Development of a Mathematical Model for the Water Distribution in Freeze-Dried Solids

Hak-Kim Chan,[1] Kwok-Leung Au-Yeung,[2] and Igor Gonda[3,4]

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Purpose. Development of a mathematical model to provide information about the amount of water associated with a protein and an excipient in a lyophilized product.

Methods. The moisture content of the product and the mass fraction of each component were used to derive a model for the calculation of the mass of water associating with each component. The model was applied to lyophilized formulations of rhDNase containing various amounts of mannitol or lactose. The total water content was investigated by thermogravimetry, crystalline properties by X-ray powder diffraction and water uptake behaviour using a moisture microbalance system.

Results. Calculations based on the model suggest that in a lyophilized rhDNase-mannitol formulation where the sugar is crystalline, most of the water is taken up by the protein. However, in the lyophilized rhDNase-lactose formulation where the sugar is amorphous, water is taken up by both the sugar and protein to a comparative extent. At high relative humidities when the amorphous sugar undergoes crystallization, the model can accommodate such a change by allowing for the formation of an additional crystalline phase.

Conclusions. The rhDNase-sugar formulations show excellent conformity to the model which provides quantitative information about the distribution of water in the lyophilized binary protein-excipient products.

KEY WORDS: water; solids; rhDNase; proteins; lyophilization.

INTRODUCTION

The importance of water affecting the physico-chemical properties including stability of pharmaceutical solids has been widely reported (1,2). Residual water affects the chemical reactivity, physical transformation and mechanical properties of pharmaceuticals, as well as supporting unwanted microbial growth. The importance of residual water in the stability of lyophilized proteins is also well recognized (3–5). Therapeutic proteins are commonly formulated as freeze-dried powders containing excipients. The total water content in lyophilized formulations is usually determined by Karl Fisher titration and thermogravimetric analysis. However, the total water content determined by these techniques does not tell us about the amount of water actually associating with the individual components (i.e. protein and excipients) of the formulation. Such knowledge is crucial to gain a fundamental understanding of how water affects stability of lyophilized proteins. Although not in the pharmaceutical context, moisture transfer among solids within a package was initially studied in dehydrated foods (6). A moisture transfer model was subsequently developed by Zografi and colleagues (7) and Kontny (8) to predict distribution of water in solid pharmaceutical systems containing separate solid entities. Kontny further extended the approach to investigate water permeation into the package (9). The solids in these systems existed as physical mixtures or as separate entities., Our present interest, however, is on solids lyophilized from solutions containing a protein and an excipient. It is not obvious that whether the previous model can be applied to the lyophilised system as the protein and excipient may interact and thus do not exist as a purely physical mixture. We have developed a mathematical model by assuming water sorption by protein to be independent of the excipient sugar, anassumption fundamentally similar to that by Zografi (7). It does not necessarily preclude the possibility of interaction between the protein and excipient, provided that the interaction does not interefere with the water sorption of the individual components. The model is applied to co-lyophilized powders containing a recombinant protein and a sugar excipient at various weight ratios (10), a situation which has never been studied before. This is important if the protein and sugar uptake moisture differently depending on the relative concentration, as the model will then be valid only for certain limited situations.

METHODS

Derivation of the Mathematical Model

For a lyophilized formulation containing two components (e.g., a drug D, and an excipient M), the relationship between the moisture content and the dry mass fraction of the components may be derived as follows.

m_D = mass of drug

m_M = mass of excipient

$m_{water,D}$ = mass of water taken up by durg

$m_{water,M}$ = mass of water taken up by excipient

$m_{water} = m_{water,D} + m_{water,M}$ = mass of water

$$x = \frac{m_D}{m_D + m_M} = \text{dry mass fraction of drug of the sample}$$

MC = moisture content of the sample, defined as

$$MC = \frac{m_{water}}{m_D + m_M + m_{water}}$$

It is straight forward to show that the moisture content (MC) is related to the dry mass fraction of drug (x) through the following relationship.

$$\frac{MC}{1 - MC} = \frac{m_{water,D}}{m_D} x + \frac{m_{water,M}}{m_M}(1 - x) \qquad (1)$$

Now if we assume that water sorption by sugar and excipient occurs independently of each other, the ratios $m_{water,D}/m_D$ and

[1] Department of Pharmacy, University of Sydney, Sydney 2006, Australia.

[2] Department of Chemical Engineering, University of California, Berkeley, California.

[3] Aradigm Corporation, Hayward, California.

[4] To whom correspondence should be addressed. (e-mail: kimc@pharm.usyd.edu.au)

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$m_{water,M}/m_M$ become constants that are independent of x. We simplify Eq. (1) by naming these two constants as

$$\alpha = \frac{m_{water,D}}{m_D} = \text{equilibrium mass ratio of water to drug}$$

$$\beta = \frac{m_{water,M}}{m_M} = \text{equilibrium mass ratio of water to excipient}$$

Substituting in α and β and rearrange Eq. (1),

$$\frac{MC}{1 - MC} = \alpha x + \beta(1 - x) = (\alpha - \beta)x + \beta \qquad (2)$$

This shows that a plot of $MC/1 - MC$ versus x will give a straight line with a slope of $\alpha - \beta$, and a y-intercept of β if the model applies to our system.

Note that this model does not assume any specific physical states of interactions between the components (and water). The fundamental assumption is that water associates only with D and M and not D coupled with M. The key experimental input parameters are dry mass fraction of each component in the formulation and the moisture content of the formulation.

A General Form

For a system with n components (where n is the number of proteins and sugars combined), the general form of the eq. can be obtained as:

$$\frac{MC}{1 - MC} = \sum_{i=1}^{n} c_i x_i = \left(\sum_{i=1}^{n-1} c_i x_i\right) + c_n x_n \qquad (3)$$

$$= \left[\sum_{i-1}^{n-1} (c_i - c_n)x_i\right] + c_n$$

m_i = mass of ith component

$m_{water,i}$ = mass of water taken up by the ith component

$$m_{water} = \sum_{i=1}^{n} m_{water,i} = \text{mass of water}$$

$$c_i = \frac{m_{water,i}}{m_i} = \text{equilibrium mass ratio of water to the } i\text{th component in a single component system}$$

$$x_i = \frac{m_i}{\sum_{i=1}^{n} m_i} = \text{dry mass fraction of the } i\text{th component}$$

MC = moisture content of the sample, defined as

$$MC = \frac{m_{water}}{m_{water} + \sum_{i=1}^{n} m_i}$$

Equation (3) can be reduced to Eq. (2) for the 2-component system.

$$\frac{MC}{1 - MC} = (c_1 - c_2)x_1 + c_2 = (\alpha - \beta)\,x + \beta$$

For a 3-component system:

$$\frac{MC}{1 - MC} = (c_1 - c_3)\,x_1 + (c_2 - c_3)\,x_2 + c_3$$

The system can be a formulation of a protein with two different excipients, or as our example in this report, a protein with a sugar existing in two physical states, crystalline and amorphous.

Using the same nomenclature system as in eq. (2) with α representing the equilibrium moisture content for protein and β representing that for excipient, the equation for a 3-component system is:

$$\frac{MC}{1 - MC} = (\alpha - \beta_{Lc})x + (\beta_{La} - \beta_{Lc})y + \beta_{Lc}$$

where $\alpha = \frac{m_{water,D}}{m_D}$ = equilibrium mass ratio of water to protein drug

$\beta_{La} = \frac{m_{water,La}}{m_{La}}$ = equilibrium mass ratio of water to *amorphous* excipient

$\beta_{Lc} = \frac{m_{water,Lc}}{m_{Lc}}$ = equilibrium mass ratio of water to *crystalline* excipient

$x = \frac{m_D}{m_D + m_{La} + m_{Lc}}$ = dry mass fraction of drug in the sample

and $y = \frac{m_{La}}{m_D + m_{La} + m_{Lc}}$ = dry mass fraction of *amorphous* excipient in the sample

y may be expressed as

$$y = \frac{m_{La}}{m_{La} + m_{Lc}} \cdot \frac{m_{La} + m_{Lc}}{m_D + m_{La} + m_{Lc}} = \frac{m_{La}}{m_{La} + m_{Lc}}(1 - x)$$

Define

$\gamma = \frac{m_{La}}{m_{La} + m_{Lc}}$ as the amorphous fraction of the excipient

$y = (1 - x)\gamma$

We obtain the following equation for the system with one protein and one sugar in two physical states.

$$\frac{MC}{1 - MC} = (\alpha - \beta_{Lc})\,x + (\beta_{La} - \beta_{Lc})\,(1 - x)\gamma + \beta_{Lc} \qquad (4)$$

The solutions to the model were obtained by iterative calculations using Microsoft® Excel to obtain the best fit between the experimental data and the model.

Preparation of Samples

Freeze-dried samples were prepared by lyophilizing aqueous solutions containing recombinant human deoxyribonuclease I (rhDNase) alone, and 12.5 mg/ml excipient (mannitol,

AR grade, Mallinckrodt, KY; lactose, ACS reagent grade, Sigma, MO) with varying concentrations of rhDNase, such that the rhDNase content in the dry powders were 100, 80, 50, 17, 9, 2 and 0 wt.%, respectively. Lyophilization was carried out at −20 °C for 35 hr for primary drying, and at 10°C for 8 hr for secondary drying, both at a vacuum of 150 μm Hg.

Physical State Characterisation

The physical state (crystalline or amorphous) of the powders was investigated by X-ray powder diffraction (D/max-B, Rigaku, Tokyo, Japan). The instrumental conditions were: CuKα radiation, 35 kV and 15 mA, angular increment of 0.05 degrees per second with a count time of 2.0 seconds per increment, scanning from 2θ = 10–30 degrees. Silicon powder (640b, National Bureau of Standards) was used as an internal standard.

The initial water content of the freeze-dried samples prior to moisture sorption measurement was determined thermogravimetrically (TGA 7, Perkin-Elmer, Perkin-Elmer, CT) by heating the samples at a rate of 2°C/min under a nitrogen purge.

Moisture Sorption Isotherm Determination

The sorption isotherms of the powders were determined on a moisture uptake balance system (model MB-300G, VTI Corp., Hialeah, Fl) at 25°C in a partial vacuum system. The samples were first dried under vacuum at room temperature (25°C) to constant weight, followed by equilibrating at different relative humidity ranging from ~0.1 to 90% with a step change of 5%RH. Residual water content of the samples at the start of the isotherm run was obtained as the difference between the weight loss of the sample after the initial drying under vacuum and the initial total water content determined by the TGA. A sampling interval of 5 min was used. A weight change of less than 5μg, which was 0.05 wt.% using 10 mg samples, was employed as the criterion for reaching the equilibrium.

RESULTS AND DISCUSSION

Lyophilized rhDNase-Mannitol Formulations

X-ray diffraction indicated that samples with rhDNase content of 17 wt.% or less were partially crystalline as compared to the raw mannitol from the supplier (which was crystalline), while samples having higher rhDNase contents (80 wt.%) were amorphous (Figure 1a). When the samples were exposed to high humidities (75–85% RH) during the moisture sorption study, further crystallization of mannitol occurred in those samples which were initially partially crystalline: The desorption isotherm curve ran below the sorption isotherm curve. On visual inspection, the sorption was accompanied by shrinking of the sample volume. This indicates a solid phase change. Subsequently, X-ray diffraction showed that crystallinity increased in these samples as evidenced both by changes in the diffraction pattern and angular intensity (Figure 1a). In contrast, crystallization at high humidities (up to 90% RH) was not evidenced in the samples that were initially amorphous (i.e., those with 80 wt.% rhDNase content or higher). It is generally believed that moisture-induced crystallisation from the amorphous state as the solid is hydrated results from an increase in molecular

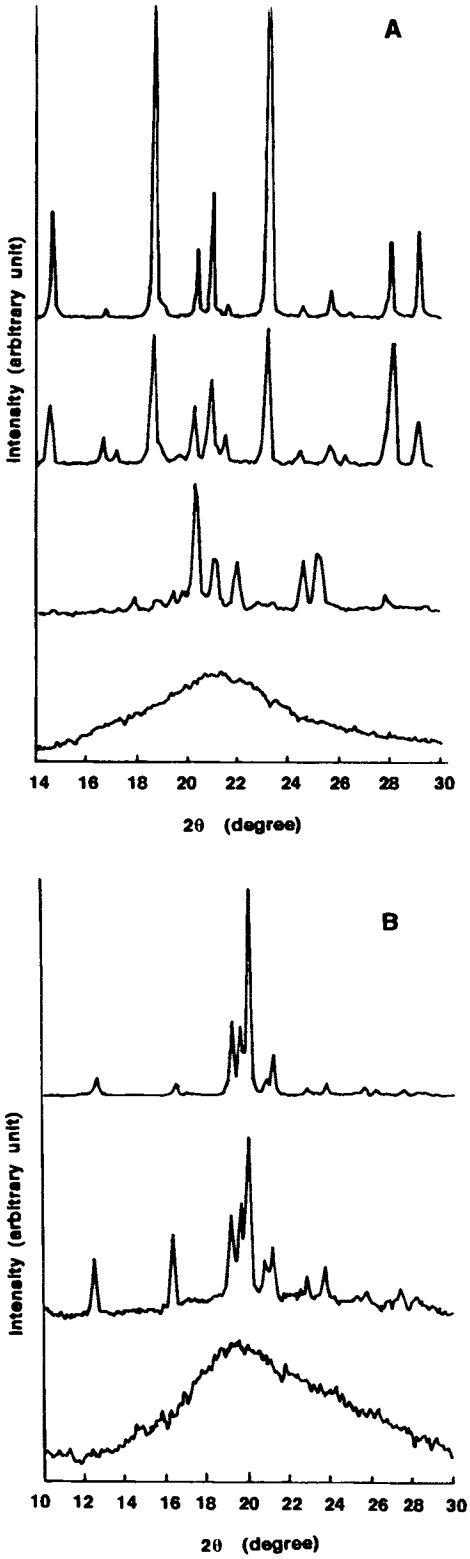

Fig. 1. (A) X-ray powder diffraction patterns of rhDNase-mannitol (from bottom to top: 80 wt.%. rhDNase, 17 wt.%. rhDNase, 17 wt.%. rhDNase after exposure to high humidities in the moisture sorption measurement, mannitol raw material from the supplier is included for comparison). (B) X-ray powder diffraction patterns of rhDNase-lactose 50:50 wt. ratio (from bottom to top: before and after exposure to high humidities in the moisture sorption measurement, lactose raw material from the supplier).

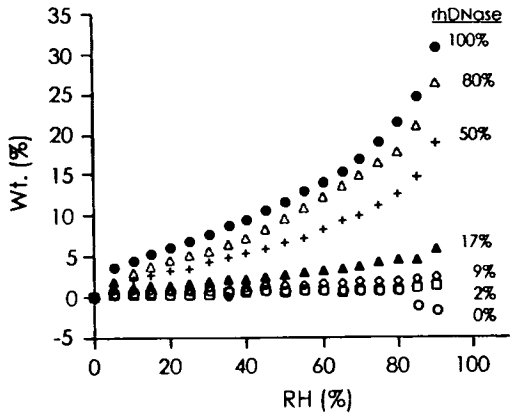

Fig. 2. Moisture sorption data of lyophilized rhDNase-mannitol formulations.

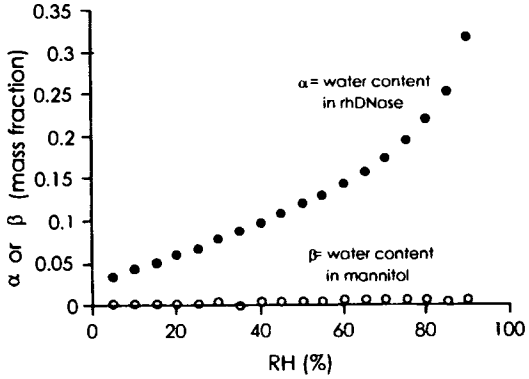

Fig. 4. Plots of α (equilibrium mass ratio of water to rhDNase) and β (equilibrium mass ratio of water to mannitol) of equation 2 *vs* relative humidities.

mobility leading to increased crystal nucleation rates (2). However, the protein present in high quantities may act as a physical barrier to prevent crystallization (11). It has been reported for another protein (bovine somatotropin) that lyophilised formulations of high protein concentration will be more resistant to sucrose crystalliation (12). As expected, pure rhDNase powder did not crystallize; rhDNase crystals require specialized preparation method (13).

Figure 2 shows the sorption data of the lyophilized rhDNase-mannitol powders. The water uptake is predominantly controlled by the protein content as lyophilized mannitol (containing 17 wt.% rhDNase or less), being partially crystalline, is non-hygroscopic until high relative humidities. The linear plots of the moisture content function *versus* the rhDNase content at various relative humidities (Figure 3) indicate that the rhDNase-mannitol systems conform to the 2-component model. Thus, the results are consistent with the model that assumes that the equilibrium mass ratio of water to protein at a given temperature and relative humidity is a constant independent of the proportion of mannitol in the sample. The plots of α (equilibrium mass ratio of water to rhDNase) and β (equilibrium mass ratio of water to mannitol) *versus* RH are, indeed, almost the same as those obtained during water uptake isotherm measurements with pure rhDNase and pure mannitol, respectively (Fig. 4). Consequently, this finding suggests that the water distribution in each component of a powder containing any rhDNase/mannitol ratio can be obtained simply from the water content of pure rhDNase and pure mannitol at a given temperature and RH. Fig. 5 indicates a close agreement between the experimental value and the model prediction of the water content associated with rhDNase.

There is a slight discrepancy between the experimental data and the theoretical model at high RH (Fig. 4 & 5) which is due to crystallization of mannitol, as discussed above. The presence of both amorphous and crystalline mannitol in the sample can be dealt with theoretically using the 3-component model. This will be illustrated quantitatively in the rhDNase-lactose systems where the water content associated with the excipient is more important in the overall interpretation of the behavior of the powders at different relative humidities.

Lyophilized rhDNase-Lactose Formulations

In contrast to the mannitol powders, X-ray diffraction indicated that all the lyophilized rhDNase-lactose samples, including the pure lactose, were initially amorphous (Fig. 1b). Further, all samples containing a lactose content of 50 wt.% or higher crystallized on exposure to ≥60% RH as indicated by a discontinuity (due to water loss on crystallization) in the

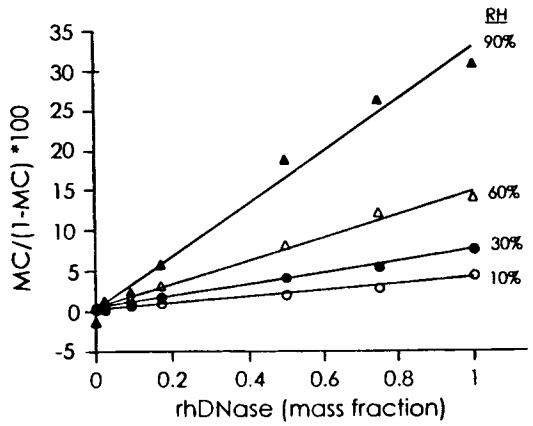

Fig. 3. Linear plots of the moisture content function *vs* the rhDNase content at different relative humidities.

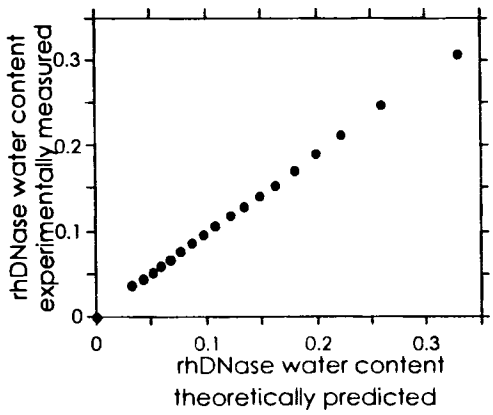

Fig. 5. Comparison between the experimental data and the model prediction of the water content in rhDNase for the rhDNase-mannitol formulations.

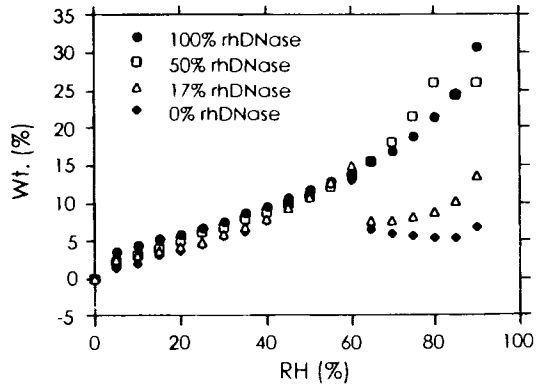

Fig. 6. Moisture sorption data of lyophilized rhDNase-lactose formulations.

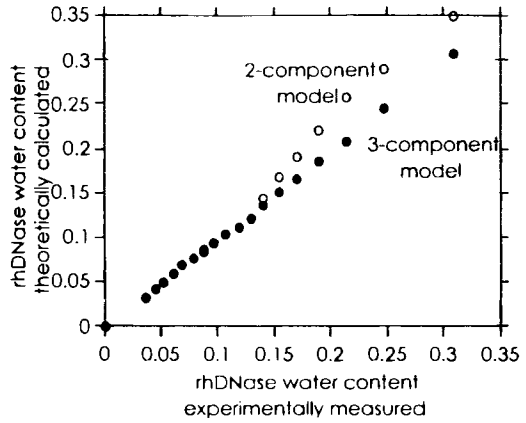

Fig. 8. Comparison between the experimental data and the model prediction of the water content in rhDNase for the rhDNase-lactose formulations.

adsorption isotherm (Fig. 6) with the subsequent desorption isotherm curve lying below the sorption curve. X-ray powder diffraction confirmed that the crystalline state was α-monohydrate lactose (Fig. 1b).

The linear plots (Fig. 7) the moisture content function *versus* the rhDNase content indicate that the rhDNase-lactose systems conform to the 2-component model at low RHs (20 & 40%). At higher RHs (≥60%) where discontinuity due to crystallization occurs, the plots are no longer linear. This, however, can be interpreted using the 3-component model by taking into account the presence of both the crystalline and amorphous phases of lactose (see Model). The assumptions we used in this situation are:

(i) Before crystallization occurs, all lactose is in the amorphous state, thus the parameter γ in equation (4) equals to unity.

(ii) For high RH (≥ 60%) where crystallization cannot be ignored, all crystalline lactose is assumed to be in the form of monohydrates, i.e. $\beta_{La} = MW_{water}/MW_{lactose} = 0.05109$, and γ is allowed to vary.

Both of these assumptions are necessary for the iterative computation to converge since the problem would be underspecified otherwise. As discussed above, both assumptions are valid as supported by X-ray diffraction. Fig. 8 shows that the rhDNase-lactose conforms well to the 3-component model. The results show that in a system containing a crystalline phase (crystalline sugar) and two amorphous phases (protein and amorphous sugar), the water content in each component can be obtained from the 3-component model.

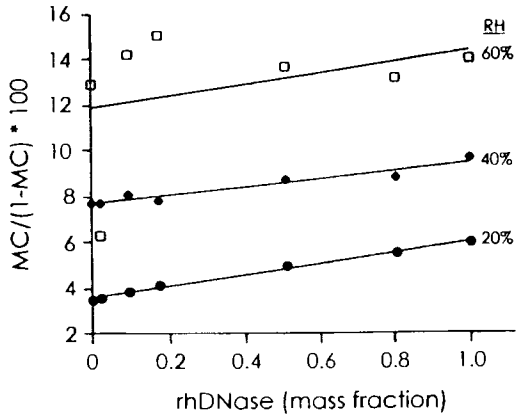

Fig. 7. Plots of the moisture content function *vs* the rhDNase content at different relative humidities.

In contrast to the rhDNase-lactose system, the 2-component model still gives good agreement with the experimental results in the rhDNase-mannitol models. This is because much of the mannitol in the initial samples with low protein content was partially crystalline and this form of mannitol does not take up water until high relative humidities. Thus, the water in the rhDNase-mannitol samples was mainly associated with rhDNase which is the amorphous phase. The finding is in close agreement with the detailed observations in water uptake by amorphous and crystalline sucrose and raffinose as reported by Zografi and colleagues (14,15).

This study has implication for the storage conditions for lyophilized or spray-dried proteins. For example, if a protein is formulated with an amorphous sugar and stored in a sealed container, moisture migration from the sugar due to crystallization may cause stability problems for the protein. Based on this model, at constant temperature storage, water would distribute according to the water affinity of each individual components. No crystallisation would occur unless the water content inside the container is raised e.g. because of moisture permeation or seal leakage in a high humidity environment. At elevated temperature, due to increased molecular mobility crystallisation is expected to occur at lower RH (16). During temperature cycling at the elevated temperature, crystallisation of the sugar could lead to physical instability of the protein-sugar formulation, depending on the RH inside the container. As the amorphous sugar crystallises it expels its sorbed water which would be taken up by the protein in the sealed container, possibly causing chemical instability of the protein. It is thus no coincidental that lyophilised proteins are generally kept at low temperature for storage. Lastly, it is worth to note that although not our objective, the 3-component model could be used to predict the extent of sugar crystallization (from the parameter γ).

CONCLUSIONS

A mathematical model was developed based on the assumption that moisture sorption by proteins and sugars occurs independently of each other. The model can be applied to provide quantitative information about the distribution of water

in a solid state formulation containing multiple components. Results in the rhDNase-mannitol and rhDNase-lactose systems show excellent conformity to the model.

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