Water-soluble Synthetic Polymers in Immunology and Biomedicine*

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In recent years the range of materials developed, tested and accepted for use in medicine and associated areas has been extended by the inclusion of a number of water-soluble synthetic polymers (WSP).[1,2] In this paper the significance and applications of these polymers in the field of biomedicine (including that of immunology) are reviewed. Although certain WSP have been developed especially for their biological activity,[1,2] this aspect will not be discussed here. The intention, rather, is to explain some of the physical and chemical principles underlying their more general applications in these areas; these principles are discussed in greater detail elsewhere.[3-5]

The review is divided into four sections. In the first section, the six most common and important WSP are listed and the structures of their monomer units compared. Secondly, certain significant differences between the general features of the WSP and natural polymers (notably, the proteins) are outlined. Thirdly, it will be shown how experimental studies on the WSPs can be used to give quantitative information on the strengths of noncovalent interactions, which are fundamentally important in molecular biology. Finally, some of the practical applications of WSP in biomedicine are listed and discussed.

SOME IMPORTANT WATER-SOLUBLE SYNTHETIC POLYMERS

For brevity and simplicity the present discussion will be restricted to the six WSP which are most common (and therefore most important) in biomedicine. Further details of the properties and behaviour of these and other WSP will be found in a number of texts.[5-7] All six of these WSP contain only one type of monomer unit in the chain, i.e. they are therefore examples of *homopolymers.* There is also an even greater diversity of WSP containing two or more types of unit in the same chain, i.e. *copolymers;* once again, the details of these will not be discussed here.[5-7]

Poly (ethylene oxide), PEO

$$-[OCH_2\,CH_2]-$$

This polymer is available not only as the true high polymer, but also as various grades with relatively short chains (i.e. oligomers) which are more commonly referred to as polyethylene glycol (PEG). There are a variety of trade names for PEO (e.g., Carbowax, Polyox); the pharmaceutically official name for PEG is Macrogol.[1] The chain has a relatively simple chemical structure, with alternate oxygen atoms and pairs of methylene (CH_2) groups. It is evidently the oxygen atoms that make the polymer water-soluble, for if they are removed then the structure becomes that of polyethylene (polythene), which is of course a water-insoluble (and indeed, a highly hydrophobic) polymer.

Poly (ethylene imine), PEI

$$-[NHCH_2\,CH_2]-$$

This polymer is similar in chemical structure to PEO, with the oxygen atoms replaced by secondary amine (NH) groups, which confer water-solubility on the polymer. The presence of these groups also means that the polymer is basic (i.e., a polybase), and it forms salts (such as the hydrochloride) which are ionised in the solid state and in aqueous solution i.e.., they are polyelectrolytes; in this form the polymer chains are positively charged (polycations).

Poly (vinyl alcohol), PVAL

$$-\left[\begin{array}{l} CH - CH_2 \\ | \\ OH \end{array}\right]-$$

This polymer typifies the general structures of both the vinyl and the acrylic group, where the basic struc-

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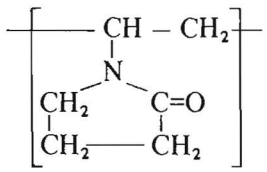
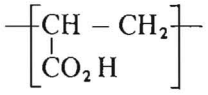
ture is that of polyethylene but with a substituent group on every other carbon atom. In the present case this is the hydroxyl group, which evidently confers water-solubility on the polymer. One special feature of this polymer is that the commonly available commercial grades are produced by hydrolysis of the water-insoluble polymer, poly (vinyl acetate), and certain of these grades of PVAL still contain an appreciable proportion of acetate groups which can greatly modify the physical properties, especially vis-a-vis water.

Polyvinylpyrrolidone, PVP



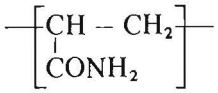
This is another vinyl polymer, and one which is quite important in the biomedical area, particularly in its pharmaceutical applications.[8] Its monomer unit structure is somewhat more complex than that of PVAL; the substituent group is in this case the five-membered pyrrolidone ring, which contains three methylene groups and an amide group. In view of the hydrophobic character of the methylene groups, it is evidently the amide group that makes this polymer water-soluble; the presence of this group also means that the monomer unit structure is somewhat similar to that of the proteins. The pharmaceutically official name for this polymer is Povidone;[1] common trade names include Kollidon, Luviskol, Plasdone and Pllyclar. Cross-linked forms of this polymer (which therefore only swell in water rather than dissolving in it) are also available under a variety of trade names (e.g., PVPP, polyclar AT, Kollidon CL).

Poly (acrylic acid), PAA



This is the prototype polymer of the acrylic group of synthetic polymers; in this case, the water-solubility is conferred by the carboxyl group. As the name and structure indicates, this polymer is acidic (i.e., a polyacid), and forms salts (e.g., sodium polyacrylate) which are ionised both in the solid state and in aqueous solution, and are thus further examples of polyelectrolytes; in this case the polymer chain is negatively charged (i.e., a polyanion). Cross-linked or branched forms of this polymer are available commercially, and have a diversity of trade names (e.g., Carbopol); the pharmaceutically official name of one such form is Carbomer.[1]

Polyacrylamide, PAAm



This is evidently related to poly (acrylic acid), with the carboxyl groups converted into amide groups, so that this is a nonionisable polymer. The amide groups not only make the polymer water-soluble, but give it such a highly hydrophilic character that it is much more resistant to precipitant by salts or organic liquids than the other WSP listed here.

DIFFERENCES IN THE FEATURES OF NATURAL AND SYNTHETIC POLYMERS

To those working in biomedicine, the most familiar macromolecular substances are the natural polymers (biopolymers) such as proteins, polysaccharides and nucleic acids. In this area, water-soluble synthetic polymers are used in association or in parallel with biopolymers, particularly the proteins, in such applications as "tracers" or "markers" or "models". It is therefore pertinant to point out a number of ways in which certain general features of WSP differ from those of the proteins, and which must be borne in mind in such applications.

Molecular weight variability

Many of the proteins are definite chemical entities, with a specific sequence of specific amino acids and hence with a definite molecular weight; these features are indeed presumed for any named protein.[9] With synthetic polymers, on the other hand, in principle there is no restriction on the length of the chain, and hence none on the molecular weight of the polymer. Accordingly, many WSP are commercially available in a variety of molecular weight grades. Thus PEO (PEG) is available in grades with molecular weights ranging all the way from the "monomer" (ethylene glycol), through the oligomers (PEG), up the true high polymers such as the Polyox grades (which have molecular weights of several million). Similarly, PVP is available in a number of molecular-weight grades, each designated by a Fikentscher K-number related to its solution viscosity, ranging from K-15 (nominal molecular weight 10,000) up to K-90 (nominal molecular weight 360,000).[8] The differences in molecular weights of these grades will affect the properties and behaviour of the polymer both *in vitro* and *in vivo*. For example, the melting/freezing point of PEO varies with molecular weight (particular in the PEG range), which is evidently important in its pharmaceutical applications. Also, when a WSP such as PVP is given parenterally, the rate of its renal excretion via glomerular filtration will evidently decrease with increase in molecular size, i.e. with increase in molecular weight. Thus in any application of these polymers it is necessary to both know and to state the molecular weight of the sample used.

Molecular weight distribution

A further complication concerning the molecular weight of synthetic polymers is that bacause of the random manner in which the molecules grow and die during the chemical synthesis, in the final product

the chains are in general not all of the same length (i.e., they are not *monodisperse*) but they have a range of lengths (i.e., they are *polydisperse*), leading to a spread or distribution of molecular weights.[9,10] This is illustrated schematically in Fig. 1, which represents the molecular weight distributions (MWD) for three different samples of polymer with the same average molecular weight. The first sample, A, is monodisperse so that the plot is a vertical line going up to 100 per cent; this applies to certain biopolymers, such as the enzymes and other specific proteins. The second sample, B, is polydisperse but with a relatively narrow MWD, such as would be obtained by the fractionation of material with a wider MWD. Finally, sample C is polydisperse with a relatively MWD, as would be obtained for a single batch of directly synthesised polymer. The breadth of the MWD of a synthetic polymer also affects the properties and behavior *in vitro* and *in vivo*. Thus a blend of two different grades of PEO will have a wider range of melting temperatures than that of the component grades with a narrow MWD. Similarly, if we consider the renal excretion behaviour of a polymer given parenterally, then whereas a sample with relatively narrow MWD (e.g. B in Fig. 1) would have an acceptable optimum residence time in the body, for one with a wider MWD (e.g. C in Fig. 1) the performance would be quite different and most probably unacceptable (since the appreciable low molecular weight fraction would be excreted too rapidly, while the appreciable high molecular weight fraction would be retained too long and possibly show undesirable interactions with other blood components or the body tissues). Thus in all these applications of WSP it is not sufficient to know the average molecular weight, but it is also necessary to have at least an estimate of the width of the MWD.

The presence of an appreciable width in MWD also means that any single-value molecular weight that is quoted must be some type of average. Furthermore, different methods of determining molecular weights give different types of average and hence genuinely different numerical values, so that the type of average should also be stated with the value. The three most common averages are the number-average, M_n (obtained from end-group or osmotic-pressure methods), the viscosity-average, M_v (obtained from viscometry), and the weight-average, M_2 (obtained from lightscattering methods).

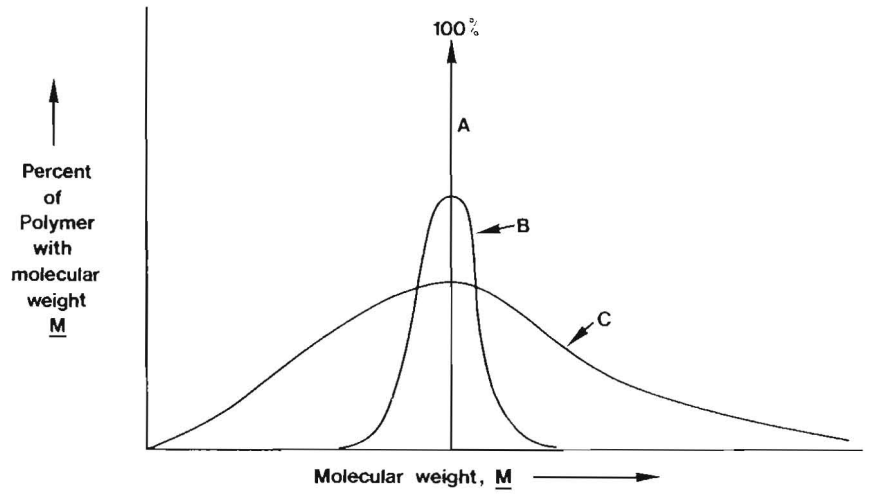


Fig. 1 Representation of the molecular weight distribuions for polymers of different dispersity: (A) Monodisperse polymer; (B) Polydisperse polymer with a relatively narrow MWD; (C) Polydisperse polymer with a relatively wide MWD.

Molecular size and shape

A further important difference between proteins and WSP lies in their effective sizes and shapes in solution.[9,10] With the globular proteins (which includes many common exmaples, such as serum albumin, haemoglobin and enzymes) the molecule in its native state has a fairly compact shape, with the chains coiled specifically and relatively tightly; in such a conformation the molecular shape thus approximates to a fully compact sphere, which with a protein of molecular weight 50,000 would have a diameter of about 6 nanometres (60 A). By contrast, the chains of synthetic polymers are in general much more flexible and mobile in solution, exploring all possible conformations through their thermal (Brownian) motion; this leads to an average shape, often referred to as a random coil conformation, which is much more expanded in its size. Thus, for the same molecular weight value of 50,000, a WSP might typically have a diameter of about 12 nm, i.e. approximately twice that of the corresponding globular protein. *In vitro*, the WSP will be characterised by a higher intrinsic viscosity than that for the protein; correspondingly, again because of its greater size, it will be eluted more rapidly from a size-exclusion chromatography (SEC) column than the protein, hence giving a falsely high value for its molecular weight if native proteins are used directly as molecular weight calibrants for the column. *In vivo*, the WSP will pass less readily and less rapidly through porous membranes (e.g., in glomerular filtration) than the protein, a feature that must be borne in mind when a WSP is being used in clinical applications as a diagnostic or therapeutic agent.

Other natural polymers

The above comparisons have been restricted to proteins as the natural polymers. By contrast, the features of synthetic polymers out-

lined above (i.e., variability and spread of molecular weight, and an expanded shape in solution) are also shown by many polysaccharides, both the purely natural ones such as dextran as well as the chemically modified ones such as methylcellulose.[9,10] In this respect, therefore, a polysaccharide such as dextran is more appropriate (although still not necessarily ideal) for the direct calibration of an SEC column that is used for molecular weight studies on WSP. Similarly, *in vivo* the renal excretion patterns of dextran and WSP should be similar (if we ignore the effect of the higher biological stability of WSP).

EVALUATION OF NONCOVALENT INTERACTIONS

Turning to some applications of the WSP, one important "theroretical" application is to the evaluation of the strengths of noncovalent interactions in aqueous solution. The term "noncovalent interactions" (NCI) refers collectively to the different types of "adhesive" or "cohesive" forces between molecules, other than true covalent bonds. The most important of these may be classified as follows:

1. Van der Waals forces, subdivided into
   (a) Dipole/dipole (Keesom forces)
   (b) Dipole/induced-dipole (Debye) forces
   (c) Dispersion (London) forces
2. Ion/ion (electrostatic, or coulombic) forces
3. Hydrogen bonds
4. Hydrophobic effects
5. Charge-transfer interactions
6. Co-ordinate (dative) bonds*

*The properties of co-ordinate bonds vary widely according to the nature of the two groups linked, especially in the biochemically important examples which involve transition metal ions complexing with inorganic ligands (including water) or organic ligands. In many cases these bonds are indeed quite weak and labile, and hence clearly classifyable as one form of NCI; in other cases they can approach the strength and stability of true covalent bonds.

Individually, these NCI are weak compared with true covalent bonds; collectively, however, they have the feature that when there is a suitable combination of complementary forces of these types (for example, between two macromolecules) then this gives an overall interaction which is at the same time *reversible* and *specific* and *strong* – three characteristics which are otherwise incompatible. Indeed, rather than being just some abstract part of physical chemistry, NCI have a fundamental importance throughout molecular biology. For example, NCI control the specific folding and aggregation of the subunits of proteins in solution, and hence determine protein stability and denaturation.[11,12] They are also accordingly fundamental in antigen/antibody interactions in solution, which are one form of protein aggregation; they are therefore responsible for the fact that the association and precipitation reactions between an antibody and its complementary antigen have the threefold characteristic (reversibility + specificity + strength) already noted. The role of various NCI in such systems was noted and discussed nearly 40 years ago by Pauling and Pressman,[13] although they did not realise (or emphasise) the importance of the solvent water in these interactions. These same NCI are likewise involved in a whole host of "complexing" and "binding" phenomena *in vivo* – enzyme/substrate, enzyme/inhibitor, serum albumin/drug, receptor/hormone, etc. Even *in vitro* they are important in biomedicine, since they are responsible for the differential staining of tissues by the dyes used in histology, and for certain types of blood cells being distinguishable as "basophils", "neutrophils", "eosinophils", etc.

Despite the extensive work that has been done by physical chemists on the theoretical basis of NCI in liquids and solutions,[14,15] and despite their evident importance in molecular biology, there is still much doubt and dispute about the actual strengths of the component interactions in biological systems. This is because such biological interactions take place predominantly in aqueous solution, and the presence of the water as solvent profoundly affects the strengths of the component interactions.[16] For example, because of the high value ($\sim$80) of the dielectric constant $\in$ of water, the forces between ions are much weaker (by the factor $1/\in$) in aqueous solution than they are in a vacuum ($\in = 1$), in the crystalline state of the solid salt, or even in a nonpolar solvent ($\in \approx 2$). Similarly, the *net* strength of a hydrogen bond between a donor group and an acceptor group is greatly reduced in aqueous solution because of the competition from the molecules of water hydrogen-bonded to (i.e., hydrating) these groups in such a solution, since at least one such water molecule will have to be removed from each of the groups before they can form their own hydrogen bond. On the other hand, the presence of the water brings into prominance the so-called hydrophobic effects, i.e. the attractive forces between nonpolar molecules, and nonpolar groups on molecules, in aqueous solution.[11,12,17,18]

Thus any experimental work which promises to give more direct information on the actual strengths of the component NCI in aqueous solution will be useful in aiding the interpretation of biological phenomena at the molecular level. Such information may be obtained from studies on WSP, both because they are water-soluble and because their structures are generally simple and well defined. There are four aspects to these studies.

Firstly, there is the behaviour of these polymers vis-a-vis water itself; this involves in particular their solubility behaviour and its dependence upon temperature and upon molecular structure (which therefore also requires the consideration of structurally related polymers that are *not* water-soluble), and also

their thermodynamic and conformational behaviour in solution.[5,6]

Secondly, information on NCI may be obtained by studying the interactions between an WSP in aqueous solution and an accompanying small molecule solute (cosolute), particularly one that specifically binds (i.e., complexes) with the polymer.[5] By varying the structure of the polymer or the cosolute in a systematic manner and observing the consequent variation in the strength of the binding, information may be obtained on the NCI between the constituent groupings of the polymer chain and the cosolute molecule. This may be illustrated by the results obtained from studies on the binding by polyvinylpyrrolidone of alkyl p-hydroxybenzoates as cosolutes; the binding equilibrium constant was observed to increase by a factor of 1.5 for each additional carbon atom (i.e., methylene group) in the alkyl chain, which may be identified as the contribution from hydrophobic interactions between this methylene group and the methylene groups on the polymer chain.[19]

Thirdly, pairs of nonionic polymers such as PAA and PEO, or PAA and PVP, form water-insoluble complexes (i.e., they mutually precipitate from aqueous solution) which are evidently held together by hydrogen bonds (the first polymer of the pairs acting as the donor and the second as the acceptor).[5] The study of these complexes hence gives us information on the effective strengths of hydrogen bonds in aqueous systems, as well as providing support for the role of hydrogen bonds in maintaining proteins in the α-helix, and DNA in the double-strand form, even in the aqueous environment of the cell.

Finally, there is the formation of water-insoluble polyelectrolyte complexes, between polyanions (from a polyacid) and polycations (from a polybase); for example, solutions of poly (acrylic acid) and poly (ethylene imine) give such a complex when they are mixed.[5] The study of these complexes can thus give us information on the effective strength of interionic forces in aqueous solution, while the complexes serve as models for the interactions between oppositely charged biopolymers in the cell, such as those between protamines and nucleic acids.

PRACTICAL APPLICATIONS

Water-soluble synthetic polymers have numerous and diverse applications in biomedicine and allied areas. As stated earlier, we confine the discussion to those applications which depend upon the physicochemical effects of the polymers rather than any more specifically biological effects; indeed, in many cases the success of the application depends upon the biological inertness of the WSP. However, frequently it is not clear what function (or functions) the WSP does play in the application. This is often the situation with work reported in the patent literature, where WSP are frequently included in a recipe or formulation; of course, it should be borne in mind that the publication of a patent claim does not make the procedures described either scientifically valid, clinically acceptable, or commercially successful.

In those applications where it is simply the macromolecular nature of the additive that is important, WSP are often used as alternatives to their natural and chemically-modified natural counterparts. In this context, WSP have the advantage that in general they are much more stable both physically, chemically and biologically; however, the high biological stability can be a drawback in internal (especially, parenteral) uses where the polymer may be retained too long in the body rather than being metabolised and hence excreted.

In applications of WSP where they come into contact with living cells, care must be taken to guard against the presence in commercial samples of the polymers of small-molecule contaminants (especially, residual monomer) which can have a toxic effect on the cells; the polymer solution may need to be exhaustively dialysed beforehand to obviate such effects.[20-22]

Pharmaceutical applications

The water-soluble synthetic polymers have been used in many different ways in drug dosage forms.[1,2]

In liquid dosage forms, WSP have been used for their general viscosifying and thickening action. They have also been used more specifically in applications depending on the propensity of polymers to be absorbed at interfaces. Examples of this are: stabilising emulsions and suspensions; inhibiting the recrystallisation of solid drugs in suspension (which otherwise leads to the undesired growth of the larger particles at the expense of the smaller); and inhibiting the adsorption of any small-molecule solutes which are present onto solid drugs in suspension.

In semisolid dosage forms (gels, pastes, ointments, etc.), WSP have been used as base materials; in the case of PEG, the consistency and melting behaviour are both important, and these will depend upon the average molecular weight and the MWD of the polymer sample. This particular polymer, although widely used for these purpose, has the disadvantage that it may induce the decomposition of certain drugs, such as hydrocortisone.[23]

Solid dosage forms have also employed WSP in a number of capacities. In tablets, they have been used as binders (in amounts between 1 and 10% of the whole formulation), as disintegrants,[24] and as stabilisers, e.g. in nitroglycerin formulations to reduce the loss of this rather volatile drug.[25-27] They have also been used more particularly to give enhanced rates of release and absorption in the body for drugs which are poorly soluble or essentially insoluble; two such

applications are in "fusion mixtures" and in "high energy coprecipitates", as discussed below.

Fusion mixtures are an application especially of PEG, which is a crystalline polymer with a convenient range of melting points for different molecular weight grades. Here, the powdered drug is dispersed in the fused polymer, in which form it may be metered into hard gelatin capsules where it is allowed to cool and solidify. This procedure is more convenient and accurate than measuring in the powdered drug itself into the capsule, while its dispersed state in the water-soluble matrix means that it is more readily released and absorbed when the fusion mixture comes into contact with the body fluids. Although this technique has been established for nearly twenty years,[28] applications continue to be developed with such drugs as the steroids,[29] cardiac glycosides,[29,30] and the antidiabetic tolbutamide.[31]

With *high energy coprecipitates* the main polymer used is PVP, which is amorphous and with an amphiphilic character (see above); the dosage form either results as a true coprecipitate on mixing the drug and the polymer in a common solvent, or alternatively the mixed solution is evaporated or spray-dried if the "coprecipitate" does not appear directly. The early work on this method was carried out by Mayerson and Gibaldi,[28] and it was also developed subsequently by Simonelli and coworkers;[29,33] it continues to be developed and applied to a diversity of poorly soluble drugs.[30,31,34,35] In this dosage form, particularly where it is obtained by evaporation of the joint solution, it is apparent that the drug is molecularly dispersed (i.e. "dissolved") in the amorphous matrix of the polymer, and that it is this feature which is responsible for the greatly enhanced rate of absorption in the body. From this viewpoint it would be more precise to refer to these systems as "amorphous solid solutions", particularly when they are obtained as evaporates rather than precipitates.[8]

Certain of the WSP, notably PVP, have been used parenterally, especially as blood-plasma expanders.[36] However, this latter application of PVP was discontinued since it was found that the higher molecular weight material is stored in the reticuloendothelial system rather than being metabolised and excreted, so that the bacterial polysaccharide dextran is preferred where a plasma substitute must be used.[37]

A further pharmaceutical application of WSP is in the iodine containing biocides referred to collectively as iodophores (or iodophors). The most notable is that containing PVP, pharmaceutically named Povidone-Iodine and widely used under a diversity of trade names as an antiseptic and disinfecting agent.[1,8]

A further biomedical application of WSP is to the development and production of covalent conjugates with proteins, particularly to make the latter more resistant to proteolytic degradation and hence have a prolonged action when given clinically. Such covalent conjugates of polyacrylamide with a number of enzymes are available commercially.[38] Covalent conjugates of PVP have also been prepared with timothy grass allergen,[39] enzymes,[40,41] and insulin.[42]

Freeze-preservation (cryopreservation) and freeze-drying (lyophilisation)

The cryopreservation process, in which solutions or suspensions are frozen and then stored in this form at a low temperature, has been used for many biomedically important materials, notably with suspensions of whole cells which must have a high rate of recovery when the suspension is thawed out eventually. Water-soluble synthetic polymers such as PVP and PEG have been used as one form of cryoprotective (cryophylactic) agent in this process, to improve the overall recovery and the viability of the recovered cells, as alternatives to other "nonpenetrating" polymeric agents (such as glycerol, dimethyl sulfoxide, or sucrose). The use of PVP in the cryopreservation of erythrocytes is well established;[20,43] a more recent application is to the preservation of leprosy bacteria.[44]

A parallel application of WSP is in the freeze-drying (lyophilisation) of drugs (including antibiotics and immunosupressive agents), vaccines (and other immunological preparations), enzymes, and reagents for clinical tests. Polymeric additives act, like the small-molecule additives used alternatively (lactose, mannitol), to bulk up the volume of the plug produced after freeze-drying; however, polymers have the particular advantage that they very efficiently bind an otherwise powdery form (which would be readily dispersed by an electrostatic effects when the vial is unsealed), while they also form a coherent and continuous film which coats the particles and protects them from chemical attack in this rather vulnerable state with a large exposed surface area.

The rational development of each of these procedures (cryopreservation and lyophilisation) to their maximum efficiency requires an appreciation of the highly complex phenomena involved in the freezing and thawing processes; the physical chemistry of water and aqueous solutions at these subzero temperatures has been recently reviewed.[45]

Clinical analysis and diagnosis

Water-soluble synthetic polymers have been used as additives in clinical analysis. Thus, PVP has been used in the determination of inorganic phosphate by the blue phosphomolybdate complex, where the polymer is stated to catalyse the formation of the complex and also to minimise interference from any proteins that may be present in the

clinical sample.[46-48]

In clinical diagnosis, WSP have been used as nonabsorbable radiolabelled markers or tracers. Thus PVP labelled with ^{125}I or ^{131}I, referred to as Iodinated Povidone Injection, is administered intravenously in the diagnosis of protein-losing gastro-intestinal disorders and for permeability studies.[1] In such applications, the radiolabel must be linked covalently and firmly to the polymer chain.

These polymers may also be used as stabilisers for suspensions of radiocolloids used for diagnostic purposes, such as the scanning of bone and of organs such as the brain, liver, lung, spleen and thyroid. This stabilising property may also be applied with other inorganic colloids. Thus, in a rather more fundamental area, but one with biomedical implications, PVP-coated colloidal gold particles have been used by Feldherr to study the permeability of nuclear membranes in the amoeba *Chaos chaos*[49,50] and *Amoeba proteus*,[51] the coating of PVP makes the gold particles inert biologically, and hence suitable as models for the nucleoproteins which are also transferred across the nuclear membranes.

Separation and purification of biomaterials

Because of the complexity of biological systems, separation and purification procedures have acquired a great importance in studying such systems and dealing with the diverse mixtures of biomaterials obtained from them. Water-soluble synthetic polymers have been found to be useful in many of these procedures.

One general application of WSP such as PEG or PVP is to the concentration of solutions of biopolymers obtained in analytical or preparative work. The liquid sample is placed in a cellophane dialysis bag and this is immersed in a concentrated solution ($\sim$50%) of a low molecular weight grade of the polymer or even simply put in contact with the solid polymer, when water is then removed osmotically from the sample. In these applications, other small-molecule components of the sample will also be lost, while at the same time small-molecule impurities in the polymer may be transferred across to contaminate the sample.

In their cross-linked form, WSP are then (like other such polymers) water-swollen rather than water-soluble, and hence may be used in bead or granular form for general separation procedures, including the removal of impurities and contaminants. Thus the ion-exchange resins that are widely used for analytic and other purposes are in many cases cross-linked forms of WSP such as poly (acrylic acid) and poly (styrenesulphonic acid).

Polyacrylamide is used in a cross-linked form in such procedures as polyacrylamide gel electrophoresis (PAGE) and size-exclusion chromatography; its particular advantage in these applications is that it is a highly hydrophilic polymer whose degree of swelling in this crosslinked form is little affected either by temperature variation, or by the presence of high concentrations either of buffer salts or of such agents as sodium dodecyl sulphate or urea which are commonly used as additives in these analytical procedures.[5]

Polyvinylpyrrolidone, especially in the cross-linked form, has been used extensively as an adsorbant for polyphenols such as tannins, particularly in work on plant extracts.[52-57] Recent applications of immunological significance include the work of Bjorksten and colleagues on the purification of apple allergens,[58] and the studies of Kutz and colleagues on byssinosis from cotton dust, where cross-linked PVP was used to remove from the dust extract polyphenols which gave a nonspecific pseudoimmune reaction.[59]

In their linear, water-soluble form, these WSP have been applied to the fractionation of biopolymers. In many cases, this depends upon the rather nonspecific "excluded volume effect" of the highly extended coil of the WSP in solution; this effect depends on the fact that since the biopolymer is excluded from the domain of the coil then its concentration in the free volume remaining to it in the solution is increased, which hence favours its transfer to another phase, such as a highly concentrated phase of precipitated biopolymer. This mechanism presumably underlies the use of PVP in the fractionation of lipoproteins.[60,61] In the wider context, a similar effect also presumably underlies the current widespread use of PEG in the production of hybridomas, where the PEG acts to force the different strains to aggregate and hence hybridise. However, this excluded volume effect can also lead to changes in the conformation of biopolymers which may give rise to misleading effects in analytical procedures. Thus, Lerman and his colleagues found that when DNA was dissolved in solutions of WSP such as PEG, PVP or sodium polyacrylate, although no actual precipitation occurred the DNA molecule was converted into a highly compact form which therefore sedimented much more rapidly in the ultracentrifuge cell.[62,63]

Other applications of WSP to fractionation and separation depend on more specific effects. Thus, the basic polymer PEI acts as a precipitant for nucleic acids and acidic proteins, and it has been used accordingly in the isolation and purification of enzymes.[64-67]

Partition in biphasic aqueous polymeric systems

This is a technique that was developed originally by Albertsson and his colleagues at Uppsala[68] and extended by Walter and colleagues in California.[69] The production of the biphasic systems that are used in this technique depends upon the phenomenon referred to specifically in polymer science as "incompatibility". It may be illustrated by

considering the behaviour of the two polymers PEG and dextran when they are placed together in water; this is a very pertinant example because such systems containing these two polymers have been used extensively in this partition technique. The basic observation is that if an attempt is made to prepare an aqueous solution to contain, say, 4% PEG 6000 and 7% dextran D68, this does not lead to a homogenous system but instead it separates into two phases of approximately the same volume, with the upper phase relatively rich in PEG (concentration about 7%) but poor in dextran (about 0.2%) whereas the lower phase is relatively rich in dextran (about 14%) but poor in PEG (about 1%). This is a quite general type of behaviour which is shown by most pairs of soluble polymers in nonaqueous media and also by many (but not all) of them in aqueous media. In thermodynamic terms, although the entropy change of mixing per unit mass for the two different polymers is still positive (i.e. favourable), it is very small simply because of their high molecular weights; thus there only needs to be a small net repulsive force between the different types of polymer molecules for the system to separate into two phases with the polymers largely segregated from one another, as observed.

These types of aqueous biphasic system containing PEG and dextran have then been used in partition techniques to separate and purify a variety of biomaterials, including enzymes and other proteins, nucleic acids, chloroplasts, and viruses. Depending on the relative affinity of the biomaterial for the two water-soluble polymers, it may concentrate mainly in the upper phase with the PEG or in the lower phase with the dextran, while certain types of particles congregate instead at the interface.

Although possibly somewhat overshadowed by more recently introduced techniques such as isopycnic centrifugation (see below), these biphasic systems have the merit that they are wholly aqueous, and do not involve any organic solvents or require either high salt concentrations or extremes of pH. Thus the conditions can be kept close to physiological so that the separation procedure is quite gentle and has little tendency to denature any of the biopolymers, or to damage or kill any cells or organelles being separated.

Isopycnic centrifugation in density gradients

This is a technique that enables cells of different types to be separated by taking advantage of even small differences in their densities (the word "isopycnic" is the name of the technique meaning "of the same density"). In its simplest form, the procedure comprises setting up a suitable density gradient in a column of liquid held in centrifuge tube (with the density naturally increasing down the column), layering the sample of cells on the top, and then centrifuging so that the different types of cells sediment to the levels of their own density. The key feature is the density gradient, and four types of system have been used in this procedure.

The first, and simplest, approach is to use a small-molecule solute such as caesium chloride or sucrose. However, it is not always possible to keep these solutions isotonic, and hence this can lead to damage to the cells from osmotic effects.

An alternative approach is to use as the solute a water-soluble polymer, e.g. a synthetic type such as PVP, or a protein such as serum albumin, or a polysaccharide such as Ficoll (a sucrose-based polymer). In this case the osmotic effects are much less because of the higher molecular weight of the polymer. However, the relatively high viscosities of these solutions can lead to difficulties in handling.

A third approach is to use a suspension of solid colloidal particles, notably silica, since suspensions of this are available commercially under such trade names as Ludox (Du Pont). Here again osmotic effects are not serious, while the viscosity is less than with polymeric solutes. However, this particular system has the drawback that the surface of the silica can be toxic to certain types of cells.

The fourth approach is then to combine some features of the second and third types, by using colloidal silica particles having their surface coated with a water-soluble polymer such as polyvinylpyrrolidone; the silica/PVP combination is commercially available under the trade name Percoll (Pharmacia).[70] This has the advantages that it has a relatively low osmotic pressure and a relatively low viscosity, while the toxicity is much less than with uncoated silica because its surface is covered by the PVP (which is itself relatively inert biologically). The material Percoll was developed by Pertoft and his colleagues at Uppsala.[71,72] It has subsequently been applied both by them and by numerous other groups with a great diversity of cell mixtures, and equally for preparative and analytical purposes. This may be illustrated with the results obtained with a mixture of three types of blood cells by Ulmer and Flad.[73] In their work, the monocytes separated out as a layer at 1.063 g/ml, the lymphocytes as a band between 1.065 and 1.069 g/ml, and erythrocytes as a layer at 1.075 g/ml. As will be seen, the cells differ little in their actual density – maximally, only 0.01 g/ml, i.e. about 1% – and yet it is quite possible to set up the small stable gradients necessary for this kind of separation.

The favourable features of a water-soluble polymer such as PVP in its application to Percoll arise partly from the flexibility of its chains which enables its molecules to be adsorbed onto the silica surface at the active (i.e. "toxic") sites while the remainder of the chains project into the solution to give physical stability to the suspension; at the same time, the polymer is al-

most inert biologically as well as being very stable physically, chemically and biologically.

Summary

The phyiscal chemistry of water-soluble synthetic polymers (WSP) is reviewed specifically in relation to their applications in biomedicine and immunology. The six WSP most commonly encountered in this context are: poly (ethylene oxide) (polyethylene glycol), poly (ethylene imine), poly (vinyl alcohol), polyvinylpyrrolidone, poly (acrylic acid), and polyacrylamide. It is pointed out that there are important differences between certain general features of WSP and those of biopolymers such as the proteins – notably, in their molecular weight variability, in their molecular weight distributions, and in the size and shape of the polymer molecules in solution. It is also shown that studies on the physical chemistry of WSP can be used to elucidate the diverse types of non-covalent interactions that are fundamentally important in molecular biology. Finally, a number of practical applications of WSP in biomedicine and immunology are listed and described. These include: pharmaceutical applications (especially in drug dosage forms); additives in cryopreservation and lyophilisation (freeze-drying); in clinical diagnostic and analytical techniques; and in procedures for the separation and purification of biomaterials such as biopolymers, organelles and cells (including partition in aqueous biphasic polymeric systems, and isopycnic sedimentation in density gradients).

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