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Review article

Water vapor sorption by peptides, proteins and their formulations

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Abstract

The interactions of pharmaceutical peptides, proteins and their formulations with environmental water vapor are reviewed. Particular attention is paid to the importance of the physical structure and chemical diversity of peptides and proteins, and comparisons are made with the mechanisms of water vapor sorption by synthetic macromolecular systems. The influences of formulation processes and additives are also considered and suggestions made for future areas of research. © 1998 Elsevier Science B.V.

Keywords: Water vapor sorption; Protein; Peptide; Formulation; Amorphous

1. Introduction

In recent years therapeutically-active peptides and proteins have increasingly been incorporated into solid-state formulations designed for parenteral and pulmonary drug delivery. The parenteral and pulmonary routes are used because of the generally poor overall oral bioavailability of most peptides and proteins. The formulations are usually produced in solid forms because of the strong tendency of these materials to chemically degrade and physically denature in aqueous solution, leading to a significant loss in therapeutic potency. Peptides and proteins generally are formulated for parenteral products as lyophilized solids intended to be reconstituted into a solution at the time of use [1–6]. For products intended for pulmonary administration, peptides and proteins are often prepared by lyophilization or spray drying for use in dry powder inhalers. It is well recognized that processes that involve the rapid removal of solvents such as lyophilization and spray drying can cause materials that might ordinarily exist in a crystalline form to become partially or fully amorphous [7,8]. One important property of amorphous materials is their ability to sorb water vapor from the atmosphere into their bulk structure, as compared to the water vapor uptake by crystalline materials that is generally limited to the particle surface (assuming no crystal hydrate formation). Depending on the polarity of the solid, and the surrounding temperature and relative humidity, we can expect significant differences in the amount of water vapor absorbed by peptide and protein formulations. This sorption of water into the bulk amorphous structure can have profound effects on properties (i.e. chemical stability, physical stability, biological activity) which depend on the structure and dynamics of the amorphous state [7,8]. With these observations in mind it is important to answer certain questions about the relationship between absorbed water vapor and peptides and proteins in the amorphous state: (1) how much water is taken up by such a system at any given temperature and relative humidity; (2) what is unique and what is similar about non-crystalline peptides and proteins in the presence of absorbed water relative to other amorphous materials such as synthetic polymers? In the remainder of this paper we will attempt to answer these questions by critically reviewing work done in this laboratory and elsewhere that can be used to interpret and predict the relationship between water

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vapor content and relative humidity in amorphous peptide and protein formulations.

2. Discussion

2.1. Background

Peptides and proteins are natural macromolecules that are produced by joining amino acid subunits with amide bonds. Unlike the single regular repeat units of homopolymers, or the two different monomers of random copolymers, many different types of amino acids are assembled in proteins and peptides, giving rise to a complex secondary structure (e.g. loops, chains, turns, β-sheets, helices) and tertiary structure (i.e. varying types of folding). The complexity of protein structure increases when a protein consists of more than one chain and thus assumes a specific quaternary structure (i.e. spatial arrangement of individual chains). Additionally, every protein molecule whether crystalline, amorphous or in solution can be considered to be a disordered array of structures some of which are more locally ordered than others. Disordered regions (e.g. random coils, turns, loops) and more ordered structures (e.g. lamellar sheets, helices) give proteins a character that is analogous to partially-crystalline synthetic macromolecules with respect to having regions of order and disorder.

Water vapor sorption isotherms have been reported for over 20 peptides, proteins and structurally related materials (Table 1) [9], as well as for several pharmaceutical protein formulations [10,11]. It is generally observed that these materials sorb considerable quantities of water under ambient conditions, and that the extent of sorption is a sigmoidal function of the relative humidity (Fig. 1), and qualitatively similar to that observed with hydrophilic amorphous polymers [9]. The effect of temperature on the water vapor sorption behavior of protein systems is usually small but significant (Table 2) [12], and large sorption/desorption hysteresis has been reported in several instances [9,13,14].

Table 1

Water vapor sorption studies reported for some poly(amino acids), peptides, proteins and related compounds

Material	Temperature (°C)	Reference
Polyglycine	31.5	[36]
Poly-L-alanine	31.5	[36]
Poly-L-glutamic acid	17, 27, 37, 57	[63]
Poly-L-lysine HBr/HCl	5, 25, 35, 40, 45	[45]
Insulin	25, 50	[22]
Ovalbumin	–	[26]
Bovine serum albumin	–	[56]
Lysozyme	25; 17, 27, 37, 47; 35	[27,44,63]
Methemoglobin	–	[18]
Somatotrophin salts	24	[48]
Chymotrypsinogen	35	[27]
Casein	25	[37]
Sodium caseinate	25, 40, 60, 80; 5, 15, 25	[12,55]
Whey protein	25, 40, 60, 80; 5, 15, 25	[12,55]
Soy protein	25, 40, 60, 80; 35; 5, 15, 25	[12,21,55]
Peanut proteins	35	[21]
Wheat proteins	35; 5, 25, 45	[21,64]
Collagens	25; 20	[25,65]
Wool/keratin	22.2	[26,66]

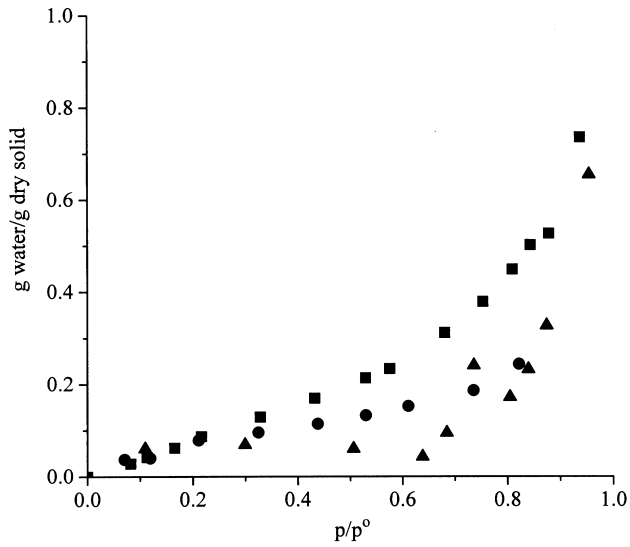

Fig. 1. Water vapor sorption isotherms for insulin at 50°C (▲), for poly(-vinylpyrrolidone) at 30°C (■) and for gelatin (as gelatin capsules) at 20°C (●). Data from Kontny and Mulski [71], Oksanen and Zografi [72], Costantino et al. [22] and Costantino et al. [23].

The interaction of water vapor with peptides and proteins is expected to be (1) complex, (2) extensive and (3) fairly strong, given the physical and chemical properties of most proteins. Water–protein interactions are expected to be complex because of the heterogeneous chemical structure of such compounds. Their large molecular weight and polar groups encourage numerous interactions, and a large number of potential hydrogen bonding sites dictates that many of the interactions will be quite strong. Specific residue–water interactions have been reported and some of these are thought to be important for maintaining the integrity of certain proteins [15]. Native proteins typically exist in a water-rich environment, so it is not surprising that water is critical for protein structure and function. It is probable that changes in the degree of hydration of proteins are responsible for significant changes in physical structure and molecular cooperativity [16].

2.2. Traditional approaches

There have been several extensive reviews of the historical attempts to model water vapor sorption by proteins and related compounds, and the reader is encouraged to refer to these for a complete perspective [9,17–19]. As a consequence of the complexity of the interactions between proteins and environmental water vapor, it would not be expected that conventional theories and models of water vapor sorption behavior which apply to inorganic and

Table 2

Glass transition temperature data reported for some poly(amino acids), peptides, proteins and related compounds

Material	Transition temperature data	Reference
Poly-L-serine	Single point	[35]
Poly-L-methionine	Single point	[35]
Poly-L-asparagine	As a function of water content	[37,67]
Single amino acids	For solutions	see [43]
Bovine serum albumin	For solutions	see [43]
α-Casein	For solutions	see [43]
Ovalbumin	As a function of water content	see [18]
α-Lactalbumin	As a function of water content	see [18]
	For solutions	see [43]
Lysozyme	Single point	[34,41]
	As a function of water content	see [18]
Chymotrypsinogen	Single point	[34,41]
	As a function of water content	see [18]
Globulin	Single point	[34]
Elastin	Single point	[41]
	As a function of water content	[68]
Collagen	Single point	[41]
	As a function of water content	[69]
	For solutions	see [43]
Gelatin	As a function of water content	see [43]
Wheat glutens	As a function of water content	[42,70]

organic solids would be very useful for describing the behavior of proteins and peptides in the amorphous state. Most early workers attempted to use water vapor sorption models where the mechanisms were very simple despite the obvious inadequacies of these models in accounting for the behavior of proteins. The most popular approach was to use models that describe the physical adsorption of water vapor on the surface of solid particles, such as the Langmuir, BET and GAB equations [20]. Apparent monolayer capacities obtained from these equations have been widely used to estimate specific surface areas, and it has been noted that unusually large surface area estimates are obtained [9,21] which do not agree with those from well proven nitrogen sorption methods [22,23]. This is not surprising, since a scenario of water molecules physically adsorbing only onto the surface of protein particles and not interacting in any other way is clearly incorrect. Several other common modes of water–solid interaction such as capillary condensation and deliquescence can be considered to be equally unlikely [24], although the formation of crystal hydrates with proteins is possible under some circumstances.

Models of stoichiometric binding between water molecules and specific functional groups in protein materials have been proposed by several authors [25–27]. These are based on the assumption that certain groups (e.g. amide groups) bind a specific number of water molecules. In the more complex models different functional groups are assigned different interaction or binding capacities which are determined by correlations drawn from a series of structurally similar compounds. These 'group contribution' approaches provide reasonable estimates of the experimental water vapor sorption isotherms in several instances (e.g. see Fig. 2) including several block copolymers [28], but they do not appear to have general applicability [13,29, 30]. This is not surprising, since it is clearly an over-simplification to assume that all protein–water interactions are controlled by the number of each type of amino acid present and are insensitive to the effects of molecular size, secondary, ternary and quaternary structure, and the degree of ordering of the system. Models which invoke the existence of water in different states (e.g. bound and unbound) at different relative humidities have also been proposed [9,31,32].

More recent approaches have considered the water–protein system to be analogous to a simple aqueous solution [9]. Early workers tested concordance with the simplest of solution models (e.g. Raoult's law, Henry's law) and concluded (not surprisingly) that protein–water systems were not behaving as ideal or regular solutions [9]. Following these reports, several investigators used solution models that had been developed for synthetic amorphous polymeric systems to describe their data. Perhaps the simplest and most widely used of these solution models is the Flory–Huggins model [33]

$$p/p_0 = \phi_1 \exp\{(1-(1/x))\phi_2 + \chi\phi_2^2\} \quad (1)$$

where p/p_0 is the partial vapor pressure, ϕ is the volume fraction of penetrant (1) or matrix (2), $\times$ is the relative molecular size of 1 and 2, and χ is a matrix–penetrant

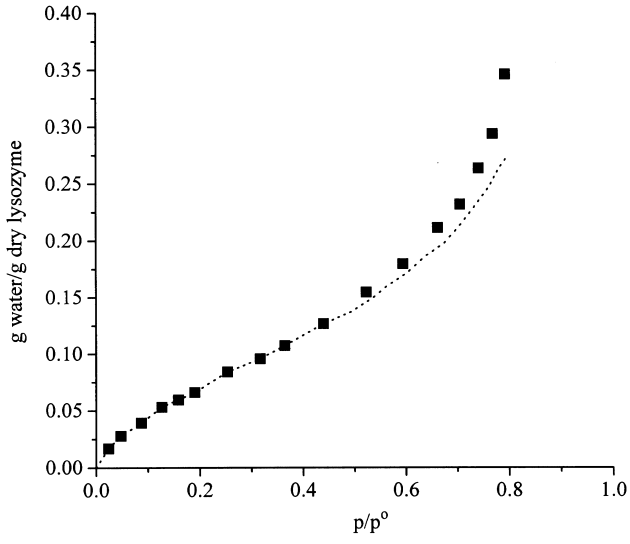

Fig. 2. Predicted (dotted line) and experimental (■) water vapor sorption isotherms at 35°C for lysozyme using the group contribution method of Leeder and Watt [27].

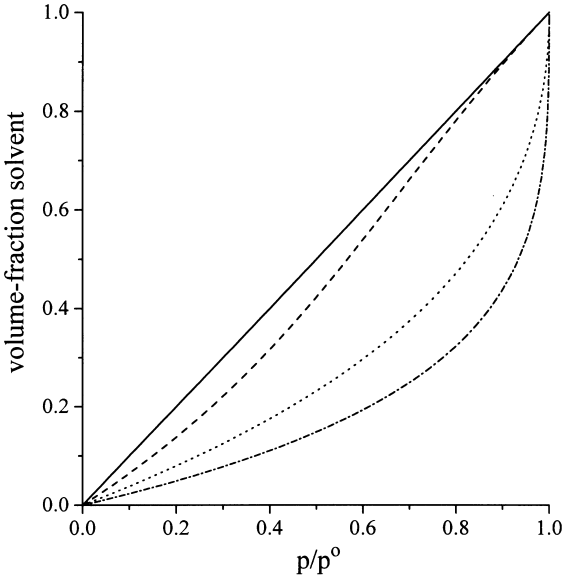

Fig. 3. Theoretical water vapor sorption isotherms predicted using the Flory–Huggins polymer solution model, showing the influences of molecular size (x) and the solid–liquid interaction parameter (χ). $x = 1$, $\chi = 0$ (solid line); $x = 1$, $\chi = 0.5$ (dashed line); $x = \infty$, $\chi = 0$ (dotted line); $x = \infty$, $\chi = 0$ (dash-dotted line).

interaction parameter. This model has the ability to account for molecular size effects and the strength of the solid–liquid molecular interactions which are particularly important in protein–water systems (Fig. 3). A much better success rate has been achieved using this approach and the shape and magnitude of the isotherms predicted are similar to those depicted by most experimental data (Fig. 1). The physical picture of the water molecules and the protein molecules mixing at a molecular level and experiencing both specific interactions and bulk structural effects is much more reasonable than those of the models used previously [9]. Whilst the agreement between theory and experiment is good at moderate relative humidities there are often significant discrepancies, particularly at lower humidities, where the system is least like a true solution and where the sorbed water has a significant effect on solid state structure and dynamics.

2.3. Protein structure and dynamics

At this point, before further discussing models for water vapor sorption by proteins, we would like to briefly discuss the relationship between the structure and dynamics of peptides and proteins and water vapor that is absorbed by peptides and proteins. Given sufficient time and exposure to certain environmental conditions (e.g. high temperature), proteins can experience molecular relaxation processes that have important effects on chemical reactivity, affinity for water, and biological function [34]. Two important characteristics of these relaxation processes are their non-exponential time dependence and their cooperativity within a single protein molecule. These properties are shared by synthetic amorphous macromolecules and help to distinguish them from conventional crystalline and liquid organic systems. Considering these similarities in dynamic processes in proteins and amorphous (or semi-amorphous) synthetic polymers, it may be possible to gain some insight into relaxation processes of proteins and their relationship to water vapor sorption by considering some model amorphous macromolecular systems [35–37].

Amorphous materials are highly energetic, disordered systems whose physicochemical properties depend strongly on temperature. They are formed when the crystallization of a material below its melting temperature is prevented. This process is termed ‘supercooling’ and it can result from (1) a large molecular size and high degree of molecular connectivity, (2) extensive random intra- and intermolecular bonding, or (3) exposure to very rapid changes in environmental conditions (e.g. temperature, solvent). Typically an amorphous supercooled macromolecule displays only short-range molecular order and is sufficiently viscous to be considered a solid. At some temperature well below its theoretical liquid-to-crystal transition the amorphous material falls out of thermodynamic equilibrium and becomes an amorphous ‘glass’. This is the glass transition temperature (T_g), and its exact location depends upon the formation conditions/processing history, the chemical structure and molecular size of the material, and the time scale of inspection. Below T_g molecular motions in an amorphous material are limited to lower order motions. Excluded from this temperature regime are large, diffusion-related motions which are responsible for main chain fluctuations in synthetic macromolecules. The glass transition temperature is usually determined experimentally by measuring either the heat capacity (DSC), volume (dilatometry) or mechanical properties of

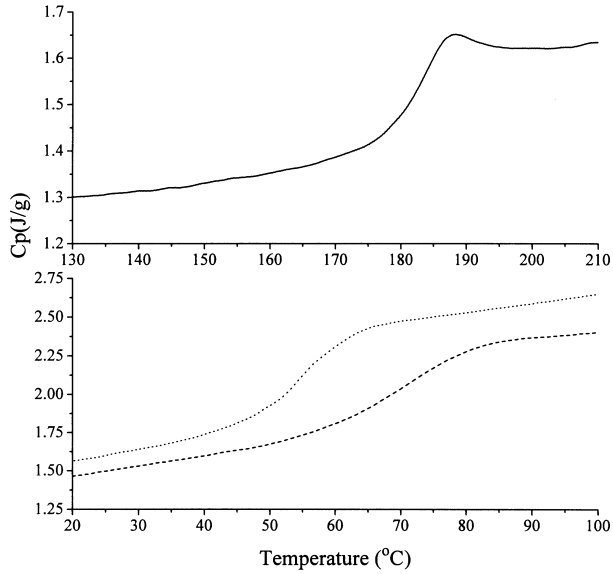

Fig. 4. DSC trace showing a step-wise change in heat capacity for two proteins, collagen (dashed line) and elastin (dotted line) compared with PVP (solid line). Data taken from Tsereteli and Smirnova [41] and Oksanen and Zografi [72].

the material as a function of temperature. It has been reported that when subjected to these methods amorphous proteins do not always demonstrate a T_g, even when they have been prepared by methods that typically yield a highly amorphous state (e.g. lyophilization [38]). This is not totally unexpected since the dynamics of molecular motions in proteins are likely to be extremely heterogeneous, even over a small temperature range, due to their high internal structural diversity. Fan et al. [39] have suggested that proteins do not always show a classical T_g because of a 'smearing' of lower temperature relaxations that consequently overlap with the primary relaxations that are responsible for T_g. There are proteins, however, that do show a second order transition that is characteristic of a T_g [40]; elastin and collagen are two classical examples (Fig. 4). Both of these proteins are 'structural proteins' and are quite 'polymeric' in nature since they are typically richer in one or two amino acids arranged in a regular, repeating sequence. Other proteins have been shown to demonstrate a step-wise change in heat capacity upon heating [18,41,42], and whether or not these changes represents a strict T_g, they do appear to depend on physical and chemical structure, thermal history and the measurement timescale/technique. For most peptides and proteins it is likely that there will be a critical temperature that is associated with a sudden increase in the number of configurational states and this may be manifested as an apparent second-order transition [6,13,18]. This transition, if experimentally detectable, can be a useful indicator of important phenomena such as increased chemical reactivity, denaturation, or aggregation [38].

In addition to a changing temperature, the sorption of water vapor can significantly alter the physical structure and molecular dynamics of amorphous materials. Sorbed water increases the overall free volume of amorphous materials and consequently promotes molecular mobility. Such changes are particularly pronounced when the amorphous material is in the thermodynamically metastable glassy state, that is, below its characteristic glass transition temperature. Under these circumstances the 'plasticizing' effect of a small level of sorbed water can be quite profound and may be enough to cause major changes in the physical or chemical integrity of the material. The effects of sorbed water are usually accompanied by a depression of T_g in those materials where this transition can be detected [42], and by a shift to lower temperatures of processes that occur above T_g. The effects of water on the T_g of poly(vinylpyrrolidone), elastin and poly(asparagine) are shown in Fig. 5. Proteins that have been rapidly dried (e.g. lyophilized, spray dried) absorb significant amounts of water vapor and the increase in their water content also appears to facilitate molecular motions that are otherwise only accessible at high temperatures [13,43,44].

2.4. Water vapor sorption and structural changes

With the effects of absorbed water vapor on the structure of amorphous peptide and protein materials in mind, it is appropriate to consider two theories that have been developed to describe the sorption of vapors by macromolecules when sorption concurrently causes changes in the physical structure of the sorbent, one of which was specifically developed to describe the sorption of water vapor by a protein. These theories each build upon the concept of the water–solid system being analogous to a solution, but they incorporate additional terms to account for the structural changes occurring in the macromolecule as its water content changes [45]. In each case there is an implicit assumption of pseudo-equilibrium conditions and a thermodynamic driving force. The approach of Vrentas and Vrentas [46,47] was originally developed to describe the sorption of organic vapors by non-polar polymers, and was then applied to water sorption by more hydrophilic pharmaceutical polymers [48]. The theory considers the changes in the free volume of the polymer as it is hydrated to be critical and the magnitude of those changes are estimated from the plasticizing efficiency of the sorbed water vapor. This leads to an equation that appears as a modified version of the Flory–Huggins polymer solution equation (Eq. (1)):

$$p/p_0 = \phi_1 \exp\{(1-(1/x))\phi_2 + \chi\phi_2^2\} \exp f \quad (2)$$

where ϕ, $\times$, and χ have the same meaning as in the Flory–Huggins equation (Eq. (1)). The final exponential term in this equation (exp f) incorporates all the terms which quantify the effect of the sorbed vapor on the structural properties of the amorphous matrix:

$$f = \{M_1 w_2^2 (C_{pg} - C_p)(dT_g/dw_1)((T/T_g) - 1)\}/RT \quad (3)$$

where M_1 is the molecular mass of the penetrant, w is the weight fraction of penetrant (1) or matrix (2), (C_{pg} − C_p) is the difference in heat capacity above and below the transi-

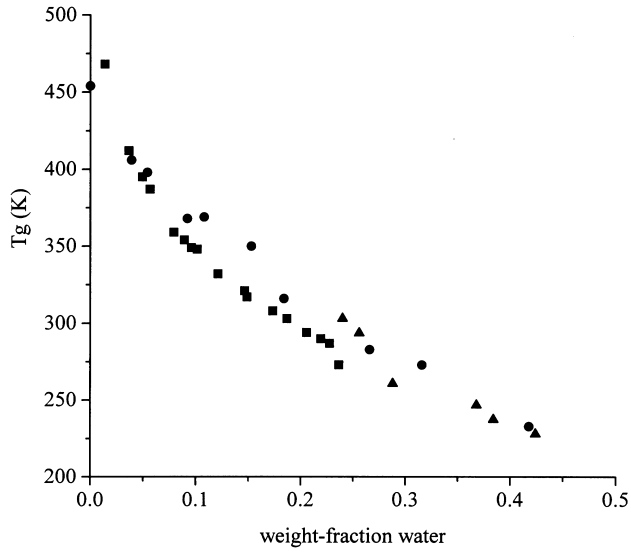

Fig. 5. Glass transition temperature as a function of water content for poly(vinylpyrrolidone) (●), elastin (■) and poly-L-asparagine (▲). Data from Oksanen and Zografi [72], Kakivaya and Hoeve [68] and Green et al. [37].

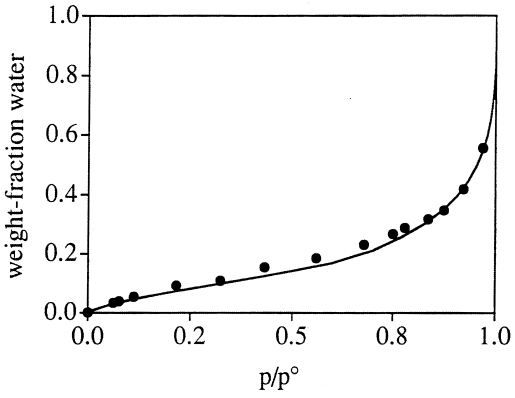

Fig. 6. Predicted (solid line) and experimental (●) water vapor sorption isotherms at 25°C for poly(vinylpyrrolidone) using the method of Vrentas and Vrentas [46].

tion region, dT_g/dw_1 is the change in the transition temperature with penetrant weight fraction, T is the operating temperature, T_g is the glass transition temperature for the pure solid, and R is the ideal gas constant. The agreement between the a priori prediction and the experimental data for a high molecular weight grade of poly(vinylpyrrolidone), a synthetic homopolymer which has been used as a model protein by previous workers, is extremely good over the entire range of humidities (Fig. 6). The predictions are also very good for other more hydrophobic synthetic polymers (e.g. poly(methylmethacrylate)) and macromolecular sugar derivatives (e.g. polysucrose). Since several of the key assumptions of this theory are the same as those used to develop the Flory–Huggins' theory, the model would not be expected to work a priori with polar aqueous systems which experience significant hydrogen bonding. The reason for the agreement between the theory and experimental data is most likely due to a cancelling out of various non-idealities. A similar explanation has been proposed for the unexpected agreement between glass transition temperature data for water–solid systems and glass transition behavior predicted by ideal mixing theories [49]. The experimental data required to use this model to predict the sorption of water vapor by proteins are not yet widely available but could be generated for some well defined model systems without too much difficulty. As with synthetic polymers, the major experimental challenges are likely to be encountered in determining the critical temperature for structural change as a function of water content, and in independently determining the penetrant–matrix interaction parameter.

The approach of Rosenbaum [50] for predicting water vapor sorption by a protein pre-dates the work of Vrentas and Vrentas by over 20 years and is based on very similar concepts. To the best of our knowledge this theory has only been used to describe one system, the keratin–water system (Fig. 7), and thus its general applicability is not yet proven. This author described the structural changes taking place upon hydration of the protein by using a compressibility term to estimate volumetric changes induced by water sorption, and by incorporating this description into a Flory–Huggins type isotherm equation:

$$\ln p/p_0 = \ln\phi_1 + \phi_2 + \chi\phi_2^2 - (1/2\beta RT)\boldsymbol{V} \quad (4)$$

where β is the compressibility, $\boldsymbol{V}$ is the differential change in volume with water content, and the other terms are as before. The generation of additional experimental data for other well-defined protein systems will be necessary to test this theory rigorously and to determine its general applicability. In this instance the experimental parameters required to test the theory can be determined from mechanical property measurements and thus may be more easily determined for proteinaceous materials [44,51].

In spite of a sound theoretical basis and some practical success in predicting water sorption isotherms in systems that experience changes in physical structure, 'structural modification' theories will have to be further developed before they can account for all of the structural changes that occur in proteins and peptides as they become hydrated, primarily because of the complex and diverse physical structure of these materials. It is probable that in most protein materials several different modes of protein–water interaction are operating during the water vapor sorption process, and to a different extent for each different humidity and each material. More sophisticated models which can probe the contributions of surface adsorption, specific group interactions, bulk solubilization mechanisms, and structural rearrangements in each different type of protein system are required, but are currently a long way from being realized. Part of the reason for this is the fact that the modes of water vapor sorption vary considerably according to the chemical composition of the protein, and the structural history of the system. The former of these influences is an inevitable consequence of studying a diverse, heterogeneous series of macromolecules which are assembled from a relatively large number of different repeat units, leading to a much more complex chemical composition than is encountered with conventional organic or polymeric systems. An additional complication of this feature is that

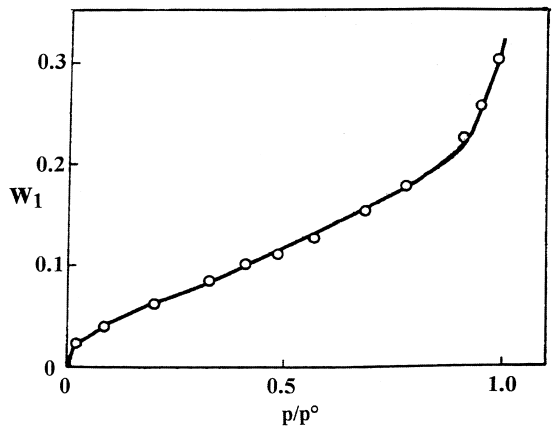

Fig. 7. Predicted (solid line) and experimental (○) water vapor sorption isotherms at 25°C for keratin using the method of Rosenbaum [50].

the higher level organization of proteins (e.g. secondary and tertiary structure) is strongly influenced by their unique primary amino acid sequence. One way forward may be to study simple peptides and poly(amino acids) in order to achieve an understanding of the importance of the positioning of amino acids in the protein.

The structural history of protein materials is a key parameter in determining water vapor sorption behavior because it has a profound effect upon the protein's structural response to exposure to water vapor (e.g. relaxation, crystallization, denaturation, collapse). Heat, cold, extremes of humidity, and various processing stresses can cause significant changes in the structure of the material which can alter its response when encountering water vapor in the environment. One means of illustrating the profound effects of structural history on the water vapor sorption behavior of peptide and protein systems is to consider the effects of experimental temperature and sample history on the form of the sorption isotherms. Considerable experimental data have been reported which indicate that in the temperature range from 5 to 70°C the effects on water sorption are small but significant (Table 1) [12]. In almost all cases there is an increase in the amount of water sorbed at all humidities as the temperature decreases indicative of an exothermic sorption process [9,17,45]. This is consistent with calorimetric data for protein hydration [9] and with many of the mechanisms of water sorption that have been proposed including surface adsorption, solution formation, structural relaxation, and specific interactions (e.g. hydrogen bonding). Without knowing the exact mechanism of the sorption process it is not possible to accurately predict the magnitude of the shift in the isotherm, and the use of the Clausius–Clapeyron equation to estimate thermodynamic parameters, which has been attempted by some workers [25,52], is not appropriate. A decrease in temperature might intuitively be expected to discourage water vapor sorption by amorphous materials by decreasing their free volume. The opposite direction of the reported isotherm shifts with temperature indicates a significant influence from either specific water–substrate interactions or through the modification of the structure of the amorphous matrix by the sorbed water according to the theories described earlier.

Water vapor sorption/desorption hysteresis is often reported to be particularly pronounced for peptides and proteins [9]. The reasons for this hysteresis in conventional materials have been studied in some detail [47,53] and are generally quite well understood. Hysteresis appears to have four main origins: (1) irreversible covalent binding of the sorbent, (2) capillary condensation in pores, (3) slow equilibration, and (4) relaxation of the sorbate structure. In surface adsorption processes the major causes of hysteresis are capillary condensation and the irreversible binding of the sorbent. For absorption processes ('dissolution' into amorphous solids) the major hysteresis effects come from slow equilibration and structural relaxation of the sorbate upon exposure to the sorbent [47]. The manifestation of this latter phenomenon is often quite striking in sorption kinetic data [45,54]. For proteins there is evidence for irreversible binding of water vapor, slow equilibration between protein and sorbed water, and structural relaxation accompanying water vapor sorption [9,53,55]. Hence it comes as no surprise that hysteresis during the measurement of water vapor sorption isotherms for proteins is often very pronounced [56]. The direction of the hysteresis will be the same whichever of these processes is responsible, but the exact magnitude of the hysteresis may not be easily predicted due to multiple causative mechanisms. It is certainly expected to vary with different proteins and under different conditions because the unique structure and history of each protein will alter the balance of those factors which cause the hysteresis. Such hysteresis can have significant impact in situations where the amorphous protein encounters varying and cyclical relative humidities upon storage.

2.5. Multicomponent formulations

The sorption of water vapor by formulations of proteins and peptides which contain a significant proportion of additives (or excipients) is considerably more complex than that of proteins and peptides alone [11,57]. The most common adjuvants are sugars, buffers and polymers which are added to protect the protein during processing, to improve the stability of the protein in the final formulation, and in the case of lyophilized injectable preparations to ensure a stable, biocompatible solution upon reconstitution [5,6]. These excipients are often in the amorphous state after the preparation of the peptide and protein formulations, usually by lyophilization or spray drying. A molecular level amorphous mixture (or solid solution) may be formed whose water vapor sorption behavior will depend upon the identity and proportion of each formulation component and the nature of any interactions between them [58]. In some instances phase separation may occur upon preparation of the formulation, or one component may crystallize within an amorphous matrix, leading to even more complex water vapor sorption behavior. Sugars are often added for their supposed 'water replacement' properties when the protein or peptide is dried below its native water content [1–6]. The use of sugars to stabilize proteins during freezing and drying stages of processing thus presupposes a strong protein–sugar interaction. This interaction may alter the affinity of these components for water vapor upon subsequent exposure to elevated humidities. It has also been suggested that amorphous additives with a high T_g can be added to protein formulations to decrease the molecular mobility of the amorphous matrix and thus to stabilize the protein or peptide against any chemical or physical instabilities [1–6,59]. This may be appropriate whilst the formulation is protected from environmental water vapor; however, a very hydrophilic amorphous additive may actually promote water vapor sorption upon exposure to higher humidities and thus actually permit a greater degree of molecular mobility

in the protein. Furthermore, exposure of the formulation to high relative humidities may lead to phase separation and/or crystallization of additives and subsequent loss of their protective antiplasticizing effects [60]. The question of how the components of such formulations interact with each other and environmental water vapor is currently being explored in our laboratories, with particular attention being given to predicting the behavior of a formulation from the properties of its individual components [58,61]. Other groups are actively investigating the mechanism of action of sugars and other additives, stabilizers and protectants for proteins and peptides [11,57,59,62] and while the current level of understanding is quite poor, it is anticipated that over the next few years the influence of formulation additives on the water vapor sorption behavior of protein and peptide formulations will become much more completely understood.

3. Conclusions

Biological macromolecules and peptides in the amorphous state sorb significant amounts of water under conditions of ambient temperature and moderate relative humidity in a manner that is qualitatively similar to synthetic amorphous polymers. In this paper we have provided a historical overview of the attempts to predict the water vapor sorption behavior of biological macromolecules using sorption models that are currently available in the literature. These models can be placed into one of three categories: (1) those derived from physical surface adsorption models; (2) models based on stoichiometric relationships between the sorbate and water; and (3) solution models. In general, these models fail to absolutely predict the sorption behavior of proteins and peptides because they lack the needed sophistication to account for the heterogeneous chemical structure of biological macromolecules and the complexity of the interactions of these molecules with sorbed water.

Although the extreme diversity of interactions with water and also the complexity of structural changes that accompany hydration of processed proteins are likely to limit the success of any one model to predict water sorption of proteins and peptides, progress in this area could by made by choosing simpler systems, i.e. peptides, polypeptides, and oligomers, to tailor existing theories to proteins and peptides. Of the three types of models available, a solution model is likely to be the best starting point for predicting sorption behavior of biomolecules. Clearly, a successful model must incorporate terms that account for the specific interactions with water and the accompanying structural changes that occur with varying degrees of hydration.

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