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The Factors Affecting the Compatibility of Serum Albumin and Pectinate in Aqueous Medium

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ABSTRACT

Compatibility is observed in aqueous solutions of serum albumin and pectin (degree of esterification 57%) at pH levels above the isoelectric point of protein. Both the variation in the pH values from 5 to 8 and the increase of ionic strength from 0·1 to 1·0 do not result in phase separation. These facts enable us to conclude that the affinity in this system is of a nonelectrostatic nature. The interaction of serum albumin and pectinate fractions with different degrees of esterification was studied by light scattering. The negative sign of A_{24} (the second virial coefficient component of the mixture associated with the interaction between different polymer molecules) means that for any degree of esterification there is affinity between pectin and serum albumin. Information concerning excess thermodynamic functions was obtained from the temperature dependence of light scattering. Mixing microcalorimetry was used for precise measurement of enthalpy. The experimental results indicate that thermodynamic compatibility of serum albumin and pectin is controlled by increase of mixing entropy, which mainly stems from dehydration of biopolymer macromolecules during contact formation.

INTRODUCTION

It was shown recently (Tolstoguzov *et al.*, 1985; Tolstoguzov, 1987), that mixtures of polysaccharides and proteins exhibit incompatibility or form soluble or insoluble complexes only at specific pH and ionic strength values. The conditions leading to complex formation or phase separation of interacting proteins and polysaccharides depend on their physical and chemical properties.

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Up to now the detailed study of incompatibility of proteins with various polysaccharides was limited to the globulin fraction of soy-bean protein and casein (Antonov *et al.*, 1975, 1980). Experiments on the incompatibility of proteins, belonging to the albumin class (according to the Osborn classification), were confined to gelatin, having under the experimental conditions ($t = 40°C$) a disordered coil conformation (Grinberg *et al.*, 1972). The interaction of serum albumin with highly charged sulphated polysaccharides at pH values lower than the isoelectric point (IEP) of protein, where the conditions of complex formation are favourable, has also been examined (Tolstoguzov *et al.*, 1985). The interaction of albumin with anionic polysaccharides above the isoelectric point (IEP) was not investigated in detail.

In the present paper we report experimental results on the interaction of serum albumin with pectin at pH values above the protein IEP.

MATERIALS

Serum albumin

We used commercial bovine serum albumin (BSA) manufactured by Minsk bacterial plant (USSR) and human serum albumin (HSA) obtained from 'Reanal' (Hungary). HPLC showed that the BSA contained by weight 92% of monomers and 8% of dimers. This sample was used without further purification. HSA contained about 20% of high molecular weight contaminants ($MW \sim 10^6$). They were removed by GPC (Sepharose CL-6b). The purified sample contained 60% of monomers and 40% of dimers and trimers. The denaturation temperatures were measured by differential scanning microcalorimetry. They were 60°C and 75°C for BSA and HSA, respectively. This proves that HSA contains a certain amount of fatty acids, while the BSA sample is almost free of them (Gumpen *et al.*, 1979). The stability of BSA and HSA solutions was monitored by light scattering. The experiments showed that the storage of albumin solutions for 10 days at 5°C did not affect their properties. In experiments we used solutions stored no longer than 10 days.

Preparation of solutions

The serial dilution for light scattering measurements and determination of specific partial volume was carried out by dilution with the solvent,

dialysed against the stock-solution for 3 days. Where necessary solutions were concentrated by membrane ultrafiltration.

Determination of concentrations

The albumin concentration was determined by absorption at 278 nm. The extinction coefficients $A^{1\%}_{1\,cm}$ were taken as 6·67 for BSA (Westphal & Harding, 1973) and 5·4 for HSA. The latter value corresponds to albumin solution containing aggregates (Westphal & Harding, 1973).

Pectin

In the present studies we used citrus pectin (Koch-Light, UK), having a degree of methoxylation of 58%. Prior to the experiment the pectin sample was reprecipitated by acidic isopropanol, according to the method reported by Plashchina *et al.* (1985). Pectin fractions having different degrees of esterification and various molecular weights were obtained by fractional precipitation from purified pectin solution. We used a mixture of isopropanol and double distilled water (9:1) as a precipitant (Yudakhina *et al.*, 1987). It can be assumed that under these conditions the fractionation by degree of esterification predominates over the molecular weight fractionation (Kantow, 1971). The pectin fractions obtained were lyophilized. We used the sample prepared from citrus pectin (Koch-Light, UK) as a pectate. This sample was prepared by de-esterification by adjusting the solution with NaOH to pH 12 for 1 h ($t = 23°C$). The alkali was then neutralized by HCl and NaCl was removed by dialysis and the pectate was lyophilized. The almost entirely methoxylated pectin was obtained by methylation of sodium pectate by diazomethane according to the method of Vollmert (1950).

Determination of uronide content and degree of esterification

The uronide content and degree of esterification were measured for all fractions, the quantities obtained were expressed relative to the total carbohydrate determined by the phenol–sulphuric acid method (Dubois *et al.*, 1956), using galacturonic acid (Serva, Germany) as a calibration standard. The uronide content was assessed according to the modified carbosol technique (Filipov *et al.*, 1973), which allows accompanying neutral sugars to be eliminated. The degree of esterification was obtained by the method of Filipov *et al.* (1971). This method measures the quantity of methanol isolated during the action of alkali on the pectin

ester groups. We used absolute methanol to obtain the calibration curve. The degree of esterification of the pectin fractions are reported in Table 1.

Determination of concentrations

The concentration of pectin in solution was measured by the phenol–sulphuric acid method (Dubois *et al.*, 1956), using galacturonic acid as a standard.

Preparation of solutions

The pectin solutions were prepared as follows. The lyophilized samples were dissolved in 0·005 M EDTA, then the ionic strength was gradually increased by dialysis against a 0·1 M solution of NaCl over a period of three days. The series of solutions for light scattering experiments and mixing calorimetry were prepared by dilution with solvent, which had been dialysed against a stock solution for three days. The concentration of solution necessary to plot the phase diagrams was obtained using ultrafiltration.

TABLE 1
Chemical and Solution Properties of Pectin and Human Serum Albumin (pH 7·0, Ionic Strength 0·1, 298 K)

Pectin fractions	*Uronic acid content (% ±0·5%)*	*Degree of esterification (% ±3%)*	M_w *(kD)*	$(R_g^2)^{1/2}$ *(nm)*	$A_{i4} \cdot 10^3$ *(m^3 mol/kg^2)*
1	88	0	150	75	11
2	74	45	500	96	3·6
3	98	47	360	95	3·2
4	88	50	170	74	4·1
5	86	57	250	92	3·0
6	94	70	250	82	2·8
7	88	76	250	90	1·9
8	89	89	380	72	1·5
9	86	98	500	69	1·5
HSA			130		0·84

$i = 2$ or 4 (2 = serum albumin, 4 = pectin).

METHODS

HPLC

Analytical HPLC was performed using a Gilson (France) chromatograph equipped with the column TSK G 2000 SW of dimensions 7·5 × 30 mm. 0·1 M NaCl (pH 7·0) was used as eluent. The injection volume was 20 ml.

Differential scanning calorimetry

The microcalorimetric investigations were carried out using a differential scanning microcalorimeter DASM-1M (Special Design Bureau of Biological Instruments, Academy of Sciences, USSR) over the temperature range 20–100°C at a scanning rate 2°C/min, and an excess pressure of 1 bar.

Sedimentation analysis

The sedimentation coefficients were measured at 25°C at 50 000 rpm using ultracentrifuge MOM 3170 B (Hungary) equipped with a Filpot-Svensson refractometric measuring system.

Nephelometric titration

Nephelometric titration was carried out using a 'Karl-Zeiss' (FRG) spectrocolorimeter equipped with a special unit to measure light scattered at 90°C. The wavelength of incident light was 436 nm, the cell volume was 30 ml. The solutions were stirred with a magnetic stirrer. They were kept for 30 min before measurement to attain the equilibrium state. The titration was performed using an automatic titrator 'Radelkis' (Hungary). The ionic strength was maintained constant by adding the required amount of concentrated NaCl. In binary systems the concentration of the polymers was equal to 2·5% while ternary systems contained equal masses of polysaccharide and protein with a total polymer concentration of 5%.

Determination of compatibilities

We used ultrafiltration to obtain concentrated solutions, containing equal amounts of serum albumin and pectin. Solutions of pectin having concentrations of 5% were very viscous. When the concentration of pectin

solution reached 16%, a gel was formed. The estimation of phase composition was carried out over the concentration range 5–20%. The mixtures obtained were intensively stirred for 3 h to attain homogeneity. If phase separation was not observed under the microscope (NU-2 'Karl Zeiss', FRG, magnification 380–800) and the solution was homogeneous after centrifugation for 1 h (140 000 *g*), we concluded that the mixture of serum albumin and pectin was thermodynamically compatible.

Light scattering

The refractive index increments for the serum albumin and pectin fractions were measured using a differential refractometer 'Shimadzu' (Japan). We obtained the following values:

$$\nu_2 = 0{\cdot}140 \pm 0{\cdot}003 \text{ cm}^3/\text{g}$$

and

$$\nu_4 = 0{\cdot}200 \pm 0{\cdot}03 \text{ cm}^3/\text{g}$$

(here 1 denotes low molecular weight solvent, 2, pectinate, 4, albumin) with statistical confidence 0·95. These results are in good agreement with the published data (Huglin, 1972). The refractive index increment of pectin does not depend on the degree of esterification.

The light scattering experiments were carried out using nephelometer FPS-3M (Unique Design Bureau of Scientific Instruments, Academy of Science, USSR) at 25°C. The wave-length of non-polarized light was 436 nm. The scattering angle varied from 40° to 150°. The light scattering measurements on the serum albumin solution were carried out at a fixed angle of 90° as no asymmetry was observed. Dust free benzene was used as a calibration standard, its Rayleigh ratio was taken as: $R = 47{\cdot}4 \times 10^{-6}$ cm^{-1} (Eskin, 1973). The intensity of light scattering was corrected using the refractive index ratio ($n_{\text{water}}/n_{\text{benzene}} = 0{\cdot}785$) and the variation in the light scattering volume. The exit walls of the sample cells had traps to minimize the effect of reflections. The intensity of excessive light scattering was calculated for every scattering angle according to the difference in intensities of light scattered in solution and the pure solvent.

All the solutions were filtered through PVH membranes (Slectron, Germany, pore size 0·45 μm) into the cells, which had previously been flushed with condensed acetone (Stark & Danz, 1982). To facilitate plotting of a Zimm diagram for pectinate fractions, we ascribed a negative value to the constant k in the argument (Eskin, 1973). The calculation of the second virial coefficient, characterizing the interaction

of different macromolecular species, was performed according to the following expression (Kratochvil & Sudelof, 1986):

$$\frac{K(C_2+C_4)}{[\Delta R_{\theta=0}^{(c)}]} = \frac{1}{\nu_2^2 M_{2W} W_2 + \nu_4^2 M_{4W} W_4}$$

$$+2\frac{\nu_2^2 M_{2W}^2 W_2^2 A_{12} + 2\nu_2\nu_4 M_{2W} M_{4W} W_2 W_4 A_{24} + \nu_4^2 M_{4W}^2 W_4^2 A_{14}}{(\nu_2^2 M_{2W} W_2 + \nu_4^2 M_{4W} W_4)^2}(C_2+C_4) \quad (1)$$

where

$K = 2\pi^2 n^2 / N_A . \lambda^4$
n = refractive index of solvent
N_A = Avogadro's number
λ = the wavelength of incident light in vacuum
ν_2, ν_4 = refractive index increments of polymers 2 and 4
W_2, W_4 = mass fractions of polymers 2 and 4
M_{2W}, M_{4W} = weight average molecular weights of polymers 2 and 4
C_2, C_4 = concentrations of polymers 2 and 4 (g/ml) in solution
$[\Delta R_{\theta=0}^{(c)}]$ = Rayleigh ratio at a zero scattering angle
A_{12}, A_{14} = second virial coefficients, characterizing interaction of polymers 2 and 4 with solvent;
A_{24} = the second virial coefficient, characterizing interaction of polymers 2 and 4 with each other.

The temperature was varied in the range 283–333 K for pectin solutions and from 283 to 303 K for albumin and albumin–pectin solutions. From 283 to 303 K the temperature was varied by increments of 4 K, and from 303 to 333 K by increments of 10 K. Prior to the experiment solutions were kept for 30 min. The temperature control was better than 0·1 K.

Calorimetry

Enthalpy of dilution of solutions of albumin, pectin and their equal mass mixtures was measured using a differential transient calorimeter DPMK (Design Bureau for Unique Biological Equipment, Academy of Sciences, USSR) having the following features. Flow rate range 0·002–0·133 cm^3/s; volume of detector cell 0·54 ml; volume of transient channel 7·8 ml and sensitivity 5×10^{-7} W. Experiments were carried out at 298 K at a flow rate $0{\cdot}133 \pm 0{\cdot}002$ cm^3/s in each detector. The ratio of the flow

rates in the two channels was close to 1, i.e. the solutions of protein, polysaccharide and their mixtures in all cases were diluted by a factor of two by the solvent. The latter was prepared by equilibrium dialysis against solutions. The resulting thermal effect (Q_Σ) was observed during dilution of albumin, pectin and their mixtures. It was determined as a sum of dilution (Q_d) and friction (Q_f) effects. This effect was measured in thermal power units (J/s). To evaluate the contribution of friction we put the resultant diluted solutions through both channels. The molar dilution enthalpy was obtained from relationship:

$$\Delta h_d = \frac{Q_d}{\Delta n_1} = \frac{Q_\Sigma - Q_f}{\Delta n_1} \tag{2}$$

where Δn_1 is the number of mols of solvent, mixed with solution per second (mol/s). Δh_d was computed to a precision better than 1×10^{-3} J/mol.

RESULTS AND DISCUSSION

Nepholometry

The present study revealed that the intensity of light scattering determined in arbitrary units in the system albumin–pectin (degree of esterification 57%)–water ($I = 0{\cdot}1$) at pH levels above the isoelectric point of the protein is of same order as the light scattered from each component for all the concentrations studied. The results of sedimentation analysis and microscopic studies proved that under these conditions the system does not undergo phase separation, which enabled us to conclude that the serum albumin is thermodynamically compatible with pectin. In order to determine if a stable soluble complex is formed we performed sedimentation and HPLC analysis with a mixture of equal weights of protein and polysaccharide. In the mixture (pH 7·0, $I = 0{\cdot}1$) we only observed sedimentation peaks corresponding to each of the individual components as found for the single components (Fig. 1). HPLC on the mixture revealed no additional peaks and the area of the serum albumin peak did not alter. All these facts suggest that the formation of a stable protein–polysaccharide complex is unlikely. It is known (Zeman *et al.*, 1972; Hsu & Prausnitz, 1974), that the compatibility of polymer solutions depends on the interaction of macromolecules with the solvent and with each other. The light scattering method appears to be the most informative in this respect (Kratochvil & Sudelof, 1986). Recently we

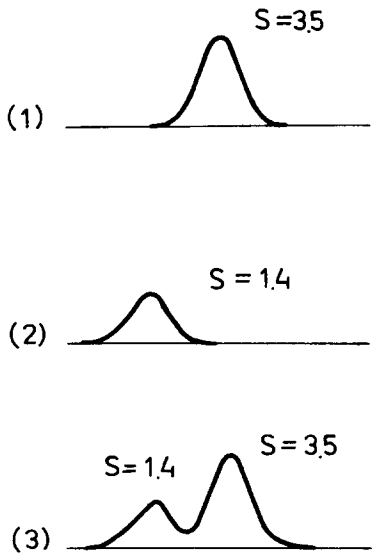

Fig. 1. Results of sedimentation analysis of separate components and their mixtures at pH 7·0 and $I = 0{\cdot}1$ M: (1) serum albumin; (2) pectin; (3) mixture of serum albumin and pectin.

proved that the affinity of pectin to a solvent depends on the degree of methoxylation (Plashchina *et al.*, 1985). It therefore seemed likely that the thermodynamic compatibility of serum albumin and pectin depends on the degree of esterification of the pectin. To clarify this point we studied the interaction of serum albumin with pectin fractions having different degrees of esterification. As already mentioned in the experimental section, the calculation of A_{24} involves the evaluation of A_{12} and A_{14}. So we began with the measurements of the intensity of light scattered in solutions of the individual macromolecules. Figure 2 represents a Zimm plot obtained for pectin fractions having a degree of esterification of 57%. Zimm plots for other pectin fractions are also linear, which facilitates the extrapolation to zero scattering angle and increases the accuracy of the calculation of the radius of gyration. Concentration dependencies of $KC_2/\Delta R_\theta$ are linear up to 0·3% for all systems. Figure 3 shows the concentration dependence of $KC_4/\Delta R_\theta$ for serum albumin. The numerical results obtained from Zimm plots are given in Table 1. First of all we point out the positive value of the second virial coefficient obtained for all pectin fraction solutions. Earlier we used the results of light scattering experiments to prove the hydrophilic properties of pectin (Plashchina *et al.*, 1985). The magnitude of the second virial coefficient significantly decreases as the degree of esterification is increased. It was

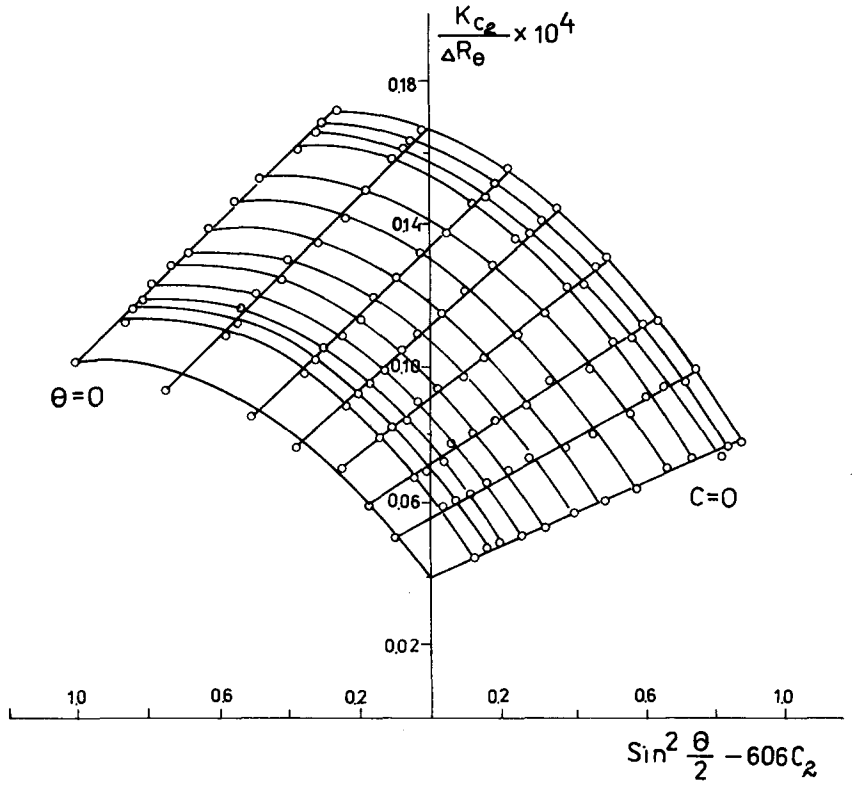

Fig. 2. The Zimm plot for the pectin fraction with a degree of esterification of 57%.

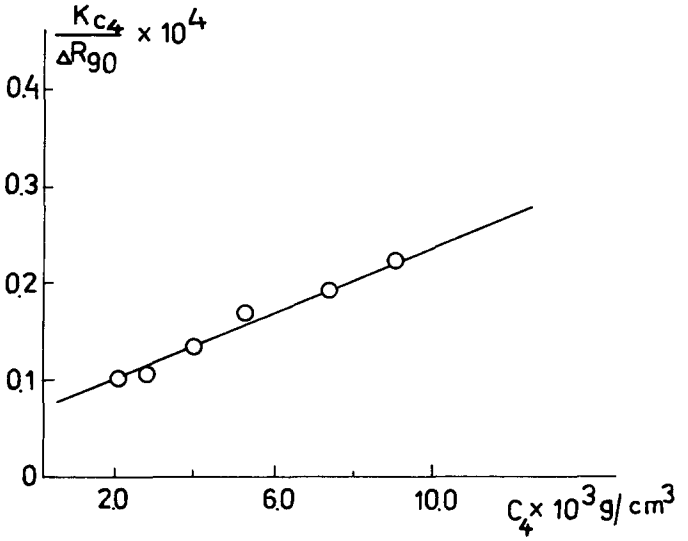

Fig. 3. The concentration dependence of $KC_4/\Delta R_{\theta=90}$ obtained for serum albumin.

shown previously (Plashchina *et al.*, 1985), that this decrease can be only partially explained by a decrease in the coulombic interaction. In other words, as the degree of esterification increases the behaviour of pectin solutions becomes more ideal. (The degree of non-ideality depends mainly on the excluded volume of the macromolecules.) The discrepancy between the magnitudes of the second virial coefficients of pectins having a degree of esterification in the range 45–70% obtained in the present study and reported by Plashchina *et al.* (1985) are likely to be accounted for by the difference in the homogeneity of the samples with respect to the degree of esterification. The data in Table 1 also suggest that pectins, having higher degrees of esterification, have higher molecular weights. This appears to reflect the association of macromolecules through interactions involving the ester groups. These associates are stable with respect to dilution in aqueous salt solution.

The increase in the degree of association between pectin macromolecules with an increase in the degree of esterification was also observed by Fishman *et al.* (1984).

The absence of a systematic dependence of the radius of gyration on the degree of esterification indicates that highly esterified pectins mainly associate side by side as suggested by Siano (1978).

We also note (Table 1), that the second virial coefficient does not depend on the molecular weight of the pectin fraction to any significant extent. It remains positive as the degree of association increases with the increasing degree of esterification. These results suggest that the affinity forces due to the ester groups mainly act inside the associates, while the surface of these associates retains hydrophilic properties. The serum albumin has a lower hydrophilicity than the pectin fractions.

Figure 4 gives an example of the angular and concentration dependence of the ratio $K(C_2 + C_4)/\Delta R_\theta$ obtained for a mixture of serum albumin and pectin. The peculiarity of these functions lies in their broad range of linearity as compared to binary solutions of pectinate fractions. The angular dependence is linear over the whole measurement range. One can deduce from eqn (1) that the concentration dependence of $K(C_2 + C_4)/\Delta R_\theta$ intercepts the ordinate axis at the point:

$$\frac{1}{\nu_2^2 M_{2W} W_2 + \nu_4^2 M_{4W} W_4}$$

If we compare this value with the value calculated from the binary system data, we get nearly the same results. This shows that the precision of the light scattering experiments in solutions containing two polymers is satisfactory.

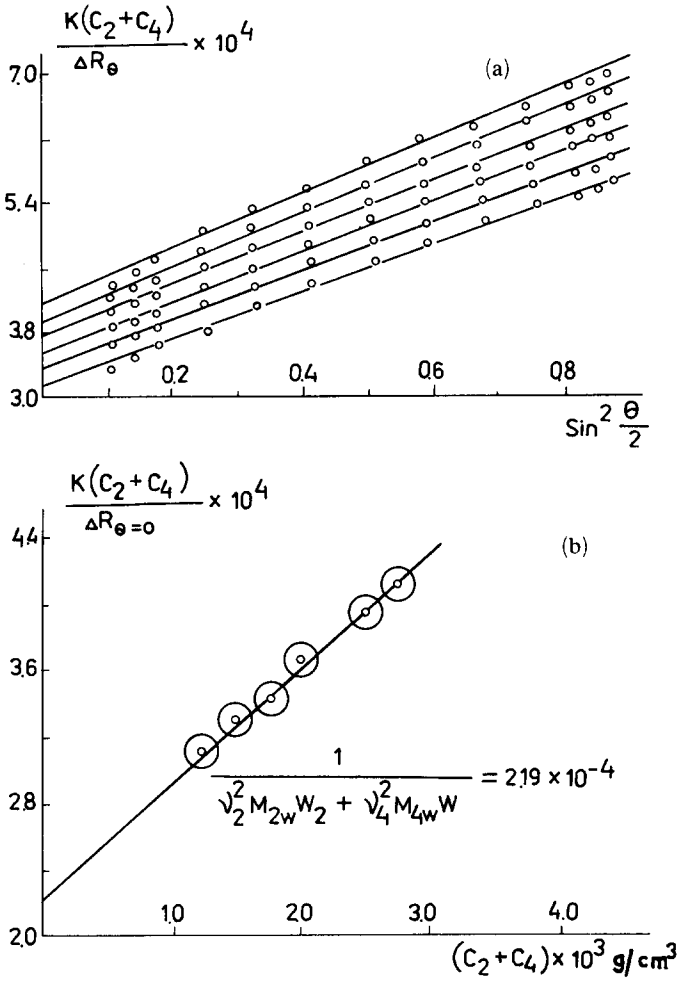

Fig. 4. Light scattering data for a mixture of serum albumin–pectin (degree of esterification 57%), pH 7·0, $I = 0{\cdot}1$ M: (a) the angular dependence $K(C_2 + C_4)/\Delta R_\theta$; (b) the concentration dependence $K(C_2 + C_4)/\Delta R_{\theta=0}$.

Table 2 displays the values of the second virial coefficient A_{24}. The negative sign of A_{24} means that for any degree of esterification there is an affinity between macromolecules of pectin and serum albumin. Moreover, the data in Table 3 show that the sufficient conditions for stability of the system with respect to diffusion (Prigozhin & Defey, 1954; Edmond & Ogston, 1968) are fulfilled for pectin samples 4–8. It was experimentally proved in the study of the phase state of mixtures comprising serum albumin and pectinate fractions at a high concentra-

TABLE 2
The Second Virial Coefficients of Systems Containing Equal Weights of Pectin and Serum Albumin (pH 7·0, ionic strength 0·1 M NaCl, 298 K)

Pectin fraction	$A_{24} \cdot 10^3$ (m^3 mol/kg^2)
1	−4·30
2	−3·84
3	−3·50
4	−1·85
5	−1·10
6	−0·82
7	−0·69
8	−0·89
9	−1·60

tion range ($C_\Sigma = 17\%$, pectin 7%, protein 10%). For the other pectin samples 1–3, 9 complex coacervation occurs.

We can deduce from Table 2 that the affinity of serum albumin for pectin decreases with increasing degree of esterification, which suggests that hydrophobic and hydrogen bonding effects are not the cause of the affinity, but a possible role for electrostatic interactions cannot be neglected. To analyse the latter mechanism we varied pH, ionic strength and concentration of added urea. Both variation in pH values from 5 to 8 and the increase in ionic strength from 0·1 to 1·0 M did not result in phase separation, allowing us to conclude that the affinity in this system is not of electrostatic origin.

On addition of urea to the solution phase separation was observed at a concentration of 6 M. Urea is known to be a protein denaturing agent (Jenks, 1972), thus we can conclude, that for compatibility the protein must be in the native conformation.

Thermodynamic parameters

The temperature dependence of light scattering gave information concerning the excess thermodynamic functions and mixing micro-calorimetry was used for precise measurement of enthalpy.

These experiments were done on human serum albumin (HSA) and pectin (degree of esterification 76%). The second virial coefficients are

TABLE 3

The Values of the Second Virial Coefficients Measured in Molal Concentration Units and First Derivations of Chemical Potentials with Respect to Concentration in the Serum Albumin–Pectin–Water System (pH 7·0, 0·1 M NaCl, 298 K, $C_2 = 10\%$, $C_4 = 10\%$)

Pectin fraction	$A_{14}.10^{-2}$ (m^3/mol)	$A_{24}.10^{-2}$ (m^3/mol)	$M_{22}/RT.10^{-2}$ (m^3/mol)	$M_{24}/RT.10^{-2}$ (m^3/mol)	$M_{22}M_{44}-M_{24}^2/(RT)^2.10^{-4}$ $(RT)^2.10^{-4}$ (m^6/mol^{-2})
1	5·0	−1·7	5·0	−1·7	−1·47
2	18·0	−5·0	18·0	−5·0	−1·99
3	8·3	−3·3	8·3	−3·3	−8·60
4	2·4	−0·8	2·4	−0·8	0·01
5	3·8	−0·7	3·8	−0·7	0·53
6	3·5	−0·5	3·5	−0·5	0·71
7	2·4	−0·5	2·4	−0·5	0·48
8	4·3	−0·9	4·3	−0·9	0·45
9	7·5	−2·1	7·6	−2·1	−2·30
HSA	$A_{12}.10^{-2}$ (m^3/mol)		$M_{44}/RT.10^{-2}$ (m^3/mol)		
	0·3		0·3		

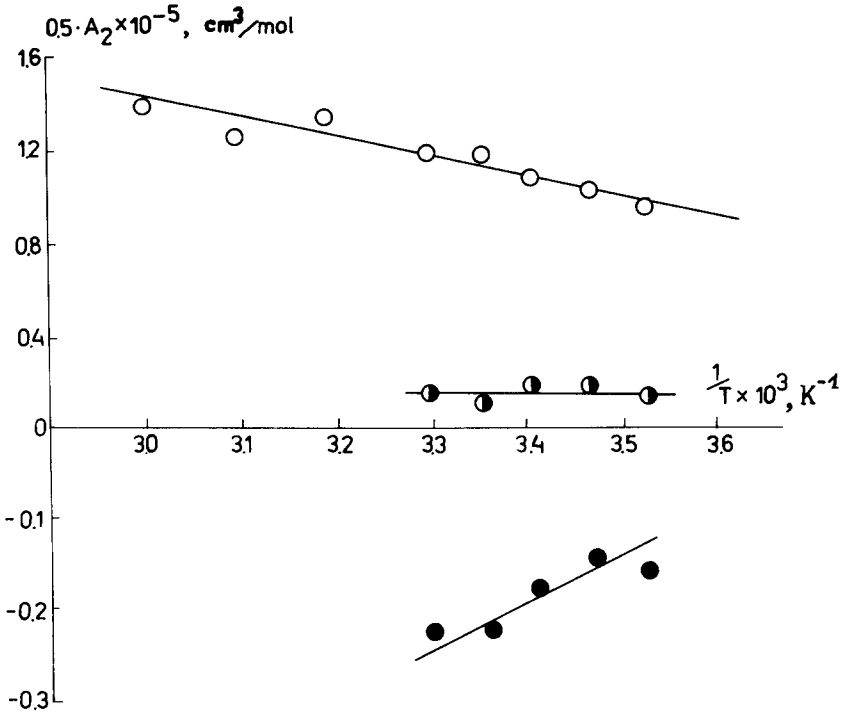

Fig. 5. The values of second virial coefficients as a function of the reciprocal of the absolute temperature: —○—, pectin solution; —◑—, HSA solution; —●—, solution of equal mass fractions of pectin and albumin.

given in Fig. 5 as functions of reciprocal temperature. The tangents to these lines are equal to the enthalpy of contact formation between macromolecules of different species (Prigozhin & Defey, 1954; Polyakov *et al.*, 1986):

$$\Delta h_{22} = R\left\{\frac{\partial A_{22}^*}{\partial(1/T)}\right\}_{m_2, m_4}; \Delta h_{44} = R\left\{\frac{\partial A_{44}^*}{\partial(1/T)}\right\}_{m_2, m_4}; \Delta h_{24} = R\left\{\frac{\partial A_{24}^*}{\partial(1/T)}\right\}_{m_2, m_4} \tag{3}$$

where Δh_{22}, Δh_{44}, Δh_{24} are the enthalpies of formation of intermolecular contacts (pectin–pectin, albumin–albumin and pectin–albumin, respectively) for $m_2 = m_4 = 1$ (in order to demonstrate the dependence of the second virial coefficient on the excessive thermodynamic functions more distinctly we assume that $m_2 = 1$ and $m_4 = 1$); R is the universal gas constant; A_{22}^*, A_{44}^*, A_{24}^* are the second virial coefficients in molal concentration units. They characterize the free energy of

intermolecular interaction (pectin–pectin, albumin–albumin and pectin–albumin); T is the temperature in K; m_2 and m_4 are concentration of pectin and albumin respectively in molal fraction units.

From the experimental data we can deduce that the process of contact formation between macromolecules of pectin is an exothermic one. The contacts between macromolecules of serum albumin are formed athermally, and macromolecules of pectinate and albumin form contacts endothermically. The absolute values of variation of molar enthalpies Δh_{22}, Δh_{44}, Δh_{24}, of interaction are listed in Table 4.

To verify the signs and magnitudes of the thermal effects leading to the formation of macromolecular contacts we carried out microcalorimetric measurements diluting solutions of protein, polysaccharide and solutions comprising equal mass fractions of protein and polysaccharide.

Figure 6 depicts the concentration dependencies of dilution enthalpy of pectinate, serum albumin and their mixtures, obtained experimentally by microcalorimetry and calculated results obtained from the values Δh_{22}, Δh_{44} and Δh_{24} measured from the light scattering experiment. Enthalpies of dilution (Δh_d) in the latter case were calculated according to equations given by (Takagi & Kimura, 1978; Polyakov *et al.*, 1986):

$$\Delta h_d = -R\frac{M_1}{1000}\frac{(K-1)}{K}0{\cdot}5\,\Delta h_{ii}m_i^2 \quad (i=2,4) \tag{4}$$

for a solution containing one polymer.

$$\Delta h_d = -R\frac{M_1}{1000}\frac{(K-1)}{K}(0{\cdot}5\,\Delta h_{22}m_2^2 + \Delta h_{24}m_2m_4 + 0{\cdot}5\,\Delta h_{44}m_4^2) \tag{5}$$

for a solution containing two polymers; where K is the degree of dilution and M_1 is the molecular weight of the solvent.

TABLE 4

The Molar Excess Thermodynamic Functions of Formation of Molecular Contacts between Similar and Different Species in Serum Albumin–Pectin–Water Systems (molal fractions of polymers are taken as equal to one. The data are obtained by light scattering. Temperature = 298 K)

	Albumin–albumin	*Pectin–pectin*	*Albumin–pectin*
$\Delta h.10^{-8}$ (J/mol)	$0 \pm 0{\cdot}05$	$-7{\cdot}40 \pm 0{\cdot}24$	$6{\cdot}24 \pm 0{\cdot}82$
$T\Delta S^E.10^{-8}$ (J/mol)	$-0{\cdot}34 \pm 0{\cdot}04$	$-10{\cdot}31 \pm 0{\cdot}91$	$7{\cdot}35 \pm 0{\cdot}99$
$\Delta g^E.10^{-8}$ (J/mol)	$0{\cdot}34 \pm 0{\cdot}04$	$2{\cdot}91 \pm 0{\cdot}35$	$-1{\cdot}11 \pm 0{\cdot}17$

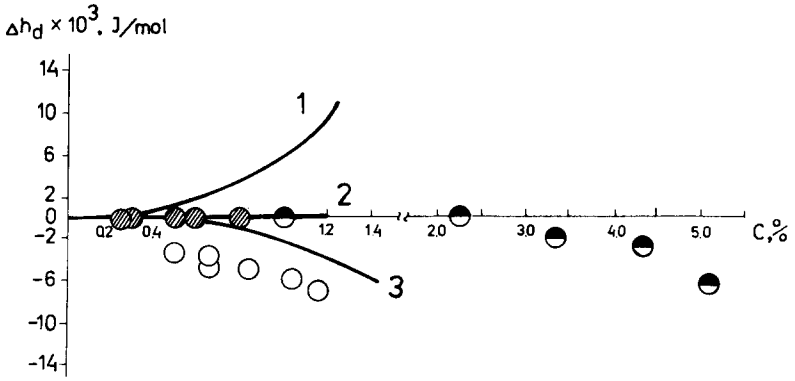

Fig. 6. The concentration dependence of dilution enthalpy calculated from the light scattering data: (1) pectin solution; (2) serum albumin solution; (3) solution of equal mass fractions of pectin and albumin. The results, obtained by microcalorimetry: —⊘—, pectinate solution; —◓—, serum albumin solution; —○—, solution of equal mass fractions of pectin and albumin.

The results obtained both by calorimetry and light scattering coincide on a qualitative basis. The quantitative discrepancy is mainly caused by restrictions imposed by both experimental procedures. However, the results prove that the mixing of components in the system under consideration is realized endothermically and this thermal effect is mainly caused by the positive enthalpy of albumin–pectin contact formation. This means that interaction of serum albumin and pectin is not favourable with respect to energy.

We showed that the observed affinity between macromolecules of serum albumin and pectin, proved by negative values of excessive free energy Δg^{E}_{24}, has an entropic origin. In order to calculate the values of molar excessive free energy of contact formation we used the following expressions (Edmond & Ogston, 1968; Polyakov *et al.*, 1986):

$$\Delta g^{E}_{22} = RT.0{\cdot}5\,A^{*}_{22};\ \Delta g^{E}_{44} = RT.0{\cdot}5\,A^{*}_{44};\ \Delta g^{E}_{24} = RTA^{*}_{24} \qquad (6)$$

The molar values of excessive entropy were obtained according to the relationship:

$$\Delta S^{E} = \frac{\Delta h^{E} - \Delta g^{E}}{T} \qquad (7)$$

The results are given in Table 4. They indicate that the entropy of interaction of pectin and serum albumin increases and determines the negative value of the free energy. Such thermodynamic behaviour is

indicative of weak molecular association in solution, which results in the building up of a new species of short lived particles (Prigozhin & Defey, 1954). Moreover, this interaction can be accompanied by dehydration of macromolecules. The dehydration is a heat consuming process and causes an increase in entropy in the system. So we can suggest that this process both controls the endothermic effect of interaction of albumin with pectin and accounts for the increase in entropy. The fact that pectins with a low degree of esterification and possessing a greater amount of hydrated water exhibit a higher affinity toward macromolecules of serum albumin, can be clearly understood from this viewpoint.

CONCLUSION

Thermodynamic affinity is observed in aqueous solutions of serum albumin and pectin irrespective of the degree of esterification of the latter.

The experimental results indicate that thermodynamic compatibility of serum albumin and pectin is controlled by increase of mixing entropy, which mainly stems from dehydration of biopolymer macromolecules during contact formation.

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