unimolecular crosslinking of eq. (14) as already outlined earlier in the paper with eqs. (4), (8), and (9). Reverting to eq. (4), from the best linear fit to the data for Pu, G4, G5, and G6, the unimolecular reduction coefficient S_1 for the present data has the value 11.2(6), the degree of substitution x now being in mole fraction rather than mole %. The four other quantities required for the calculation of the numerical factor Q in eq. (9) are as follows:

a. MHS parameters for Pu/water. $\alpha = 0.664$, $K_\eta = 2.16 \times 10^{-2}$ cm^3 g^{-1} (g mol^{-1})$^{-0.664}$. The data are from Yamaguchi and Shima for water at 25°C;[39] the use of aqueous NaCl (0.1 M) as solvent in the present MCM studies[11] should not change these values appreciably. The fractional exponent on the units for the value of K reflects the α-value, and should be included to get the correct units in the final results.
b. Monomer molecular weight. $M_0 = 162$ g mol^{-1} (anhydroglucose unit).
c. Monomer unit length. $b = 515 \times 10^{-10}$ cm. This is taken as one half of the length of the diglucose unit in the cellulose crystal, from the lattice spacing (another b-quantity) of 10.3 Å (1.03 nm).[42,43]
d. Flory-Fox universal viscosity parameter. $\Phi = 2.1(2) \times 10^{23}$ mol^{-1}. There is some uncertainty in the assignment of this value, since it depends on the molecular weight distribution of the polymer,[21–23] with higher values quoted for fractionated samples, but the present starting material (pullulan PI-20) apparently has a broad distribution[35] for which the quoted value is therefore most appropriate.

Substituting these values in eq. (9) gives

$$Q(\mathrm{Pu}) = 89 \times 10^{-3}\ \mathrm{M}^{-1} \tag{16}$$

and hence using the value derived for S_1 this gives:

$$K_1 = 1.0(1)\ \mathrm{M}^{-1} \tag{17}$$

One remarkable feature of this value is that such a small association constant can give the marked reduction in IV seen in Figure 5. For example, for the sample G3, the presence of only five groups per hundred monomer units gives a halving of the IV value, that is, a halving in the hydrodynamic volume of the polymer molecule. Likewise, by extrapolation in Figure 5, a content of nine such groups would be sufficient to shrink the molecule to a compact coil with very small IV, although in practice the polymer would already have become insoluble before this degree of substitution had been reached because of the intermolecular crosslinks.

Putting this value of K_1 into context, it may be correlated with the values obtained for the bimolecular constant K_2 for the systems already discussed (PVAL-VAC and alkyl HECs), which gave an essentially linear increase in free energy of interaction with the length of the alkyl chain as shown in Figure 1. Interpolating from this data, the present value corresponds essentially to the hydrophobic interaction between two chains each of five CH_2 units.

INTERPRETATION OF THE MCM PULLULAN DATA—MOLECULAR INTERACTIONS

Molecular interactions involved

The two notable features of the present results are that first, the adeninylbutyryl and thyminylbutyryl groups seem to give essentially the same degree of crosslinking; and second, this effect is unimolecular, that is, the group R is attracted more to another part of the same chain rather than to another group of the same type. The first observation, although it depends on somewhat fragmentary data, would suggest that the effects reside not in the heterocyclic (purine or pyrimidine) ring, but in the butyryl chain, which was intended presumably only a spacer between the heterocyclic rings and the main chain. The discussion therefore centers on the competition between two possible modes of interaction: one R group interacting with a distantly connected part of the same pullulan chain, and one R group interacting with another such group on a distantly connected part of the same chain. To this end, the

behavior of model small-molecule systems is examined, as dealt with in the following sections: (a) hydrophobic effects as indicated by octanol–water partition coefficients; (b) stacking interactions between nucleobase (including alkylnucleobase) molecules; (c) hydrophobic interactions in saccharides (mono-, oligo- and polysaccharides); (d) nucleobase–saccharide interactions.

Hydrophobic effects and the octanol-water partition coefficient *P*

To quantify the interactions expected for the HSWSP in general, and compare this with the experimental values for the present samples, we need to have a measure of the hydrophobic character of the molecules and groups concerned. One very widely used measure of the hydrophobic character of a molecule is its partition coefficient between 1-octanol and water, P, i.e., for a molecule Z the equilibrium constant for the transfer process from water (aq) to octanol (oc):

$$Z(\mathrm{aq}) \rightleftharpoons Z(\mathrm{oc}) \tag{18}$$

There are now extensive databases of values of P,[44–46] while this parameter has also received official recognition in connection with environmental protection.[47] The wide use of this parameter in the biochemical and pharmaceutical/medicinal areas suggests that it should also be a useful parameter for interpreting hydrophobic interactions in water-soluble polymers, where it does not seem to have been considered or applied before. Some general features and correlations for this parameter are therefore discussed here from the present viewpoint.

Evidently, the higher the value of P, the higher is the hydrophobic character of the molecule Z. The value of log P is then related to the standard free energy of transfer of Z from water to octanol, $\Delta G(Z\text{: aq}\rightarrow\text{oc})$:

$$\Delta G(Z : \mathrm{aq} \rightarrow \mathrm{oc}) \equiv -R\ T \ln P \equiv -2.303\ R\ T \log P \tag{19}$$

where R is the gas constant and T is the absolute temperature. In the simplest case, this free energy change may be taken to be sum of independent contributions from the component groups on the molecule.[44,45] This is well shown in the case of homologous series, as illustrated by the plots in Figure 6 for log P versus carbon number n_C for six such series that are relevant to the present case. The series range from the highly hydrophobic *n*-alkanes to the highly hydrophilic alkylglucosides (which in the present case has to use the literature data for the alkylgalactosides for the higher members). In all

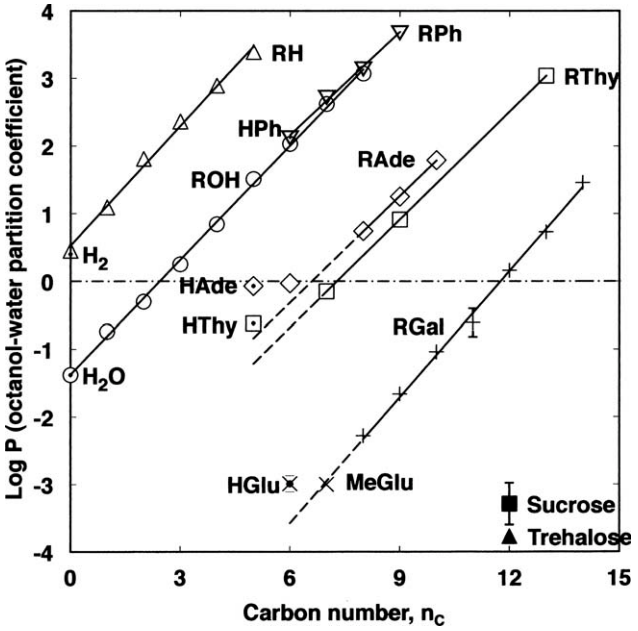

Figure 6 Octanol-water partition coefficients, P, for *n*-alkyl homologous series.[44,46] Plots of log P versus total carbon number n_C for: △ alkanes RH; ○ 1-alkanols ROH; ▽ alkylbenzenes RPh; ■ 9-alkyladenines RAde; □ 1-alkylthymines RThy; x alkylglucosides RGlu, and + alkylgalactosides RGal (common correlation line); ■ sucrose; ▲ sigma; trehalose. The parent members (H_2, H_2O, etc.) are dotted for emphasis. The horizontal chain dotted line at log $P = 0$ represents the "hydrophilic-hydrophobic" boundary. See text at eqs. (18) to (20) for interpretation of the constant-slope lines in relation to hydrophobic interactions with alkylnucleobases and saccharides.

cases, the plots are essentially linear, with an essentially constant slope of 0.56(4); in particular, the individual slopes do not seem to correlate with the nature of the end group. If we interpret this as relating to the transfer of the methylene group from water to octanol:

$$> \mathrm{CH_2(aq)} \rightleftharpoons\ > \mathrm{CH_2(oc)} \tag{20}$$

then the constant increment in log P corresponds (for an assumed temperature of 298 K) to an essentially constant value of $-3.2(2)$ kJ mol^{-1} for the free energy of transfer $\Delta G(>\mathrm{CH_2}\text{: aq}\rightarrow\text{oc})$.

This may be compared with the value, obtained earlier, of 0.7 kJ mol^{-1} for a single methylene group entering in hydrophobic interaction with another hydrophobic species in water. The ratio of these two ΔG values, 4.5(5) may be interpreted taking a simple lattice picture for these systems, with the CH_2 group in an alkyl chain in aqueous solution having (say) four to five molecules of water as well as the neighboring CH_2 groups on the same chain. Then in the hydrophobic interaction, one of the water molecules is replaced by the interacting CH_2 group on the other hydrophobic species, whereas in the aq→oc

transfer all of the (four to five) water molecules are replaced by CH_2 groups on the octanol.

It should be evident that plots of log P versus some molecular characteristic, such as carbon number as used in Figure 6, provide a powerful method for displaying and interpreting partition coefficient data normally only presented in tabular form, and giving a visual form to the various correlation equations.[44–47]

Of particular interest in the present case are the data in Figure 6 for the 1-alkylthymines and the 9-alkyladenines, since these are models for the behavior of the corresponding substituent groups (Figs. 3 and 4) on the pullulans studied by Mocanu et al.[11]

On this log P scale, thymine (HThy) is effectively hydrophilic (log $P = -0.5$) while adenine (HAde) is on the borderline, with log P close to zero. However, a better comparison would be the propyl derivatives in each case, modeling the butyryl groups intervening between the nucleobase and the pullulan chain (Figs. 3 and 4); the difference between the log P values (interpolated for the thymine case) is 0.36, giving a factor of 2.3 difference in the values of P itself. This should give a corresponding difference in any hydrophobic contribution to crosslinking ability in the pullulan derivatives.

Limitations of the octanol-water partition coefficient as a hydrophobicity parameter

Because the octanol–water partition coefficient is used widely as a way of characterizing hydrophobic interactions, and should therefore be applicable to these interactions that are presumed to occur in these HSWSP, it is necessary to emphasize some limitations of this parameter:

a. Source: As with the values of other parameters listed in databases, the P-values have generally been obtained as an incidental to a research program, rather than as part of a specific program for such data.
b. Variability: In many cases, where a number of values for a particular solute are available, these show a wide variation, often by more than one unit in log P.[46] Indeed, individual values should be treated with caution; their main strength is in correlations such as the homologous series shown in Figure 6, where the goodness of fit to the correlation lines (assumed to be linear) then gives more confidence in the individual values concerned.
c. Averaging: The log P value is a global measure for hydrophobic character of the molecule as a whole, and represents only an average of the different hydrophobic and hydrophilic characters of the component groups on the molecules. In the case of the nucleobases, in particular, there is a distinction between the peripheral region where the hydrogen bonding to the water takes place, and the regions above and below the molecules, which might be expected to be somewhat hydrophobic because of the absence of such direct bonding (Figs. 3 and 4).
d. Alkyl hydrophobic character: The value of log P parameter only represents what may be called the alkyl hydrophobic character of the molecule, that is, the balance of the interactions of the component groups with water molecules on the one hand, and with the methylene groups of the octanol on the other. It does not reflect other types of attractive effects that may lead to association in aqueous solution, such as dipole/induced dipole interactions (between a polar polymer such as PVP and polarizable molecules such as the phenols already discussed), or the stacking interactions that occur with the nucleobases in the present case as are discussed below.

Association behavior of (alkyl)nucleobases

One important requirement in interpreting the present results is to estimate how strongly the nucleobase parts of the substituent groups might be expected to associate in aqueous solution, so as to decide how such association might contribute to the viscosity effects seen with the substituted pullulans (Fig. 5). In evaluating such association data from the literature, it is necessary to distinguish between (a) the present nucleobases as used for heterocyclic ring compounds derived from pyrimidine (e.g., thymine) and purine (e.g., adenine), (b) the derived *nucleosides* (e.g., adenosine = adenylriboside), and (c) the derived *nucleotides* (e.g., adenine monophosphate) that form the nucleic acids.

Regarding interactions between these molecules and groups in aqueous solution, the noncovalent self-association of a molecule Z may be characterized by the equilibrium

$$2Z\ (\mathrm{aq}) \rightleftharpoons Z \sim Z\ (\mathrm{aq}) \qquad (21)$$

will be governed by an association constant K_{ZZ}. In practice, with the free nucleobases this stacking interaction does not stop at the dimer stage but evidently continues to form multimers through face-to-face stacking, but this may effect be neglected if the concentration is low.

In general terms, compounds derived from the purine nucleobases (e.g., adenine) show a stronger self-association than those from the pyrimidine types

(e.g., thymine), as would be expected from their larger ring system.[48–59] Without going into the details of individual cases, these data show that the alkylthymine derivatives have association constants around 1 M^{-1}, and the adenine derivatives around 10 M^{-1}, with the values increasing somewhat in each case with increasing length of the alkyl chain.

By applying these data for the adenines to the pullulan viscometry results, it is possible to estimate what would be the strength of bimolecular association involving the adenine substituent group AdeBu (Fig. 4). Using the stacking constant $K_{ZZ} = 10\ M^{-1}$, estimated for the adenines, as the value of the bimolecular association constant K_2, and the value of $Q(Pu) = 89 \times 10^{-3}\ M^{-1}$ from eq. (16) this gives the expected bimolecular shrinkage coefficient S_2 defined by the equivalent of eq. (6):

$$V \equiv [\eta]_x/[\eta]_0 = 1 - S_2 x^2 \tag{22}$$

where from this quoted data, $S_2 = 112$. The expected parabolic form of behavior from eq. (22) in plotted as the dotted curve in Figure 5. It is seen to differ markedly from the observed linear form for the experimental data.

Hydrophobic interactions in saccharides

Here, the term "saccharides" is used as general term for to mono-, oligo-, and polysaccharides. Although water-soluble polysaccharides are normally considered to be purely hydrophilic, the occurrence of hydrophobic effects in the interactions within and between the chains of these polysaccharides is supported by much work cited in the literature, as has been discussed notably in a recent review by Sundari and Balasubramanian.[60]

In this review, it was noted that in starch (amylose) and dextrin chains of the oligomaltose type, the orientation of the successive units is such as to present a surface of methine (CH) units forming a weakly hydrophobic environment (Ref. [60], Fig. 11). This applies to free chains, as in amylose that forms helices enclosing a diversity of molecules, notably iodine (as the polyiodide ion) but also a variety of hydrophobic cosolutes.[61] It also applies to the cyclodextrans (CD), which are cyclic maltose oligomers with 6, 7 or 8 glucose units, and which are well known to form inclusion complexes within their cavities. It is significant, in the present context, that this complexing occurs between β-CD (7-membered ring) and adenosine 5′-monophosphate (AMP), indicating again an interaction between the maltose-type cyclic chain and the nucleobase.[62] Since pullulan has sequences of maltotriose units (Fig. 2) then these may also be expected act as hydrophobic species.

Looking at the partition coefficient data for saccharides in Figure 6, the data for saccharides above monosaccharides seems to be confined to that for the two disaccharides sucrose (fructosylglucose) and trehalose (1→1 linked glucose dimer) as plotted. The values for these disaccharides would be expected to be much lower judged by the separation between the correlation lines for the alkanols and the alkylglycosides in Figure 6, suggesting indeed that there is some hydrophobic character arising when the monosaccharides are linked. However, for application to pullulan, this needs to be confirmed further with log P data for maltose, maltotriose and the maltodextrins as the closer analogs.

The expected hydrophobic effects were in fact observed in the early data obtained by Janado and coworkers on effect of saccharides on hydrophobic cosolutes.[63,64] In the first of these papers,[63] data were obtained for the effects of five saccharides (glucose, maltose, sucrose, maltotriose, dextran) on the solubility of octanol, and on the critical micelle concentration (CMC) of sodium dodecyl sulfate (SDS). The criterion of a hydrophobic effect in the first case is, on the simplest picture, an increase in the solubility of the octanol through complexing with the saccharide. In the second case, it is an increase in the CMC of the surfactant by a similar complexing with the SDS ions, since this means that a higher total concentration is required to attain the free concentration for micelles to be formed. In each case, the most significant effects were seen with the maltotriose, with maltose showing little effect and glucose having a reductive effect; for maltotriose, since the increases are linear in the saccharide concentration, this is consistent with the formation of a 1 : 1 complex:

$$X + Y \rightleftharpoons X \sim Y \tag{23}$$

where X is the alkyl compound and Y is maltotriose. Using this picture, the data[63] gives values for the association constant K_{XY}/M^{-1} of 0.25 for octanol at 40°C and 2.2 for the dodecyl sulfate anion in 0.1 M NaCl at 25°C. In the second paper,[64] three aromatic hydrophobes (benzene, naphthalene, and biphenyl) were used. Most significant from the present viewpoint were the solubility studies on naphthalene with the maltose oligomers Glu_n from $n = 1$ (glucose) to $n = 6$. These all gave linear increases in solubility with saccharide concentration which may be interpreted as 1 : 1 complexing according to eq. (23), with K_{XY}/M^{-1} values ranging from 0.07(4) for glucose up to 0.85 for the maltohexaose, showing the increasing hydrophobic character with increasing number of saccharide units. Of particular significance is the fact that maltotriose had a $K_{XY} = 0.6\ M^{-1}$ and that similar values were obtained for

the α- and β-methylglucosides, indicating that the two extra glucose units in the chain are equivalent to one methyl substituent group. Although these results with naphthalene are not strictly applicable to the case of purely hydrophobic interactions, they may be applicable to adenine because of its aromatic character as discussed below. Judging from the review already cited,[60] this early work[63,64] does not seem to have been followed up.

This type of binding by pullulan involving hydrophobic bonding is also indicated by its enhancement of the fluorescence of the cosolute 2-*p*-toluidinylnaphalene-6-sulfonate anion (TNS), which is widely used as a hydrophobic probe.[65] It does not seem possible to deduce an association constant for the TNS/maltotriose from the data reported, other than that the effect here is smaller than that seen in the same studies with amylose.

Nucleobase–saccharide interactions

The partition coefficient data for the nucleobases seem to indicate that they do not have any hydrophobic character, with log *P* close to zero (Fig. 6). However, there may be effects that are more specific with saccharides, from the nucleobase aromatic rings or their dipoles. The experimental work in this area seems to be limited to the early studies of Lakshmi and Nandi[66] on the solubility of adenine and thymine in aqueous saccharide solutions, which indicated a marked difference in the behavior of the two bases. Although only mono- and disaccharides were studied (ribose, xylose, glucose, sucrose), the adenine solubility was in general increased by saccharides, and essentially linearly with the saccharide concentration, whereas the thymine solubility was essentially unchanged. Using again the simple assumption of the formation of a 1 : 1 complex according to eq. (23), one may deduce the values of the association constants K_{XY}/M^{-1} with adenine as: ribose, 0.1; xylose, 0.1; glucose, 0.4; sucrose, 0.7. For thymine, the value is evidently essentially zero in each case. These fragmentary data suggest that the maltotriose units in pullulan would interact more strongly still with adenine.

Molecular interactions in the pullulan derivatives

The above results for the association between alkyl groups, nucleobases, and saccharides, can be brought together to interpret the MCM intrinsic viscosity data discussed earlier.

For pullulan, the significant fact is that this polysaccharide contains the maltotriose units (albeit interrupted by α-1,6-linkages) that seem to be the minimum required for hydrophobic effects (Fig. 2). The present data suggest that this is sufficient to give the environment to attract the present substituent groups by the butyryl chain in the unimolecular interaction of eq. (14), and more than enough to compete with the direct interactions for a pair of such groups in a bimolecular interaction of eq. (15).

It is also significant that the two types of nucleobases show markedly different stacking constants K_{ZZ}, being about 1 M^{-1} for the thymine-type and 10 M^{-1} for the adenine type, and that even the smaller of these is comparable to the value of 1.0(1) M^{-1} deduced for the unimolecular association constant K_1. It therefore remains unclear, why there should not have been an appreciable bimolecular contribution from these pairs of nucleobases, particularly for the adenine type, leading also to a marked divergence between the behaviors of the two types of samples, rather the close similarity seen in Figure 5. It can only be concluded that the free-molecule association constant K_{ZZ} does not reflect the strength of the corresponding interaction when these groups are covalently linked to a polymer. Possibly even the butyryl linker group is not sufficiently long to give the mobility of the attached nucleobase required for it to take its preferred orientation either to another such group in a bimolecular stacking interaction, or to the pullulan chain to contribute to the observed unimolecular association.

The deduction must therefore be made that it is only the butyryl "spacer" group with its three methylene groups that is active, and that these are able to interact with enough methine (>CH—) groups on the maltotriose units to give the "5-CH_2" equivalence suggested by the interpolation in Figure 1.

SCOPE FOR FURTHER WORK

Further scope for intrinsic viscosity measurements in associating polymer systems

The present article serves to emphasize both the need to have direct data on the interactions between groups in polymers, and the suitability of IV measurements to obtain this data. The measurement of IV has the benefit of simplicity—involving a thermostat, stopwatch, and Ubbelohde-type suspended level dilution viscometer—although the stopwatch may be replaced by automatic photoelectric detection of the flow time.[21,22] This simplicity is attractive when resources (time, apparatus, materials, personnel, and finance) are limited. There has also been a recent advance with the development of microchip techniques for measuring IV.[23]

The treatment above has shown how these results, using substituted copolymers with defined contents of the group of interest, may be used to define the mode of the interaction (unimolecular, bimolecular, etc.) and the equilibrium constant of the interaction

process; the limitation is the need to know the MHS parameters for the parent polymer.

In the present context of nucleobase-substitution, a further candidate for IV studies could be the nucleobase derivatives of PVAL that have been synthesized and studied for their UV characteristics by Yu and Carlsen.[67]

Further work also needs to be done with other substituted pullulans. For example, the simple alkyl derivatives of pullulan have been known (and patented) for some years.[68] Likewise, the interactions in cholesterol-substituted pullulans that lead to the formation of nanoparticles[6] need to be investigated using smaller-molecule analogs of such steroids, notably alicyclic types (cyclohexyl, decahydronaphthyl, etc.).

Indeed, there is much scope for the study of simple aromatic derivatives (phenyl, biphenyl, naphthyl, etc.) of the water-soluble polymers in general, since these promise to give direct data on the hydrophobic association forces in aqueous solution that is lacking for even these simple groups.

With these aromatic groups, and with the nucleobases, the length of any "spacer" group (e.g., number of methylene groups) should also be varied to show the effect of this on the interactions. An alternative approach would be to use one or more ethoxy groups (—CH_2CH_2O—) as the spacer; such groups, while still providing a flexible linkage, would show a hydrophilic rather than a hydrophobic effect. This is indicated by the effect of such groups on the partition coefficient; for example, the log P value falls by 0.75 units for the ethoxy group insertion $H_2 \rightarrow CH_3CH_2OH$ (Fig. 6).

Copolymer production

The copolymers for studying interactions in HSWSP need to be obtained using substitution processes on a fixed parent polymer, as in the examples cited here, to give the same degree of polymerization throughout. The alternative method that is widely used to obtain HSWSP is the copolymerization of the main monomer with small amounts of hydrophobic comonomer; however, this is not appropriate for the present purpose, because copolymers produced in this way lack the fixed degree of polymerization necessary for applying the relations introduced earlier in the paper.

Cosolute binding studies

These IV studies, insofar as they indicated the unimolecular interactions of eq. (14), suggest that the free substituent groups, such as the butyric acid RH or its salts related to the present R groups in Figures 4 and 5, should be bound reversibly to pullulan. This could be studied by standard methods, particularly thermodynamic methods such as equilibrium dialysis and cosolute solubility.[69] This is also suggested by the association seen between maltotriose and alkyl compounds discussed earlier.[63] Indeed, viscosity measurements should also be applicable to the binding of such ionic cosolutes, through the expansion of the coil from repulsions between the bound ions, as well as to those that lead to intramolecular crosslinks such as the phenols and the nucleobases.

Such studies would further clarify the association forces occurring in these HSWSP, as noted with the PVP/phenols studies discussed earlier.[35,36]

Spectroscopic studies

These association effects might be expected to give effects on the UV spectra of the nucleobases because of the change in their environment, for the intramolecular association would remain even as the system is diluted to the levels required for the UV analysis. Such UV measurements were used in the MCM work to determine the substituent group content, but apparently no such effects (notably, any shift in λ_{max}) were reported,[11] although the degree of crosslinking may be too low to give detectable effects in this case. However, covalent attachment to the polymer chain of such fluorescence probes as TNS, which as discussed earlier is known to bind reversibly to the pullulan,[65] may provide independent measure of the extent of intramolecular interactions.

Rheological studies

As already noted at the start of this article, one prominent feature of the HSWSP is the thickening effect that they have, which sets in most markedly at a fairly specific critical polymer concentration c^*. One application of the IV measurements would therefore be to correlate the value of this parameter c^* with the value of the intramolecular crosslinking association constant (K_1 or K_2) obtained as outlined earlier. One limitation is that, inasmuch as the thickening region around c^* refers to multiple association, such as into a micellar structure, the actual effect may be greater than that expected from the low-concentration value for the association constant. However, some guidance may be obtained here by the correlations between hydrophobic effects and micellisation discussed in earlier work.[31]

Thermodynamic aspects

The present article has concerned itself only with measurements at a single temperature, reflecting the fact that the IV measurements on the polymers concerned have generally been limited to one

temperature (25°C). However, to fulfill the thermodynamic aspects promised in the title, it is necessary to extend this to a range of temperatures, giving the division of the free energy change (from K_1 or K_2 as appropriate) into enthalpy and entropy parameters. This means that alongside the IV studies on the substituted polymers over a range of temperatures, it would be necessary to have the MHS parameters K_η and α in eq. (2) for the parent polymer to calculate the numerical parameter Q in eq. (9) for that temperature, to obtain the corresponding association constant. However, so long as these MHS parameters are known at one main temperature (e.g., 25°C) and three or more fractions are available of the polymer, then IV measurements on these fractions at the other temperatures (alongside those on the substituted polymers) would give the MHS parameters required.

Use of denaturing agents

One diagnostic method for the presence of hydrophobic interactions, especially in polymer systems, is to add a denaturing agent that effectively breaks such interactions. Such agents have been widely used with proteins and other biopolymers, but they have also been applied with synthetic polymers.[70] The aim here is to add sufficient of the agent to progressively annul the hydrophobic interactions so that the properties revert to that of the parent polymer; it is convenient to retain the term denaturation for this same effect. As a reference, it would be necessary to carry out such addition with the parent polymer to see what effect the agent has in this case. The denaturant concentration range over which the denaturing effects (increase, and then levelling off, in the IV) occur would be diagnostic of the strength of the hydrophobic effects.

There seems to have been only a few examples of the use of such agents in the published work on the present HMWSP. Two such examples are discussed below.

In the first example, Gelman and Barth[33] have used methanol as such an agent with in the hydrophobically-substituted hydroxethylcelluloses discussed earlier (Fig. 1), where the denaturing effect seems to occur in the region around 50% MeOH content. The consequent increases in IV parallel the decreases seen for the original substitution. It was also shown that the MeOH had no appreciable effect on the IV of the parent HEC. However, such water-miscible organic solvents would evidently only be useful in cases where the parent polymer is soluble in the solvent, or at least in the water-solvent mixture effective for denaturation.

As a second example, Karlson et al.[71] have used cyclodextrans (CD) as denaturing agents with hydrophobically-modified ethylhydroxyethylcellulose (HM-EHEC), where the effect clearly is the complexing of the CD with the alkyl substituent groups, as discussed earlier in connection with the hydrophobic character of saccharides.[60] The studies used 1% polymer concentration, where the reductive effect of the CD on the solution viscosity was used to estimate the strength of complex formation between the CD and the hydrophobic group. In fact, similar studies on the effect of the CD on the IV of the polymers would have enabled them to determine the strength of the hydrophobic interactions, as discussed earlier. This use of CD is clearly more selective than, say, organic solvents, but it may have the disadvantage of not being able to complex with less accessible groups, such as the linking alkyl chains in the present MCM work.[11]

In practice, the most common denaturing agents that are used are the two structurally-related compounds, urea and guanidinium chloride.[70] One interpretation of the effect of these agents is that they bind to the hydrophobic groups and made them effectively hydrophilic. Such as effect with guanidinium chloride would convert the polymer into a polyelectrolyte, with more complex IV behavior as already noted, so that urea is therefore the preferred agent in this case. In general, urea is commonly added at concentrations up to 8 M, but higher levels up to the solubility limit of about 20 M could be used.

It is clear that such an agent, for preference urea, should be used routinely in this way in all studies of the solution behavior of hydrophobically substituted water-soluble polymers, as a diagnostic test of the presence and strength of presumed hydrophobic interactions. This would apply both with studies on very dilute polymer solutions for the measurement of IV, and with those in more concentrated solutions on the behavior around the critical thickening concentration c^*.

CONCLUSIONS

- The need for intrinsic viscosity studies on non-ionic hydrophobically substituted water soluble polymers, to provide basic information for interpreting the rheological behavior of these and their ionic counterparts, has been emphasized.
- The manner in which intrinsic viscosity measurements may be used to quantify the noncovalent interactions in these polymers has been detailed.
- The intrinsic viscosity data of Mocanu et al.[11] on pullulan substituted by thyminylbutryl and adeninylbutyryl ester group show the shrinkage in its hydrodynamic volume because of reversible intrachain interactions.

- The results are interpreted to show that the shrinkage is due to unimolecular reversible crosslinking, that is, each crosslink takes place between a substituent group and a distantly connected part of the same chain. This is discussed in terms of the amphiphilic character of the pullulan chain, related to the hydrophobic character of the component maltotriose units.
- The scope and limitations for using octanol–water partition coefficients to characterize such hydrophobic interactions of species in aqueous solution have been discussed.
- The apparent absence of the expected bimolecular interactions, that is, between pairs of the nucleobase substituent groups is also discussed, using literature data on the association (stacking interactions) between alkylnucleobases.
- The fact that the strength of the observed unimolecular (substituent group/polymer chain) interactions is essentially the same for the two types of substituent group suggests that the nucleobases are not involved in the interaction. This is therefore ascribed to the butyryl linking group, through its sequence of three methylene groups hydrophobically bonding with the maltotriose units on the pullulan chain.
- It is suggested that by using modified substituent groups, different linking groups, and different water-soluble polymers, the present pullulan studies may be clarified and further information obtained on hydrophobic and other interactions in these systems.
- The application of cosolute binding studies, using the free-molecule analogs of the substituent groups is also suggested to further clarify these interactions.
- It is also recommended that a denaturing cosolute, for preference urea, should be used routinely as an additive in studies of these HSWSPs to provide diagnostic information on the presence and strengths of the presumed hydrophobic interactions.

NOMENCLATURE

A	cosolute molecule
Ade	adenine/adeninyl
aq	aqueous solution
b	monomer unit span along the polymer chain
Bu	3-butyryl
CD	cyclodextran
CMC	critical micelle concentration
Gal	(anhydro)galactose
Glu	(anhydro)glucose
HEC	hydroxyethylcellulose
HSWSP	hydrophobically-substituted water soluble polymer
IV	intrinsic viscosity ([η])
k_H	Huggins' viscosity slope parameter—eq. (1)
K	association constant for noncovalent interactions [M^{-1}]
K_1	K-value for unimolecular interaction in a HSWSP chain—eq. (5)
K_2	K-value for bimolecular interaction in a HSWSP chain—eq. (7)
K_{XY}	K-value for the noncovalent association X ∼ Y
K_η	MHS prefactor—eq. (2)
M	molar concentration (mol dm^{-3})
M_0	monomer unit relative molar mass
MHS	Mark-Houwink-Sakurada relation—eq. (2)
n	alkyl chain length
n_C	carbon number (for whole molecule)
oc	octanol solution
P	octanol–water partition coefficient—eq. (18)
Ph	phenyl/phenylene group
Pu	pullulan
Q	numerical factor in eq. (9)
r	degree of cosolute binding (mole/basemole polymer)
R	alkyl substituent group
S	binding site on polymer chain
S_1	unimolecular shrinkage coefficient—eq. (4)
S_2	bimolecular shrinkage coefficients—eq. (6)
Thy	thymine/thyminyl
V	intrinsic viscosity ratio, $[\eta]_r/[\eta]_0$ or $[\eta]_x/[\eta]_0$
VAC	vinyl acetate
VAL	vinyl alcohol
x	degree of covalent substitution (mole/base mole polymer)
α	MHS exponent [eq. (1)]
Φ	Flory-Fox universal viscosity parameter
[η]	intrinsic viscosity value ($cm^3\ g^{-1}$)
$[\eta]_0$	[η] value for parent polymer
$[\eta]_r$	[η] value for polymer with bound cosolute
$[\eta]_x$	[η] value for covalently-substituted polymer
∼	noncovalent interaction (X ∼ Y)

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