Absorption of Water by Proteins and Related Compounds

D. K. RAMSDEN, F. WOOD, and G. KING, *Wool Industries Research Association, Torridon, Leeds, England*

Synopsis

Isotherms have been obtained for the absorption of water by proteins and polyamides and by simple amides closely related to the repeat chemical unit of the polymers. The differential heat of absorption of water at zero water content has also been determined for these substances, from isosteres and from heats of mixing. In addition the nature of the hydrogen bonding of water to these compounds has been investigated by infrared spectroscopic methods. The large differences found between the heats of absorption by the polymers and the simple model compounds are discussed.

Introduction

The differential heat of absorption of water $(\Delta \bar{H})$ by a polar polymer has generally been regarded[1,2] as the difference in energy between the hydrogen bonds made and broken during the sorption process. Water molecules entering the polymer phase can form hydrogen bonds either with the hydrophilic groups in the polymer or with adjacent water molecules, the observed value of $(\Delta \bar{H})$ depending on the relative numbers of these bonds and the hydrogen bond energies. At low water concentrations the proportion of water–water bonds will decrease, and the differential heat of absorption at zero water content $(\Delta \bar{H}_0)$ may be regarded as a measure of the sorbent–water interaction energy less the latent heat of evaporation of water. This would be expected to have a similar value for the polymer nylon 66 and the compound N-n-butylacetamide which closely resembles one repeat chemical unit of the polymer.

In addition to the chemical reaction already discussed, there has arisen the concept of polymer constraint. This is a resistance to swelling imposed by the long-chain network as distinct from that of a monomeric liquid, and Warburton[3] suggests that this could amount to several hundred calories per mole of water for keratin or cellulose. The differential heat of absorption of water by a simple model liquid such as N-n-butylacetamide, for example, would therefore be expected to have a larger exothermic value than that for nylon 66, by an amount equal to the heat of swelling of this polymer.

This paper reports the measurement of differential heats of absorption of water, together with absorption isotherms for several proteins, polyamides, and simple amides, and the results for long- and short-chain compounds are

compared.   Infrared spectroscopic investigations were also carried out on the same compounds in order to assess bond strengths for water–polymer and water–monomer hydrogen bonds.   Identical studies on polymers and related monomers possessing other hydrophilic groups have been reported elsewhere[4] by one of us.

EXPERIMENTAL

Materials

The N-methylacetamide (NMA), N-n-butylacetamide (NBA), and N,N-dimethylacetamide (DMA) used in this work were of the highest purity grade available commercially.   They were dried over anhydrous calcium sulfate and fractionated twice under reduced pressure before use.   Nylon 66 monofilament to the following specifications was kindly supplied by British Nylon Spinners Ltd.: TiO$_2$ content, 0%; NH$_2$ ends, 33 equiv./10^6 g.; COOH ends, 100 equiv./10^6 g.; intrinsic viscosity, 0.72.

Samples of Bombyx mori silk were kindly supplied by Mr. J. T. B. Shaw of the Cotton, Silk and Man-made Fibres Research Association, and were degummed before use by the method described by Lucas et al.[5] Poly-N-vinylacetamide was prepared by the method described by Reynolds and Kenyon[6] and poly-N-vinylbutyramide by a similar method.   N-Vinyl-N-methylacetamide was prepared by the method of Hanford and Stevenson,[7] and this compound was polymerized by heating at 100°C. for 1 hr. in a stoppered tube with 1% azobisisobutyronitrile, followed by dialysis and freeze drying.

Determination of Differential Heats of Absorption

The differential heats of absorption of water by all the polymers used in this work, were obtained by an isosteric method based on that used by Urquhart and Williams.[8] In the case of the liquid amides, values were ob-
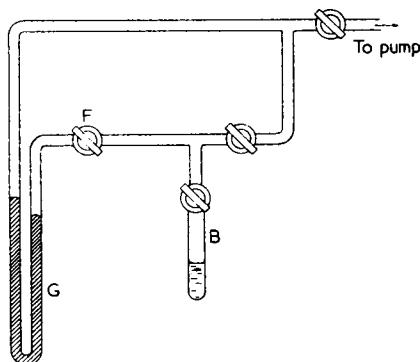

Fig. 1. Apparatus for determining the differential heat of absorption of water by polymers.

tained both by this method and from heats of mixing data. The apparatus employed to obtain the isosteres is shown in Figure 1. B is an evacuated water reservoir, and G is a manometer 20 cm. in length and containing Silicone Fluid 703 (Edwards High Vacuum Ltd.). In measuring vapor pressures with the oil manometer, corrections were made for density changes over the temperature range employed. Polymers in a dispersed state, either as fiber or thin film were packed inside the vacuum tap F. Because of the dispersed state of the polymer samples, equilibrium was rapidly attained with the water vapor. Polymers in powder form were placed inside a porous polyethylene tube which was stoppered at each end. At least 1.25 g. of polymer could be accommodated by these methods. The whole apparatus was immersed in a thermostatically controlled water bath at the maximum temperature to be employed and the polymer evacuated for 2 days. Air adsorbed on the polymer surface was flushed off by saturating the sample with water vapor for 8 hr. followed by evacuation. This was done three times.

Water vapor was introduced to the sample and when equilibrium had been attained the vapor pressure was measured to 0.002 cm. Hg and the bath temperature read to 0.01°C. The temperature was then lowered by successive intervals of about 2°C. and the new readings taken. The volume occupied by sample and vapor was about 10 cc.

The logarithm of the equilibrium relative humidity was plotted against reciprocal absolute temperature and $\Delta \bar{H}$ determined from the relation

$$d(\ln r)/d(1/T) = \Delta \bar{H}/R \tag{1}$$

where r is the relative humidity, T is the absolute temperature, and R is the gas constant. A series of values of $\Delta \bar{H}$ at low r values was obtained and $\Delta \bar{H}_0$ at zero water content obtained by extrapolation.

Because of the small volume of the vapor space, the water content of the sample remained sensibly constant over the temperature range employed. This ensured that eq. (1) applied to the system accurately. Essentially the same method was used for the liquid amides. To avoid contamination of the manometer oil by traces of amide vapor, an all-glass Bourdon gage[9] was used to measure vapor pressures.

Determination of the Heat of Mixing of Liquid Amides with Water

The calorimeter is shown in Figure 2. A known amount of water was sealed in the glass bulb B immersed in the liquid amide in the container M. The latter was immersed in oil in the Dewar vessel F, the oil being stirred by stirrer S. The Dewar vessel was enclosed in a wooden box packed with glass wool. The temperature produced by breaking B and allowing mixing to take place was measured by thermocouples. The calorimeter was calibrated by an electrical heating coil. The whole was placed in an air thermostat controlled at 25 ± 0.02°C. and this in turn was situated in a temperature-controlled room.
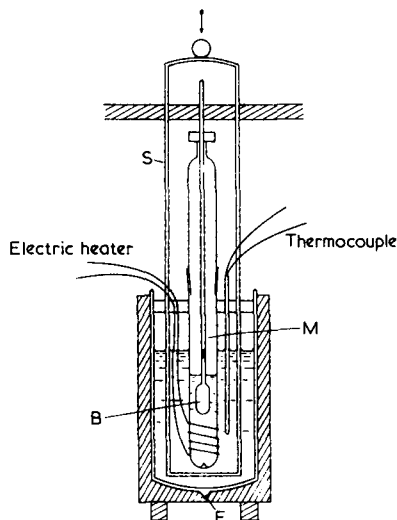
Fig. 2. Heat of mixing apparatus.

Determination of the Isotherms

The water absorption isotherms for the polymers were determined by a sorption balance method,[10] and the isotherms for the liquids by using the apparatus shown in Figure 3. The liquid amide was contained in the vessel E fitted with a magnetic stirrer, and the last traces of water removed by
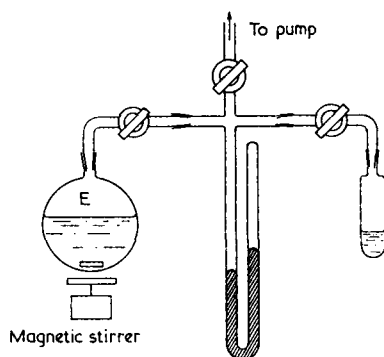

Fig. 3. Apparatus for the determination of the isotherm of a low vapor pressure liquid.

evacuation; this was possible because of the low vapor pressure of the amides. E was removed and weighed, after which increasing amounts of water were distilled into E and the equilibrium water vapor pressure measured in each instance at the temperature of the thermostat. Where necessary, a correction was made for the vapor pressure of the amide, assuming Raoult's Law to hold.

Infrared Spectroscopic Investigation

All spectra were recorded on a Grubb-Parsons S3 spectrometer fitted with a DB3 double-beam radiation unit with a lithium fluoride prism. The spectrum of absorbed water in the sample amides was recorded by using thin films of the mixture held between calcium fluoride windows. In the case of DMA, which has itself no absorption in the 3μ region, the OH vibration of the water could be observed directly. On the other hand NBA has an N—H absorption band in this region, and this was compensated for by a thin film of dry NBA placed in the reference beam. Exact compensation was assumed to occur when no trace of positive or negative C—H absorption bands could be detected at 3.4 μ.

The $3\text{-}\mu$ absorption spectrum of water in keratin, and in the corresponding polyvinylamides was recorded by using the vacuum technique described by Wood and King.[11] In the case of keratin, thin films were prepared as described elsewhere,[11] and each film was mounted in a cell with calcium fluoride windows, and the two cells placed in the two beams of the spectrometer. The cell in the reference beam was connected directly to a vacuum pump and was kept pumped out throughout the subsequent procedure. The cell in the sample beam was connected to a reservoir containing water and to a manometer as previously described.[11] After pumping out both cells for several hours to ensure that the samples were dry, the spectrum of the balanced keratin was recorded. The films could usually be chosen to give a reasonably flat trace on the recorder chart. The sample beam side of the apparatus was then isolated from the pump, and the water reservoir immersed in carbon dioxide–acetone mixture in a Dewar flask, at a temperature calculated from a predetermined isotherm to give a relative humidity required to produce a regain of about 2% in the sample. Water vapor was then admitted to the sample, and after a time sufficient to allow equilibrium to be attained (about 1 hr. for films about 5 μ thick), the spectrum was recorded with the water present. The temperature of the reservoir was raised slightly, and a new spectrum recorded at about 5% regain. This procedure was repeated at intervals up to a regain of about 30%.

Similar methods were used with the other polymers, but these were examined in the form of thin films cast on glass microscope slides from aqueous solution. Poly-N-vinyl-N-methylacetamide has no peak in the $3\text{-}\mu$ region, so that in this case no compensation other than a clean piece of microscope slide in the reference cell was necessary; the other vinyl polymers required compensation with a film of identical thickness.

RESULTS AND DISCUSSION

The molecule of NMA, $CH_3CONHCH_3$, is chemically very similar to the repeat unit of the silk fibroin molecule (I) and the keratin molecule.

$$\left(\begin{array}{cc} -CHCONHCH- \\ | \qquad\quad | \\ R \qquad\quad R \end{array}\right)_n$$

I
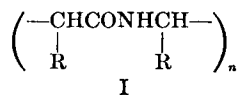
Unfortunately a direct comparison of the sorption behavior of NMA and silk fibroin is complicated by the fact that a considerable proportion of the fibroin is inaccessible to water.   In this respect, a better polymer for comparison with NMA is poly-N-vinylacetamide (II) which is apparently completely accessible

$$\left(\!-CH_2-\!\!\underset{\substack{|\\NH\\|\\COCH_3}}{CH}-\!\!\right)_n$$
II
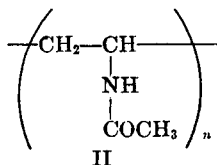
to water and readily dissolves in it at room temperature.   Similarly, the inaccessibility to water of a high proportion of nylon 66 precludes a simple comparison of this polymer with NBA which closely resembles a repeat unit in the nylon molecular chain; again, the completely accessible poly-N-vinylbutyramide (III) is more suitable for comparison purposes.

$$\left(\!-CH_2-\!\!\underset{\substack{|\\NH\\|\\COC_3H_7}}{CH}-\!\!\right)_n$$
III
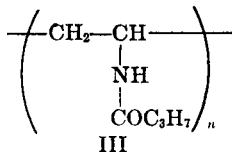
Another pair of compounds related in this way are DMA and poly-N-vinyl-N-methylacetamide (IV).

$$\left(\!-CH_2-\!\!\underset{\substack{|\\N-CH_3\\|\\COCH_3}}{CH}-\!\!\right)_n$$
IV
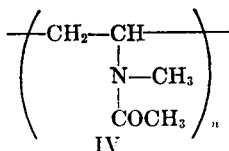
For each of these pairs of compounds the reaction between water and the hydrophilic groups would be expected to be chemically identical.

The variation of the differential heat of absorption of water by these substances with change in relative humidity is shown in Figure 4, and the cor-

TABLE I
Differential Heats of Absorption of Water at Zero Water Content ($\Delta \bar{H}_0$)

Absorbent	ΔH_0, kcal./mole
Wool keratin	-3.6 ± 0.5[a]
Silk	-3.5 ± 0.5
Poly-N-vinylacetamide	-5.0 ± 0.5
NMA	-1.0 ± 0.1
Nylon 66	-3.5 ± 0.5
NBA	-0.75 ± 0.1
Poly-N-vinyl-N-methylacetamide	-4.0 ± 0.5
DMA	-1.3 ± 0.1

[a] Calculated from the results of Hedges.[12]

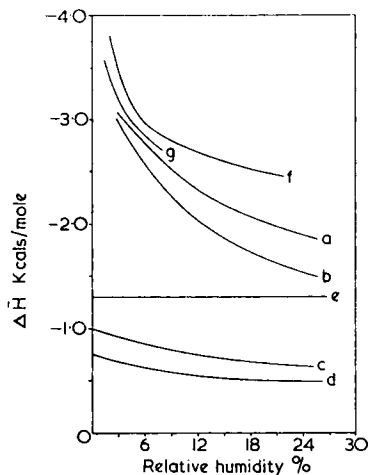Fig. 4. Differential heats of absorption for (a) silk, (b) nylon 66, (c) N-methylacet-amide, (d) N-butylacetamide, (e) N,N-dimethylacetamide, (f) poly-N-vinylacetamide, and (g) poly-N-vinyl-N-methylacetamide.
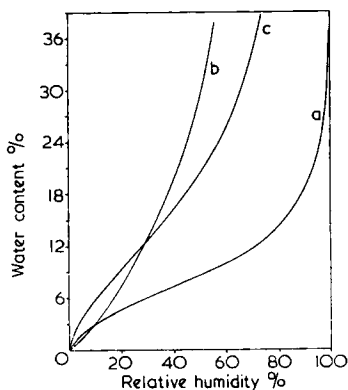

Fig. 5. Isotherms for (a) silk, (b) N-methylacetamide, and (c) poly-N-vinylacetamide; 25°C.

responding isotherms are shown in Figures 5–7. The differential heats of absorption at zero water content ($\Delta\bar{H}_0$) obtained from Figure 4 are given in Table I.

It is apparent from these results that the absorption of water by the polymers at low water contents is a more exothermic process than the absorption by the liquid amides. This conclusion is supported by the isotherm results wherein the polymers give sigmoid isotherms characteristic of exothermic absorption. The simple amides on the other hand show isotherms largely convex to the vapor pressure axis.

These results may be compared with those obtained by King[13] and by Bailey[14] concerning the differential heat of absorption of formic acid by
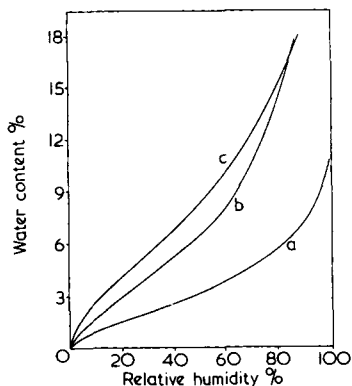
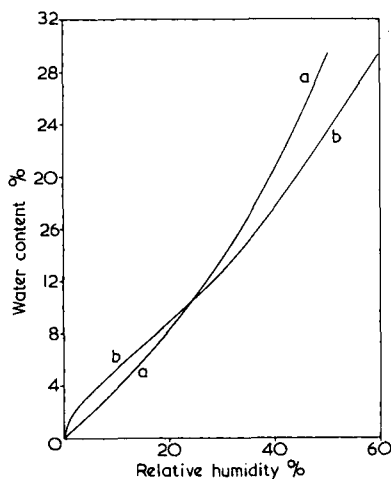Fig. 6. Isotherms for (a) nylon 66, (b) N-butylacetamide, and (c) poly-N-vinylbutyl-acetamide; 25°C.

Fig. 7. Isotherms for (a) N,N-dimethylacetamide and (b) poly-N-vinyl-N-methyl-acetamide.

simple amides and related polymers. The same trend is evident as shown in Table II.

TABLE II
Differential Heat of Absorption of Formic Acid at Low Acid Content

Absorbent	ΔH_0, kcal./mole
Keratin	-6.7 ± 0.5[a]
NMA	-2.5[b]
Nylon 66	-6.5 ± 0.5[a]
NBA	-2.0[b]

[a] Data of King.[13]
[b] Data of Bailey.[14]

The divergence between the differential heats of absorption of polymers and of corresponding amides could be simply ascribed to the formation of stronger hydrogen bonds with the hydrophilic groups in the polymer. This aspect was examined by infrared spectroscopic methods in which the frequency of the water OH band was measured while in the absorbed state. The results may be summarized as follows: (1) in most cases, the OH vibration frequency of the absorbed water occurred at a higher frequency than for liquid water but shifted towards the value for liquid water as the amount of absorbed water was increased; (2) at low water contents, around 5%, the OH vibration frequency of the absorbed water varied with the absorbent used, but values for a given polymer and the corresponding model amide were almost identical. Examples are quoted in Table III.

TABLE III
Peak OH Frequency of Absorbed Water at Low Water Contents

Absorbent	OH frequency, cm.$^{-1}$
Poly-N-vinylbutyramide	3440
N-n-Butylacetamide	3420
Poly-N-vinyl-N-methylacetamide	3490
N,N-Dimethylacetamide	3480

Now it is generally accepted that the A–H stretching vibration is a measure of the strength of the A—H- - -X hydrogen bond.[15] The results in Table III therefore suggest that equally strong hydrogen bonds are formed with water for the polymers and corresponding monomers.

One curious feature of these results is that all the OH frequencies are higher than that for liquid water—about 3380 cm.$^{-1}$ In view of the exothermic nature of the absorption one would have expected the bonds with the absorbent to be stronger and that the OH frequency would be less than 3380 cm.$^{-1}$.[15] This difficulty in reconciling heats of absorption results with infrared and proton magnetic resonance results has previously been encountered by Dinkard and Kivelson.[16] No satisfactory explanation has as yet been put forward.

An alternative possible explanation of the divergence between heats of absorption of polymers and of simple amides involves the possible presence of partially bonded water in the mixture. If there were a greater proportion of free OH in the water–amide system than in the water–polymer system the net evolution of heat would be smaller in the former case, all other contributions remaining the same. This possibility was examined by looking for the presence of the free OH stretching vibration for water at 3700 cm.$^{-1}$, but it was not found.

In the absence of any other explanation we are then left with the hypothesis that molecular cavities are present in the polymers in which absorbed molecules may be accommodated. Barrer and his co-workers[17] have suggested such a possibility to explain the high negative heats of absorption

of hydrocarbons by ethyl cellulose. If cavities, not necessarily as large as the absorbate molecules, exist in the polymer, less energy will be required to accommodate the absorbate and a more exothermic reaction will result.

The concept of molecular cavities in protein polymers is not new and is suggested by the values derived for the partial molar volume of adsorbed water at low water contents.[18] For wool, silk, and nylon this lies between 8 and 10 cc., whereas in NBA it is 15 cc.[4] The small value for water in the polymers was associated with the exothermic nature of the absorption by analogy with the water–sulfuric acid system.[18] However this explanation appears to be ruled out by the spectroscopic evidence presented in the present paper which suggests that the hydrogen bonds formed between proteins and water are similar to those in liquid water.

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Résumé

On a obtenu des isothermes pour l'absorption d'eau par les protéines et les polyamides et par des amides simples homologues de l'unité périodique chimique des polymères. L'enthalpie différentielle d'absorption d'eau à teneur en eau nulle a également été déterminée pour ces substances au départ des isostères et des chaleurs de mélange. En outre, la nature de la liaison hydrogène de l'equ à ces substances a été étudiée par des méthodes spectroscopiques infra-rouges. Les grandes différences entre les valeurs des enthalpies d'absorption par les polymères et les composés modèles simples sont discutées.

Zusammenfassung

Absorptionsisothermen für Wasser durch Proteine und Polyamide sowie durch einfache, den Polymerbausteinen eng verwandte Amide wurden erhalten. Die differentielle Absorptionswärme von Wasser beim Wassergehalt Null wurde für diese Substanzen aus

den Isosteren und den Mischungswärmen bestimmt. Zusätzlich wurde die Natur der Wasserstoffbindung des Wassers an diese Substanzen mit infrarotspektroskopischen Methoden untersucht. Die grossen Unterschiede zwischen der Absorptionswärme bei den Polymeren und den einfachen Modellverbindungen werden diskutiert.