Technical paper

Determination and Modelling of Moisture Sorption Isotherms of Chitosan and Chitin

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Abstract

Moisture sorption isotherms of chitosan and chitin in granular powder form were determined at 4 °C using the static desiccators' technique. Ten saturated salt solutions were used to provide constant relative humidity environments. The isotherms were found to be sigmoid type and of BET classification II. The GAB model was used to fit the experimental data, this model was found to be adequate for accurate prediction of moisture sorption isotherm of chitosan and chitin with high value of coefficient of determination (R^2) and lower values of root mean square (*RMS(%)*) and mean relative percentage (*E(%)*). The value of monolayer moisture content of chitosan and chitin varied according to the method of regression used**.** The performance of the regression method depends on the product used.

Keywords: Sorption isotherms, water activity, GAB equation, modeling.

1. Introduction

Chitin is the second most abundant natural biopolymer after cellulose and is a β (1→ 4) linked glycan, but is composed of 2-acetamido-2-deoxy-β-D-glucose (N-acetylglucosamine), which is one of the most abundant polysaccharides named polyβ (1 → 4)-2-acetamido-2-deoxy-D-glucose.[1] Chitosan is the name used for low acetyl substituted forms of chitin and is composed primarily of glucosamine, 2-amino-2-deoxy-β-D-glucose, known as (1 → 4)-2-amino-2-deoxy-(D-glucose). Chitosan has three types of reactive functional groups; an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively.[2] Chemical modifications of these groups have provided numerous useful materials in different fields of application. Chitin is the major structural component of the exoskeleton of invertebrates and the cell walls of fungi.[3] The use and the application of their derivatives in different field is interesting.

Chitin and its deacetylated form, chitosan, have been of interest in the past few decades due to their potential broad range of industrial application.[4] In that sens, these biopolymers offer a wide range of unique applications including bioconversion for the production of value-added food products,[5] preservation of foods from microbial deterioration,[6] formation of biodegradable films,[7] and purification of water.[8] Chitosan inhibit the growth of several fungi,[9,10] and can be used as water absorbent or film for modified atmosphere packaging of horticultural commodities.

The important developments related to foods and pharmaceutical applications of chitinous products necessitate the determination of their isotherm sorption. These sorption properties (equilibrium moisture content, monolayer moisture, heat of sorption) are essential for the design and optimization of many processes such as drying, packaging and storage.[11] Moisture sorption isotherms of chitin and chitosan, in powders forms, are of particular importance in the design of a food dehydration process, especially in the determination of a drying end point which ensures economic viability and microbiological safety. They give important information about the physical properties of powders. The measurement of physical properties of powder is important because these intrinsically affect its behaviour during storage, handling and processing.[12] In order to optimise quality attributes in new food formulations, data on physical properties including equilibrium moisture content are needed which may be expressed in moisture sorption isotherms.[13]

The moisture sorption isotherm of products can be described by numerous mathematical models with two or more parameters. However, models with more than three parameters are too complex for interpretation or use.[14] Among the most efficient equations for prediction of the equilibrium data, the GAB equation can be mentioned. The main advantage of GAB model are its need for only three parameters (with physical meaning) and is suitable for water activity up 0.9.[15–18]

The objectives of the present work were to determine the adsorption isotherms of chitin and chitosan over a wide range of values for relative humidity (0–100%), evaluate the suitability of the Guggenheim-Anderson-Boer (GAB) equation in describing the sorption isotherms, determine the critical storage parameters such as monolayer moisture content, and select the best regression method used for fitting performance of the GAB equation.

2. Materials and Methods

2. 1. Materials

Crab-shell chitin (purity = 95%) and chitosan used throughout this work were purchased from ICN In. (Cleveland, OH). Chitosan deacetylation degree was 92% with molecular weight 600 kDa. They were in granular powder form.

2. 2. Water Sorption Isotherm

2. 2. 1. Experiments

The water sorption isotherms were determined by gravimetric technique, in which the weight was monitored discontinuously within a standard static system of thermally stabilized desiccators. This method was recommended by the COST 90 project.[19] 2 ± 0.001g of samples were placed in a petri-dish inside desiccators. Standard solutions of lithium chloride (a_w = 0.11), potassium acetate (a_w = 0.24), magnesium chloride (a_w = 0.34), potassium carbonate (a_w = 0.43), calcium nitrate (a_w = 0.56), sodium nitrite (a_w = 0.65), Sodium chloride (a_w = 0.75), potassium chloride (a_w = 0.88), potassium nitrate (a_w = 0.96), potassium sulphate (a_w = 0.98) were used to maintain the specified relative humidity inside the desiccators at constant temperature (4 °C).[20] For the water activity of 0 and 1, the desiccants and distilled water were used respectively. At high relative humidities (aw > 0.7), toluene (1.5 ml) was placed in desiccators to prevent microbial growth.The prepared desiccators were kept in temperature controlled cabinets at constant temperature of 4 °C ± 0.5. The samples were weighed within interval 24 h, and were allowed to equilibrate until there was no discernible weight change, as evidence by constant weight values (± 0.001 g). The samples were equilibrated for approximately 4 weeks to reach a constant weight.

The total time required for removal, weighing and replacing the samples in desiccators was approximately 25 s. This minimized the degree of atmospheric moisture sorption during weighing. Each experiment was carried out in triplicate. The dry mass was determined gravimetrically by drying in a convectional oven at 105 °C for 8–10 h.[21]

2. 2. 2. Modelling of Moisture Sorption Isotherm

Several isotherm equations have been proposed for the correlation of the equilibrium moisture content with the water activity (a_w) of food products.[14] Among the most efficient equations for the prediction of the experimental data, the GAB can be mentioned. The main advantages of the GAB model are its viable theoretical background, need for only three parameters (with physical meaning) and its capacity to describe the sorption of water vapour in foods up to water activity 0.9 by just multiplying the activity by a constant less than unity.[15–18] The GAB equation is normally written in the following form:[22]

$$M = \frac{M_o C K a_w}{\left[(1 - K a_w)(1 - K a_w + C K a_w)\right]} \tag{1}$$

where M is the moisture content of material on a dry basic (g/100g d.b.), C is the Guggenheim constant related to heat of sorption, a_w the water activity, K is the constant related to multilayer molecules properties and M_o is the moisture content of monolayer in BET theory (g /100g d.b.).

After transformation, the GAB equation has an equivalent form to the Hailwood et al.[23] equation which is also called the single-hydrate sorption model:

$$a_w / M = \left(b_3 a_w^{\ 2} + b_2 a_w + b_1\right) \tag{2}$$

from the parameters b_1, b_2, b_3, the values of K, C and M_o were calculated through the following relations:

$$K = \frac{\sqrt{b_2^{\ 2} - 4 b_1 b_3} - 2}{2 b_1} \tag{3}$$

$$C = \frac{b_2}{b_1 K} + 2 \tag{4}$$

$$M_o = \frac{b_2}{b_1 K C} \tag{5}$$

The root mean square *RMS(%),* recommended by Maroulis et al.[24] is the conditional standard deviation of the dependent variable and has the form:

$$RMS(\%)=\sqrt{\frac{\sum\left[\left(M_{\exp}-M_{pr}\right)/M_{\exp}\right]^{2}}{df}}*100 \qquad (6)$$

where M_{exp} and M_{pr} are the experimental and predicted values, respectively and *'df'* is the degrees of freedom of the fitting equation. The number of degrees of freedom as follows N-n_p where N is the number of data points and n_p is the number of parameters.[20]

The mean relative deviation *E(%)* is an absolute value that was used because it gives a clear idea of the mean divergence of the estimated data from the measured data:

$$E(\%)=\left[\sum_{n=1}^{n}\frac{\left|M_{\exp}-M_{pr}\right|}{M_{\exp}}\right]*\frac{100}{N} \qquad (7)$$

The mean relative percentage deviation modulus *E(%)* is widely adopted throughout the literature, with a modulus value below 10% indicative of a good fit for practical purposes.[14]

In general terms, high values of R^2**,** low values of *RMS(%)* and *E(%)* mean that the model is able to explain the variation in the experimental data.[22]

2. 3. Surface Area

The surface area σ (m^2/g) was determined from the monolayer moisture content, using the following relationship:[25]

$$\sigma = (A_{H2O}N_{Avogadro}M_0)/\ M_{H2O} = 3530M_0 \qquad (8)$$

σ = the specific surface of the solid in (m^2/g d.b.); M_0 is the moisture content of the monolayer in (g/100g d.b.); M_{H2O} is the molar mass of water (18 g/mol); $N_{Avogadro}$ is Avogadro's number (6 × 10^{23} molecules/mol) and A_{H2O} is the water molecule surface area (1.06 × 10^{-19} m^2)

2. 4. Regression Method and Statistical Studies

The isotherm fitting were obtained by extrapolation using the single-hydrate sorption model.[19] The data were transformed by dividing the water activity (a_w) with the equilibrium moisture contents (M) earlier. A quadratic curve of polynomial function was obtained from these data points. A graphical illustration of the ratio a_w/M against activity water (a_w), using the polynomial regression, is shown and was deduced the values b_1, b_2, b_3 (table 2) and R^2 obtained. The statistical proportion of variation, R^2, is used to explain the regression line whereby higher or lower R^2 would indicate the goodness of fit to regression line.

The non linear regression using the method of evaluation based on the parameters bound by a non linear relation by the method of Newton, permitted to determine the constants. The single-hydrate sorption model is the simplified form of GAB. Its can easily be used.

The goodness of fit for each isotherm was quantified through three standards: the correlation coefficient R^2, the root mean square *RMS(%)* and the mean relative deviation *E*(%).

3. Results and Discussion

3. 1. Experimental Sorption Isotherms

Figure 1 shows moisture adsorption isotherm of chitin and chitosan at 4 °C. The water sorption isotherms are of sigmoid shape and of BET type classification II. The determination of sorption at 4 °C is necessary for their use in modified humidity packaging of several fruits and vegetables as the hygroscopic materials. The values of equilibrium moisture content (EMC) in the water activity range from 0.11 to 0.75 were found to be varying between 5.86 to 18.24 and 2.36 to 12.10 g/100 g d.b. for chitosan and chitin, respectively. At higher level of water activity (a_w = 1) the EMC was observed to vary in range 18.24 to 54.60 and 12.10 to 38.75 for chitosan and chitin, respectively.

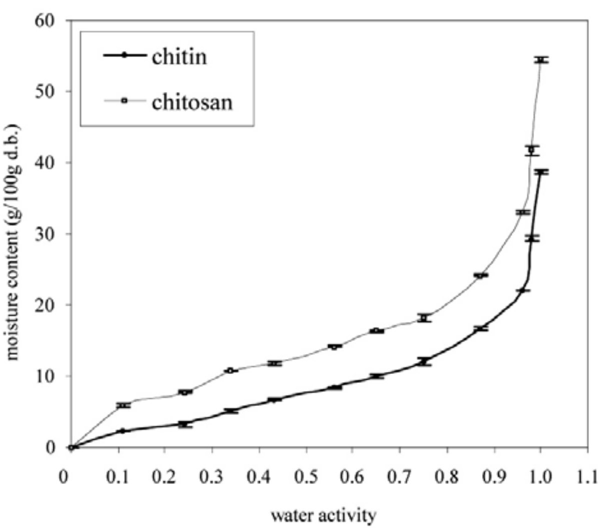

Figure 1: Comparison of experimental moisture sorption of chitosan and chitin at 4 °C.

Chitosan was more hygroscopic than chitin. Chitosan can carry a large number of amine groups on its chain, while chitin has a large number of N-acetyl groups on its chain. The deacetylation of the chitin destroys its residual crystallinity and increase the accessibility of the water in the sites of sorption.[26] The chitosan which is the deacetylated form of chitin is less crystalline than it, therefore more hygroscopic. This result was similar to Kurita et al.[27] work, substituting nonaoyl groups on chitosan. This high hygroscopicity of chitosan would be due to the presence of the pair of free electrons of the amine group supposed to be the origin of the dative bonds. However, there may be interaction due to the simple phenomenon of adsorption, electrostatic attraction or ion exchange. Chitosan absorbs more water because it is more polar (it is a glucosamine polymer with free amino groups). Whereas chitin is a polymer of acetylated glucosamine, i.e., amino groups are acetylated, and hence it is less polar.

3. 2. Modelling of Moisture Sorption Isotherm

Figure 2 and 3 illustrates a comparison of experimental and calculated data for adsorption isotherm for chitin and chitosan at the temperature 4 °C. The results show that at low water activities (a_w = 0.1–0.6) fitted isotherms was much below experimental points. This was similar for Lewicki[28] work on food sorption data.

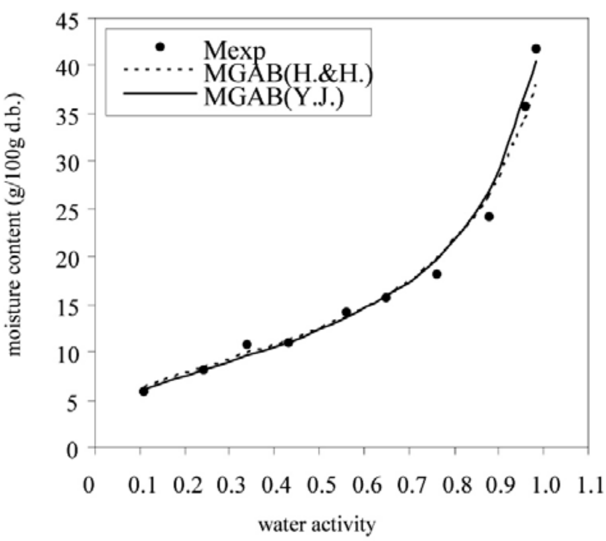

Figure 2: Moisture sorption of chitosan fitted by GAB (non linear regression method (continuous curve), polynomial regression method (discontinuous curve)) model to the experimental data (symbols).

Sorption isotherms of chitin and chitosan were described by the GAB model, and the goodness of the fit was measured by calculation of the mean standard deviation (Mean s.d.), the mean relative deviation *E(%)* and the root mean square *RMS(%)*.

The non linear regression and the polynomial regression were used to fit tested equation to experimental isotherms. Products tested are collected in table 1 and statistical measures were calculated from the equation (6) and (7) were calculated using Excel solver (Microsoft).

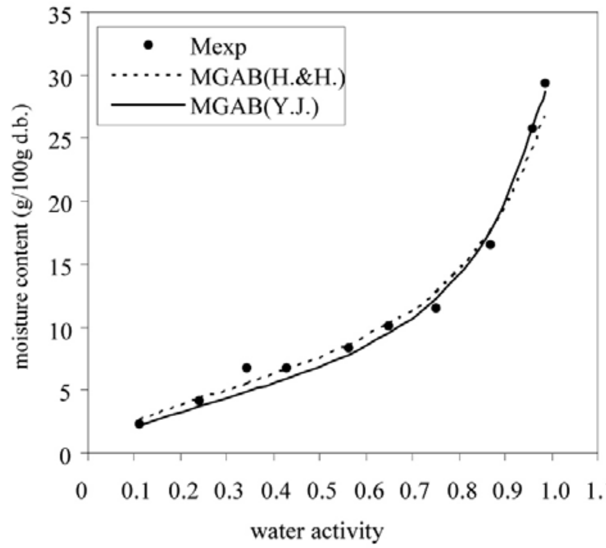

Figure 3: Moisture sorption of chitin fitted by GAB (non linear regression method (continuous curve), polynomial regression method (discontinuous curve)) model to the experimental data (symbols).

Table 1: Mean relative percentage and standard error estimated obtained by the two types of regression.

	E $(\%)_{Y.J.}$	*E* $(\%)_{H\&H}$	*RMS* $\%_{Y.J.}$	*RMS* $\%_{H\&H}$	Mean s.d. (%)
chitin	8.20	6.83	12.80	9.95	21.83
chitosan	4.64	5.53	7.16	7.58	22.50

The evaluation of goodness of fit was as follows: the mean relative deviation *E*(%) and the root mean square *RMS(%).* Examination of results indicates that the GAB equation is able to adequately describe the chitosan and chitin data, with mean relative error and the root mean square *RMS(%)* in the predicted moisture content values (table 1). The coefficients of chitosan are better than those obtained using the chitin. The GAB model fitted the moisture sorption isotherms of the chitosan and chitin, quite well (table 1.). Boquet et al.[29] considered *E(%)* values below 10% as indicating a very good fit for practical purposes. The GAB model has been used successfully by other workers.[30]

Analysis of data presented in table 1 shows that chitosan presents the best fit with *RMS(%)* smaller than *RMS(%)* of chitin. The root mean square *RMS(%)* on the whole are smaller than the mean standard deviation (Mean *s.d.* (%)). The fitting is considered as good.

3. 3. Surface Area

The specific surface is very important in determining the water binding properties of material particles, including the monolayer moisture content. As it's shown in table 2 and 3, the surface area values of the polynomial regression were higher than those obtained by the non linear regression method.

3. 4. Fitting Performance of the GAB Equation

Fitting performance of the GAB equation to sorption data depends on the regression method used. Schar et al.[31] tested fitting of the transformed GAB equation (second degree polynomial) and the ordinary GAB equation (equation 1) on the same data. The tests resulted in larger mean relative errors for the former equation and the authors suggested the use of the latter equation. Maroulis et al.[24] compared direct and indirect non-linear regressions of GAB equation for sorption of dried fruits at different temperatures. They did not recommend using of the indirect method. Samaniego-Esguerra et al.[32] conducted a similar work on dried fruits and vegetables, and Maroulis et al.[24] corroborated adding that the direct method seems adequate and simple to use.

The moisture adsorption data of chitin and chitosan were fitted to GAB model. Tables 1, 2 and 3 show the values of model coefficients and constants fitted to the experimental data along with their R^2, *E(%)* and *RMS(%)*.

Figures 2 and 3 show the predicted values of equilibrium moisture content of chitin and chitosan with two regression methods (non linear regression and polynomial regression) at 4 °C. The fitting of the regression method was based on minimizing average *E(%)* value. It can be seen from table 1 that for the second polynomial regression (table 2), the value of root mean square *RMS(%)* was 7.58 and 9.95, and the relative deviation percentage *E(%)* was 5.53 and 6.83 for chitosan and chitin, respectively. However, for the non linear regression (Newton method) (table 3), the values of root mean square *RMS(%)* was relatively higher with chitin (12.80). The values of relative deviation percentage were lower with chitosan *E* (4.64%). Between the two methods used to fit experimental data, second polynomial regression was found to be best for chitin as exhibited by low *RMS* (9.95%) and low *E* (6.83%).

Table 2: Values of parameters and constants obtained by polynomial regression method for chitin and chitosan at 4 °C.

		chitin	chitosan
parameters	b_1	0,029	0,005
	b_2	0,143	0,122
	b_3	–0,136	–0,103
constants	M_0(g/100g d.b)	5,27	7,69
	C	8,05	32,10
	K	0,82	0,76
	R^2	0,84	0,93
	$\sigma_{H\&H}$(m^2/g)	186,18	271,53

Table 3: Constant obtained by non linear regression method.

	Mo (g/100g d.b)	*C*	*K*	$\sigma_{Y,J}$(m^2/g)
chitin	4,68	7,06	0,86	165,24
chitosan	7,56	25,28	0,83	267,01

Contrary to chitin, the non linear regression was found to be best for chitosan *RMS* (7.16%) and *E* (4.64%). The non linear regression gave the best performance (*E* = 4.64%) of GAB model. This result was similar to some researchers who showed that the non linear method may give better results.[26,24]

There is not meaningful difference between *E(%)* of chitin and the chitosan with the polynomial regression, whereas non linear regression gives a high difference between the *E(%)* of chitin and the chitosan. This meaningful difference would be due to the specificity of non linear regression. This regression, because of its specificity cannot give better results for all products. On the contrary the polynomial regression is standard for most products and gives good results on the whole. We recommend the use of the non linear regression in the case of the chitosan and its derivative products. For the other product the use of the polynomial regression is more adapted. This regression gives the best values of the moisture content of monolayer and the surface area compared to the results of Nadarajah et al.[33]

3. 5. Monolayer Moisture Content

The monolayer moisture content M_0 is recognized as the moisture content according the longest time period with minimum quality loss at given temperature.[34] It corresponds to the amount of moisture adsorbed by a single layer to the binding sites in the product. The value of monolayer moisture content of a product gives an indication of total number of polar groups binding water and the level of hydration, at which the mobility of small molecules become apparent.[35]

Fitting of this model to results is of particular value given the physical significance of the parameters. Table 2 and 3 shows the monolayer moisture content M_o of chitosan and chitin obtained with the two method of regression. The polynomial regression gave the highest value. For the chitosan and chitin, the values of monolayer moisture content determined by GAB correspond respectively to water activity around 0.24 and 0.34 at 4° C. For most dry product, the rate of quality loss due the chemical reaction is negligible below the monolayer value.[36] These values are particularly important in storage of the product, since level the water does not act as a solvent, being biologically inert.

The constants *C* and *K,* which relate to the interaction energies between the water and food, also vary according to the method of regression used. The polynomial regression gave the highest values of *C,* but the values of *K* were smaller.

4. Conclusion

Chitosan and chitin showed the isotherms type II in classification of BET. The water adsorption of chitosan according water activity was higher than water adsorption of chitin. The chitosan was significantly hygroscopic than chitin.

The GAB model can be used to predict the sorption behavior of chitosan and chitin. This model was more applicable to the experimental data of chitosan than to the experimental data of chitin.

The polynomial regression method was more adequate to the chitin whereas the non linear regression applies better to chitosan.

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Povzetek

Z uporabo statične eksikatorske tehnike smo določili adsorpcijske izoterme vlage v granulatih chitosana in chitina pri 4 °C. S pomočjo desetih nasičenih raztopin soli smo pripravili atmosfere s konstantno relativno važnostjo. Dobljene izoterme so sigmoidnega oblike in ustrezajo BET II modelu. Eksperimentalne podatke smo obravnavali s pomočjo GAB enačbe, ki se je za obravnavana sistema izkazala kot najbolj primerna. Ugotovili smo, da je delež vlage, za katerega lahko predvidevamo adsorpcijo v eni plasti, odvisen od uporabljene regresijske metode.