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Physicochemical Aspects of the Conversion of Proteins into Food Products

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A general physicochemical approach to the problem of the conversion of protein into food products is examined. The results of studies on processes involving the formation of gels from proteins and polysaccharides—the principal macromolecular components of food—are analysed. The phenomena involving the non-equilibrium formation of complexes by proteins and anionic polysaccharides as well as the limited thermodynamic compatibility in protein-polysaccharide-water and protein-protein-water systems are discussed. The applied aspects of the phenomena indicated are briefly noted.
The bibliography includes 127 references.

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I. INTRODUCTION

The conversion of proteins and other foods into high quality food products is carried out to ensure a wide-scale application of these substances in man's diet. The preparation of new forms of food (NFF), i.e. food products manufactured by the industrial processing of natural foods, has developed vigorously in recent years.[1] The problem of the economically rational employment of traditional and non-traditional food resources has been solved thereby. The problem of the processing of proteins and polysaccharides as the principal macromolecular components of food belongs to the field of the physical chemistry of polymer solutions; it also has a direct bearing on the modelling of multicomponent biopolymer systems and in addition on the synthesis and employment of polymers for medical purposes.

The questions associated with the conversion of protein into various forms of food can be divided into two groups: (1) structure formation in proteins mixed with other foods in order to obtain food products with a specified structure and physicochemical properties; (2) the imparting of the necessary colour, flavour, and smell to the products with the aid of food dyes and flavouring and aromatic agents and also their enrichment in aminoacids, vitamins, and other essential dietary factors.

This subdivision is, however, arbitrary because the flavour, colour, and smell of a product are associated with its structure and physicochemical properties. Thus the perception of taste arising when food is eaten depends on its mechanical properties, swelling, syneresis, wetting, etc. The vapour pressure of aromatic substances over a food product is determined to a large extent by the sorption of the substances in the bulk of the lipid and aqueous phases, including solubilisation by proteins, and also on the internal and external interfaces. It is natural that the introduction of various additional components into the product can induce the desorption of aromatic, flavouring, and dyeing agents and can alter the structure and physicochemical properties of the product.

In this review only problems concerning the first group, i.e. the physicochemical aspect of the conversion of the protein into NFF will be examined. These questions are analysed from the standpoint of the general physicochemical approach to the problem of the processing of protein developed during recent years. The development of this approach was based on two self-evident considerations. Firstly, food products are multicomponent systems and, secondly, in most cases they are solids and at the same time contain a large amount of water (more than 50%). This is a combination of composition and properties characteristic of gels. In other words, the majority of solid food products can be regarded as gels of proteins and other foods from the physicochemical standpoint. Hence it follows that the principal task faced by investigators is to obtain gels with the necessary composition, structure, and physicochemical properties.[1]

However, it appeared necessary to supplement this general physicochemical task by two others. It is natural to wish to convert any one kind of protein into a wide assortment of food products, which necessitates the preparation of liquid and gel-forming systems with a wide variety of properties. Hence follows the task of regulating the solubility of a protein, its ability to stabilise disperse systems, its ability to form gels, etc. This complex set of physicochemical characteristics, determining the structural functions of protein in non-processed systems, is designated by the concept "functional properties of the protein".[2,3]

The converse problem, namely the preparation of a given dietary product from different proteins, is also important. The fundamental physicochemical phenomena and characteristics common to all proteins are therefore of greatest interest. Processes based on phenomena of this kind should apparently be distinguished by their simplicity, should be susceptible to technological treatment, and should be "universal with respect to the protein".

This review discusses three fundamental physicochemical phenomena underlying the modern methods for converting protein into NFF: the formation of gels from proteins, polysaccharides, and their mixtures, the formation of complexes of proteins and anionic polysaccharides, and the limited thermodynamic compatibility in solution of proteins and polysaccharides and also of proteins with one another. The applied aspects of the problem are only briefly considered in this review. They are discussed in greater detail in a monograph.[1]

II. THE FORMATION OF GELS FROM PROTEINS AND POLYSACCHARIDES

The main physicochemical task arising in the manufacture of NFF consists in the development of methods for the preparation of gels (single-phase and two-phase) with a specified and controllable composition, structure, and mechanical and other physicochemical properties.

The problem of the preparation of NFF is to some extent close in its character to other physicochemical problems arising in the manufacture of polymer articles via a solution—in particular in the processing of technical polymers.[4,5] This analogy is, however, by no means complete. In the first place the fundamental multicomponent nature of the processed food systems and of the final products, together with the wide variety of the functions performed by individual components, is responsible for the complexity and the multiplicity of aspects of the physicochemical problems associated with the regulation of the composition and properties of the products, which is not usually characteristic of technical polymers. The second important feature of food systems, which distinguishes them from technical polymers, is that in the former case the gel state is characteristic not only of the intermediates but also of the final products. Accordingly, there arises a complex set of requirements which must be met by the physicochemical properties of the finished food products as gels. These properties determine in their turn the food value and the organoleptic and technological characteristics of the products.[6]

Three types of processes for the conversion of liquid solutions of biopolymers into the gel state are used in the preparation of NFF: thermotropic, ionotropic, and lyotropic gel formation. In the first case the change in the temperature of the solution plays the main role, in the second case it is the change in the ionic composition of the system, and in the third the change in the concentration of the biopolymer. The last type of gel formation constitutes the basis of the technology of the manufacture of protein products with a porous structure, but the nature of the processes occurring under these conditions has been very little studied.[6]

An example of ionotropic gel formation is the preparation of calcium alginate and pectinate gels. The technology of the manufacture of pasta, cereals, and potato products is based on these processes.[7-12] In the preparation of three-dimensional food products based on calcium alginate and pectinate gels, the main difficulty consists in ensuring the homogeneity of their structure, because the high rate of the interaction of calcium ions with the molecules of polyuronides precludes a satisfactory mixing of the reactants. The known methods for the preparation of isotropic calcium alginate and pectinate gels are apparently based on the regulation of the activity of calcium ions in the reaction system.

The ionotropic gel formation process has found a wider application in the preparation of one- and two-dimensional gels, i.e. fibres and films.[13,14] The diffusion of precipitant ions into solutions of polyuronides is used in such cases. Gels with a well developed phase structure, reproducing the structure of many biological tissues, are obtained in this way.[1,15-18] Among the methods for the ionotropic fibre formation, the matrix spinning process has been studied systematically. This procedure makes it possible to process proteins regardless of their spinnability and to regulate the functional properties of the fibres within wide limits.[19-21] The calcium ion-stabilised interfacial absorption layers containing proteins and polyuronides ensure an exceptionally high resistance of emulsified food products to coalescence.[22]

A method whereby the boundary conditions for ionotropic gel formation can be characterised has been developed.[23] It consists in the determination of the minimum concentrations of reactants sufficient to fix the shape of a drop of the polyelectrolyte solution introduced into a solution of a polyvalent metal salt. Fig.1 illustrates gel–liquid solution diagrams for sodium alginate–polyvalent metal chloride systems. At comparatively low concentrations of the cross-linking agent, namely the polyvalent metal ion, the boundary gel formation conditions are determined by the ion-exchange equilibrium constant. With increase in the concentration of the cross-linking agent, conditions are reached under which the boundary concentration of the polyelectrolyte ceases to depend on the concentration of the polyvalent metal ion. This polymer concentration has been called the "critical gel formation concentration". It apparently depends on the molecular weight and the conformation of the macromolecules. The method indicated is particularly convenient for the determination of the conditions for the formation of gels from anionic polysaccharides in the presence of proteins,[22] which is important in the solution of many applied problems, for example, in the conversion of the polysaccharide phase of a two-phase liquid protein–polysaccharide–water system into the gel state (see below).

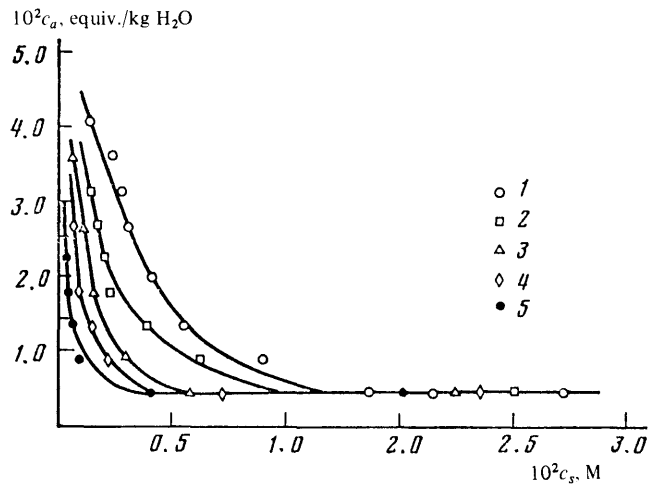


Figure 1. The boundary conditions for gel formation in the sodium alginate–polyvalent metal chloride systems;[22] c_a = concentration of sodium alginate; c_s = concentration of polyvalent metal chloride: *1*) $CaCl_2$; *2*) $SrCl_2$; *3*) $BaCl_2$; *4*) $CuCl_2$; *5*) $LaCl_3$.

Thermotropic gel formation has been investigated in relation to soya bean globulins (SBG), ovalbumin, and also gelatin and many polysaccharides. The thermotropic gel formation from solutions of proteins and polysaccharides has also been investigated, which constitutes the next step in the modelling of multicomponent food systems.[6]

1. The Thermotropic Formation of Gels from Globular Proteins

Thermotropic gel formation is a consequence of the thermal denaturation of globular proteins.[24-28] The study of the thermal denaturation of SBG in a dilute solution by the

method of precision scanning calorimetry[29] showed that the 7*S*-component of the globulin fraction denatures at lower temperatures than the 11*S*-component (Fig.2).[30] The temperature and enthalpy of the denaturation of the principal SBG components increase linearly with increased NaCl concentration. The slope of this relation (for the denaturation temperature), which is equal to the lyotropic constant K_S, is unusually high—about 20 K mol^{-1} litre^{-1}. The ratio of the lyotropic constant to the denaturation temperature, which characterises the magnitude of the lyotropic effect, increases linearly with increasing mole fraction of the hydrophobic units in the protein molecule. The unusually strong influence of NaCl on the denaturation temperature of the principal SBG components can be explained by the comparatively high content of hydrophobic units and their complete masking in the native state. The differences between the enthalpies and entropies of transport of the denatured and native forms of the principal SBG components from water to an NaCl solution are positive and also increase linearly with increasing salt concentration. This shows that the influence of the salt on the thermal denaturation of SBG is based on the decreased degree of hydration of the protein.

The influence of the gel formation conditions on the structure of thermotropic SBG gels has been investigated by the stress relaxation and electron microscopic methods.[31,32] It has been established with the aid of the multifactorial extreme experimental design method that the variation of the modulus of shear of the above gels as a function of temperature and the duration of heating has a single maximum and that its position (95° ± 5 °C; 35 ° 5 min) is independent of the protein concentration. At the same time the relaxation properties of SBG gels depend exclusively on the heat treatment temperature. The explanation is that the temperature (but not the duration) of the heat treatment determines the composition of the SBG components involved in the gel formation process.

The relaxation properties of thermotropic SBG gels can be characterised unambiguously by the parameter $\bar{H} = -[\partial F(\tau)/\partial \ln(\tau)]$, where $F(\tau)$ is the normalised relaxation function, representing the ratio of the relaxation moduli at a certain time τ and at $\tau_0 = 1$ min. In the range from 70° to 115 °C the dependence of the parameter $\bar{H}$ on the heat treatment temperature is linear (Fig.2). According to scanning calorimetric data, the content of the denatured forms of the 11*S*-globulin increases monotonically with increase in temperature over this range and hence also the degree of its involvement in the gel formation process. The deviation of the $\bar{H} = f(T)$ relation from linearity above 115 °C is apparently induced by the increasing contribution of degradative processes.

Experimental data on the isothermal stress relaxation in SBG gels at different concentrations have been generalised in the form of a concentration-invariant relaxation curve as a result of its reduction with respect to the modulus but without its reduction with respect to time.[33] Analogous results have been obtained also in the study of the rheological properties of thermotropic ovalbumin gels and also the gels of a number of anionic polysaccharides.[34] The absence of reduction with respect to time shows that the rate of relaxation processes in gels is independent of the polymer concentration, which is a characteristic of a microheterogeneous gel structure, in many respects similar to that of micellar surfactant solutions. An increase of the polymer concentration in a solution with a microheterogeneous structure leads to an increase in the number of structural elements—associated macromolecular species, without altering the composition and structure of such species. The latter factor determines the concentration invariance of the rate of relaxation processes as well as other intensive properties of the solution. Thus a study[35] of the concentration dependence of the relative activity coefficient of counterions† in solutions of anionic polysaccharides showed that, after the tentative transition to the region of semidilute solutions, this quantity becomes constant.

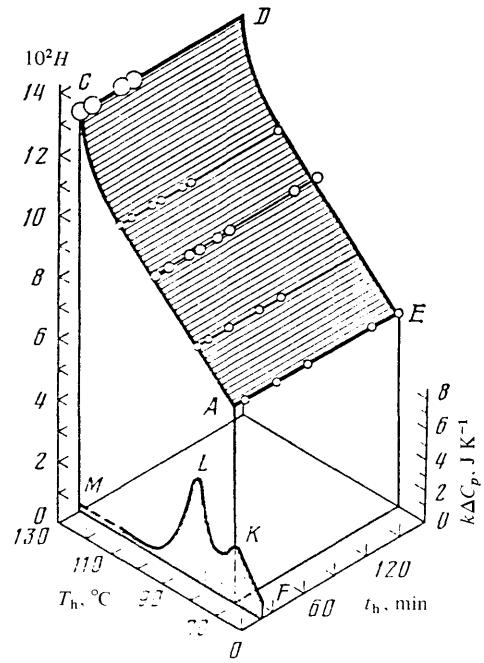


Figure 2. Dependence of the slope of the normalised relaxation function for the thermotropic gels of the globulin fraction of soya beans at 25 °C ($\bar{H}$) (left-hand ordinate axis) on the temperature and duration of the heat treatment in the gel formation process (the *ACDE* surface). Thermogram for a solution of the globulin fraction is plotted on the facing plane (the right-hand ordinate axis; k = scaling factor, the value of which is unimportant) obtained at a scanning rate of 2 K min^{-1} (the *FKLM* curve).[30]

The parameters for the reduction of the relaxation curves with respect to the modulus is proportional to the equilibrium modulus of elasticity. Operating with this parameter, it is possible to determine the scaling relations for the equilibrium modulus of elasticity despite the fact that this modulus cannot be measured for the majority of the protein and polysaccharide gels investigated owing to the low rate of the relaxation process. The concentration dependence of the equilibrium modulus of elasticity of SBG,[33] ovalbumin, as well as a gelatin and agar[36] gels is described by the theoretical Hermans relation[37] derived for a model of the gel formation process as a random reversible association of linear

† The relative activity coefficient of an ion is the ratio of its activity coefficient in the test solution to its activity coefficient in the solution of its chloride at the same equivalent concentration.

identical macromolecules without cyclisation and with formation of network nodes via a bimolecular mechanism.

Hydrophobic interactions play a leading role in the thermotropic formation of gels from SBG. Thus thermotropic SBG gels are soluble in 8 M urea solution, but are insoluble in 0.01 M solution of β-mercaptoethanol, a substance which ruptures disulphide linkages. Under the influence of urea, thermotropic SBG gels and native proteins decompose into virtually identical subunits or their dimers. On the basis of ^{1}H NMR data, it has been suggested that the packing densities of the polypeptide chains in the structural elements of gels and in the subunits of native proteins are comparable. In the 17.5% gel, one structural element consists of ~20 subunits.

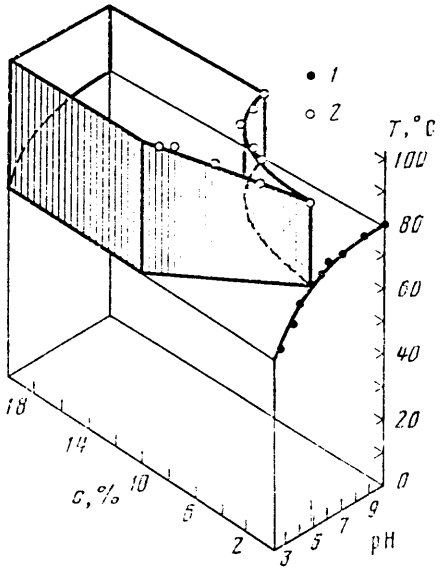


Figure 3. The phase diagram for the ovalbumin–water system at different temperatures, pH, and protein concentrations [*1*) pH dependence of the denaturation temperature; *2*) pH dependence of the gel formation threshold]; the region of the gel state is shown shaded and the isoelectric point is indicated by an arrow.

Taking into account the decisive importance of hydrophobic interactions in the formation of SBG gels, one can assume that the gel-forming capacity of these proteins is correlated with the Tanford–Bigelow hydrophobic parameter, which is defined as the average free energy for the transfer of a polypeptide chain unit from the hydrophobic interior of the protein macromolecule to an aqueous environment.[38,39] According to this criterion, the gel-forming capacity of the monomeric forms of the basic soya bean globulins should decrease in the sequence 2,8*S*-globulin > *B* > β > α > *A*, where *A* and *B* are respectively the acid and basic subunits of 11*S*-globulin and α and β are the heavy and light subunits of the 7*S*-globulin. Analysis of the sol fraction showed that the acid subunits of the 11*S*-globulin do indeed have the lowest gel forming capacity among the monomeric forms of the basic soya bean globulins and accumulate in the sol fraction during gel formation.

According to the results of Maleev and Gasan,[40] the denaturation temperature of ovalbumin gels is almost independent of the ovalbumin concentration in solution. The phase diagram for the ovalbumin–water system is illustrated in Fig.3#. The dependence of the critical gel formation concentration of albumin on the pH passes through a sharp minimum near the isoelectric point (IEP), which means that the rate of gel formation and the functionality of the ovalbumin molecules at the IEP are maximum. It is known[41] that the negative contribution of the charge fluctuations to the chemical potential of the protein in solution is significant precisely at the IEP.

As in the case of SBG gels, the dependence of the modulus of elasticity of the ovalbumin gels on the temperature and the duration of heat treatment has a single maximum§. The optimum gel formation time (30 min) is independent of pH, but the optimum temperature, which is 80 °C under conditions remote from the IEP, increases sharply in the pH range 4.0–5.5. Gels formed under conditions remote from the IEP consist of a single phase, while those produced in the vicinity of the IEP consist of two phases. Each of these groups is characterised by its own mechanical relaxation function. Since the temperature and duration of the heat treatment of the solution, the pH, and the protein concentration (*c*) influence only the equilibrium modulus of elasticity, all the relaxation curves obtained can be described by two normalised relaxation functions corresponding respectively to single-phase and two-phase gels.

The form of the generalised relaxation curve for ovalbumin gels¶ (Fig.4) makes it possible to assign these gels to the systems at the beginning of the zone corresponding to the transition from the vitreous to the highly elastic state.[42] The mechanical relaxation curves for the gelatin,[43] maltodextrin,[44] and a number of anionic polysaccharide[45–48] gels have an analogous character. The form of the relaxation curves shows that the relaxation processes localised in the associated macromolecular species making up the three-dimensional network are retarded.[34] On the other hand, during a time interval corresponding to about seven decadic orders of magnitude, the temperature-invariant relaxation curve for the SBG gel[33] has the form characteristic of amorphous cross-linked polymers at the end of the transition zone or at the beginning of the zone corresponding to the plateau.[42]

2. The Thermotropic Formation of Gels from Polysaccharides

In constrast to the formation of globular protein gels, the processes involving the thermotropic formation of polysaccharide gels are in most cases reversible. By virtue of the relative simplicity of the chemical structure of polysaccharides, an inter-relation between the chemical structure

#N.V.Grinberg, T.M.Bikbov, V.Ya.Grinberg, T.S.Chaika, I.A.Vaintraub, and V.B.Tolstoguzov, Int.J.Biol.Marcomol., 1985, 7, 152.

§N.V.Grinberg, T.M.Bikbov, V.Ya.Grinberg, and V.B.Tolstoguzov, Colloid and Polymer Sci., 1985, 33 (In the press).

¶V.Ya.Grinberg, N.V.Grinberg, T.M.Bikbov, and V.B.Tolstoguzov, "Materialy V Vsesoyuznogo Soveshchaniya 'Protsessy Studneobrazovaniya v Rastvorakh Polimerov'" (Proceedings of the Vth All-Union Conference on 'The Gel Formation Processes in Polymer Solutions'), 1985, Part 1, p.96.

of biopolymers of this class, their conformation, and their gel-forming capacity can be traced.[49-51] Like other thermotropic gels, particularly gelatin gels, the thermotropic polysaccharide gels are formed when the quality of the solvent is impaired. Gel formation must be preceded by a stage in which macromolecular associated species are formed and the gel is produced as a result of the combination of these species in a three-dimensional network.[52] Certain features of the relaxation behaviour of the polysaccharide gels, which follow from the gel formation mechanism described, are discussed in the previous Section.

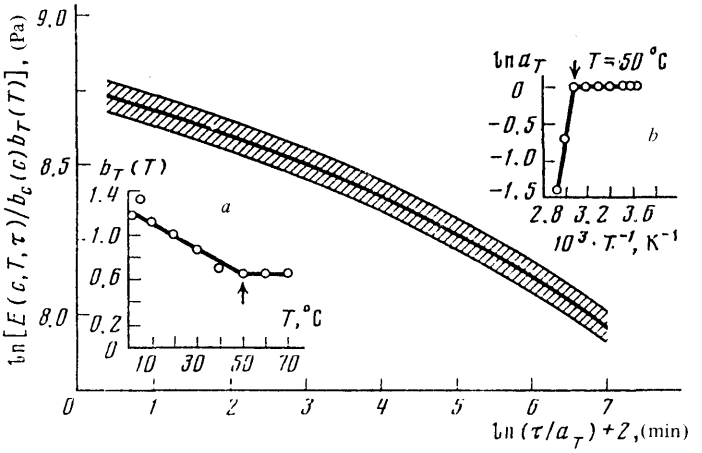


Figure 4. $c-T$ invariant generalised relaxation curve for single-phase thermotropic ovalbumin gels reduced to the state with c_0 = 10% and T_0 = 50 °C [E = relaxation modulus, c = protein concentration, T = temperature, τ = time, $b_c(c) = E(c, T, \tau)/E(c_0, T, \tau)$, and $b_T(T) = E(c, T, \tau)/E(c, T_0, \tau)$ are the parameters of the reduction of the modulus with respect to the concentration and temperature, and a_T = parameter of the reduction of time with respect to temperature]; the half-width of the shaded band is equal to the standard deviation of the experimental points from the average curve; the temperature variations of the parameters of the reduction of the modulus and time with respect to temperature are shown in the inserts *a* and *b*.

The thermotropic formation of pectin gels takes place at pH 4 in the presence of substances which reduce the activity coefficient of water (sucrose, glycerol, etc).[55] The gel-forming capacity of pectins depends critically on the degree of their esterification (DE). This dependence is determined by at least two factors: (1) the influence of the DE on the flexibility of the pectin chain, which is responsible for the possibility of the appearance of the optimum number of contact zones in gel formation; (2) the influence of the DE on the free energy of the intermolecular interactions in the contact zones. Apparently the first factor is of decisive importance, since the maximum strength and moduli or elasticity of the gels are attained for DE ≅ 50%,[56,57] i.e. when the flexibility of the pectin chain is a maximum.[35,58] The rate of gel formation for this DE is a minimum.[56] Spectroturbidimetric study of the structure of the gels showed# that, under these conditions, systems with minimum dimensions of the structural elements, which corresponds to the maximum strength of the gel, are formed (Fig.5).

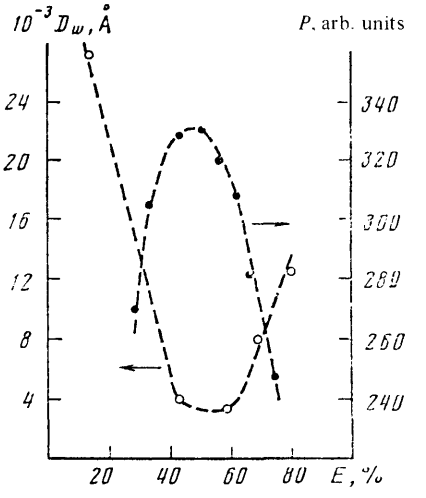


Figure 5. Dependence of the effective diameter of the structural elements (D_w) and of the stability (P)[57] of pectin gels on the degree of esterification (E) of pectin; pectin concentration 1%; pH 2.2; solvent—1:1 water–glycerol mixture.

The study of the time variation of the structure and properties of gels has shown that there exist two types of networks: the thermodynamically stable and metastable networks. The latter type includes the maltodextrin[44] and methylcellulose hydrogels†. On prolonged storage, these gels pass into a past-like state, which is manifested, in particular, by a decrease of the heat of fusion of maltodextrin gels. According to electron microscopic data,[44] the structural elements of the maltodextrin gel network rearrange after a time and form isodiametric associated species. The opposite direction of the structure-forming process has been observed for gelatin gels.[53] The stability of such gels increases with time.[54] The moduli of elasticity of pectin[45] and other anionic polysaccharide gels vary analogously. For these polymer–solvent systems, the gel state is thermodynamically stable.

†E.V.Grishchenkova, E.E.Braudo, and V.V.Tolstoguzov, Colloid and Polymer Sci., 1985, **263** (in the press).

The standard enthalpy of decomposition of the pectin gel network nodes in mixtures of water with sucrose[45] and glycerol[48] is 7–39 kJ mol^{-1}. Such low enthalpies are characteristic of solutions of semirigid-chain polymers incapable of conformational transformations under gel formation conditions. The formation of network nodes in such systems is not accompanied by appreciable losses of conformational entropy. The gel formation process therefore becomes thermodynamically favourable even for a small decrease of enthalpy. This type of gel formation has been called non-cooperative.[34]

An analogous picture has been observed for maltodextrin gels. For a 20% gel, the decomposition of the network nodes is characterised by the following thermodynamic

#I.G.Plashchina, Abstract of Candidate's Thesis in Chemical Sciences, Institute of Organoelemental Compounds, USSR Academy of Sciences, Moscow, 1984.

parameters: $\Delta H^0 = 25$ kJ mol^{-1},[34,44] $\Delta S^0_{25\,°C} \cong 32$ J mol^{-1} K^{-1}; $\Delta G^0_{25\,°C} \cong 15$ kJ mol^{-1}.

The formation of gels from gelatin proceeds quite differently. The standard enthalpy of decomposition of the network nodes of a 5% gel is 300 kJ mol^{-1}.[34,43] In this case (the cooperative mechanism of gel formation) the appearance of network nodes is accompanied by the formation of helices from the flexible macromolecules capable of conformational transformations. The high enthalpy of the gel formation process compensates for the large losses of conformational entropy.

Among the anionic polysaccharides investigated, the galactans converted into the sulphate form, namely κ-carrageenan and furcellaran, are conformationally labile. On cooling their solutions to room temperature, both polysaccharides undergo the coil–helix conformational transformation.[49] The formation of these polysaccharide gels is promoted by certain metal ions, including potassium ions.

In the existing theories the formation of helices from κ-carrageenan macromolecules is regarded as the primary process in gel formation,[59-61] as happens in the formation of gelatin gels.[62] The function of the metal ions promoting the formation of the κ-carrageenan gel reduces to promoting helix formation[63] and to participation in the formation of contacts between the helical sections of the macromolecules. However, it has been shown that the κ-carrageenan macromolecular associated species and gel network are formed under the influence of potassium ions above the temperature range of the coil–helix conformational transition. Thus the effective size of the κ-carrageenan macromolecular associated species does not change in the conformational transition.[64]

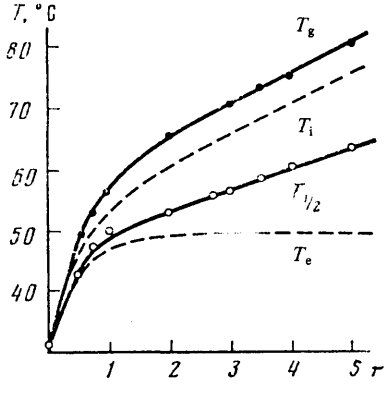


Figure 6. Dependence of the gel formation temperature (T_g) and the temperature of the onset (T_i), middle ($T_{1/2}$), and end (T_e) of the coil–helix transition of a 1.5% solution of κ-carrageenan on the KCl concentration; r = ratio of the equivalent concentrations of KCl and κ-carrageenan.

According to Plashchina et al,[46] the melting point of the 1.5% κ-carrageenan gel exceeds the temperature of its complete conversion into the helical form by 13°. Fig.6 illustrates the dependence on the KCl concentration of the gel formation temperature of the 1.5% κ-carrageenan solution and also the temperature of the onset, the middle, and the end of the coil–helix transition in this solution§. Evidently, at [KCl] > 3.7×10^{-2} M, the gel formation temperature is above the helix formation temperature range. It has been suggested that the κ-carrageenan gel network is formed as a result of the cross-linking of the polysaccharide macromolecules by the potassium ions, forming coordinate bonds with the hydroxy-groups. An essential condition in the formation of the gel network is the neutralisation of the charge of the sulphate groups by the potassium ions.

The conformational state and the stability of the conformations of the macromolecules determine the thermorheological behaviour of the κ-carrageenan gels§. Fig.7 illustrates the temperature dependence of the highest Newtonian viscosity of the 1.5% gel in terms of the variables of the De Guzman–Arrhenius equation. It is seen that the experimental temperature range can be divided into three regions:

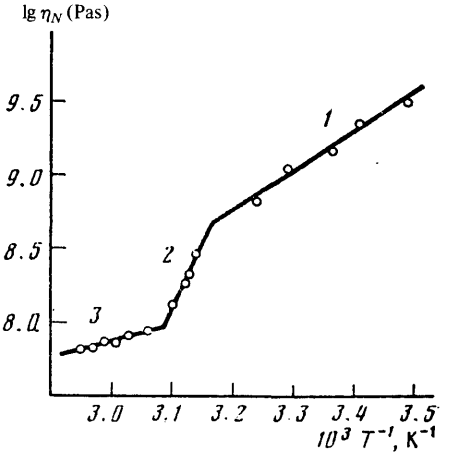


Figure 7. Temperature dependence of the maximum Newtonian viscosity (η_N) 1.5% κ-carrageenan gel.

(1) t < 41 °C—the temperature range in which the helical conformation of the macromolecules is stable (ΔH^0 = 36 kJ mol^{-1}, $\Delta S^0_{25\,°C} \cong 135$ J mol^{-1} K^{-1}; $\Delta G^0_{25\,°C} \cong -4$ kJ mol^{-1} [34]);

(2) 41 °C ≤ t < 52 °C—the region of the helix–coil conformational transition ($\Delta H^0 = 235$ kJ mol^{-1}; $\Delta S^0_{41\,°C} \cong 775$ J mol^{-1} K^{-1}; $\Delta G^0_{41\,°C} \cong -9$ kJ mol^{-1} [34]);

(3) 52 °C ≤ t ≤ 66 °C—the temperature range in which the κ-carrageenan gel network is formed by macromolecules which have lost their helical structure ($\Delta H^0 = 11$ kJ mol^{-1}).

It is seen from the thermodynamic parameters presented that in region 2 the character of the gel formation is cooperative. In regions 1 and 3 the gel formation is non-cooperative: in the former case this can be accounted for by the stability of the helical conformation, as a result of which the polysaccharide assumes the properties of a semirigid-chain polymer, while in the latter case it can be explained by the impossibility of the appearance of the helical conformation.

Apparently the higher stability of the helical conformation of furcellaran, due to the lower content of sulphate groups compared with κ-carrageenan, is responsible for the non-cooperative character of the formation of this polysaccharide gel in a mixture of sucrose (70%) and water up to 50 °C ($\Delta H^0 = 19$ kJ mol^{-1}).[34,47]

§I.G.Plashchina, I.R.Muratalieva, E.E.Braudo, and V.B.Tolstoguzov, Carbohydr.Polymers, 1985, 5 (in the press)

Thus non-cooperative gel formation is largely characteristic of the polysaccharides investigated. It has been observed for a polysaccharide with a highly rigid chain (pectin), for polysaccharides with a helical conformation stable under the experimental conditions (maltodextrin, furcellaran, and κ-carrageenan below 41 °C), and also in gel formation above the temperature range of the helix–coil transition (κ-carrageenan above 52 °C).

3. The Formation and Properties of Thermotropic Gels Containing Proteins and Polysaccharides

The following types of multicomponent gels have been differentiated:[1,65,66]

(1) Filled gels in which one of the macromolecular components functions as the gel-forming agent, while the others play the role of fillers. Gels filled with particles of a disperse phase can also be included in this group.

(2) Mixed gels in which there are two or more networks formed by different gel-forming agents. The necessary conditions for the formation of mixed gels are thermodynamic compatibility of the gel-forming agents in solution and the absence of a specific interaction between them.

(3) Complex salts whose three-dimensional network is formed by the products of the interaction of different macromolecular components.

The properties of the mixed and complex gelatin and calcium and sodium alginate and pectinate gels have been summarised in a review[66] and in a monograph.[1]

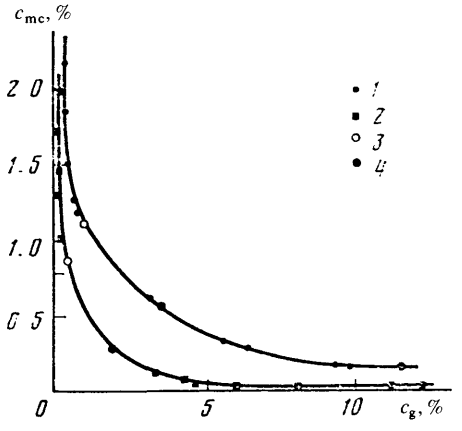


Figure 8. The phase diagram of the gelatin–methylcellulose–water system at 35 °C;[67] c_{mc} and c_g are the concentrations of methyl cellulose and gelatin: *1*) binodal curve at pH 3.0; *2*) binodal curve at pH 4.75 (isoionic point of gelatin); *3*) threshold point; *4*) critical point.

The formation of mixed and single-phase unfilled gel is limited by the low compatibility of the macromolecular components in the absence of complex formation between them. Fig.8 presents the phase diagram for the gelatin–methylcellulose–water system at 35 °C (at this temperature none of the components form a gel).[67] It is seen from the figure that the compatibility of gelatin with methylcellulose increases somewhat as the pH of the system shifts from the isoelectric point of gelatin. This can be explained by the suppression of the self-association of gelatin induced by the Coulombic interaction due to charge fluctuations as a consequence of the increasing electrostatic repulsion.[68] The introduction of NaCl does not lead to an appreciable increase of compatibility, since the decrease in the degree of self-association of gelatin is compensated by the increase in the degree of self-association of methylcellulose under the influence of NaCl. The asymmetry of the gelatin–methylcellulose–water phase diagram, which is characteristic of quasi-binary protein–polysaccharide systems, is striking.[69]

In the absence of complex formation, an enhancement of structure formation in individual components of single-phase multicomponent gels, i.e. the development of intramolecular and intermolecular contacts between the macromolecules of one component at the expense of the polymer–solvent contacts, is observed. This phenomenon is due to the excluded volume effect.[6,70,71]

An important example of a process determined by the development of intramolecular polymer–polymer contacts is provided by the coil–helix transition. Fig.9 illustrates the influece of dextran on the mutarotation of gelatin in solution at 6 °C. It is seen that the presence of only 0.2% of dextran, which is 5% of the concentration of gelatin, induces a sharp acceleration of the conformational transition of the latter. There is a simultaneous increase of the rate of gel formation. The coil–helix transition leads to the expansion of the region of incompatibility of gelatin and dextran.[72]

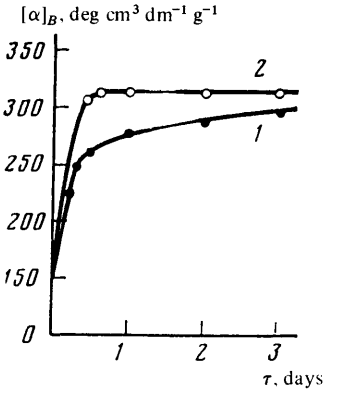


Figure 9. The influence of dextran on the mutarotation of a 4% gelatin solution;[72] $[\alpha]_B$ = specific optical rotation at λ = 436 nm: *1*) water; *2*) 0.2% dextran solution.

The enhancement of structure formation in the components of a mixed gel is manifested also by a decrease of the critical gel formation concentration¶. Fig.10 shows that a mixed gel of gelatin and agarose is formed at concentrations of the polymeric components such that each separately is still incapable of forming a gel network. An analogous phenomenon has been noted for mixed thermotropic agar and bovine serum albumin gels.[73]

The study of the viscoelastic properties of gelatin gels containing globular proteins or a polysaccharide (dextran) showed that the presence of fillers affects the effective

¶A.M.Gotlieb, I.G.Plashchina, E.E.Braudo, and V.B.Tolstoguzov, Colloid Polymer Sci., 1985, 263 (in the press).

number of gel network nodes (the equilibrium modulus of elasticity), while the rate of relaxation processes remains unaltered in most cases.[72,74]

The properties of multicomponent gels naturally depend on their phase state. In certain cases extrema appear on the gel property–composition curves, which may be attributed to a transition from the single-phase to the two-phase state. Such a transition in the region of the filler concentration corresponding to the minimum pliability (the maximum modulus of elasticity) has been established for the gelatin–dextran–water system by electron microscopy.[72] The decrease of pliability in the single-phase state can be accounted for by the increase of the effective number of gelatin gel network nodes under the influence of the filler (dextran) by virtue of the excluded volume effect. In the two-phase state the drops of the phase enriched in dextran form defects in the gelatin gel network.

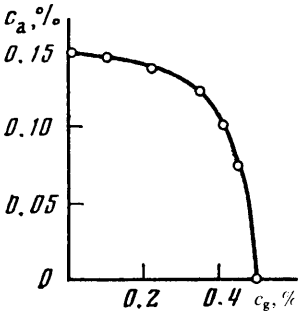


Figure 10. Dependence of the critical concentration for gel formation on the composition of the gelatin–agarose–water mixture at 8 °C; c_g = gelatin concentration; c_a = agarose concentration.

It has been suggested that in the general case the region of filler concentrations corresponding to the onset of the increase in the pliability and in the degree of swelling of gels be called the limit of the structural compatibility of the filler and the gel matrix.[6] This limit depends both on the nature of the matrix and on the molecular characteristics of the filler.[74,75] The character of the influence of the molecular weight of dextran on the limits of its structural (thermodynamic) compatibility with the gelatin gel matrix can be seen from Fig.11.[72]

In the region of the two-phase state each of the polymeric components is concentrated in one of the phases. In this case the properties of the mixed gels are determined primarily by those of the continuous phase. When the composition of this system is varied within wide limits, phase inversion, accompanied by a change in the properties of the gel, takes place.[6,36,75,76]

III. COMPLEX FORMATION IN SOLUTIONS OF PROTEINS AND ANIONIC POLYSACCHARIDES

The interaction between proteins and anionic polysaccharides has been studied by many workers and certain aspects of this phenomenon have been surveyed in a number of reviews.[77–81] The first systematic studies, which were devoted mainly to the nature of the phase separation in solutions of proteins and anionic polysaccharides (i.e. to the formation of complex coacervates or insoluble complexes), showed that the protein–polysaccharide complexes (PPC) are stabilised mainly by electrostatic forces. On the other hand, the complex formation between anionic polysaccharides and proteins, demonstrated in a number of studies at a pH above the isoelectric point of the protein (where the macroreactants bear like charges),[82–86] as well as the complex formation between neutral polysaccharides and proteins (not lectins),[87,88] indicate a significant role of the secondary non-electrostatic interactions in the stabilisation of protein–anionic polysaccharide complexes.

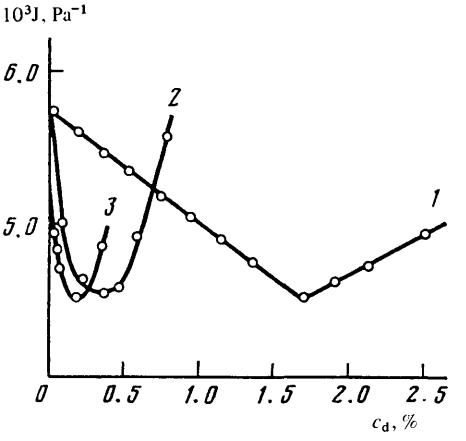


Figure 11. The influence of the dextran concentration (c_d) on the pliability J of 10% gelatin gels;[72] molecular weight of dextran: *1*) 20 kD; *2*) 65 kD; *3*) 500 kD.

The functional properties of the PPC, such as solubility, surface activity, and rheological characteristics, differ significantly from the analogous properties of the initial macroreactants and, what is especially important, can be regulated within wide limits.

Water-soluble PPC are of interest for the food technology, but their structures and properties have been little studied. The investigation of the equilibrium nature of the PPC, the nature of the forces stabilising them, the molecular structure of the PPC and the bound protein, and the functional properties of the complexes is urgently required.

1. The Non-Equilibrium Complexes

Studies of the formation of the PPC have shown that, at sufficiently low pH and ionic strengths, the latter do not correspond to equilibrium: their properties and in particular their solubilities† depend on the method of preparation.[89] Thus, when solutions of the macroreactants are mixed under the conditions of vigorous complex formation (at a pH below the IEP), "mixing complexes" (M-complexes), which are sparingly soluble in water, are formed. On the other hand,

† The terms "soluble" and "insoluble" which we use in relation to the non-equilibrium complexes are arbitrary, since strictly speaking they should refer to a stable thermodynamic equilibrium.

on slow titration of the solution, when conditions in the medium change in such a way that the interaction gradually intensifies, readily soluble "titration complexes" (T-complexes) are produced.

The difference between the solubilities of the M- and T-complexes are clearly manifested in nephelometric titration (Fig.12). The turbidity of the system serves in this instance as a semiquantitative characteristic of the yield of the insoluble PPC.

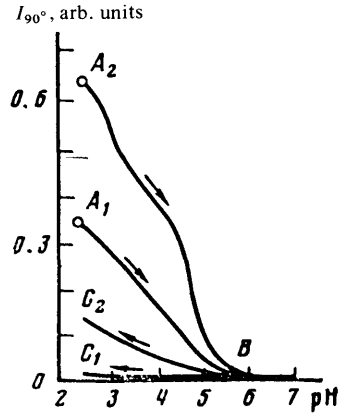


Figure 12. Nephelometric titration curves for solutions of complexes of bovine serum albumin and dextran sulphate in proportions, by weight, of 1:1 and 2.3:1 (the A_1BC_1 and A_2BC_2 curves); overall concentration of macroreactants 0.1 g litre^{-1}.

The differences between the solubilities for the M- and T-complexes (aggregation hysteresis) are particularly marked for the PPC of highly sulphated polysaccharides such as dextran sulphate and heparin. Furthermore, a high aggregation stability is characteristic of the solutions and suspensions of these PPC: the light scattering and the wave exponent (sensitive to changes in the size of the scattering particles) can be constant for several days at room and elevated (40–50 °C) temperatures.

The non-equilibrium PPC have been studied in greatest detail in relation to the complexes of the high-molecular-weight dextran sulphate with proteins, in particular with bovine serum albumin (BSA).[90] The character of the interaction of BSA with dextran sulphate varies sharply as a function of the pH. In the range $5.6 \leqslant pH \leqslant 8.5$ (the range corresponding to a relatively weak interaction) the properties of the PPC are independent of the method of their preparation, which indicates the equilibrium character of the complex formation. Analysis of the composition of the water-soluble complexes at pH 5.6 showed that the sorption of BSA by dextran sulphate is non-cooperative, i.e. the protein–protein interaction does not have an appreciable influence on the binding of the protein to the polysaccharide. Under these conditions, the PPC are not aggregated: each particle consists of one dextran sulphate molecule.

In the vicinity of the isoelectric point of BSA ($5.0 < pH < 5.6$) the composition of the soluble complexes is also independent of the way in which they are prepared, but does depend strongly on the pH. At the same time, in the above pH range the aggregation hysteresis is already observed (Fig.12)#.

The fact that in the above pH range a transition is observed from a uniform distribution of the protein molecules between the dextran sulphate chains to a non-uniform distribution is of particular interest. Two discrete fractions of the complex appear in the solution—the rapidly sedimenting α-fraction and the slowly sedimenting β-fraction. Analysis by the method of high-speed sedimentation and gel chromatography showed that the ratio of these fractions varies steadily: with increase of the fraction of BSA in solution, the proportion of the α-fraction rises and that of the β-fraction falls. At the same time the sedimentation coefficients of these fractions also increase. They both consist of a BSA–dextran sulphate complex but differ in composition: the α-fraction is enriched in the protein while the β-fraction is depleted in the latter. The non-uniform distribution of the protein between the polysaccharide chains can be accounted for by the cooperative character of its sorption.[91,92] The transition from the non-cooperative sorption of the BSA to the cooperative sorption is apparently due to the intensification of the interactions between the protein molecules in the PPC species or the decrease of the pH which can be accounted for in its turn by the decrease of the overall charge of the protein, hindering, at a higher pH, the mutual approach of the protein globules.[93,94]

At $pH \leqslant 5.0$ the sorption of the BSA becomes irreversible. Under these conditions, the α-fraction of the M-complexes of any composition is precipitated. On the other hand, the α-fraction of the T-complexes is readily soluble, provided that the ratio of BSA and dextran sulphate does not exceed 4:1 by weight (critical mixture composition). For a BSA : dextran sulphate ratio of 4:1 and above, the T-complexes are fully precipitated. This ratio also corresponds to the complete disappearance of the β-fraction, which is soluble regardless of the way in which the complexes have been prepared.

The aggregation of the protein–dextran sulphate complexes and the aggregation hysteresis are observed also in concentrated urea solutions. Since the aggregation of proteins in this medium is suppressed, presumably the secondary interactions (hydrogen bonds and hydrophobic interactions) do not play a decisive role in stabilising the aggregates of the protein–dextran sulphate complexes (both equilibrium and non-equilibrium). The formation of non-equilibrium aggregates is apparently due to electrostatic interactions. At the same time the number of ionic bonds between the macroreactants is independent of the way in which the complexes have been obtained, since hysteresis in the sorption of protons is not observed in the potentiometric titration of BSA–dextran sulphate solutions. One can therefore assume that the differences between the M- and T-complexes are caused by the different distributions of the protein molecules between the polysaccharide chains: the T-complexes consist of species with a single dextran sulphate chain (monomatrix complexes), while the M-complexes (the α-fraction) consist of three-dimensional intertwined networks made up of dextran sulphate chains cross-linked by the protein (polymatrix complexes).

Thus mixing of BSA and dextran sulphate solutions at $pH < 5.0$ (for all the ratios investigated) leads to the appearance of a highly aggregated α-fraction, produced as a result of the cross-linking of the random network of the dextran sulphate chain by the protein, together with the readily soluble β-fraction. In the T-complexes

#V.B.Tolstoguzov, V.Ya.Grinberg, and A.N.Gurov, J.Agric.Food Chem., 1985, 33, 151.

obtained when the complex formation equilibrium is "frozen" at pH ~5.0 with subsequent transition to the same conditions as those for the M-complexes, the α-fraction remains soluble up to the protein : dextran sulphate ratio of 4 : 1 by virtue of the electrostatic repulsion between the species bearing like charges and containing one dextran sulphate chain.

2. The Influence of Temperature on the Structure of the Protein Bound in a Complex with the Polysaccharide

The formation of complexes with anionic polysaccharides makes it possible to prevent the thermal coagulation of the protein,[95] which can evidently be explained by the immobilisation of the protein molecules in the complex, preventing the formation of aggregates on denaturation. For example, the coagulation of the BSA–dextran sulphate complex and the absence of the free protein is not observed up to 100 °C (at pH 5.6). The circular dichroism method makes it possible to observe the denaturation of the bound protein to the same high extent as that of the free protein. However, the denaturation of the bound protein is non-cooperative, in contrast to that of the free protein, and is therefore not accompanied by a thermal absorption peak characteristic of the thermal denaturation of free proteins.

3. The Use of the PPC for the Regulation of the Surface Properties of the Protein

The complexes of proteins with anionic polysaccharides have a number of advantages over the initial proteins by virtue of their surface activity. Thus they can ensure a higher stability of emulsions of the "oil–water" type.[96] Fig.13 presents stability diagrams for model emulsions of n-decane in aqueous solutions of BSA and its complexes with dextran sulphate (the latter shows no surface activity and does not itself stabilise emulsions). It can be seen that, over a wide range of protein concentrations, the stability of the emulsions of n-decane in solutions of the complexes is significantly greater. The differences between the emulsifying properties of the protein and its complexes with dextran sulphate diminish with increasing ionic strength, disappearing completely under conditions where the complexes are decomposed.

An analogous increase of the stability of emulsions when the PPC is used instead of the protein has been observed for casein–carrageenan, casein–pectin, human serum albumin–dextran sulphate, and certain other complexes.

It is important to note that the PPC make it possible to regulate the rheological characteristics of the emulsions within wide limits. In particular, one of the versions of the highly viscous (stuffing-like) emulsions stabilised by protein–anionic polysaccharide complexes has been used in the manufacture of cooked sausages.[97]

The properties of the interfacial adsorption layers formed by the PPC have not so far been investigated. However, presumably the structural organisation of the PPC at the interfaces is similar to that which is obtained in complex gels. The formation of the latter and their properties have been investigated in greatest detail in relation to gels of the gelatin–sodium alginate[98,99] and gelatin–pectin[100] complexes, for which significant differences from gelatin gels have been observed. In contrast to the latter, the gelatin–sodium alginate complexes do not melt in the range 30–40 °C and exhibit only an abrupt increase of pliability while retaining other elastic properties up to 100 °C. It is significant that the gel formation in PPC solutions can be observed under conditions where the individual macroreactants do not form gels. An example is provided by the thermoreversible complex gel of casein and sodium alginate§.

IV. THERMODYNAMIC COMPATIBILITY OF PROTEINS AND POLYSACCHARIDES IN SOLUTION

Multicomponent mixtures of polymers are as a rule of a heterophase type; compatibility is attained only when the mixing is accompanied by the evolution of heat. From the standpoint of the technology of the preparation of new forms of food, the study of the thermodynamic compatibility in solutions of the principal classes of food polymers, namely proteins and polysaccharides, was of great interest.

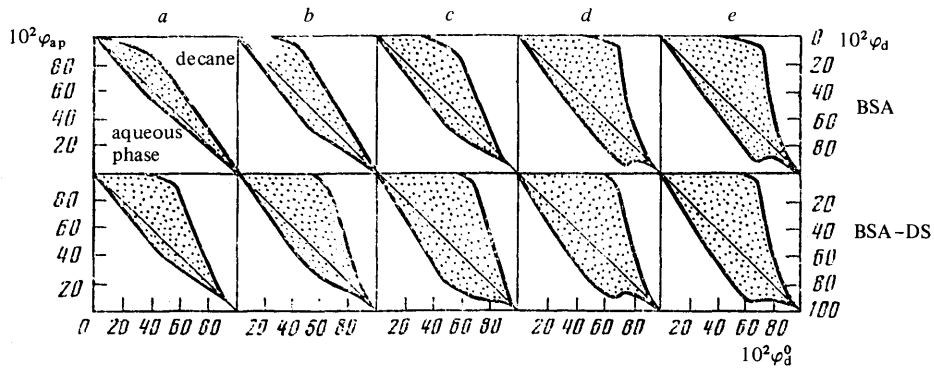


Figure 13. Stability diagrams for n-decane emulsions in aqueous solutions of bovine serum albumin (BSA, upper row) and its complex with dextran sulphate in proportions, by weight, of 1 : 1 (BSA–DS, lower row) at pH 4.0 and an ionic strength of 0.01; ϕ_d^0 = volume fraction of n-decane in the system, ϕ_{ap} = volume fraction of separated aqueous phase, ϕ_d = volume fraction of separated n-decane; BSA concentration (%): *a*) 0.025; *b*) 0.05; *c*) 0.10; d) 0.30; *e*) 0.50.[96]

§M.A.Mukhin, Abstract of Candidate's Thesis in Chemical Sciences, Institute of Organoelemental Compounds, USSR Academy of Sciences, Moscow, 1984.

1. Thermodynamic Compatibility in Protein–Polysaccharide–Water Systems

Although the limited thermodynamic compatibility of gelatin and polysaccharides in solution was observed as early as the end of the previous century[101] and since then has been the subject of a series of studies,[68,102–108] the systematic investigation of thermodynamic compatibility in solutions of polysaccharides and proteins, in the first place globular proteins, was begun only in recent years.

The phenomenology of the limited thermodynamic compatibility in solution has been investigated[68,106,109–114] for the principal classes of proteins and polysaccharides. The fact that the phenomenon of limited thermodynamic compatibility is general has been demonstrated with more than 80 protein–polysaccharide–water systems. All these systems undergo liquid-phase separation with the preferential concentration of the macromolecular components in different phases. This occurs at a fairly high overall concentration of the macromolecular components (usually above 4%) under conditions ensuring the suppression of the association of heterotypical macromolecules and favouring the association of isotypical macromolecules. The principal conditions governing limited thermodynamic compatibility differ for systems containing neutral, carboxylated, and sulphated polysaccharides.

The thermodynamic compatibility of casein[110,111] and the globular fraction of soya beans[112] with different polysaccharides has been studied in greatest detail. Data concerning the influence of the most important thermodynamic variables (pH, ionic strength, temperature, and the molecular weight) on the compatibility of protein and polysaccharides have been obtained. They have been published[110–113] in the form of three-dimensional phase diagrams. As a rule the compatibility diminishes as the pH approaches the isoelectric point of the protein and also with increase of the concentration of the low-molecular-weight salt, temperature, and the molecular weight of the polysaccharide. The sulphated polysaccharides are a known exception. In this case the compatibility increases as the pH approaches the isoelectric point of the protein. The region of total compatibility exists in the vicinity of this point.

The protein–neutral polysaccharide–water and protein–sulphated polysaccharide–water systems have a lower consolute point below a certain concentration of the low-molecular-weight salt. If the salt concentration is below this threshold value, the protein and the polysaccharides indicated are completely compatible. The threshold concentration of the salt depends on its composition and on the pH. The influence of the salt on the thermodynamic compatibility of proteins and neutral polysaccharides is not generally correlated with the positions of the salts in the lyotropic series.

In order to elucidate the nature of thermodynamic compatibility a thermorheological study of the single-phase protein–neutral polysaccharide–water systems has been carried out at different ionic strengths corresponding to the complete or limited compatibility of the macromolecular components at higher concentrations.[114] The excess activation energies for viscous flow, determined by the protein–polysaccharide interaction, were calculated. Positive values of this parameter are characteristic of the conditions ensuring the complete compatibility of the components in the system while negative values are characteristic of systems with limited compatibility, which indicate the predominance of the attraction and repulsion forces respectively between heterotypical macromolecules. Thus it was possible to demonstrate the formation of weak protein–neutral polysaccharide complexes in media with a low ionic strength, where the components are thermodynamically compatible, and their decomposition at a higher ionic strength, i.e. under the conditions of the limited thermodynamic compatibility of proteins and neutral polysaccharides.

2. Thermodynamic Compatibility in Protein–Protein–Water Systems

Data concerning the thermodynamic compatibility of proteins have also appeared recently. It has been shown for nine systems[115] that under certain conditions proteins belonging to different classes in Osborn's classification are compatible only to a limited extent in an aqueous medium. The phase diagrams for certain protein *1*–protein *2*–water systems¶, obtained by the method of the phase volume ratio,[116] are presented in Figs.14 and 15.

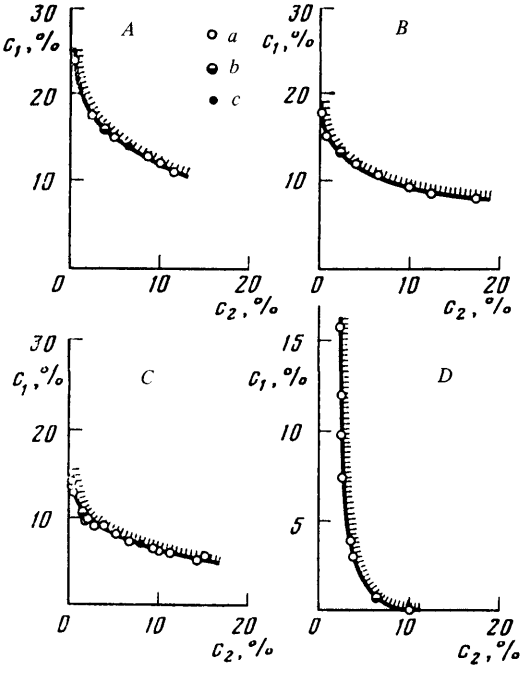


Figure 14. Phase diagrams for certain protein (1)–protein (2)–water systems at 20 °C: *A*) ovalbumin (1)–casein (2)–water (pH 6.6); *B*) ovalbumin (1)–globulin fraction of soya beans (2)–water (pH 6.6); *C*) casein (1)–globulin fraction of soya beans (2)–water (pH 6.9); *D*) casein (1)–gliadin (2)–water (pH 11); c_1 and c_2 are the weight concentrations of proteins (1) and (2) in the system: *a*) points on the binodal curve; *b*) layer separation threshold; *c*) consolute point.

Analysis of the equation of state for polymer *1*–polymer *2*–solvent systems by the Flory–Huggins method leads to characteristics which determine the form of their phase diagrams.[117] In particular, the layer formation threshold, i.e.

¶ V.I.Polyakov, O.K.Kireeva, V.Ya.Grinberg, and V.B.Tolstoguzov, Nahrung, 1985, 29, 153.
V.I.Polyakov, I.A.Popello, V.Ya.Grinberg, and V.B.Tolstoguzov, Nahrung, 1985, 29 (in the press).

the point at which the binodal curve is tangential to the straight line making equal intercepts on the coordinate axes, should be displaced towards the axis corresponding to the polymer with the lower molecular weight, while the consolute point (the point of intersection of the binodal curve and the rectilinear diameter) should be displaced towards the axis of the polymer with the lower lyophilicity. These features are valid for mixtures of proteins in a common solvent.

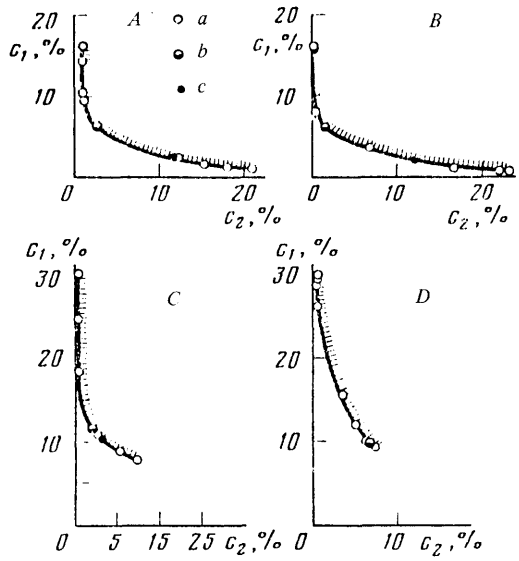


Figure 15. Phase diagrams for certain protein (1)–protein (2)–water systems: *A*) gelatin (1)–globulin fraction of fodder beans (2)–water (t = 40 °C, pH 7.0); *B*) gelatin (1)–11*S*-globulin from fodder beans (2)–water (t = 40 °C, pH 7.0); *C*) ovalbumin (1)–thermotropic ovalbumin cluster (2)–water (t = 20 °C, pH 6.8); *D*) bovine serum albumin (1)–thermotropic ovalbumin cluster (2)–water (t = 20 °C, pH 6.8); conditions in the preparation of the thermotropic ovalbumin cluster: temperature 100 °C, duration of heat treatment 60 min, protein concentration 5%, method of concentration—lyophilisation.

The above result shows that the equation of state indicated is applicable regardless of the model (freely jointed chains on a lattice[118,119]) on the basis of which it has been obtained.

The possibility of regulating the thermodynamic compatibility of proteins is of some interest. One of the effective ways of achieving such regulation is thermotropic aggregation of the protein. It is known[120] that heating above the denaturation temperature leads to the formation of soluble clusters of protein molecules when the concentration of the protein does not exceed the gel formation threshold.[121] The size, shape,[122] and probably the hydrophilic properties of the accessible surfaces of these clusters can be altered by varying the conditions in the heat treatment of the protein solution. The extent to which the heat treatment influences the compatibility of proteins is indicated by the phase diagrams *C* and *D* in Fig.15. In the native state BSA and ovalbumin are fully compatible in an aqueous medium. However, the thermotropic ovalbumin clusters are incompatible not only with BSA but also with native ovalbumin.

3. Practical Utilisation of Two-Phase Liquid Systems

(a) Concentration and purification of proteins. A low layer-formation threshold and a high ratio of the protein and polysaccharide concentrations at the consolute point are typical for the phase diagrams of protein–polysaccharide–water systems. In other words, the protein–polysaccharide–water systems separate into different phases which differ sharply in the concentrations of the macromolecular components, namely into a highly concentrated phase enriched in the protein (the polysaccharide content is not more than 0.5%) and a dilute polysaccharide phase. A new method of concentrating protein solutions, called membrane-free osmosis, is based on this phenomenon.[69] In particular, it has been used to concentrate proteins of defatted milk with the aid of apple pectin. The concentration of milk proteins to 30% is achieved at a polysaccharide concentration in the system of 1%. The high productivity, by virtue of the large area of the interface, the simplicity of the apparatus, and the relatively high energy efficiency may be included among the advantages of the process.

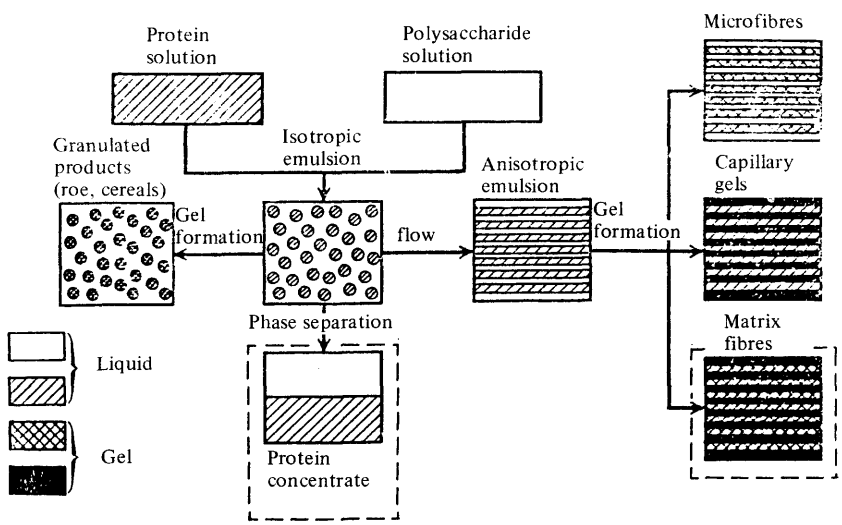


Figure 16. Scheme for the employment of two-phase protein–polysaccharide–water systems in the processing of protein.[1,66]

Yet another possibility of the practical utilisation of the phenomenon of the limited thermodynamic compatibility of proteins and polysaccharides in solution is based on the fact that protein–lipid complexes are less compatible with polysaccharides than lipid-free proteins. This effect has been used to remove lipids from the globulin fraction of baker's yeast proteins.[123,124] The lipid content in the protein preparation was thus reduced to 0.1%.

(b) The manufacture of new forms of food by processing two-phase liquid systems. The extensive possibilities for the preparation of NFP by processing two-phase liquid systems of the "water in water" type have been demonstrated in recent years. Such systems are formed by thermodynamically incompatible solutions of proteins and polysaccharides or different proteins. A general scheme for the processes involved in the processing of two-phase liquid systems to produce NFP[1,66] is illustrated in Fig.16. It includes the deformation of the two-phase liquid system in a flow and the conversion of one or both phases into a gel.

The possibility of attaining high degrees of deformation of the two-phase liquid system is ensured by the low interfacial tension; an approximate equality of the viscosities of the two phases is also necessary.[125] The ratio of the viscosities of the phases together with the dimensions of the particles of the disperse phase and the rate of shear determine the structure of the two-phase liquid systems in a stream and the possibility of processing them.[126]

On passing to the gel state in a flow, the particles of the disperse phase form protein microfibres. Depending on the volume fraction of the disperse phase, it is possible to obtain both short fibres and infinitely long fibres. In the latter case the formation of a gel is preceded by the coalescence of the deformed disperse particles. This process has been called spinning without a drawplate.[126] For high degrees of deformation, granulated products can be obtained in which the grains are anisodiametric to different extents.

When the dispersion medium is converted into a gel in a flow, gels filled with liquid cylinders oriented in the direction of the flow, the so-called gels with a capillary structure, are produced. The stability of these gels at right angles to the direction of orientation of the capillaries increases with increasing rate of shear.[125,127] For high degrees of filling, the continuous phase consists of the fibre cross-sections. Finally, there is a possibility that both phases of the deformed two-phase liquid system are converted into a gel. Gels filled with fibres oriented in the direction of the deformation, which are anisotropic in their mechanical properties, are obtained in this way.

All the principal versions of the processing of proteins in two-phase liquid systems under the conditions of high degrees of deformation of the disperse particles (not less than 10) are of practical importance in the preparation of analogues of meat products—fibres and textured materials.

REFERENCES

1. V.B.Tolstoguzov, "Iskusstvennye Produkty Pitaniya. Novyi Put' Polucheniya Pishchi i Ego Perspektivy. Nauchnye Osnovy Proizvodstva" (Artificial Foodstuffs. A New Method of Obtaining Food and Its Likely Usefulness. Scientific Principles of the Manufacture), Izd.Nauka, Moscow, 1978.
2. K.Kinsella, CRC Crit.Rev.Food Sci.and Nutr., 1976, **7**, 219.
3. W.B.Tolstoguzov, E.E.Braudo, and A.N.Gurov, Nahrung, 1981, **25**(231), 817.
4. S.P.Papkov, "Fiziko-Khimicheskie Osnovy Pererabotki Rastvorov Polimerov" (Physicochemical Principles of the Processing of Polymer Solutions), Izd.Khimiya, Moscow, 1971.
5. S.P.Papkov, "Studneobraznoe Sostoyanie Polimerov" (The Gel State of Polymers), Izd.Khimiya, Moscow, 1974.
6. W.B.Tolstoguzov and E.E.Braudo, J.Text.Stud., 1983, **14**, 183.
7. W.B.Tolstoguzov, Ju.I.Tschimirow, E.E.Braudo, E.S.Wainerman, and E.P.Kosmina, Nahrung, 1975, **19**, 33.
8. W.B.Tolstoguzov, Ju.I.Tschimirow, B.P.Suchanow, E.E.Braudo, A.D.Ignatjew, and E.P.Kosmina, Nahrung, 1976, **20**, 329.
9. W.B.Tolstoguzov, Nahrung, 1979, **23**, 649, 821.
10. W.B.Tolstoguzov, L.V.Lemisova, Ju.I.Chimirov, E.E.Braudo, V.A.Tsyrjapkin, A.I.Volnova, and E.P.Kosmina, Nahrung, 1980, **24**, 951.
11. Ju.I.Chimirov, E.E.Braudo, and V.B.Tolstoguzov, Nahrung, 1981, **25**, 719.
12. Ju.I.Chimirov et al., Nahrung, 1982, **26**, 741.
13. R.W.Moncrieff, "Man-Made Fibres" (Translated into Russian), Izd.Legkaya, Industriya, Moscow, 1964, p.227.
14. V.A.Ershova, V.B.Tolstoguzov, E.E.Braudo, N.V.Mikheeva, and E.S.Bondareva, Trudy Moskov. Inst.Narod.Khoz.im.Plekhanova, 1968, **58**, 125.
15. H.Thiele, "Hystolyse und Hystogenese; Gewebe und ionotrope Gele. Prizip einer Strukturbildung", Akademische Verlagsgesellschaft, Frankfurt/M, 1967.
16. V.B.Tolstoguzov, A.I.Mzhel'skii, N.V.Grinberg, B.P.Rysev, E.E.Braudo, D.B.Izyumov, and N.M.Shel'pova, Vysokomol Soed., 1973, **15A**, 2703.
17. E.S.Obolonkova et al., Colloid and Polym.Sci., 1974, **252**, 526.
18. E.S.Obolonkova et al., Colloid and Polym.Sci., 1978, **256**, 133.
19. V.V.Suchkov, V.Ya.Grinberg, and V.B.Tolstoguzov, Nahrung, 1980, **24**, 893.
20. Yu.A.Antonov, V.Ya.Grinberg, N.A.Zhuravskaya, G.Schmidt, H.Schmandke, and V.B.Tolstoguzov, Nahrung, 1982, **26**, 9.
21. V.V.Suchkov, V.Ya.Grinberg, and V.B.Tolstoguzov, Carbohydr.Polym., 1981, **1**, 39.
22. S.G.Semenov, E.E.Braudo, Yu.F.Yaroshenko, G.G.Zharikova, and V.B.Tolstoguzov, Nahrung, 1982, **26**, 21.
23. V.P.Yuryev et al., Starch/Stärke, 1979, **31**, 121.
24. M.P.Tombs, Faraday Discuss.Chem.Soc., 1974, **57**, 158.
25. M.G.Bezrukov, Angew.Chem.Int.Ed.Engl., 1979, **18**, 599.
26. A.H.Clark and C.D.Tuffnell, Int.J.Peptide and Protein Res., 1980, **17**, 339.
27. A.H.Clark, D.H.P.Sanderson, and A.Suggett, Int.J.Peptide and Protein Res., 1981, **17**, 353.
28. A.H.Clark et al., Int.J.Peptide and Protein Res., 1981, **17**, 380.
29. P.L.Privalov et al., J.Chem.Thermodyn., 1975, **3**, 631.
30. T.M.Bikbov, V.Ya.Grinberg, A.N.Danilenko, T.S.Chaika, I.A.Vaintraub, V.B.Tolstoguzov, Colloid and Polym.Sci., 1983, **261**, 346.
31. T.M.Bikbov, V.Ya.Grinberg, and V.B.Tolstoguzov, Nahrung, 1979, **23**, 403. 487.

32 A.A.Olekhnovich, E.A.Kamenskaya, E.M.Belavtseva, T.M.Bikbov, V.Ya.Grinberg, and V.B.Tolstoguzov, Biofizika, 1981, **26**, 439.
33. T.M.Bikbov, V.Ya.Grinberg, H.Schmandke, T.S.Chaika, I.A.Vaintraub, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1981, **259**, 536.
34. E.L.Braudo, I.G.Plashchina, and V.B.Tolstoguzov, Carbohydr.Polym., 1984, **4**, 23.
35. V.P.Yuryev et al., Carbohydr.Polym., 1981, 1, 139.
36. A.H.Clark et al., Macromolec., 1983, **16**, 1367.
37. J.Hermans, Jr., J.Polym.Sci., A, 1965, **3**, 1859.
38. C.Tanford, J.Amer.Chem.Soc., 1962, **84**, 4240.
39. C.C.Bigelow, J.Theor.Biol., 1967, **16**, 187.
40. V.Ya.Maleev and A.I.Gasan, Biopolym., 1974, **13**, 2409.
41. C.J.Tanford, "The Physical Chemistry of Macromolecules" (Translated into Russian), Izd. Khimiya, Moscow, 1965, p.270.
42. J.D.Ferry, "Viscoelastic Properties of Polymers" (Translated into Russian), Inostr.Lit., Moscow, 1967, p.267.
43. E.E.Braudo, I.G.Plashchina, N.S.Kuz'mina, and V.B.Tolstoguzov, Vysokomol.Soed., 1974, **16A**, 2240.
44. E.E.Braudo, E.M.Belavtseva, E.F.Titova, I.G.Plashchina, V.I.Krylov, V.B.Tolstoguzov, F.R.Schierbaum, M.Richter, and G.Berth, Starch/Stärke, 1979, **31**, 188.
45. I.G.Plashchina, O.A.Fomina, E.E.Braudo, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1979, **257**, 1180.
46. I.G.Plashchina, N.V.Grinberg, E.E.Braudo, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1980, **258**, 939.
47. I.G.Plashchina, N.A.Fishkina, E.E.Braudo, and V.B.Tolstoguzov, Nahrung, 1980, **24**, 543.
48. I.G.Plashchina, A.M.Gotlieb, E.E.Braudo, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1983, **261**, 672.
49. D.A.Rees, Adv.Carbohydr.Chem.and Biochem., 1969, **24**, 267.
50. D.A.Rees and E.J.Welsh, Angew.Chem.Int.Ed. Engl., 1977, **16**, 214.
51. D.A.Rees, Biochem.Soc.Trans., 1981, **9**, 25.
52. L.Z.Rogovina and G.L.Slonimskii, Uspekhi Khim., 1974, **43**, 1102 [Russ.Chem.Rev., 1974(6)].
53. E.F.Titova, E.M.Belavtseva, E.E.Braudo, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1974, **252**, 497.
54. V.N.Izmailova, Samir Abu Ali, and V.A.Pchelin, Symposium, "Fiziko-Khimicheskaya Mekhanika Dispersnykh Struktur" (The Physicochemical Mechanics of Disperse Structures) (Edited by P.A.Rebinder), Izd.Nauka, Moscow, 1966, p.77.
55. D.A.Rees, Chem.Ind., 1972, 630.
56. H.S.Owens, H.A.Swenson, and T.H.Schultz, Adv.Chem.Ser., 1954, 11, 10.
57. J.B.Smith and E.F.Bryant, J.Food Sci., 1968, **33**, 262.
58. O.Smidsrod and A.Haug, Biopolym., 1971, **10**, 1213.
59. E.R.Morris, D.A.Rees, and G.Robinson, J.Mol. Biol., 1980, **138**, 349.
60. O.Smidstrod, I.L.Andersen, H.Grasdalen, B.Larsen, and T.Painter, Carbohydr.Res., 1980, **80**, C11.
61. O,Smidstrod and H.Grasdalen, Carbohydr.Polm., 1982, **2**, 270.
62. A.Weiss, "Macromolecular Chemistry of Gelatin" (Translated into Russian), Izd.Pishch.Prom., Moscow, 1971.
63. E.R.Morris, D.A.Rees, I.T.Norton, and D.M.Goodall, Carbohydr.Res., 1980, **80**, 317.
64. N.V.Grinberg, I.G.Plashchina, E.E.Braudo, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1980, **258**, 1038.
65. W.B.Tolstogusow, Nahrung, 1974, **18**, 523.
66. W.B.Tolstogusow, E.E.Braudo, and E.S.Wainerman, Nahrung, 1975, **19**, 973.
67. E.V.Grishchenkova, Yu.A.Antonov, E.E.Braudo, and V.B.Tolstoguzov, Nahrung, 1984, **28**, 15.
68. V.Ya.Grinberg and V.B.Tolstoguzov, Carbohydr. Res., 1972, **25**, 313.
69. Yu.A.Antonov, V.Ya.Grinberg, N.A.Zhuravskaya, and V.B.Tolstoguzov, Carbohydr.Polym., 1982, **2**, 81.
70. T.Laurent, in "The Chemical Physiology of Mucopolysaccharides" (Edited by G.Quintarelli), Little, Braun and Co., Boston, 1968, p.153.
71. E.A.Bekturov, "Troinye Polimernye Sistemy v Rastvore" (Ternary Polymer Systems in Solution), Izd.Nauka, Alma-Ata, 1975, p.168.
72. V.B.Tolstogusov, V.P.Belkina, V.Ja.Gulov, V.Ya.Grinberg, E.F.Titova, and E.M.Belavzeva, Starch/Stärke, 1974, **26**, 130.
73. A.H.Clark, R.K.Richardson, G.Robinson, S.B.Ross-Murphy, and A.C.Weaver, Progr.Food and Nutr.Sci., 1982, **6**, 149.
74. E.E.Braudo, and W.B.Tolstogusov, Nahrung, 1974, **18**, 173.
75. M.Watase and K.Nishinari, Rheol.Acta, 1980, **19**, 220.
76. H.Moritaka, K.Nishinari, H.Horiuchi, and M.J.Watase, Text.Stud., 1980, **11**, 257.
77. H.L.Booiy and H.G.Bungenberg de Jong, "Biocolloids and Their Interactions", Springer-Verlag, Vienna, 1956.
78. T.N.Evreinova, "Kontsentrirovanie Veshchestv i Deistvie Fermentov v Koatservatakh" (Concentration of Substances and the Action of Enzymes in Coacervates), Izd.Nauka, Moscow, 1966.
79. K.B.Serebrovskaya, "Koatservaty i Protoplazma" (Coavervates and Protoplasm), Izd.Nauka, Moscow, 1971.
80. W.B.Tolstogusow and E.S.Wajnermann, Nahrung, 1975, **19**, 45.
81. D.A.Ledward, in "Polysaccharides in Food" (Edited by J.M.V.Blanshard and J.R.Mitchell), Butterworth, London, 1979, p.205.
82. S.Sasaki and H.Noguchi, J.Gen.Physiol., 1959, **43**, 1.
83. H.Noguchi, J.Phys.Chem., 1960, **64**, 185.
84. T.E.Thompson and W.M.McKernan, Biochem.J., 1961, **81**, 12.
85. Th.H.M.Snoeren, T.A.J.Payens, J.Jeunink, and P.Both, Milchwissenschaft, 1975, **30**, 393.
86. M.A.Rozenfel'd, K.L.Erzinkyan, and L.A.Piruzyan, Izv.Akad.Nauk SSSR, Ser.Biol., 1975, 419.
87. E.Ponder and R.V.Ponder, J.Gen.Physiol., 1959, **43**, 753.
88. W.D.Comper and T.C.Laurent, Biochem.J., 1978, **175**, 703.
89. A.N.Gurov, E.S.Wajnerman, and W.B.Tolstoguzow, Stärke, 1977, **29**, 186.
90. A.N.Gurov, N.A.Larichev, and N.V.Lozinskaya, Symposium, "Vodorastvorimye Polimery. II Vsesoyuznaya Konferentsiya. Tezisy Dokladov" (Water-Soluble Polymers. The IInd All-Union Conference. Abstracts of Reports), Irkutsk, 1982, p.84.

91. D.E.Olins, A.L.Olins, and P.H.von Hippel, J.Mol. Biol., 1967, **24**, 157.
92. J.D.McGhee and P.H.von Hippel, J.Mol.Biol., 1974, **86**, 469.
93. V.A.Kabanov, V.P.Evdakov, M.I.Mustafaev, and A.D.Antipina, Mol.Biol., 1977, **11**, 582.
94. V.A.Kabanov, M.I.Mustafaev, V.V.Belova, and V.P.Evdakov, Biofizika, 1978, **23**, 789.
95. A.N.Gurov, N.A.Larichev, V.I.Krylov, and V.B.Tolstoguzov, Studia Biophysica, 1978, **72**, 7.
96. N.A.Larichev, A.N.Gurov, and V.B.Tolstoguzov, Colloids and Surfaces, 1983, **6**, 27.
97. E.S.Tokaev, I.A.Rogov, A.N.Gurov, and V.B.Tolstoguzov, Myasnaya Industriya SSSR, 1981(6), 22.
98. M.A.Muchin, E.S.Wajnerman, and V.B.Tolstoguzov, Nahrung, 1976, **20**, 313.
99. M.A.Muchin, Z.A.Streltsova, E.S.Wajnerman, and V.B.Tolstoguzov, Nahrung, 1978, **22**, 867.
100. G.Ja.Tschumak, E.S.Wajnerman, and V.B.Tolstoguzov, Nahrung, 1976, **20**, 321.
101. M.W.Beijerinck, Zentralbl.Bakteriol., Abt.2, 1896, **2**, 627, 628.
102. O.Bütschli, Untersuch.Strukturen, 1898, 251.
103. M.W.Beijerinck, Koll.-Z., 1910, **7**, 16.
104. Wo.Ostwald and R.H.Hertel, Koll.-Z., 1929, **47**, 258, 357.
105. K.Doi, Biochim.Biophys.Acta, 1965, **94**, 257.
106. V.B.Tolstoguzov, V.Ya.Grinberg, and L.I.Fedotova, Izv.Akad.Nauk SSSR, Ser.Khim., 1969, 2839.
107. V.Ya.Grinberg, V.B.Tolstoguzov, and G.L.Slonimskii, Vysokomol.Soed., 1970, **12A**, 1593.
108. V.Ya.Grinberg, K.-D.Shvenke, and V.B.Tolstoguzov, Izv.Akad.Nauk SSSR, Ser.Khim., 1970, 1430.
109. V.B.Tolstoguzov and V.Ya.Grinberg, Izv.Akad. Nauk SSSR, Ser.Khim., 1970, 1423.
110. Yu.A.Antonov, V.Ya.Grinberg, and V.B.Tolstoguzov, Starch/Stärke, 1975, **27**, 242.
111. Yu.A.Antonov, V.Ya.Grinberg, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1977, **255**, 937.
112. Yu.A.Antonov, N.V.Lozinskaya, V.Ya.Grinberg, and V.B.Tolstoguzov, Colloid and Polym.Sci., 1979, **257**, 1159.
113. Yu.A.Antonov, V.Ya.Grinberg, and V.B.Tolstoguzov, Nahrung, 1979, **23**, 207, 597, 847.
114. E.P.Varfolomeeva, V.Ya.Grinberg, and V.B.Tolstoguzov, Polym.Bull., 1980, 2, 613.
115. V.I.Polyakov, V.Ya.Grinberg, Yu.A.Antonov, and V.B.Tolstoguzov, Polym.Bull., 1979, 1, 593.
116. V.I.Polyakov, V.Ya.Grinberg, and V.B.Tolstoguzov, Polym.Bull., 1980, 2, 757.
117. C.C.Hsu and J.M.Prausnitz, Macromolec., 1974, **7**, 320.
118. M.L.Huggins, J.Chem.Phys., 1941, **9**, 440.
119. P.J.Flory, J.Chem.Phys., 1941, **9**, 660.
120. V.V.Mozhaev and K.Martinek, Mol.Biol., 1982. **16**, 676.
121. T.M.Bikbov, V.Ya.Grinberg, Yu.A.Antonov, V.B.Tolstoguzov, and H.Schmandke, Polym.Bull., 1979, 1, 865.
122. T.Kamata, Int.J.Biol.Macromolec., 1979, 1, 33.
123. T.Ya.Bogracheva, V.Ya.Grinberg, and V.B.Tolstoguzov, Carbohydr.Polym., 1982, **2**, 163.
124. T.Ya.Bogracheva, V.Ya.Grinberg, and V.B.Tolstoguzov, Nahrung, 1983, **27**, 735.
125. V.B.Tolstoguzov, A.I.Mzhel'skay, and V.Ya.Gulov, Colloid and Polym.Sci., 1974, **252**, 124.
126. Yu.A.Antonov, V.Ya.Grinberg, N.A.Zhuravskaya, and V.B.Tolstoguzov, J.Text.Stud., 1980, **11**, 199.
127. V.B.Tolstoguzov, A.I.Mzhel'skii, and V.Ya.Gulov, Vysokomol.Soed., 1973, **15B**, 824.

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