

# Effect of acetonitrile on the hydration of human serum albumin films: a calorimetric and spectroscopic study

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## Abstract

A new experimental approach based on the combination of calorimetric and FTIR spectroscopic measurements was proposed to study simultaneously the sorption of water and organic solvent, and corresponding changes in the structure of protein films in the water activity range from 0 to 1.0. Enthalpy changes ( $\Delta H_{\text{tot}}$ ) on the interaction of water with the dried human serum albumin (HSA) in the presence and absence of acetonitrile (AN) have been measured using a Setaram BT-2.15 calorimeter at 298 K. Spectroscopic data on water and organic solvent vapor sorption by the HSA films and the corresponding changes in the protein secondary structure were determined by means of a Bruker Vector-22 FTIR spectrometer. By using a water activity-based comparison we characterised the effect of acetonitrile on the hydration and structure of the HSA films. Acetonitrile (AN) sorption isotherm resembles a smooth curve. HSA film binds about 250 mol AN/mol protein at the lowest water activities. As the water activity increases from 0 to 0.8, the sorption of AN gradually decreases from 250 to 150 mol AN/mol HSA. At  $a_w > 0.8$ , the sorption of AN sharply decreases to zero. Acetonitrile decreases markedly the water content at a given  $a_w$ . This behavior suggests that the suppression in the uptake of water is due to a competition for water-binding sites on the HSA films by acetonitrile. Changes in the secondary structure of HSA were determined from infrared spectra by analyzing the structure of amide I band. Acetonitrile increases the intensity of the  $1654 \text{ cm}^{-1}$  band that was assigned to the  $\alpha$ -helix structure. Changes in the intensity of the  $1654 \text{ cm}^{-1}$  band agree well with the decrease in water uptake in the presence of AN. An explanation of the acetonitrile effect on the hydration and structure of the HSA films was provided on the basis of hypothesis on water-assisted disruption of polar contacts in the initially dried protein.

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**Keywords:** Protein films; Protein hydration; Protein structure; Human serum albumin; Organic solvent; Acetonitrile; Sorption; Isothermal calorimetry; Interaction enthalpies; Fourier transform infrared spectroscopy (FTIR spectroscopy)

## 1. Introduction

It is well known that the interaction of water with proteins plays a key role in determining the structure and functions of proteins [1–3]. Knowledge of processes occurring upon hydration or dehydration of proteins is very important in various practical applications of proteins such as their use as biocatalysts [4–6], biosensors [7,8] and selective adsorbents [9,10] in low water organic solvents or as thin films in bionanotechnology [11]. Hence, for a better understanding of the intermolecular interactions and conformational rearrangements

that occur upon hydration of solid proteins in various environments there is a clear need of the experimental methods by which both the thermodynamic and structural properties of the hydration process in the presence of some additives, including organic solvents, may be obtained simultaneously.

The interaction enthalpies of proteins with the water–organic mixtures might be a very informative property of the intermolecular interactions in the above mentioned systems. Calorimetry is a reliable method to determine quantitatively this thermodynamic property. For example, based on the calorimetric measurements two different mechanisms of the interaction of the hydrated human serum albumin with organic solvents were proposed [12–14]. It was found that in low water pyridine, dioxane,

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1-propanol and 1-butanol, the water sorption is the only process contributing to the heat effects of interaction of solid HSA with water–organic mixtures. The additional exothermic process was observed when some critical water content was reached. This process was considered to include the rupture of the protein–protein contacts in the solid phase induced by protein–organic component or/and protein–water interactions. On the immersion of the hydrated HSA into binary mixtures of water with acetonitrile, dimethyl sulfoxide, methanol or ethanol, these two processes occur simultaneously over the whole water activity range.

Infrared spectroscopy is one of the effective methods for analyzing the structure of proteins in various environments, including aqueous and non-aqueous media [15]. This method has been successfully used in studying the secondary structure of proteins in various states [16–21], including protein films with various degrees of humidity and solid protein preparations immersed into pure organic solvents and water–organic mixtures. Infrared spectroscopy is also effective in studying the hydration of proteins [1,2,21]. The relationship between hydration and dioxane sorption by the human serum albumin films was recently studied by IR spectroscopy [22].

The combination of the calorimetric and IR spectroscopic data has a great potential in understanding factors governing the state and structure of solid proteins in the presence of organic solvents. By means of the combined calorimetric and FTIR spectroscopic measurements the structure and stability of the dehydrated chymotrypsin and HSA films were recently examined in a series of anhydrous organic liquids, including hydrocarbons, alcohols and hydrogen bond accepting solvents [23,24]. It was shown that solvent potential to form hydrogen bonds appears to be an important factor controlling the stability of dehydrated protein films in organic media. However, no attempt has been made to study simultaneously both the hydration of protein films in the presence of organic solvent and the corresponding structural changes over the whole range of water activity.

In the present work the water and organic solvent vapor sorption on protein films were investigated by IR spectroscopy in the water activity range from 0 to 1.0 at 298 K. The sorption data were compared with the corresponding structural and enthalpy changes that occur on the interaction of solid protein with water and organic molecules. The aim of this combined calorimetric and IR spectroscopic study is to elucidate the effect of organic solvent molecules on the hydration, structure and thermochemical properties of solid protein.

Acetonitrile (AN) was selected as a probe organic compound because it is capable of forming strong hydrogen bonds with various hydrogen donors. However, in contrast to water, it has no evident hydrogen bond donating ability. Serum albumin is the most abundant in blood serum and plays a number of important biological roles, including the divalent cation transport, fatty acid and drug complexation and transport [25]. Serum albumin is also widely used in studying

the molecular basis of the phenomenon of ‘molecular memory’ in organic solvents [9,10]. Besides, serum albumin and acetonitrile were the subjects of our previous thermochemical studies of protein behavior in non-aqueous solvents and water–organic mixtures [12,23,26].

## 2. Experimental

### 2.1. Materials

Human serum albumin (Sigma, Product No. A 1887, essentially fatty acid free) was used without further purification. Acetonitrile (reagent grade, purity >99%) was purified and dried according to the recommendations [27] and was stored over molecular sieves (3 Å) for at least 24 h prior to use. Water used was doubly distilled.

### 2.2. Calorimetric measurements

The enthalpy changes on the immersion of the dried HSA into pure liquid water or binary water–organic mixtures were measured at 298 K with a Setaram BT-2.15 calorimeter according to the described procedure [14,27]. Typically, the sample of 5–10 mg of HSA contacted with 4.0 ml of a solvent in the calorimetric cell. Calorimeter was calibrated using the Joule effect and tested with dissolving sodium chloride in water according to the recommendations [28].

The dried protein preparation (zero hydration level) was obtained by drying under vacuum using a microthermoanalyzer ‘Setaram’ MGD TD-17S at 298 K and 0.1 Pa until the constant sample weight was reached. Water content of the dried protein was estimated as  $0.003 \pm 0.002$  g of water/g of protein by the Karl Fischer titration method according to the recommendations [14,27].

Solid protein samples for the determination of the interaction enthalpies of HSA with water in the absence of acetonitrile were equilibrated at 298 K for 1 week in tightly closed desiccator over saturated salt solutions ( $\text{CaCl}_2$ ,  $\text{Mg}(\text{NO}_3)_2$ ,  $\text{NaCl}$ ,  $\text{BaCl}_2$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ). Water activity over saturated salt solutions was taken from [29]. Water content of the samples after equilibration was measured by drying under vacuum using a microthermoanalyzer ‘Setaram’ MGD TD-17S at 298 K and 0.1 Pa until the constant sample weight was reached. Salts for the conditioning of the samples were of analytical pure grade. The conditioned samples were then taken from the desiccator and equilibrated in the calorimetric cell at 298 K before the experiment.

### 2.3. FTIR spectroscopic measurements

The infrared spectra were measured at 298 K with a Vector 22 FTIR spectrometer (Bruker) at  $4 \text{ cm}^{-1}$  resolution as described previously [22–24]. The infrared spectra were obtained with glassy like protein films casted from 2% (w/v) water solution onto the  $\text{CaF}_2$  window at room humidity and temperature. After mounting the window in the sample cell,

the films were dehydrated by flushing air dried over  $P_2O_5$  powder. Water activity over  $P_2O_5$  at 298 K does not exceed 0.01 [30]. The protein films were flushed until no further spectral changes were detected in the  $3450\text{ cm}^{-1}$  water absorbance region and amide. A contour in this side represented a smooth line without any visible shoulders. The spectrum of this sample was used as a reference spectrum for calculation of the difference spectra. The difference spectra were obtained according to the criteria described previously [31].

Then, the sample was in situ exposed to pure water vapor or water–organic vapor mixtures. In the first case, pure water vapor consecutively flowed through the saturator and cell containing the protein sample. The water activity ( $a_w$ ) in vapor phase was adjusted by changing the difference between the temperature of the saturator and cell. Data on the water vapor pressure at various temperatures were taken from [32].

In the second case, the air (dried over  $P_2O_5$ ) flowed through the saturator filled with water–organic mixture and then through the measuring cell. The temperature of the cell and saturator was 298 K. The relative pressure of water vapor was adjusted by changing the water activity in the liquid water–organic mixture.

Water sorption by human serum albumin films was controlled in the region of OH stretching vibration band at  $3450\text{ cm}^{-1}$ . Acetonitrile sorption by protein films was controlled at  $2252\text{ cm}^{-1}$  (stretching vibration band of the nitrile group).

Supposing that during water sorption the protein film swells predominantly due to increasing thickness, the protein hydration and sorption of acetonitrile vapor were calculated from Eq. (1):

$$A = \frac{2.3S_{\text{solv}}\varepsilon_P}{B_{\text{solv}}D_P} \quad (1)$$

where  $A$  is the protein hydration or sorption of organic sorbate (mol/mol protein),  $S_{\text{solv}}$  is the area of water or organic solvent absorbance band ( $\text{cm}^{-1}$ ),  $\varepsilon_P$  is the protein molar extinction at the maximum of amide I band ( $\text{mol}^{-1}\text{ cm}^{-1}$ ),  $B_{\text{solv}}$  is the water or organic solvent integral molar extinction coefficient,  $D_P$  is the optical density at the maximum of amide I band. For pure water, it was taken that  $B_w = 96,000 \pm 1000\text{ mol}^{-1}\text{ cm}^{-2}$  [33]. The integral absorption extinction coefficient for acetonitrile ( $B_{\text{AN}}$ ) was calculated from the area of the absorption band of the pure substance in a cell with layer thickness of  $10\text{ }\mu\text{m}$ :  $B_{\text{AN}} = 887 \pm 8\text{ mol}^{-1}\text{ cm}^{-2}$ . The molar absorption extinction coefficient of HSA was determined measuring the amide I spectra of protein solutions in heavy water. The molar absorption extinction coefficient of human serum albumin ( $\varepsilon_P$ ) is  $237,600 \pm 300\text{ mol}^{-1}\text{ cm}^{-1}$ . The molecular weight of human serum albumin was taken as 66,000 Da.

#### 2.4. Thermodynamic activity of water in organic solvent

Water activity ( $a_w$ ) in organic solvent was calculated using Eq. (2):

$$a_w = \gamma_w x_w \quad (2)$$

where  $x_w$  is the mole fraction of water in the solution and  $\gamma_w$  is the activity coefficient of water (in mole fractions; the standard state is pure water). Water content in acetonitrile ( $x_w$ ) was measured using Karl Fisher method according to the recommendations [27,34].

Water activity coefficients in organic solvents  $\gamma_w$  were calculated from the published data on the vapor–liquid equilibrium [32] by Eq. (3):

$$\gamma_w = \frac{y_w P_t}{x_w P_0} \quad (3)$$

where  $y_w$  is the measured mole fraction of water in vapor phase,  $P_t$  is the total pressure,  $P_0$  is the saturated vapor pressure of pure water at the same temperature and  $x_w$  is the mole fraction of water in the liquid phase.

### 3. Results and discussion

#### 3.1. Acetonitrile and water sorption

Fig. 1 (curve 1) shows the acetonitrile vapor sorption isotherm for human serum albumin. The initial state of the protein film (zero hydration level) was obtained by drying in air at water activity less than 0.01 (film A). On maintaining this ‘dry’ sample in equilibrium with a vacuum of 0.1 Pa and at 298 K for 3 h, it lost about 0.3% of its weight, which for human serum albumin implies that at the zero hydration level there are about 11 water molecules strongly bound to each protein molecule.

At low water activities, HSA binds about 250 mol AN/mol protein. As the water activity increases from 0 to 0.8, the sorption of AN gradually decreases from 250 to 150 mol AN/mol HSA. At  $a_w > 0.8$ , the sorption of AN sharply decreases to zero. This sharp decrease agrees well with the cooperative change in the state of water in the presence of acetonitrile (Fig. 1) that was investigated by means of IR spectroscopy in [33]. It was found that water molecules in acetonitrile can

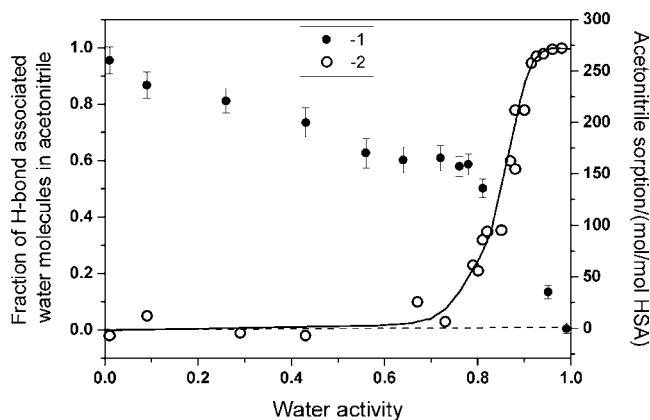


Fig. 1. (1) Sorption of acetonitrile vapor by human serum albumin film as a function of water activity; (2) fraction of H-bond-associated water molecules as a function of water activity in acetonitrile. Modified data from [33].

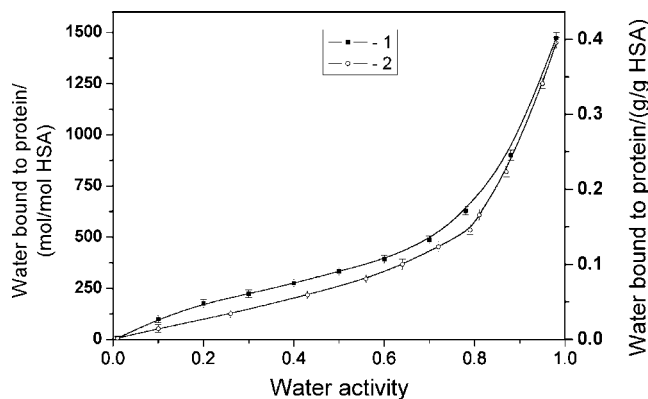


Fig. 2. (1) Water sorption isotherm for human serum albumin in the absence of acetonitrile; (2) water sorption isotherm for human serum albumin in the presence of acetonitrile. Solid lines were fitted by a set of polynomials.

exist either as associated (H-bonded) molecules or as single molecules complexed with organic molecules that depends markedly on the thermodynamic activity of water. A cooperative transition between these states occurs within a relatively narrow range of water activity from 0.8 to 0.9.

Fig. 2 shows the water sorption isotherms for human serum albumin in the presence and absence of acetonitrile. The initial state of HSA for studying the water sorption was a film A. As can be seen from Fig. 2, the water sorption isotherms resemble sigmoid shaped curves. This result is in accordance with those for serum albumin and other proteins [1,3].

The presence of organic molecules markedly affects the ability of HSA to bind water. The effect of acetonitrile on the water sorption was characterised by the difference ( $\Delta h$ ) in water uptake in the presence and absence of organic solvent (Fig. 3). As can be seen from Fig. 3, a considerable decrease in the uptake of water was observed over the whole range of water activity. This behavior suggests that the suppression in the water uptake is due to a competition for water binding sites on HSA by acetonitrile molecules. The most pronounced suppression was observed in the water activity

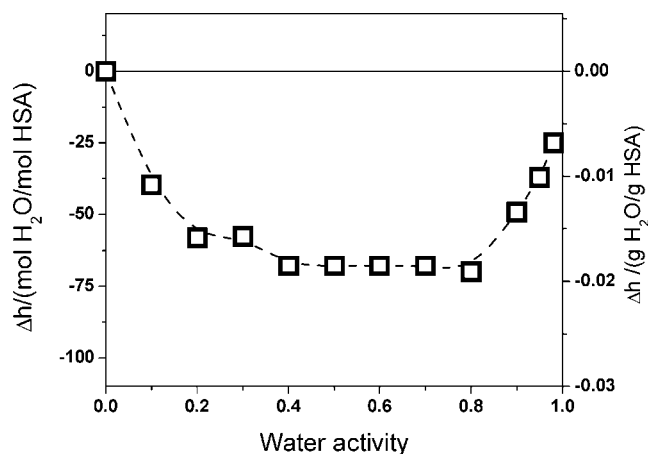


Fig. 3. Differences in the water uptake between the water sorption isotherms in the presence and absence of acetonitrile.

Table 1

Parameters of water sorption by serum albumin estimated by Eq. (4)<sup>a</sup>

$h_m$ (mol water/mol protein)	$K$	$s_0^b$
In the absence of acetonitrile		
174.3 (3.1)	12.1 (4.3)	3.3
In the presence of acetonitrile		
149.7 (4.0)	4.0 (0.8)	7.1

The values of confidence interval of the parameters calculated by Eq. (4) are given in parentheses.

<sup>a</sup> The water activity range of applicability is 0–0.6.

<sup>b</sup>  $s_0$  is the residual standard deviation.

range from 0.6 to 0.8. A sharp increase in the  $\Delta h$  values was found at  $a_w > 0.8$ . This increase agrees well with the cooperative increase of the fraction of the H-bond-associated water molecules in the presence of acetonitrile (Fig. 1).

The BET model [35] is widely used for describing the sorption ability of various solids, including proteins [1,3,36,37] (Eq. (4)):

$$h = h_m \left[ \frac{K a_w}{1 + K a_w} + \frac{a_w}{1 - a_w} \right] \quad (4)$$

where  $h$  is the hydration of solid protein (mol water per mol protein),  $h_m$  is the number of water binding sites (mol water per mol protein) and  $K$  is the equilibrium water sorption constant.

It was found that the BET equation (Eq. (4)) describes the isotherms displayed in Fig. 2 up to a water activity no more than 0.6. This result is in close agreement with the data obtained in previous studies on serum albumin and other proteins [1,3,36,37].

The water sorption parameters estimated from Eq. (4) are presented in Table 1. As can be seen from Table 1, the value of sorption constant  $K$  is markedly smaller in the presence of acetonitrile than in the absence of organic sorbate. This implies that acetonitrile molecules suppress markedly the water sorption on the HSA films.

### 3.2. Analysis and band assignment of protein infrared spectra

The infrared spectra of HSA films (Section 3.1) in the conformationally sensitive amide I region, which is due predominantly to the C=O stretching vibration of the protein backbone, are presented in Figs. 4 and 6. Fig. 4A and B shows the absorbance and second-derivative spectra of HSA film in the absence of acetonitrile in the amide I region. The assignment of individual components to secondary structure was performed as described earlier [16–21]. As can be seen from Fig. 4A and B, the most dominant band component of the HSA spectra is the band at  $1654 \text{ cm}^{-1}$ , which is usually attributed to  $\alpha$ -helix structure [18,20,21]. The bands at  $1630 \text{ cm}^{-1}$  and in the  $1680\text{--}1695 \text{ cm}^{-1}$  region were assigned to  $\beta$ -sheet structure [20,21]. Minor component in the  $1675\text{--}1665 \text{ cm}^{-1}$  region was assigned to irregular secondary structures ( $\beta$ -turns, random coil and extended chains).

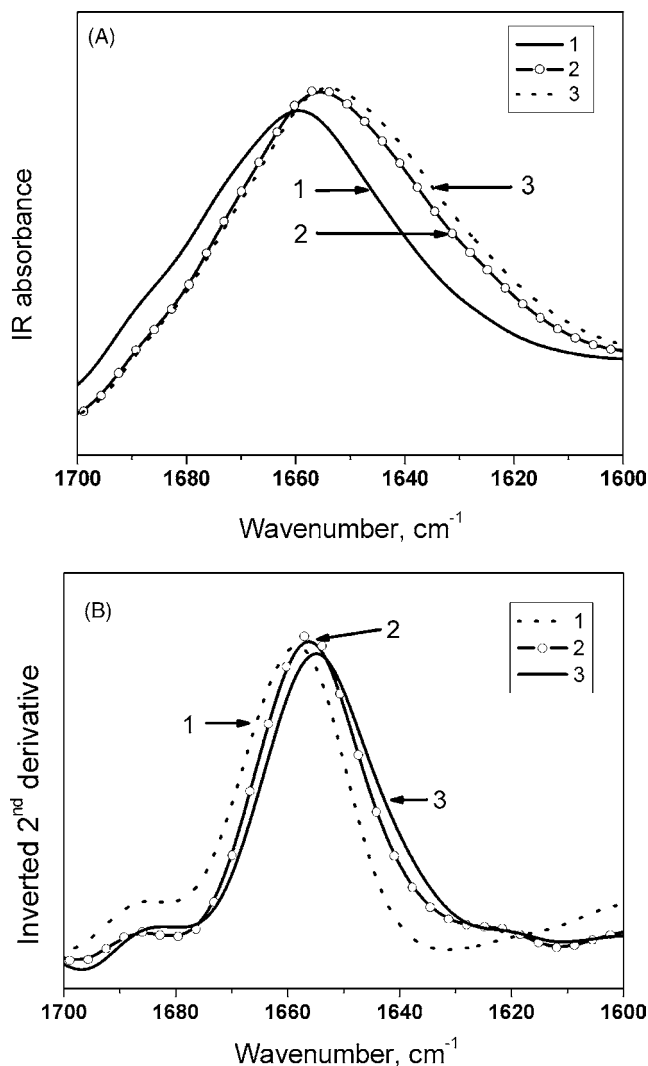


Fig. 4. (A) Absorbance spectra of human serum albumin in the amide I region at water activity of: (1) 0.01; (2) 0.78; (3) 0.98. (B) Second-derivative spectra of human serum albumin in the amide I region at water activity of: (1) 0.01; (2) 0.78; (3) 0.98. All the spectra were normalised to the amide I band intensity of film A at water activity of 0.01.

As can be seen in Fig. 4A and B, the infrared spectra of human serum albumin are grossly altered relative to that for the initial dried protein. There are increases in bandwidths and shifts in band positions and relative absorbances, which are indicative of protein conformational changes.

The hydration in the absence of acetonitrile markedly alters the secondary structure of HSA as revealed by the changes in relative intensity at  $1654\text{ cm}^{-1}$  (Fig. 5, curve 1). At low water activities ( $h < 0.1\text{ g/g protein}$ ), the hydration process induces pronounced structural rearrangements. The  $D_{1654}/D_0$  curve reaches a plateau at water activity of 0.5 ( $h > 0.1\text{ g/g protein}$ ), indicating that the conformational changes are largely completed. These results are in agreement with previously published results [1,2].

Fig. 6A and B shows the absorbance and second derivative spectra of HSA in the presence of acetonitrile in the amide

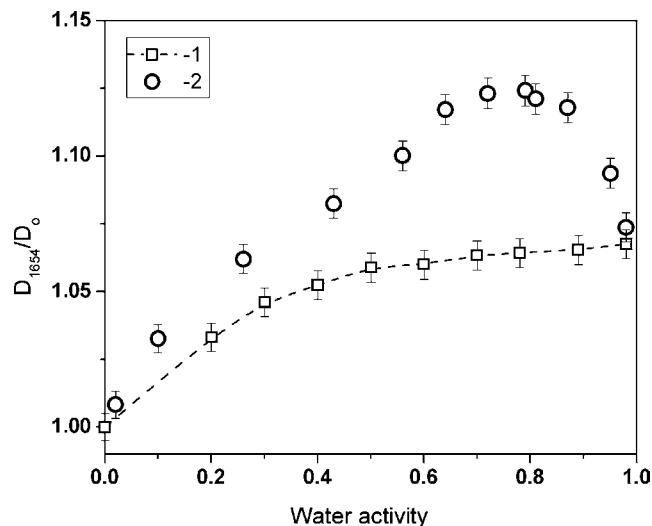


Fig. 5. Absorbance at  $1654\text{ cm}^{-1}$  as a function of water activity: (1) in the absence and (2) in the presence of acetonitrile.  $D_0$  is the absorbance value at  $1654\text{ cm}^{-1}$  for a film A at water activity of 0.01.

I region. As can be seen from Fig. 6A and B, the spectra of HSA are very different from those in the absence of acetonitrile. Acetonitrile increases markedly the intensity of the  $1654\text{ cm}^{-1}$  band.

The effect of acetonitrile on the HSA structure was characterised by the changes in the relative intensity at  $1654\text{ cm}^{-1}$  (Fig. 5, curve 2). The  $D_{1654}/D_0$  function obtained in the presence of AN has been compared with that determined for HSA in the absence of AN. This comparison can show any effects of the organic solvent molecules on the protein structure. The differences between the  $D_{1654}/D_0$  values obtained in the presence and absence of AN are presented in Fig. 7. As can be concluded from Fig. 7, the  $\Delta D_{1654}/D_0$  function is consistent with the changes in the  $\Delta h$  values (Fig. 3). This result indicates that the effect of AN on the protein secondary structure is due to a competition for water binding sites on HSA by organic solvent molecules.

At the lowest water activity values, the  $\Delta D_{1654}/D_0$  values are close to zero (Fig. 7). Hence, it may be concluded that the presence of AN has little effect on the structure of the initially dried protein film. This result agrees well with previously published results [23].

The most pronounced effect of AN on the structure was observed for the hydrated HSA ( $h > 0.1\text{ g/g protein}$ ) in the water activity range 0.6–0.8 (Fig. 7). It is well known that at a hydration level between 0 and 0.1 g/g, water is strongly bound to charged and polar groups on the protein [1,2]. This implies that the primary hydration process (the hydration of polar and charged protein groups) is sufficient for the expression of the acetonitrile-induced structural rearrangements.

A sharp decrease in the  $\Delta D_{1654}/D_0$  values was found at  $a_w > 0.8$ . This decrease agrees with the cooperative increase in the fraction of the H-bond-associated water molecules, sharp decrease in acetonitrile sorption (Fig. 1) and increase in the  $\Delta$  values (Fig. 3).

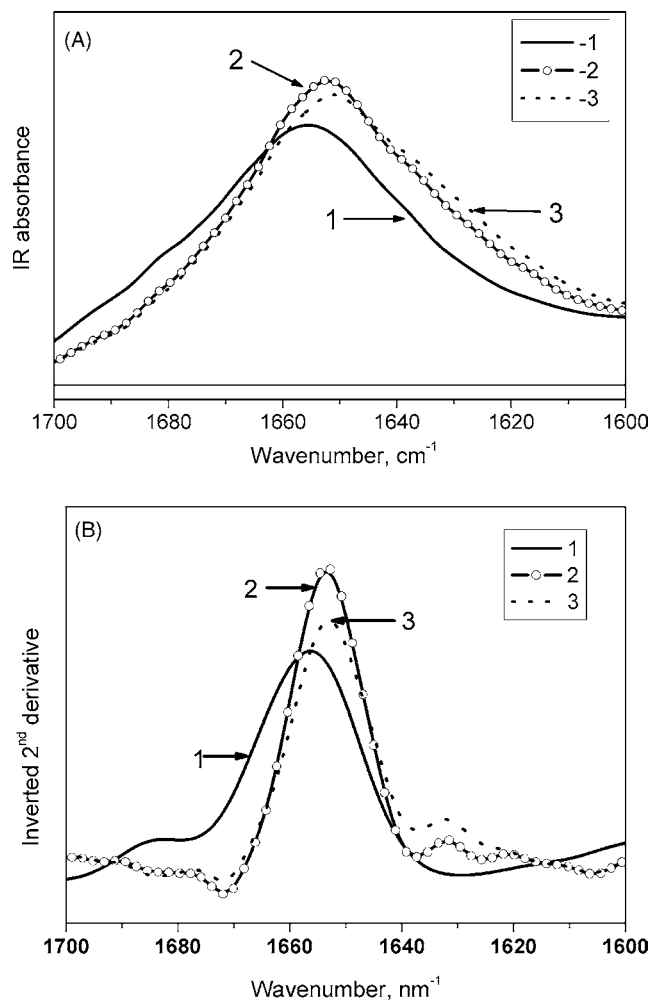


Fig. 6. (A) Absorbance spectra of human serum albumin in the amide I region at water activity of: (1) 0.01; (2) 0.79; (3) 0.98. (B) Second-derivative spectra of HSA in the amide I region at water activity of: (1) 0.01; (2) 0.79; (3) 0.98. All the spectra were normalised to the amide I band intensity of a film A at water activity of 0.01.

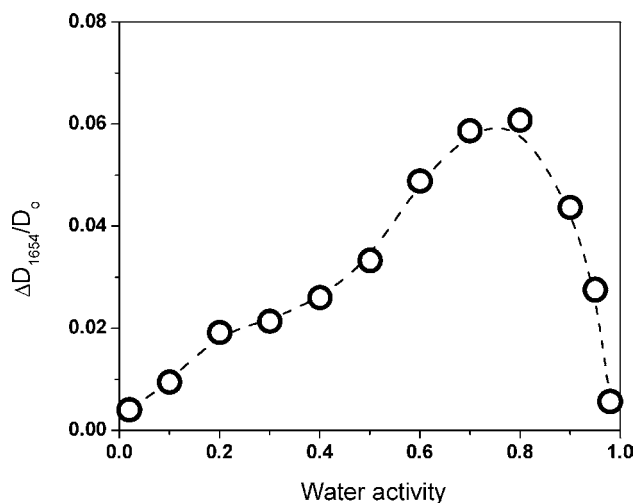


Fig. 7. Differences between the  $D_{1654}/D_0$  values obtained in the presence and absence of acetonitrile as a function of water activity.

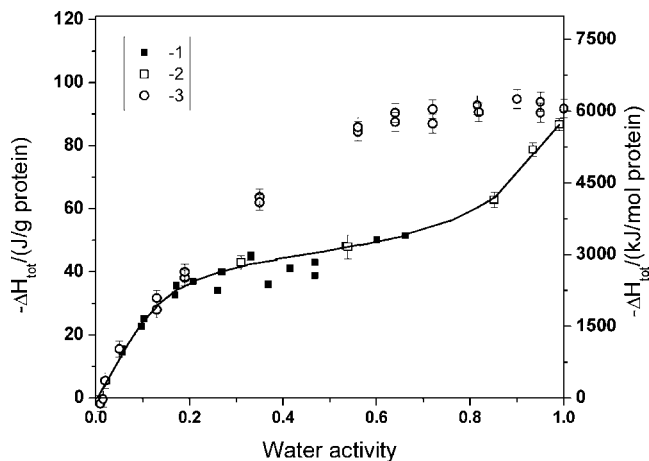


Fig. 8. The interaction enthalpy changes of water with HSA in the absence (1, 2) and presence (3) of AN.

### 3.3. Enthalpy changes on the interaction of water with HSA in the absence and presence of acetonitrile

The interaction of water with HSA in the presence as well as in the absence of AN was characterised by the  $\Delta H_{\text{tot}}$  values (Fig. 8) which were defined as follows:

- The initial state (zero hydration level) for this thermochemical parameter was the protein obtained by drying in air at water activity less than 0.01.
- The final state was the protein immersed into water–organic liquid with the varied water activity or the protein equilibrated with water vapor (with the varied water activity) that was taken from pure liquid water at the atmospheric pressure and at 298 K. This enthalpy change ( $\Delta H_{\text{tot}}$ ) represents the difference between the partial molar enthalpy of a protein in the system with the varied water activity ( $\bar{H}_P$ ) and in the dried state ( $\bar{H}_P^0$ ) (Eq. (5)):

$$\Delta H_{\text{tot}} = \bar{H}_P - \bar{H}_P^0 \quad (5)$$

Two types of calorimetric data were used to determine the  $\Delta H_{\text{tot}}$  values in the absence of acetonitrile:

- (1) The  $\Delta H_{\text{tot}}$  values were calculated using the water vapor sorption enthalpies for bovine serum albumin (the  $\Delta H_{\text{hydr}}$ , J/mol water) which were taken from [38] (Fig. 8, Data set N.1) (Eq. (6)):

$$\Delta H_{\text{tot}} = (\Delta H_{\text{hydr}} - \Delta H_{\text{cond}})h \quad (6)$$

where  $h$  is the water content of serum albumin (mol water/mol protein) at which water vapor sorption enthalpy ( $\Delta H_{\text{hydr}}$ ) was measured and  $\Delta H_{\text{cond}}$  is the enthalpy of condensation of water ( $-43.7$  kJ/mol water).

- (2) Enthalpy changes ( $\Delta H_{\text{tot}}$ ) on the interaction of water with HSA (Fig. 8, Data set N.2) were derived from the data of immersion calorimetry (Table 2) where the HSA samples equilibrated at a number of salt saturated solutions were dissolved in pure liquid water at 298 K using Eq. (7):

Table 2

Heat effects on immersing the HSA preparations equilibrated over salt saturated solutions into pure liquid water at 298 K and atmospheric pressure<sup>a,b</sup>

No.	Salt	Water activity	Water content, % (g water/g protein)	Immersion heat effect, $\Delta H_{\text{imm}}$ (kJ/mol protein)
1	CaCl <sub>2</sub>	0.31	8.1 (0.5)	−3175 (139)
2	Mg(NO <sub>3</sub> ) <sub>2</sub>	0.52	10.1 (0.6)	−2090 (250)
3	NaCl	0.76	22.1 (1.2)	−1921 (165)
4	BaCl <sub>2</sub>	0.88	28.9 (2.5)	−871 (145)
5	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	0.97	50.5 (3.7)	−330 (125)

<sup>a</sup> The data are presented as the average of 3 independent determinations.<sup>b</sup> The values of the standard errors are given in the parenthesis.

$$\Delta H_{\text{tot}} = \Delta H_{\text{sol}}^0 - \Delta H_{\text{sol}} \quad (7)$$

where  $\Delta H_{\text{sol}}^0$  is the enthalpy change on the solution of the dried HSA in pure liquid water at 298 and atmospheric pressure (J/mol protein). This enthalpy change was earlier measured in [23]: −91.8 (2.8) J/g of HSA or −6059 (185) kJ/mol HSA;  $\Delta H_{\text{sol}}$  the enthalpy change on the solution of the HSA preparation equilibrated over the salt saturated solutions in pure liquid water (J/mol protein).

Fig. 8 (Data set N.3) shows the  $\Delta H_{\text{tot}}$  values determined on the interaction of the dried HSA with the water–acetonitrile mixtures. Calorimetric curve shows a smooth dependence on the water activity in acetonitrile. Similar calorimetric dependencies were previously obtained on the interaction of hydrated (10% of water) HSA preparation with the binary mixtures of water with acetonitrile, ethanol and methanol [12,13].

At low water activities, the  $\Delta H_{\text{tot}}$  values are close to zero. This result is in close agreement with the data obtained in [23] where no significant heat evolution and structural rearrangements were found on the interaction of dehydrated HSA with anhydrous acetonitrile.

At high water activities the  $\Delta H_{\text{tot}}$  values reach a level, which is close to the solution enthalpy of the dried HSA in water (−6.019 kJ/mol HSA). This  $\Delta H_{\text{tot}}$  value is in good agreement with the previously obtained result [23].

The effect of acetonitrile on the interaction enthalpies of water with the dried HSA was characterised by the differences in the  $\Delta H_{\text{tot}}$  values obtained in the presence and absence of organic solvent at a given  $a_w$  (Fig. 9). The  $\Delta H_{\text{tot}}$  function obtained in the presence of AN has been compared with the  $\Delta H_{\text{tot}}$  values determined for HSA in the absence of AN. This comparison can show any effects of the organic solvent molecules on the interaction enthalpies of HSA with water. The differences between the  $\Delta H_{\text{tot}}$  values obtained in the presence and absence of AN ( $\Delta\Delta H_{\text{tot}}$ ) are presented in Fig. 9. As can be concluded from Fig. 9, the  $\Delta\Delta H_{\text{tot}}$  function is consistent with changes in the  $\Delta h$  (Fig. 3) and  $\Delta D_{1654}/D_0$  (Fig. 7) values.

At the lowest water activity values, the  $\Delta\Delta H_{\text{tot}}$  values are close to zero (Fig. 9). Hence, it may be concluded that the presence of AN has little effect on the hydration of the initially dried protein film.

The most pronounced effect of AN on the thermochemical and structural properties of HSA was observed in the water activity range from 0.6 to 0.8. The acetonitrile-induced

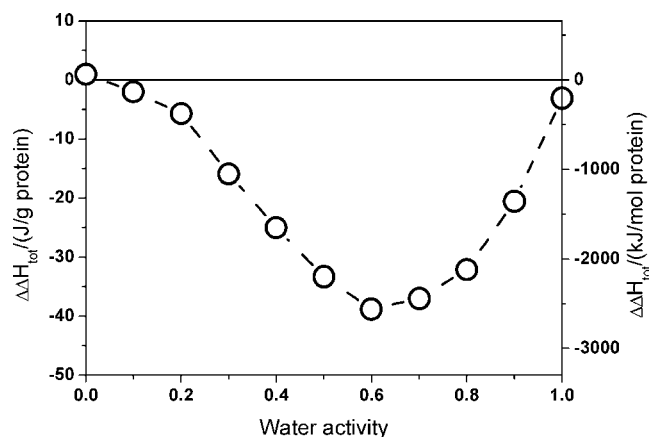


Fig. 9. The differences in the interaction enthalpies ( $\Delta H_{\text{tot}}$ ) between the calorimetric data measured in the presence and absence of AN.

increase in the content of  $\alpha$ -helix structure (the ordered structure with strong hydrogen bonds) (Fig. 7) is accompanied by the excess exothermic heat effects compared with that for pure water (Fig. 9).

### 3.4. Effect of acetonitrile on the hydration and structure of HSA

An explanation of the acetonitrile effect on the hydration and structure of HSA may be provided on the basis of earlier hypothesis of the water-assisted disruption of the dehydration-induced polar contacts in the solid protein phase [22].

Like for many proteins, dehydration of HSA leads to the formation of protein–protein contacts due to proton-transfer phenomena and hydrogen bonding between polar and ionizable protein groups. These processes result in a rigid, condensed structure in the dried state. Therefore, certain moieties of the dried protein are unavailable for sorption due to strong interactions between them resulting in sorption hysteresis (for e.g. [22]).

It was previously shown that the potential of a solvent to form hydrogen bonds is an important factor that controls the state and structure of dehydrated proteins at room temperature [23,24]. Hence, H-donating and H-accepting properties of AN are expected to be important for estimating the possible effect of organic molecules on the protein structure and hydration. When a hydrogen-bond-mediated protein–protein

contact is disrupted, sorbate molecules (water or acetonitrile) may differentiate between H-donating and H-accepting fragments of the disrupted contact. Water (H-donor and H-acceptor) is able to solvate both H-accepting and H-donating groups. An H-accepting acetonitrile molecule is expected to prefer H-donating groups, while the remaining H-bond of H-accepting partner will be solvated by water more effectively. Hence, it is expected that acetonitrile molecules are not effective in disrupting the dehydration-induced protein–protein contacts alone. Therefore, no acetonitrile-induced structural rearrangements (Fig. 7) and additional heat effects (Fig. 9) were observed at the lowest water activity values.

According to this model, by penetrating into the initial dried protein, water molecules hydrate the polar groups of protein–protein contacts and create new sorption sites at the hydrated (disrupted) contacts. There is a competition between water and acetonitrile molecules for these water-binding sites. This competition results in considerable differences in water sorption, structure and heat effects compared with that for pure water.

Cooperative increase in the fraction of the H-bond-associated water molecules in the presence of acetonitrile occurs in a narrow water activity range from 0.8 to 0.9. This cooperative transition results in sharp changes in sorption (Figs. 1 and 3), structural (Fig. 7) and thermochemical (Fig. 9) properties of human serum albumin at water activity above 0.8.

#### 4. Conclusions

- The combined study based on the calorimetric and IR spectroscopic measurements provides an informative tool in monitoring the molecular processes that occur on hydration of solid protein in the presence of organic solvent.
- The obtained results demonstrate that the hydration and structure of the human serum albumin films depend markedly on how protein films has been hydrated—whether in the presence or in the absence of organic solvent vapor.

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#### References

[1] R.B. Gregory (Ed.), *Protein–Solvent Interactions*, Marcel Dekker, New York, 1995, pp. 191–264.

[2] J.A. Rupley, G. Careri, *Adv. Protein Chem.* 41 (1991) 37–172.

[3] I.D. Kuntz, W. Kauzmann, *Adv. Protein Chem.* 28 (1974) 239–345.

[4] A.M. Klivanov, *Nature* 409 (2001) 241–246.

[5] G. Carrea, S. Riva, *Angew. Chem. Int. Ed.* 39 (2000) 2226–2254.

[6] M.N. Gupta (Ed.), *Methods in Non-aqueous Enzymology*, Birhäuser Verlag, Basel/Boston/Berlin, 2000.

[7] K. Ramanathan, B.R. Jonsson, B. Danielsson, *Anal. Chem.* 72 (2000) 3443–3448.

[8] O. Fatibello-Filho, I. Cruz Viera, *Fresen. J. Anal. Chem.* 368 (2000) 338–343.

[9] K. Dabulis, A.M. Klivanov, *Biotechnol. Bioeng.* 39 (1992) 176–185.

[10] C.J. Slade, *J. Mol. Catal. B: Enzyme* 9 (2000) 97–105.

[11] C. Lowe, *Curr. Opin. Struct. Biol.* 10 (2000) 428–434.

[12] M.D. Borisover, V.A. Sirotkin, B.N. Solomonov, *Thermochim. Acta* 284 (1996) 263–277.

[13] V.A. Sirotkin, M.D. Borisover, B.N. Solomonov, *Biophys. Chem.* 69 (1997) 239–248.

[14] M.D. Borisover, V.A. Sirotkin, B.N. Solomonov, *J. Phys. Org. Chem.* 8 (1995) 84–88.

[15] P.I. Haris, F. Severan, *J. Mol. Catal. B: Enzyme* 7 (1999) 207–221.

[16] S.J. Prestrelsky, N. Tedeschi, T. Arakawa, J.F. Carpenter, *Biophys. J.* 65 (1993) 661–671.

[17] A. Dong, S.J. Prestrelsky, S.D. Alison, J.F. Carpenter, *J. Pharm. Sci.* 84 (1995) 415–424.

[18] K. Griebenow, A.M. Klivanov, *Proc. Natl. Acad. Sci. U.S.A.* 92 (1995) 10969–10976.

[19] G. Vecchio, F. Zambianchi, P. Zacchetti, F. Secundo, G. Carrea, *Biotechnol. Bioeng.* 64 (1999) 545–551.

[20] M. van de Weert, P.I. Harvez, W.E. Hennink, D.J.A. Crommelin, *Anal. Biochem.* 297 (2001) 160–169.

[21] J. Grdadolnik, Y. Marechal, *Biopolymers* 62 (2001) 40–53.

[22] V.A. Sirotkin, D.A. Faizullin, *Thermochim. Acta* 415 (2004) 127–133.

[23] V.A. Sirotkin, A.N. Zinatullin, B.N. Solomonov, D.A. Faizullin, V.D. Fedotov, *Biochim. Biophys. Acta* 1547 (2001) 359–369.

[24] V.A. Sirotkin, A.N. Zinatullin, B.N. Solomonov, D.A. Faizullin, V.D. Fedotov, *Thermochim. Acta* 382 (2002) 151–160.

[25] D. Carter, J.X. Ho, *Advances in Protein Chemistry*, vol. 45, Academic Press, New York, 1994, pp. 153–203.

[26] M.D. Borisover, V.A. Sirotkin, D.V. Zakharychev, B.N. Solomonov, in: E.N. Vulfson, P.J. Halling (Eds.), *Methods in Biotechnology Book No. 15*, vol. 2, *Enzyme in Non-aqueous Solvents*, 2001, pp. 183–202.

[27] D.D. Perrin, W.L.F. Armarego, D.R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1980.

[28] I. Wadso, R.N. Goldberg, *Pure Appl. Chem.* 73 (2001) 1625–1639.

[29] B.P. Nikol'skii (Ed.), *Spravochnik khimika (Chemist's Handbook)*, vol. 1, Goskhimizdat, Leningrad, 1963.

[30] R.C. Weast (Ed.), *Handbook of Chemistry and Physics*, 58th ed., CRC Press, Cleveland, 1977–1978.

[31] A. Dong, P. Huang, W.S. Caughey, *Biochemistry* 29 (1990) 3303–3308.

[32] V.B. Kogan, B.N. Fridman, V.V. Kafarov, *Ravnovesie mezhdu zhidkost'ju i parom: Spravochnoe posobie (Equilibrium between Liquid and Vapor Handbook)*, Nauka, Moscow, 1966.

[33] V.A. Sirotkin, B.N. Solomonov, D.A. Faizullin, V.D. Fedotov, *J. Struct. Chem.* 41 (2000) 997–1003.

[34] H.A. Laitinen, W.E. Harris, *Chemical Analysis*, 2nd ed., McGraw-Hill, New York, 1975.

[35] S. Brunauer, P.H. Emmet, E. Teller, *J. Am. Chem. Soc.* 60 (1938) 309–319.

[36] M. Luscher-Mattli, M. Ruegg, *Biopolymers* 21 (1982) 403–418.

[37] H.B. Bull, *J. Am. Chem. Soc.* 66 (1944) 1499–1507.

[38] C.H. Amberg, *J. Am. Chem. Soc.* 79 (1957) 3980–3984.