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POSTER ABSTRACTS

Experimental Investigation of Cellulose and Cellulose Derivatives in Mesomorphic Solutions.

By P. Navard & J.-M. Haudin, Ecole Nationale Supérieure des Mines de Paris, Centre de Mise en Forme des Matériaux, Valbonne, France.

A lyotropic polymer solution is a complex system which can only be studied by using different experimental techniques on the same material. In this presentation, we investigate the structure and some physical properties of cellulose/methylmorpholine *N*-oxide and cellulose triacetate/trifluoroacetic acid solutions with several experimental techniques, for which the results and interpretations obtained with the above solutions are given. Optical microscopy gives a quick demonstration of the existence of optical anisotropy and a measure of several relaxation times. Spectrophotometry and light diffraction give the pitch of the helix of the cholesteric structure as well as information on the substructures (or texture). A good measure of the critical concentration is given by refractometry. Rheometry and calorimetry provide some important information on the ordered-disordered phase transition.

The possibilities of these techniques are examined and what could possibly be deduced for a better understanding of these solutions is exposed.

Complex Formation Between Cellulose and Tertiary Amine Oxides.

By S. Perez, Centre de Recherches sur les Macromolécules Végétales, CNRS, Grenoble, France.

Combined crystallographic investigations and computer simulation have been performed in order to arrive at an understanding of the mechanism of dissolution of cellulose in tertiary amine oxide systems such as *N*-methyl morpholine *N*-oxide (MMNO). It is shown that tertiary amine oxides can be characterised as 'structuring', 'stiffening' and 'protective' agents.

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The structuring effect emanates from the polar N→O bond of the molecule which can act as the receptor of one or two hydrogen bonds, and can interact with one or two hydroxylic oxygen atoms of cellulose. When the molecular ratio of water bonded to MMNO is less than 2, such a bonding scheme is quite feasible. On the other hand a molecular ratio equal or greater than two would be sufficient to prevent further hydrogen bonding by the N→O linkage to cellulose and therefore would hamper any solvent character.

The formation of strong complexes by the cellulose chains with tertiary amine oxide molecules (two hydrogen bonds per glucose unit) stiffens the molecule due to steric hindrance and restricts variability in the chain conformation. This may explain the formation of cellulose mesophases in amine oxide systems. Such a view is strongly supported by the unperturbed chain dimensions and spatial organisation of the cellulose-MMNO complexes.

Dissolution can be arrived at only if a segmental recombination of the cellulose chains is prevented for which role MMNO appears to be particularly well suited. The complexing of MMNO with cellulose chains is such that the apolar moiety of MMNO acts as a protective layer. Since this protective layer does not allow for any strong intermolecular interactions, the MMNO cellulose entities are free to move with respect to one another. Such a freedom of motion is a necessary step towards a dissolution process.

Phase Behaviour of The System *N*-Methyl Morpholine *N*-Oxide, H_2O and Cellulose.

By H. Chanzy, S. Nawrot & P. Smith, Centre de Recherches sur les Macromolécules Végétales, CNRS, Grenoble, France.

In the past decade there has been increasing interest in the development of new solvents for cellulose, with the final goal of the production of cellulose films and fibres by means of a simple process giving no pollution. Among the various diluents studied *N*-methyl morpholine *N*-oxide (MMNO) stands out as a powerful cellulose solvent. This compound occurs in the form of several hydrates with melting points inversely related to the water content. Typically, the onset of melting of anhydrous MMNO is observed at 172°C, whereas in the monohydrate this occurs at 74°C. The strongly dipolar N→O moiety of the MMNO molecule is responsible for its excellent solution properties. The oxygen is able to form one or two hydrogen bonds with hydroxylated substances such as H_2O and cellulose. When the dissolution of cellulose occurs in the presence of water, which leads to a lowering of the melting point of the solvent, there is a competition between water and cellulose for the MMNO molecules. The influence of water on the MMNO/cellulose system is not only definitive during the dissolution of the cellulose system but also when cellulose solutions are cooled down and allowed to crystallise. At least two types of texture can be obtained during such crystallisation experiments, depending on the presence or absence of water in the cellulose solution. In both cases the morphology consists of a cellulose skeleton embedding solvent crystallites. By subsequent removal of the solvent crystals it is possible to obtain interesting porous cellulose structures.

To gain a better understanding of the dissolution process and of the origin of the various morphological features, the crystallisation and melting behaviour of MMNO/H_2O/cellulose mixtures has been investigated by differential scanning calorimetry, optical and electron microscopy and X-ray scattering, and the MMNO-rich part of the phase diagram of this quasi ternary system has been constructed.

Interpretation of the Chain Length Dependence of the Unperturbed Dimensions of the Cellulose and Amylose Tricarbanilates in Terms of a One-Dimensional Order–Disorder Transition. By B. Hsu, C. A. McWherter & D. A. Brant, Department of Chemistry, University of California, Irvine, USA; and W. Burchard, Institüt für Makromolekulare Chemie, Universität Freiburg, West Germany.

Dissolved cellulose (CTC) and amylose (ATC) tricarbanilate both exhibit temperature and solvent induced transitions between forms of differing stiffness and chain extension. The transition temperatures are strongly dependent on chain length, suggesting that the transitions are very cooperative (Gupta *et al.* (1979). *Macromolecules*, **12**, 281). It is shown in this work that the chain length dependences of the unperturbed dimensions of CTC and ATC (Burchard (1971). *Br. Polym. J.*, **3**, 214) also reflect the cooperative conformational transitions inferred from other kinds of measurements.

The method of Miller & Flory ((1966). *J. Mol. Biol.*, **15**, 298) is used to fit the observed chain length dependency of the unperturbed dimensions using realistic chain models for the two polymers. Zimm–Bragg cooperativity parameters σ of the order of 10^{-5} and 10^{-6} are required for CTC and ATC, respectively, to match the slow convergence of the characteristic ratios to their asymptotic values at high chain length. We have been unable to fit the data in question with any model that does not involve a *cooperative* transition from a more flexible form of the chain skeleton, which predominates at low chain length, to a less flexible form, the stability of which is enhanced at high chain length.

The theoretical models providing the best fits to the unperturbed dimension data are used to calculate persistence lengths of 110 Å and 103 Å, respectively, for CTC and ATC. The models are also used to demonstrate, in terms of the calculated directional correlation function of the terminal bonds of the chain, that directional correlations persist over a substantially larger number of residues in ATC than in CTC.

Complexing Properties of Substituted Cyclodextrins and Cyclodextrin Polymers in Aqueous Solutions. By J. Szejtli, Biochemical Research Laboratory of Chinoin Pharmaceutical and Chemical Works, Budapest, Hungary.

Cyclodextrins form inclusion complexes in aqueous solutions with a number of compounds. A characteristic of such complexes is the strongly reduced solubility in water. If, however, the cyclodextrins are modified in their hydroxyl groups, e.g. methylated, or crosslinked with appropriate bifunctional reagents up to a molecular weight of 10 000 the solubility of the derivatives as well as of their inclusion complexes strongly increases. Aqueous solutions of remarkably high concentrations can be prepared by complexing such water insoluble compounds as, for example, benzene, *p*-xylene, cholecalciferol, triglycerides, etc. While no more than 1·8 g β-cyclodextrin is soluble in 100 ml water at room temperature, a clear solution can be prepared with 40 g/100 ml concentration of a soluble cyclodextrin polymer while a 50 g/100 ml solution can be easily prepared from heptakis-/2,6-di-*O*-methyl/-β-cyclodextrin. In aqueous media the physical properties and chemical reactivity of the complexed molecules reveal quite surprising features: a 40 g/100 ml β-cyclodextrin polymer solution dissolves 1·4 g benzene; a 30 g/100 ml cyclodextrin polymer solution dissolves 4 g/100 ml benzoic acid while a 50 g/100 ml heptakis-/2,6-di-*O*-methyl/-β-cyclodextrin solution dissolves 0·9 g cholecalciferol, etc. In such a solution, benzene, a typically achiral compound, induces circular dichroism. The dissociation of organic acids in the complexed form is suppressed while the diffusion rate is also strongly reduced. The enzyme catalysed reaction of poorly water soluble substrates is accelerated up to 10 times.

Dynamic Light Scattering from Malto-Oligosaccharides. By A. Reiner, W. Burchard, W. Emmerling & B. Pfannemüller, Institüt für Makromolekulare Chemie, Universität Freiburg, West Germany.

Avebe amylose was partially degraded by the α-amylase, from *Bacillus subtilis* and the product was subsequently fractionated at 50°C by GPC using BIO-Gel P4. Separation of the oligomers up to a DP of 21 (G21) became possible.

By repeated fractionation oligomers up to G13 could be obtained on a preparative scale. Only the middle fraction of the individual peaks were taken for diffusion coefficient measurements by dynamic light scattering.

The dynamic light scattering measurements were first carried out with a Kr laser by spectra physics with the red $\lambda = 647$ nm line of 550 mW power. Rather high concentrations of the oligomers were needed (10% for G1 to G6 and 4–5% for G7 to G13). Reliable results could be obtained after application of a sophisticated clarification procedure. Comparison with the conventional boundary spreading technique revealed much lower diffusion coefficients obtained with the dynamic light scattering technique. The concentration dependence of D could be measured for glucose and maltose and for α-cyclodextrin when the green line ($\lambda = 514$ nm) of a coherent Ar laser with a power of 3 W was applied. A strong concentration dependence was found for G1 and less pronounced for G2 and α-cyclodextrin. An excellent agreement was found after extrapolation to zero concentration with the data obtained by Longsworth using the boundary spreading technique. The D values are still about 10–15% lower than those obtained from the Monte Carlo calculations carried out by Cesàro and Brant. Deviations of similar magnitude have been detected, however, with PMMA and polystyrene and are probably caused by the Kirkwood diffusion equation which apparently takes insufficient account of the hydrodynamic interaction.

A higher D coefficient was found for the cyclic than for the linear hexamer in good agreement with theory.

Theoretical Studies on β-Maltose Conformers in Solution.

By I. Tvaroška, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

The present paper is concerned with the conformational equilibria of β-maltose conformers in dilute solution. The stability of four conformers (Melberg & Rasmussen (1979) *Carbohydr. Res.*, **69**, 27.) in 12 solvents is compared by using the continuum reaction field method in which the solvation energy consists of electrostatic, dispersion and cavity terms (Tvaroška & Kožár (1980). *J. Amer. Chem. Soc.*, **102**, 29). The calculated population of four conformers (designated as M1, M2, M3 and M4) significantly depends on the medium, e.g. in dioxan, M1 : M2 : M3 : M4 = 53·3 : 20·3 : 17·7 : 8·7; in dimethyl sulphoxide, 40·1 : 21·8 : 22·8 : 15·3; in water, 25·7 : 17·5 : 26·3 : 30·5. These results indicate that the conformation adopted by β-maltose in the crystalline form is not the one preferred in solution. The role of individual contributions to the solvation energy and the magnitude of the mean residual optical activity and proton–carbon coupling constants will also be discussed.

Excess Thermodynamic Properties of Aqueous Solutions of Mono- and Disaccharides and Derivatives at 25°C.

By G. Barone, P. Cacace, G. Castronuovo, D. Doucas & V. Elia, Istituto Chimico, University of Naples, Italy.

The excess Gibbs free energies and enthalpies of aqueous solutions of mono- and disaccharides and their derivatives have been obtained by using the isopiestic gravimetric method and flow and batch microcalorimetry, respectively. New data relating to:

(a) D-ribose, D- and L-arabinose and D-lyxose;
(b) 2-deoxy-D-ribose, 2-deoxy-D-galactose and 2-deoxy-D-glucose;
(c) α-methyl-D-xylopyranose, -galactopyranose, -glucopyranose and -mannopyranose;
(d) cellobiose, maltose and trehalose;

are reported and compared with other previously published results (D-xylose, D-fructose, D-galactose, D-mannose, lactose, raffinose, L-fucose and L-rhamnose) or those found in the literature (D-glucose and sucrose). Our results underline the existence of 1. the well known enthalpy-entropy compensation; 2. the unfavourable solute-solute interactions, prevented by the solute-water ones; 3. a regular dependence, at least of the excess enthalpies, on the detailed stereochemistry of the solute, in the case of isomeric monosaccharides. The data were interpreted as due to a reduction of hydration on increasing the solute concentration. With disaccharide solutions the properties do not simply depend on the nature of the constituent monomers. For instance in the case of the three isomeric glucosyl-glucosides the different mutual orientations of the two pyranoid moieties, due to the different glycosidic linkages, play an important role, probably related to the existence, or not, of intramolecular H-bonds. This is of interest for the study of the corresponding polysaccharides.

Separation of Starch Components by Ligand Induced Adsorption of Amylose on Cellulose. By Y. Y. Talib, M. S. Karve, S. V. Bhide & N. R. Kale, Department of Chemistry, Division of Biochemistry, University of Poona, India.

Amylose the linear component of starch is known to form a helical complex with a variety of ligands such as 1-butanol, 1-pentanol, cyclohexanol, thymol, sodium dodecyl sulphate (SDS), etc.

The ligand induced adsorption of amylose on a cellulose column (equilibrated with solvent system (I), containing acetate buffer (pH 4·8, 0·1 M), the complexing ligand and urea (2 M)), is used for the quantitative separation of amylose and amylopectin from starch. The amylose-ligand complex is retained on the cellulose column while the amylopectin-ligand complex appears in the effluent. The column is washed free of the amylopectin-ligand complex with the same solvent system (I) and the adsorbed amylose is eluted by gradient elution by decreasing the ligand concentration. Samples containing 300–400 mg of starch can be successfully fractionated on a column (1·5 x 25 cm) packed with defatted cellulose (25 g), with a recovery of 95% (± 2%). The elution profile reveals the heterogeneity of native amylose. The separated components are characterised by determining their blue value and β-amylolysis limit.

This method may be extended to the separation of amylose and amylopectin from starch on a micro-scale on filter paper and also may be scaled up for its use on a preparative scale.

It is interesting to know that amylose has no affinity for cellulose but the helical amylose-ligand complex is retained with high affinity on cellulose.

Resonance Raman Spectroscopy of Short-Chain Amylose-Iodine Complexes. By B. Pfannemüller & G. Ziegast, Institüt für Makromolekulare Chemie, Universität Freiburg, West Germany.

The Raman spectra of solutions of amylose-iodine complexes derived from enzymatically synthesised samples (DP = 25–200), maltooligomers (DP = 3–18) and cyclodextrins have been determined. Particular attention was focused on the minimum length of the helical complex formation and a possibly preferred length of an uninterrupted polyiodine chain. In the Raman spectra of the synthetic samples four fundamentals were observed at 162, 112, 52 and 24 cm^{-1}. Measurements at excitation frequencies of 647·1, 514·5, 457·9 and 363·8 nm showed, in agreement with our earlier studies, that the signals at 162 and probably also at 24 cm^{-1} are resonance enhanced at an excitation wavelength close to the long wavelength absorption maximum. Thus, these vibration modes are dependent on the electronic transition(s) underlying the 'blue' band, but they are obviously independent of the length of the polyiodine chain, since in the range of DP 25-200 there is no change of the Raman lines position with λ_{max}. The 112 cm^{-1} Raman line, generally assigned to the symmetrical stretching vibration of I_3^-, was shown to arise both from free I_3^- (enhanced at 363·8 nm) and from bound I_3^- (relatively most intense at 457·9 nm excitation). In the series of the linear maltooligomers the main signal at 162 cm^{-1} is detectable with maltooctaose, but not with maltohexaose. It is, however, clearly seen with cyclic maltohexaose. It may be concluded that the subunit of the aligned polyiodine chain is formed by fitting the iodine into one helical turn. Binding to such a short chain can hardly be reconciled with previous assignments of the 162 cm^{-1} line to I_5^- or I_3^-/I_2 subunits, stretching over a distance of about 15 Å. It is remarkable that identical spectra are obtained when using I_2/KI or I_2 solution initially free of I^- ions for complex formation. This finding and the position of the 162 cm^{-1} Raman line located between molecular I_2 (207 cm^{-1}) and I_3^- (112 cm^{-1}) can be taken as arguments for a slightly polarised I_2 as the main complexed iodine species.

Small Angle X-Ray Scattering Studies on Aqueous Amylose and Amylose-Iodine Complex. By E. Ferracini*, A. Ferrero*, D. Braga & A. Ripamonti, Istituto Chimico 'Ciamician', Università di Bologna, Italy; D. A. Brant, Department of Chemistry, University of California, Irvine, USA; and A. Cesàro, Istituto di Chimica, Università di Trieste, Italy.

The preliminary results of an investigation of the small angle X-ray scattering (SAXS) of aqueous solutions of amylose and the amylose-iodine complex was presented. The work is directed to explore the details of the chain conformation of aqueous amylose (Jordan, Brant & Cesàro (1978). *Biopolymers*, **17**, 2617; Cesàro & Brant (1980. In: *Solution Properties of Polysaccharides*, ACS Symposium Series No. 150, chapt. 34) and the stoichiometry and dimensions of the bound iodine-triiodide sequences in the dissolved amylose-iodine complex (Cesàro, Jerian & Saule (1980). *Biopolymers*, **19**, 1491; Cesàro, Konic & Brant (1981). In: *Solution Properties of Polysaccharides*, ACS Symposium Series No. 150, chapt. 32).

A sample of enzymatically synthesised amylose has been converted to sparsely substituted carboxymethyl amylose (DS = 0·08) to enhance the solubility in water.

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* Centro di Studio per la Fisica delle Macromolecole del CNR.

The sample has $\bar{M}_w = 3{\cdot}9 \times 10^5$ daltons and is of quite narrow MWD ($\bar{M}_w/\bar{M}_n = 1{\cdot}10$). Solutions of the complex were prepared by equilibration of the aqueous polymer solution with an iodine-saturated chloroform solution.

The scattering intensities, registered with a high resolution Kratky camera, have been corrected for background scattering and slit smearing effects with standard procedures. The data are reported as Guinier plots (log intensity versus scattering vector squared) at several polymer concentrations (1·0–7·5 g/litre) and at degrees of saturation of the iodine complex varying from 0·0 to 0·4.

The scattering curves in the absence of iodine display two linear (Gaussian) regions from which radii of gyration of 28 and 240 Å can be obtained. The larger value corresponds to the expected overall dimension of the amylose chains; we cannot yet provide a structural interpretation of the smaller dimension. The plots also display a broad concentration-dependent maximum reminiscent of observations made with SAXS on other polyelectrolytes in the absence of supporting electrolyte. In the presence of iodine the 28 Å component of the scattering curve is preserved. Other features of the curves under these conditions are under continuing investigation.

Small Angle X-Ray Scattering from Solution of *S. Salivarius* Levan. By S. S. Stivala & B. Khorramian, Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Hoboken, New Jersey, USA.

A fractionated sample of a levan from *S. salivarius* was examined in water at 25°C by small angle X-ray scattering. The high molecular weight of $16{\cdot}9 \times 10^6$ and low radius of gyration of 395 Å suggest that this levan is branched. The mass per unit length, $(m_u)_b$, for this sample was found to be 72·94 daltons/Å.

Based on the molecular weight of the monomer unit (2,6-anhydro-β,D-fructofuranose) and its length, the mass per unit length of a linear levan, $(m_u)_l$, was calculated as 28·94 daltons/Å. The observation of $(m_u)_b > (m_u)_l$ is a positive indication of branching. The *g*-factor, a measure of branching, was computed as 0·39 from the derived expression, $g = (m_u)_l/(m_u)_b = L/L_l$, where L and L_l are the hydrodynamic length (total stretched length) of the backbone of the branched levan and linear levan, respectively. Values of 42·79 Å for the persistence length, $L = 2{\cdot}313 \times 10^5$ Å, and 22 Å for the radius of gyration of the cross-section were also obtained for the branched levan.

Physico-Chemical Properties of Gum Arabic. By J. C. Fenyo, Laboratoire de Chimie Macromoléculaire, Faculté des Sciences de l'Université de Rouen, France.

Some properties of this plant exudate have been reinvestigated due to the lack of satisfactory agreement between previously published data; in addition, some new results are presented.

Molecular weights determined by low angle laser light scattering on individually identified nodules of gum arabic from *Acacia senegal kordofan* lie between 3×10^5 and 2×10^6 daltons, the maximum of the distribution being at about $4\text{-}5 \times 10^5$. The discrepancies between previous literature data can be mainly assigned to differences in the heterogeneity of the samples.

The chiroptical properties have been investigated on purified, ultrafiltered samples. The ORD curves can be fitted by a single term Drude equation with a λ_c of about 166 nm (in the region of absorption of alcohol groups). Ionisation of the COOH function affects the DC spectra below 260 nm. The effect of pH is similar to that observed for D-glucuronic acid which is one of the components of the gum. DC of Na and Ca arabate are similar; and Ca ions do not drastically affect the viscosity. This suggests weak electrostatic binding of divalent ions as observed in other glucuronic acid containing gums, such as peach gum.

The pK_a varies quasi-linearly with ionisation in salt-free solutions. The small amplitude between $\bar{\alpha} = 0$ and 1 can be attributed to the relatively low-charge density of the chains (equivalent weight = 1250). Theoretical models for polyelectrolyte solutions can account fairly well for the titration behaviour if a structural length of 11 Å is assumed, but this can only be considered a mean value as the chains are highly branched.

Preliminary results from GPC on Sepharose CL-4B are consistent with the hetero polymolecularity of the gum which may consist of a mixture of branched chains of various compositions.

Further studies are in progress.

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Role of the Sialic Acid in the Conformational Control of the Capsular Polysaccharide Antigens of Group B *Streptococcus*.

By H. J. Jennings, C. Lugowski, K.-G. Rosell, R. Roy & D. L. Kasper, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada, and Harvard Medical School, Boston, Massachusetts, USA.

The types 1a and III polysaccharides of Group B *Streptococcus* are isomeric, both consisting of linear repeating unit trisaccharide backbones with disaccharide branches of α-NeuAc*p* (2→3) β-D-Gal*p* and α-NeuAc*p* (2→6) β-D-Gal*p*, respectively. Although common to both polysaccharides these terminal sialic acid residues play no direct role in either of the determinants responsible for the serological specificity of these native polysaccharides. However, these sialic acid residues remain immunodominant in the sense that they exercise remote conformational control over these determinants which are responsible for the human immunological response to the pathogenic types 1a and III *Streptococci*. The conformational dependence of these determinants was established in a series of serological experiments using both the native and modified native types 1a and III polysaccharides. ^{13}C and ^{1}H NMR spectroscopic studies on the same polysaccharides also enabled the mode of conformational control to be determined. In both the types 1a and III polysaccharides, the terminal sialic acid residues control the orientation (torsion angles) of the penultimate branch β-D-galactopyranosyl residues to their respective backbones.

Complexes of Heparin with Polyalkylenimines: Studies of Competitive Binding with Methylene Blue. By B. Casu, G. Torri & M. Legramandi, Istituto di Chimica e Biochimica 'G. Ronzoni', Milan, Italy; and P. Righetti, Department of Biochemistry, University of Milan, Italy.

Binding of heparin (HEP) and HEP fractions with oligo- and polyalkylenimines having the general formula $H_2N(CH_2{-}CHR{-}NH)_nH$, where $R = H$ or CH_3, was investigated by Vis. spectroscopy, by evaluating the competition of the amines and the metachromatic dye methylene blue for the anionic sites of HEP. The strongest binding was observed at pH 3·5, with the essentially linear triethylenetetramine (TETA) and the slightly branched tetraethylenepentamine (PEHA) giving the most stable complexes. For N (number of nitrogen atoms per molecule) > 5, a decrease in the binding ability of the amine was observed. The apparent stoichiometry of the complexes was a function of the relative concentrations of HEP and the amine, indicating an equilibrium between two types of complexes. Beef lung HEP and a HEP fraction predominantly consisting of trisulphated disaccharide blocks gave stronger complexes than the more heterogeneous pig mucosal HEP and a HEP fraction of lower sulphate content. The results are interpreted in terms of polyelectrolyte-type associations involving sulphate groups on adjacent residues of the HEP chain and sequences of charged nitrogen atoms on the polyamine.

Solutions and Gels of *N*-Carboxymethyl Chitosan*. By R. A. A. Muzzarelli, F. Tanfani, M. Emanuelli, S. Mariotti & M. G. Muzzarelli, Institute of Biochemistry, Faculty of Medicine, University of Ancona, Italy.

Chitin and chitosan are known to be insoluble in most aqueous systems, and in all common organic solvents: however, some of their derivatives form gels and homogeneous solutions. In fact, chitosan, $|(1\rightarrow4)$-2-amino-2-deoxy-β-D-glucan$|$, which can also be obtained industrially from the by-products of the fish processing and fermentation industries, lends itself to many chemical modifications.

Chitosan has been previously *O*-carboxymethylated but a number of chemical side reactions occur that limited interest in the product. In this communication we report on the *N*-carboxymethylated product. In the new *N*-carboxymethyl chitosan, each

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* Further reading: Muzzarelli (1977). *Chitin*, Pergamon, Oxford; Muzzarelli & Pariser (1978). *Proc. 1st Int. Conf. on Chitin & Chitosan*, MIT Press, Mass.; Muzzarelli (1981). Domanda di brevetto italiano No. 22780 A/81 del 7 Luglio.

anhydroglucosidic unit carries a $-NH-CH_2-COOH$ group on position 2, i.e. a glycine residue. Therefore, this new polymer is a polysaccharide attached by a covalent linkage to the nitrogen of an amino acid. It carries both the functions of the secondary amine and of a carboxylic group plus a primary amine function if the degree of substitution is not complete.

Our approach to the *N*-carboxymethylation is new and original and is intended to preserve the integrity of both the primary and secondary alcoholic functions present on each anhydroglucosidic unit, and hence the hydrophilic properties of the polymer. The reaction also preserves the basicity of the amine since it does not disactivate its power of forming dative covalent linkages. The mild reaction conditions used (room temperature, short reaction time) do not cause chain degradation. The resulting polymer is characterised by high molecular weight, high degree of substitution (that can be up to 100%), and a regular distribution of the functional groups.

N-Carboxymethyl chitosan is therefore soluble in water and for certain degrees of substitution it is soluble at all pH values. As the pH of the solution approaches the isoelectric point of the polymer there is an increase in viscosity and a tendency for gelation to occur. This is due to 'competition' between the tendency to coagulate at the isoelectric point and hydrophilicity of the anhydroglucosidic chains.

On comparing the well-known EDTA (ethylenediamino tetraacetic acid) with *N*-carboxymethyl chitosan, it is found that both carry the $>N-CH_2-COOH$ group, that allows the formation of pentaatomic rings while reacting with metal ions. *N*-Carboxymethyl chitosan has in fact the two main characteristics of EDTA, being a chelating compound for metal ions and a blood anticoagulant. *N*-Carboxymethyl chitosan has the additional advantage of being polymeric and a secondary instead of a tertiary amine.

The fact that different functional groups on different chains can take part in complexating single metal ions causes the crosslinking of the polymer. In the presence of ions of transition metals, *N*-carboxymethyl chitosan-metal chelates are immediately precipitated. The various chelates have typical colours. It is found experimentally that the polymer capacity for the different ions (at very low concentrations in water) is exceptionally high.

In this communication the following are described: (i) the methods of organic synthesis starting from chitosans of different origins; (ii) comparisons between products of different degrees of substitution; (iii) the instrumental characterisation of the products in relation to the different uses. Data referring to infrared spectrometry, visible and ultraviolet spectrophotometry, viscometry, alkalimetry and gas-chromatography are given in detail.

While it seems that *N*-carboxymethyl chitosan can be used for several applications in different fields, information is given about metal chelation and the prevention of blood coagulation.

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Solutions of Chitin. By D. Gagnaire, J. St.-Germain and M. Vincendon, Laboratoires de Chimie, Centre d'Etudes Nucléaires de Grenoble, France.

Chitin, a poly-$\beta(1\rightarrow4)$*N*-acetyl D-glucosamine is known to be highly insoluble in chemical solvents. The dissolution of this kind of natural polymer can occur through different pathways either by classical solvation or by formation of a complex or soluble derivative. NMR spectroscopy has been used to characterise the nature of solutions of chitin in saturated lithium thiocyanate *N,N*-dimethylacetamide containing 5% lithium chloride and pure formic acid. Both ^{13}C and ^{1}H NMR allow one to differentiate between the mechanism of dissolution of chitin in solvents containing lithium salts and the dissolution in formic acid, which occurs through the formation of the soluble derivative formylchitin.

A Light Scattering Study of a Series of Xanthan Fractions in Aqueous Solution. By G. Paradossi & D. A. Brant, Department of Chemistry, University of California, Irvine, USA.

A series of fractions of xanthan polysaccharide (Kelco Keltrol) has been prepared by ultrasonic degradation and subsequent molecular weight fractionation on preparative scale size exclusion chromatography columns packed with controlled pore glass (Electronucleonics CPG-10). The samples were converted to the sodium salt form and studied in 0·1 M aqueous NaCl solution. They were characterised by NMR, ORD, and ultraviolet absorption spectroscopy and are believed to be chemically equivalent to the undegraded starting material. Twelve xanthan samples covering a range of $\bar{M}_w$ of 0·8–19·5 $\times 10^5$ daltons were studied by Rayleigh light scattering. The radii of gyration and second virial coefficients were found to depend on molecular weight in the way expected for samples of molecularly dispersed, semi-flexible macromolecules.

For the samples of higher molecular weight the angular dependence of the scattering intensity exhibited asymptotic behaviour at high angles and could be analysed directly to give a linear mass density of about 1900 daltons/nm. Assuming that the samples of lower molecular weight behave as rigid rods, it was also possible to analyse the scattering curves for these samples in the limiting (low angle) region to yield the linear mass density. Although these data are more scattered due to uncertainties arising from the correction for molecular weight heterogeneity, they yield a linear mass density fully consistent with the value obtained from the asymptotic behaviour of the high molecular weight samples.

Specificity in the Interaction Between Xanthan and Iron (III) Complex Ions in Aqueous Solution. By M. Barteri, O. Mantovani & B. Pispisa, Istituto di Chimica Fisica, Università di Roma and Istituto Chimico, Università di Napoli, Italy.

The binding process between $[Fe(tetpy)(H_2O)OH]^{2+}$, $[Cu(tetpy)]^{2+}$, Fe^{3+} and Cu^{2+} ions and xanthan (Xn), around pH 6, has been studied by means of chiroptical and calorimetric measurements. The polysaccharide was found to undergo a disorder-order transition on increasing the amount of bound counterions. The affinity of Xn for the ionic species follows the order: $[Fe(tetpy)(H_2O)OH]^{2+} \gg Fe^{3+} > [Cu(tetpy)]^{2+} > Cu^{2+}$. Induced chiroptical effects on the bound achiral complex ions together with melting temperature data on the ordered polyelectrolyte solutions clearly suggest that forces other than coulombic ones play a significant role in the formation of the $[Fe(tetpy)(H_2O)OH]^{2+}$-xanthan association complex. In this case a site binding takes place which very likely involves a coordination bonding between an apical site of the Fe(III) complex ions and a carboxylate group of the side chains of the polymer. On the contrary, despite the similarity in charge density as well as in chemical structure, the different symmetry of the Cu(II) complex ions does not allow such a mode of binding. Finally, at variance with spectroscopic data, the enthalpic changes as a function of complex-to-polymer ratio happen to exhibit a monotonic trend. In all cases the binding is weakly endothermic (around 4·2 kJ/mol) which indicates that the process is entirely entropy-driven probably because of the release of solvent molecules from the hydration shells of the interacting species.

Different Modes of Interaction of Copper(II) Ions with Natural Polyuronates as Revealed by Potentiometric and Polarographic Methods. By G. Manzini, A. Cesàro, F. Delben, S. Paoletti & E. Reisenhofer, Istituto di Chimica, Università di Trieste, Italy.

The equilibrium interaction of Cu^{2+} with two alginate samples of different origin (algal and bacterial) and monomer composition has been investigated by potentiometry and polarography. The binding isotherms determined by means of the two techniques are different. This finding has been ascribed to the fact that potentiometry can detect electrostatic binding in addition to covalent binding, since the electrode is sensitive only to free Cu^{2+}, whereas in the polarographic technique electrostatically bound Cu^{2+} is reduced as well as free Cu^{2+}. Cooperativity between Cu^{2+}-binding sites on the same polymer chain and/or different chains is shown by the data. Both the extent of interaction and the degree of cooperativity can be traced back to the monomer composition of the alginates, as shown by the analogous data obtained with three representative fragments: poly(Gul*p*A), poly(Man*p*A) and a mainly alternating poly(Gul*p*A, Man*p*A).

Thermodynamics of the Interaction of Divalent Cations with Alginates and Their Block Fragments. By A. Cesàro, A. Ciana, F. Delben & S. Paoletti, Istituto di Chimica, Università di Trieste, Italy; and V. Crescenzi, Istituto di Chimica Fisica, Università di Roma, Italy.

Calorimetric, dilatometric, osmotic pressure and phase separation experiments have been performed on aqueous salt solutions of alginates of different composition, following the addition of various amounts of Cu^{2+} and Ca^{2+} ions.

Both ΔH and ΔV values for Cu^{2+} binding are positive and large, and do not show any difference between the two alginate samples considered. The results indicate that the favourable interaction of copper(II) with the polymers is completely entropy driven. This can probably be ascribed to a large desolvation of the interaction species.

Osmotic pressure data show that the formation of soluble interchain aggregates (probably dimers) is present at all metal-to-polymer ratios. This precedes more extensive aggregation and phase separation.

In the case of the interaction between Ca^{2+} ions and alginates, a marked cooperativity is shown by both the dilatometric and the calorimetric curves. A small difference between the two polymer samples is revealed by the calorimetry.

While ΔV values of calcium binding are still positive, ΔH values are exothermic. This clearly reveals a favourable, specific interaction between the cation and some sites on the polymeric chain.

Monomer Composition and Acetyl Content as the Principal Determinants of the Ionisation Behaviour of Alginates. By F. Delben, A. Cesàro & S. Paoletti, Istituto di Chimica, Università di Trieste, Italy; and V. Crescenzi, Istituto di Chimica Fisica, Università di Roma, Italy.

Calorimetry, potentiometry and optical activity demonstrated marked differences in the dissociation process between sample A (algal alginate, commercial sample from Fluka) and sample B (bacterial alginate, from *Azotobacter vinelandii*).

The different acidity of the two samples at low degrees of ionisation was attributed to their different content of β-D-mannuronic acid residues.

The dilatometric data for dissociation, on the other hand, showed once more the insensitivity of the differential solvation of polysaccharides to different conformational situations in water.

The heat of dilution measured at high degrees of dissociation (α), showed 'anomalous' behaviour for alginate B. The deacetylation of B, however, yielded a sample behaving quite similarly to sample A and to other ionic polysaccharides.

In conclusion, while the different content in β-D-mannuronic acid and α-L-guluronic acid seems to govern the thermodynamic parameters of dissociation in water at low α values, the acetyl content (8%) of B can be considered responsible for its 'anomalous' properties at high α values.

Physico-Chemical Properties of Pectic Acid: Thermodynamic Evidence of a pH-Induced Conformational Transition in Aqueous Solution. By A. Cesàro, A. Ciana, F. Delben, G. Manzini & S. Paoletti, Istituto di Chimica, Università di Trieste, Italy.

On the basis of measurements of enthalpy of dissociation and of dilution, an intramolecular conformational transition induced by a change in pH has been demonstrated for pectic acid in aqueous solution. Additional evidence is given by potentiometric, viscometric and chiroptical results. The transition from the more rigid, probably H-bonded, structure prevailing at low pH to a more extended one at around neutrality is accompanied by a ΔH value of about 2·1 kJ equivalent and a ΔS value of 6·7 J equivalent K in water at 25°C. The addition of salts increases the stability of the rigid conformations without changing the general features of the phenomenon. Dilatometric measurements suggest that the transition is accompanied by practically no change in the overall solvation of the polymer chain.

Conformational Transitions and Molecular Aggregation in Aqueous Pectate Solutions: Interaction with Divalent Cations. By S. Paoletti, F. Delben & A. Cesàro, Istituto di Chimica, Università di Trieste, Italy.

We have reported that a pH-induced conformational transition occurs in aqueous pectate solutions (Cesaro *et al.* (1982). *Biopolymers*, in press).

A study of the interaction between pectate and divalent cations (namely, Ca^{2+} and Cu^{2+}) in 0·05 M $NaClO_4$ has been undertaken: (i) to understand the thermodynamics of binding of these cations; (ii) to test if a conformational transition accompanies the binding of metal ions by sodium pectate.

The interaction of both counterions are characterised by positive and large volume changes and by exothermic values of the enthalpy changes.

The sign and the size of the ΔH of the binding values seem to confirm the suggestion (Gidley *et al.* (1979). *J.C.S. Chem. Soc.*, 990) that divalent cations are able to induce a conformational change in the pectate chain.

Phase separation (precipitation) is observed at around stoichiometric equivalence for Ca^{2+} and at a lower metal-to-polymer ratio for Cu^{2+}.

Prior to phase separation, formation of supramolecular (dimeric?) aggregates occurs in the presence of Cu^{2+}, as shown by osmotic pressure data.

Calorimetric and Light Scattering Investigation of the Gelation of κ-Carrageenan. By C. De Jonghe, H. Berghmans & H. Reynaers, K. U. Leuven, Laboratory of Macromolecular and Organic Chemistry, Celestijnenlaan, Heverlee, Belgium.

The gelation of κ-carrageenan in water was studied by differential scanning calorimetry and light scattering. The behaviour of pure κ-carrageenan, Smith degraded samples and samples from which 6-sulphate was removed and converted into 3,6-anhydrogalactose, was compared.

No difference was observed between the calorimetric behaviour of rigid κ-carrageenan gels and solutions of Smith degraded samples at the same concentration in

0·1 M KCl. The maximum of the melting endotherm and the minimum of the gelation exotherm occur at 96°C and 73°C, respectively. The heat involved in these transitions is the same. After chemical modification a higher heat of transformation is obtained but melting and gelation occur at 89°C and 64°C, respectively. In the absence of potassium chloride, endothermic and exothermic signals are also obtained, but at much lower temperatures. The heat exchange is the same for the Smith degraded samples, but is much lower for the non-degraded samples.

The influence of potassium and lithium chloride on gelation was compared. While in the presence of potassium chloride rigid gels are already formed at relatively low salt concentrations, much higher concentrations are needed with lithium chloride to obtain the same result. In both cases and at every salt concentration endothermic and exothermic signals are obtained. In the presence of lithium chloride, these signals appear at a much lower temperature than in the presence of potassium chloride. The heat involved is also much lower for the first samples. The melting and gelation temperatures increase strongly in both cases with increasing salt concentration. Typical changes of melting points are from 83°C (0·1 M KCl) to 118°C (0·4 M KCl) and 59°C (0·1 M LiCl) to 95°C (0·4 M LiCl). The change of the gelation temperature of these samples is from 49°C to 60°C and from 28°C to 44°C. A concurrent decrease in the exchanged heat is observed.

The DSC results suggest that thermal transitions can be observed whether or not the regular sequences responsible for these transitions are connected into a polymer chain. When a three dimensional network can be formed, the behaviour is strongly affected by the environment and by the concentration of the different constituents.

From light scattering data it can be concluded that at high temperatures transparent gels are formed, while at low temperatures opaque gels are obtained. Combination of these results with a detailed analysis of the DSC curves suggests a two step mechanism of gelation.

Ionic Strength–Melting Temperature Relationships for Polyelectrolytes: Relevance for the Conformational Properties of Carrageenans in Solution.

By S. Paoletti, Istituto di Chimica, Lab. Chimica delle Macromolecole, Università di Trieste, Italy; and O. Smidsrød & H. Grasdalen, Institute of Marine Biochemistry, University of Trondheim, Norway.

Appropriate expressions have been derived from the counterion condensation theory of polyelectrolytes (Manning (1969). *J. Chem. Phys.*, 51, 924; Manning (1978). In: *Ions in Macromolecular & Biological Systems*, Colsten Papers No. 29, p. 157) to describe the ionic strength dependence of the melting temperatures of polyelectrolytes with charge densities around the counterion condensation threshold.

Both iota and kappa carrageenans solutions and gels exhibit temperature-induced conformational transitions, depending on polymer concentration, counterion specificity and, in particular, on the ionic strength. A domain model based on double helix formation followed by aggregation of double helices (Morris *et al.* (1980). *J. Mol. Biol.*, **138**, 349) and another based on an intramolecular helix formation followed by side-by-side dimerisation (Smidsrød *et al.* (1980). *Carbohyd. Res.*, **8**, C11) have been proposed to account for the observed disordered-ordered conformational transition.

On the basis of the combined experimental results of the ionic strength dependence of the melting temperatures and the measured heats of melting, and by application of the proper equations derived from Manning's theory, it is shown that the linear charge densities predicted by the double helix model largely disagree with the experimental results, while those predicted by the intrachain ordering model are in good agreement, for both kappa and iota carrageenans. This result is supported by independent measurements.

A Comparative Analysis of Counterions Binding in Aqueous Solutions of ι- and κ-Carrageenans*. By V. Crescenzi & R. Rizzo, Istituto di Chimica Fisica, Università di Roma, Italy; and C. Airoldi, Universitade de Campinas, Brasil.

It is well known that different counterions influence the gelling tendency of carrageenans in water. It has been proposed that the gel-formation process in carrageenans systems is controlled by a counterion induced disorder-order conformational transition although the exact nature of the ordered conformation is far from being well established.

In order to understand better the thermodynamics of ion binding of different mono- and divalent cations by carrageenans, we have carried out calorimetric experiments using both batch and flow LKB instruments. In this work we have taken into account the interactions between both ι- and κ-carrageenan segments and several ionic species (Mg^{2+}, Ca^{2+}, Cu^{2+}, K^{+}, Cs^{+}). For both polysaccharides the isothermal (25°C) binding plots of the enthalpy (calories per equivalent of polysaccharide), as a function of the ratio (R) of counterion to polysaccharide concentration, show a qualitatively similar trend characterised by an inversion of the sign of the enthalpy, which passes from a positive to a negative value as the ratio increases.

This behaviour is due to a conformational transition of the polysaccharidic chains. A comparison with the ion-binding data for dextran sulphate, a polymer which cannot assume any regular structure, led us to an evaluation of the enthalpy change value for the counterion induced conformational transition of both ι- and κ-carrageenans.

Unusual behaviour has been found in the case of the Cu^{2+}/κ-carrageenan system. In fact the K^{+} salt form of the polysaccharide does not show any inversion of the sign of the enthalpy and Cu^{2+} ions binding is distinctly endothermic all over the R range. In contrast, the tetramethylammonium salt of κ-carrageenan shows the endothermic-exothermic trend.

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* Further reading: Crescenzi *et al.* (1979). *Polym. Bull.*, **1**, 777; Crescenzi *et al.* (1981). *Makromol. Chem.*, **182**, 219; Crescenzi *et al.* (1981). ACS Symposium Series No. 150, chapt. 23.

Polyelectrolytic Behaviour of the Periodate Oxidation Products of Cellulose and Amylose. By V. Crescenzi, M. Dentini & C. Meoli, Institute of Physical Chemistry, University of Rome, Rome, Italy.

The sodium salts of the stereoregular polycarboxylic acids, derived from cellulose (NaDCC) and from amylose (NaDCA) by oxidative splitting of the pyranose rings (details of sample preparation, as well as physico-chemical characterisation, are given in the communication from Casu *et al.* (p. 283 of this issue)) exhibit unusual binding properties toward Ca^{2+} and Mg^{2+} ions in dilute aqueous solution. The results of our microcalorimetric and circular dichroism measurements indicate that the interaction of both NaDCC and NaDCA with Ca^{2+} ions induces a conformational change of the polyelectrolyte chains, at critical counterion/polymer equivalent concentration ratios. With NaDCC, higher Ca^{2+} concentrations than this critical level lead to gelation. In contrast, Mg^{2+} ions seem unable to trigger similar conformational changes.