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WATER SORPTION ON BIOLOGICAL MATERIALS AT LOW WATER ACTIVITY

by

Etienne CORTET

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Master of Science
IN
Food Engineering

Department of Food Science

EDMONTON, ALBERTA
Fall 1986

ISBN 0-315-32575-5

THE UNIVERSITY OF ALBERTA

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DEGREE FOR WHICH THESIS WAS PRESENTED MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED FALL 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **WATER SORPTION ON BIOLOGICAL MATERIALS AT LOW WATER ACTIVITY** Submitted by Etienne **CORTET** in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** in **FOOD ENGINEERING.**

....
Supervisor

....

....

Date...July 24, 1986.......

To Fabienne and Grégoire

ABSTRACT

At present no sorption methodology is fully reliable for the determination of water adsorption isotherms in the low a_w range (0-0.1) and also for the determination of water adsorption isotherms of low capacity products (less than 0.1%H_2O/d.b. on the 0 - 0.5 a_w range. In consequence, the suitability of a volumetric technique for the precise determination of water sorption isotherms has been studied. The volumetric technique is based on the measurement of volumes, temperatures and pressures for the determination of the adsorbate uptake from the sample using the perfect gas law.

This study includes the design, calibration, methodology and applications of a volumetric sorption apparatus for the determination of water sorption isotherms of biological materials.

The precision and accuracy of the equipment, determined through water adsorption measurements on microcrystalline cellulose (reference material), are respectivelly ±0.14 and less than ±0.1% H_2O/d.b..

THe application of the method includes the surface characterization of low moisture capacity materials (Biologically active immobilized molecules) and the determination of the net isosteric heat of water sorption

on potato starch in the range 0-0.1 a_w.

The volumetric technique allows the determination of very low levels of adsorption (less than 0.1% H_2O/d.b.(dry basis). However, the interpretation of sorption data for the characterization of active sorption sites requires certain assumptions.

The precise determination of adsorption isotherms of potato starch at different temperatures in the 0-0.15 a_w was possible with the use of an integral method of adsorption. It was found that the net isosteric heat of water sorption of starch is constant for the primary bound water. the value of 581 kJ/kg water for the net heat of sorption is similar to the values obtained from calorimetric studies.

ACKNOWLEDGEMENTS

I express my gratitude to my supervisor, Dr. Marc Le Maguer, for his support and encouragement throughout the course of this study.

I gratefully acknowledge the financial support offered by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Alberta Agriculture Reseach Trust (AART), and the Department of Food Science in the form of Graduate Research and Teaching Assistantships.

I would like to thank CHEMBIOMED Ltd. for the technical support in providing the sample products for this research.

Many thanks to my fellow students and other members of the Department of Food Science whose friendship and encouragement meant so much.

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I. INTRODUCTION

Water is the most abundant component of the majority of biological materials and has a dominant influence on many characteristics, especially those relating to texture. The importance of water is not only related to its abundance but more to its state in biological materials and the manner in which it interacts with them.

Because of a world wide interest in the water properties in foods, from both scientists and food processors; in the late 1960's, international research interest groups were founded to study the matter with financial support from governments and institutions (Hallstrom, 1983). Since then, a large amount of work has been published and in the field of water sorption, standard methodologies and procedures are now well accepted.

At present, many reliable techniques are available to study the state of water in biological materials; Nuclear Magnetic Resonance (N.M.R.), Infra-Red (I.R.) spectroscopy, calorimetric and sorption techniques, the latter ones being refered as thermodynamic techniques (Franks, 1975).

Water sorption studies have both practical and theoretical applications. In Food Processing, sorption isotherms are used for a number of purposes in the fields of drying, mixing, packaging and storage. However, from the theoretical standpoint, a severe limitation to the thermodynamic interpretation of sorption isotherms of biological materials arises from the fact that most materials are multicomponent and multiphase systems exhibiting non-equilibrium conditions. This makes numerical values of thermodynamic functions highly questionable (Gal, 1983). In addition, no sorption methodology have been shown to be totally successful for the determination of water sorption isotherms at low water activity levels; that is at moisture levels at which the interactions of water with the solid (primary bound water) contributes the most to the physical properties of the material studied. It is now well known that the physical properties of water near solid interfaces are very different from those of bulk water (density, viscosity, heat capacity). More specifically, vicinal water is thought to possess greater "ice-like" characteristics near interfaces (Etzler, 1982).

It is apparent that no single technique is likely to provide an exact specification of the location, dynamics and energetics of primary bound water, but a careful combination of two or more experimental approaches may give an adequate description of the state and properties of water in complex systems.

Using a thermodynamic approach to the study of water properties in biological materials (sorption measurement), we applied a volumetric technique for the precise determination of water adsorption isotherm in the low water activity range (0-0.15). This technique has been widely used in surface chemistry for specific surface area determinations from adsorption of inert gas on inorganic materials (Gregg & Sing, 1967).

The present work focusses on the suitability of the volumetric technique for the determination of water sorption isotherms. Two applications of the method were studied:

- The determination of sorption isotherms of low moisture capacity products and the characterization of their surface regarding water (active adsorption sites).

- The determination of the net isosteric heat of sorption of starch at low water activity. Starch was chosen due to its abundance in food systems and because it is representative of most of the powdery products encountered in the food field.

II. LITERATURE REVIEW

1. Water and food

1.1. Macroscopic considerations

Moisture is an important factor controlling certain properties of foodstuffs (Kapsalis,1975; Multon & Bizot,1978) and the nature of its interactions with the chemical components present determines the behavior of that food in many processing operations and during storage (Moreyra & Peleg,1981).

Scott (1957) introduced the concept of water activity (a_w); a_w being an important indicator of the water availability for the different deteriorative reactions of foods. It is only since the development of Intermediate Moisture Foods (I.M.F.) that the importance of a_w has been emphasized for both food and preservation (Troller & Christian,1978).

For the food microbiologist, the water activity gives indications of the amount of water in a food which is available for microbial growth. This does not apply only to microorganisms that cause spoilage but also to those which are desirable (e.g. fermented food, cheese ripening). Most microorganisms occuring in food proliferate at high a_w (over 0.9), only a few require a low a_w for growth (Leistner & Rodel,1976).

The concept of water activity enables valuable comparisons in terms of alterations between products of different composition and nature. However, the water activity is not the only factor inhibiting the microbial growth in a food; interactions with the pH, the temperature and preservatives may also influence the phenomenon (Richard-Mollard et al,1982).

Most of the preservation techniques frequently have adverse effects on the organoleptic characteristics of a food. Among the parameters which influence the food degradation (temperature, time of heat treatment, pH, a_w), the water activity is of importance in non-enzymatic browning reactions, the maximum reaction velocity occuring in the intermediate moisture range (0.6-0.7 a_w) (Fox et al,1983); lipid oxidation (Labuza et al,1972) and the protein denaturation. Enzyme activity is also strongly affected by the water activity (Troller & Christian,1978).

The operations carried out in the food processing industry involve heat and moisture tranfers; this is the case in heating, cooling, freezing and drying. In packaging, heat and moisture transfers are also important for the prediction of the storage life (shelf life) of a food. With a knowledge of the physical characteristics of a food product (thermal conductivity, specific heat, bulk density, thermal and moisture diffusivities, water activity) and their interdependance through mathematical modelling (Singh & Lund,1984), one can predict and optimize an industrial process.

1.2 Microscopic considerations

(water interactions in biological materials)

The affinity of water for biological systems is primarely due to its chemical structure. The water molecule is a strongly polar molecule. The dissymetry of the positive and negative charges in the molecule allows the participation of the molecule in up to four hydrogen bonds.

The hydrogen bonds can occur between water molecules and are responsible for molecular aggregates in liquid and solid water. Water molecules can also interact with polar organic groups such as carboxyl, amine, hydroxyl and somewhat less aldehyde and ketonic groups.

The major hydrophilic macromolecules forming the biological materials are the proteins and the carbohydrates. Those compounds are not all dissolved in the constituent water of foods but present numerous polar sites which interact with water in the hydrophilic areas.

The reversible interaction of water with food solids is referred as the sorption phenomenon and embraces both terms; desorption when water is removed (drying) and resorption when water is brought to the solid material (humidification). Because of hysteris phenomena between the two sorption directions, this distinction is somehow necessary (Van den Berg,1981). The nature of the interactions between water and food includes physical adsorption, capillary condensation and the formation of liquid and solid solutions. As a result of the binding, the properties of water in biological materials differ from those of pure water; this affects especially the vapour pressure and thus the water activity.

It is common to distinguish three orders of binding of water depending on the strengh with which water is attached to the solid material.

i. 1st order water.

This water is strongly bound and corresponds to a very low moisture content. The interactions are a result of a surface phenomenon involving hydrogen bonds on polar sites; it is a "localized adsorption" on a rigid substrate where water cannot act as a plasticizer (Van den Berg & Bruin,1981). The enthalpy of vaporization of water in that state is about 10 to 15% higher than that of pure water (Soekarto & Steinberg,1981).

ii. 2nd order water

This class of constituent water is a continuous transition between the first and third order water (Van den Berg & Bruin,1981). Additional molecules of water are superposed on the original ones and are retained by hydrogen bonds of lesser energy; other forces such as dipole-dipole forces, ionic bonds (H_3O^+ or OH^-), Van der walls forces are involved.

3rd order water

The physical properties of this water, also referred as free water, are similar to that of pure water with very little excess heat of binding. The water acts as a solvent and Raoult's law effects can apply. In addition, water penetrates the structure of the material and capillary phenomena (Kelvin effects) occur.

2.Definition of water activity

The notion of water activity is based on thermodynamic principles.

Defined by Lewis (1907), the activity of a component is equal to the ratio of its actual fugacity to the fugacity at a standard state under the same conditions of temperature and pressure.

At ambient temperature, the vapour pressure of water is low and it behaves almost perfectly. Hence fugacities are equal to pressures and it is common to define the water activity as the ratio of the partial vapour pressure of water in the system P_w to the saturation vapour pressure of pure water P_w^* under the same conditions of temperature and pressure.

$$a_w = P_w / P_w^* = \%E.R.H./100 \qquad (2.1)$$

where E.R.H. is the Equilibrium Relative Humidity.

Van den Berg (1981) mentions an investigation by Gal (personal communication) on the difference between water activity and the commonly used equilibrium relative humidity. Gal showed that at ambient conditions, this difference can be neglected; e.g. at 50°C and total pressure of 100 KPa the difference has a maximum of 0.2% (relative), and at 100°C and 500 KPa it is 1% (relative) at most.

For more detail concerning thermodynamic functions, the reader is referred to textbooks on chemical and engineering thermodynamics (e.g. Smith & Van Ness,1975; Van Ness & Abott,1982).

3.Measurement of water activity

Numerous methods and instruments to determine the water activity of foods have been reviewed (Labuza et al,1976; Prior,1979; Troller,1983); they are based on the colligative properties of solutions; freezing point depression determination on liquids and equilibrium relative humidity measurements on liquids and solids.

The freezing point depression method is suitable for water activities above 0.8 (Prior,1979) where aqueous solutions behave almost ideally.

The determination of the equilibrium relative humidity (E.R.H.) valid for both liquids and solids can be made from direct partial vapour pressure measurements (Bizot & Multon,1978; Troller,1983) or from dew point measurements or indirectly by sensors whose physical or electrical characteristics are altered by the relative humidity (Troller,1977; Favetto et al,1983). The graphical interpolation of Landrock and Proctor is also used (Multon et al,1980) and the isopiestic technique using a standard material (protein, microcrystalline cellulose) as a sensor has been successfully used in the high a_w range (0.8 - 0.99) (Vos & Labuza,1974; Fett,1973).

A collaborative study on the calibration of water activity measuring ~~instruments~~ and devices has been done by Stollof (1978) and more recently Schurer (1985) compared the precision and accuracy of sensors for the measurement of air humidity.

The precision of a piece of equipment or method represents the repeatability or the difference between replicates in a series of identical measurements, whereas the accuracy determines the degree of agreement between the "true" and the measured value.

In studies comparing the performances of different equipment and techniques some authors (Labuza et al,1976; Bousquet-Ricard et al,1980) found no significant differences regarding their precision and accuracy. The choice of a method depends strongly on its range of application, price, and conditions of use (vacuum need, calibration frequency); however, indirect methods are the most commonly used procedures in laboratories (Troller,1983).

Although expensive, electric hygrometers are easy to use with a short equilibration time (30 minutes) and cover a wide range of water activities (0.02-0.99 a_w by changing the sensor). An accuracy of less than 1% and a precision of ± 0 to 0.005 a_w can be expected. Some hygrometers are subject to contamination and require frequent calibration for best accuracy.

The performance of the isopiestic technique is comparable to that of the hygrometric one, it is inexpensive but lengthy, requiring 24 hours equilibration time. The accuracy and repeatability of the extrapolation technique (about ± 0.01 a_w) is suitable for routine purposes allowing a water activity determination to within 5 to 10 minutes (Multon et al,1980). The water activity determination by direct measurement of the water vapor pressure along with a good temperature control of the sample (better than $\pm 0.1^{o}$c) can be used as a reference method (Bizot & Multon,1978). This method is rapid (20 to 25 minutes), inexpensive and does not require a particular calibration. The accuracy and precision are reported to be less than 1% and ± 0.004 a_w respectively (Troller,1983).

4.Water sorption isotherm

Water content and solute concentration describe very little about the properties of water in food. On the other hand, water activity and equilibrium relative humidity are good indicators of the availability of the water to participate in reactions (Troller & Christian,1978). When water content and water activity are graphically related one obtains a water sorption isotherm which indicates the nature of the water binding in the product studied.

Most biological materials exhibit a sigmoidal shaped water sorption isotherm (fig 1); type II in the B.E.T. classification (from Brunauer, Emmet and Teller) (Gregg & sing,1967) due to the combination of the three types of interactions of water with the substrate; surface effect, capillary condensation and solvent effect (Raoult's law). Also observed with some materials are the type I and type III isotherms (fig 1).

The type III isotherm is characterized by its convexity to the water activity axis. This trend suggests a tendency for a tetrahedral arrangement of the water molecules to occur in the adsorbed layer (Gregg & Sing,1967). Adsorption forces are weak. At water activities corresponding to the intermediate range (0.6 to 0,8 a_w), the solvent properties of water prevail and a rapid increase in the moisture content is observed.

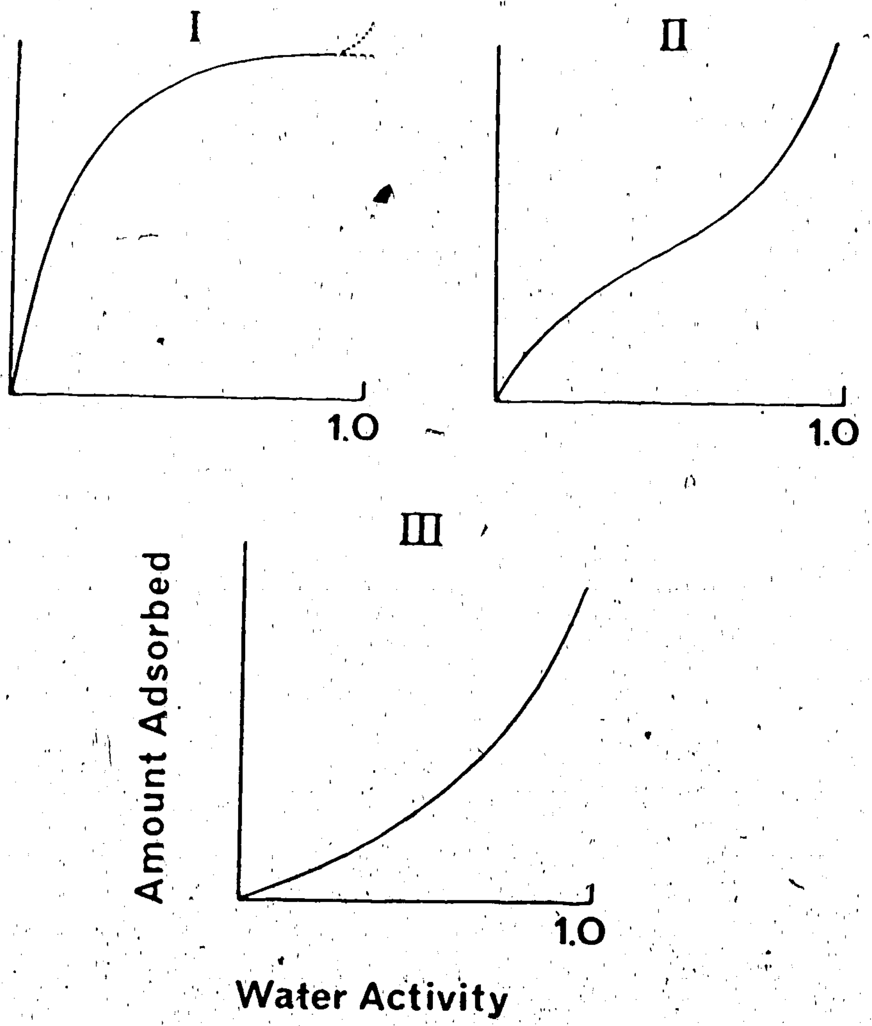


Figure 1 : Three types of adsorption isotherms for biological materials in the Brunauer, Deming, Deming and Teller (also called BET) classification (adapted from Gregg and Sing, 1967).

Pure crystalline substances are typical of type III isotherms. The presence of hydroxyl groups on these substances will promote hydrogen bonding with the molecules of water and one will observe a change from type III to type II isotherm (Gregg & Sing,1967).

The type I isotherm behavior is a result of strong water-substrate interactions. The beginning of the plateau corresponds to the completion of the monolayer capacity where the water molecules form a completely filled, single layer on the surface of the solid. Any subsequent addition of water is a result of weak interactions with the substrate (free water) and one observes a large water activity increase.

5. Sorption models

The study of the fundamental aspect of the sorption phenomenon, drying and storage processes through sorption measurements is necessarily carried out with the use of sorption models.

Any sorption isotherm (sigmoidal curve for most products) could be fitted using a suitable exponential function with a few logarithms and sufficient parameters (Van den Berg & Bruin,1981). However, a good model has to be a simple equation with a limited number of meaningful parameters accounting for temperature dependence and hysteresis effect (Van den Berg,1981).

None of the actual sorption theories are fully satisfactory and this is due, for the most part, to the heterogeneity of structure and complexity of biological materials regarding water.

The first sorption theories were based upon adsorption measurements of inert gas on inorganic materials and accounted for substrates, the surface of which do not change during sorption. It is now well known that this assumption is not valid for the adsorption of water on foods.

Chirife & Iglesias (1978) and Van den Berg & Bruin (1981) published extensive reviews of equations for fitting water sorption isotherms of foods. Van den Berg & Bruin (1981) found altogether 75 isotherms equations from the literature. Those models are either empirical, semi-empirical or theoretical and if they fit reasonably well some specific sorption data, they only concern a part of the water activity range and cannot be applied to all systems (Iglesias & Chirife,1982).

One of the most popular sorption theories is that of Brunauer, Emmet and Teller (1938) which extended the Langmuir theory to second and higher molecular layers of adsorption. The Langmuir and the B.E.T. theory have been extensively reviewed in the literature (Gregg & Sing,1967,1982; Labuza,1968).

In the Langmuir theory, the surface of the solid is seen as an array of identical and independent sorption sites (homogeneous surface), each of which is able to accomodate one molecule of adsorbate only. The process of adsorption is approached kinetically, the molecules of adsorbate continuously condensing and evaporating at the surface of the solid in a dynamic eqilibrium. The Langmuir equation reproduces the general shape of the type I isotherm.

In many food systems, at the monolayer capacity corresponding to a low moisture content, the adsorption is expected to be of a heterogeneous type. Different water molecules will have different energies of binding (Van den Berg,1981). Thus heterogeneous models are preferred to fit water sorption isotherms in the low water activity range. Synthesizing the work of various authors, Van den Berg & Bruin (1981) give an accurate description of the lowest part of the isotherm using the Freundlich equation.

An heterogeneous surface can be seen as an array of infinitesimal patches of different energy that adsorb independently (Langmuir approach). The resultant isotherm correspond to the sum of the Langmuir isotherms for the individual patches, each with its own heat of adsorption.

Brunauer, Emmet and Teller (1938), in applying the Langmuir theory, assumed that the adsorbed water molecules beyond the first ones had the properties of bulk liquid. Like the Langmuir model the B.E.T. model is incomplete and does not account for lateral interactions between adjacent adsorbed molecules.

Although criticized, the B.E.T. model has the ability to fit all sigmoidal shaped isotherms up to about a_w=0.35. In addition the monolayer concept is very useful because of its practical relationship with some aspects of the physical and chemical deterioration of dehydrated foods (Iglesias & Chirife,1976).

A modification of the B.E.T. equation, considered at present as the best isotherm equation for the description and interpretation of food isotherms is the G.A.B. equation (from Guggenheim, Anderson and De Boer) (Van den Berg,1985). The additional constant in the model is a factor correcting the properties of the multilayer molecules with respect to the bulk liquid.

Bizot (1983) developed a computer program based on the G.A.B. model to fit and draw sorption isotherms automatically from experimental data. The model seems to fit any type II isotherms and requires a minimum of four experimental points over the 0.1 - 0.9 a_w range.

Other theories have been developed to describe the sorption phenomenon. They are based on different approaches including a potential approach using the Gibbs equation and a capillary condensation approach using the Kelvin equation. Labuza (1968) reviewed the application of these theories to the sorption phenomenon in foods. The capillary condensation approach predicts the shape of the isotherm in its upper part, but beyond that it is not practical. The description of adsorption isotherms using the Gibbs approach focusses on the properties of the adsorbed film and more specifically the surface pressure of that film. This method is particularly interesting for the lower part of the isotherm to evaluate the site occupation of the adsorbate on the solid. However, it cannot be used for the estimation of the monolayer capacity (Gregg & Sing,1967; Labuza,1968).

6.Experimental determination of complete water sorption isotherms

For the last few decades, the need for sorption data has been recognized in the food field and some related areas and a lot of effort and imagination has been put into developing methods and apparatus for the determination of water sorption isotherms generally (Gal,1975,1981) and at different temperatures (Weisser,1985).

The determination of complete sorption isotherms combines water activity measurements and moisture uptake measurements from the sample.

The methods used for the determination of complete isotherms require a means of maintaining constant water vapour pressure thus constant relative humidities in the space surrounding the sample during the sorption measurement.

6.1.Relative humidity control

6.1.1.Aqueous solutions

Unsaturated solutions, saturated solutions and mixtures of solid hydrates can give relative humidities which are very little affected by the temperature (Wylie,1965).

Salt slurries have been particularly recommended as an inexpensive, easy and accurate way to provide saturated solutions of known water activity as there is no measurement of concentration needed (Young,1967; Stollof,1978): Saturated salt solutions are stable and their water activity is only slightly affected by the temperature variations; 0 to 0.3% R.H./°C depending of the salt (Young,1967).

Despite the apparent stability of most salts at a given temperature, it appears that all laboratories do not agree on the relative humidity of standard salts. Recently Resnik et al (1984) presented a world survey on the matter concerning five selected salts solutions at 25°C. Chirife et al (1983) used a theoretical model based on the thermodynamic properties of electrolytes to predict the water activity of saturated salt solutions in the intermediate moisture range. An exellent agreement was found with the literature values.

Unsaturated aqueous solutions like sulfuric acid-water mixtures have the great advantage of producing innumerable isotherm points (Labuza,1984) and have long been considered as the best way to define known relative humidities.

The thermodynamic properties of aqueous sulfuric acid solutions and hydrates have been correlated from 15 to 300 ^{o}K (Giauque et al,1960). More recently, Ruegg (1980) developed a computer program for the calculation of the water activity of such solutions at different temperatures and acid concentrations.

Due to the hazardous nature of acid solutions and the adsorption of sulfuric acid molecules onto solid surfaces reported by Kawasaky & Kanou (1965), this method is seldom used today.

6.1.2.Bithermal equilibrium method

Relative humidity is controlled by a source of pure water. A change in relative pressure is effected simply by varying the temperature of the water. The highest a_w precision with this method is achieved at low vapour pressures where the temperature dependence is minimal.

With the development of dynamic techniques for the determination of water sorption isotherms, special methods have been described to obtain an air stream of controlled relative humidities; the mixing of two air streams (one dry and one saturated with water vapour) and the bubbling method in which a dry air stream is passed through pure water at a controlled temperature.

6.1.3.Two pressure method

This method is based on Dalton's law. A saturated water vapour pressure is produced in a saturator where the total pressure is above the atmospheric pressure. By isothermal expansion of the mixture (air-water vapour) to the atmospheric pressure, the saturated water vapour pressure becomes unsaturated (Dalton's law) and defines a known relative humidity equal to the ratio of the total pressures before and after expansion. The range of relative humidities to be set with this method is limited to the optimum over pressure attainable in the saturator (Multon et al,1971).

6.2.Methods

It is common to classify the methods used for the determination of sorption isotherms into two groups:

i. methods involving the study of the solid phase

In a humidity controlled atmosphere, the amount of water adsorbed may be calculated from the variations of the weight of the material measured in a static or discontinuous way (gravimetric techniques).

ii. methods involving the study of the gas phase surrounding the sample.

The amount of water adsorbed may be calculated from the variations of the water vapour pressure in a specified volume (volumetric technique) or from a known moisture content product, the water activity can be determined by measurement of the partial pressure of water (manometric technique) or indirectly by the mean of electric hygrometers (hygrometric techniques). The chromatographic technique is a case of dynamic adsorption; the water vapor carried by an inert gas is passed through the sample, used as the stationary phase.

6.2.1.Gravimetric techniques

The gravimetric techniques have been extensively used for the last few decades and are still used today for reference purposes (Wolf et al,1985). The principle of the gravimetric determination of water sorption is identical for all gravimetric methods. The methods are based on the determination of weight changes of samples in equilibrium with different relative humidity conditions.

i.Discontinuous techniques

These methods commonly involve conditioning samples in small enclosures at different known relative humidities. The weight changes of the samples and moisture content calculations are determined by suitable methods in a separate operation.

The oven method (drying of the sample) is the most common method used to determine the moisture content of a material. The chemical method of Karl Fisher is also used but has the disadvantage of destroying the sample. In addition, solid samples have to be ground and a potential water loss can occur during the grinding process.

In a discontinuous method, the samples are generally placed in dessicators over saturated salt solutions or sulfuric acid mixtures as humidity control. This technique has been chosen as a reference method by the COST project 90 and 90 bis on the physical properties of foodstuffs. (COST: cooperation in the field of scientific and technical research in Europe). Details of the method, precautions and procedural advice are presented by Spiess & Wolf (1983) and Wolf et al (1985).

The sorption phenomenon is a slow process and to hasten the hygroscopic equilibrium, it is common to work under a vacuum or by circulating the air in the sample chamber.

ii. Continuous techniques.

The variations of the mass of the sample are continuously monitored, thus the weighing equipment is part of the entire experimental set-up.

In his review on sorption methodology, Gal (1975) put the emphasis on continuous gravimetric techniques with the use of electrobalances (Cahn, Sartorius). Although expensive, these methods allow the use of the microcomputer for data acquisition (Bolliger et al,1972; Gal,1981; Cortet,1983). Electrobalances also allow the use of very small samples. Usually, different mass ranges are available and the mean relative precision of the mass measurements for any ranges is ±0.02%.

The apparatus used are evacuated for a better diffusion of the water molecule in the gas phase.

to avoid the use of expensive features (fittings, pumps, pressure gauges) inherent to the choice of a vacuum technique, some laboratories developed dynamic systems where humid air is circulated through the system. To a certain extent, the circulation of the air enables a better control of the temperature in the vicinity of the sample during the adsorption (exothermic phenomenon). Bolliger et al (1972) et Cortet (1983) studied in details such systems.

Spring balances are relatively cheap to set up and have been shown to be very accurate. The variation of the mass of the sample is measured in terms of a spring elongation with the help of a cathetometer. A maximum deviation of ±0.1% (by weight on dry basis for 100 mg of sample) on the moisture content determination is reported (Weldring et al,1975).

6.2.2.manometric and hygrometric techniques

For obtaining complete sorption isotherms with the aid of direct or indirect vapor pressure measurements, samples with different moisture content must be available.

As it is difficult to achieve an homogeneous distribution of moisture in a given sample, this method is not usually used for sorption isotherm determinations.

6.2.3.Chromatographic techniques

This technique is also referred as Inverse Gas Chromatography (I.G.C.) because the study is carried out on the non volatile or stationnary phase in relation to the mobile phase which contains water (Gilbert,1984). When water is introduced into the carrier stream, its linear transport is retarted by its interactions with the stationnary phase (sample studied) and the mobile phase, thus, the interactions and in particular the sorption of the water by the stationnary phase can be studied. The calculation of the adsorption isotherm is derived from the chromatographic peak shape (Neumann,1976).

This method has been found to be rapid and reliable for the determination of water sorption isotherms on homogeneous solids (e,g, sugar crystals)(Smith et al,1981). Studies of dry bakery products (heterogeneous material) has been recently performed (Helen & Gilbert,1985); they found a reasonable agreement between the sorption isotherms obtained with a conventional method and IGC measurements. Water activity levels down to 0.03 can be obtained with this method (Smith et al,1981).

6.2.4.Volumetric techniques

The volumetric technique is an original method in which the amount of adsorbate uptake from the sample is determined through volume, temperature and pressure measurements using the perfect gas law.

The principle underlying the volumetric technique is as follows: the pressure, volume and temperature of a quantity of adsorbate is measured and the number of moles present calculated. This material is then brought into contact with the adsorbent and, when constant pressure, volume and temperature readings show the system to have attained equilibrium, the number of moles present in the gas phase is again calculated. The difference between the number of moles present initially and finally represents the adsorbate 'lost' from the gas phase to the adsorbed phase.

A study of this technique would be incomplete without a mention of the extensive review of Robens (1968) on volumetric apparatus.

The class of volumetric techniques has been completely ignored in food studies for the last decades as there has been no apparent applications (Gal, 1975). Although, these methods are prefered in surface chemistry research for adsorption of gases (e.g. N_2, Kr, He) on inorganic materials for porosity and specific surface area determination purposes (Young & Crowell, 1964; Ross & Olivier, 1964; Gregg & Sing, 1967). Nitrogen is usually prefered for the determination of specific surface areas greater than 5 m^2/g of material while Krypton which vapour pressure is about 3 mm Hg at liquid nitrogen temperature can be used for the determination of surface areas as low as 50 cm^2/g with a good precision (Fries, 1959). The precision figures given in the literature for this method are generally expressed in term of an error on the surface determination. Precision and accuracy on the amount of adsorbate uptake are chiefly dependent on the pressure, volume and temperature measurements and vary wich each apparatus (range of application, nature of adsorbate and adsorbent).

The volumetric technique is particularly recommended for adsorption studies in the BET relative pressure range (up to 0.35) and in the limit of precision of the pressure readings, innumerable relative pressures can be obtained.

Volumetric techniques have often been used for the determination of the adsorption isotherms of vapours which are condensable at or near room temperature and below atmospheric pressure. However, numbers of problems, not encountered with permanent gases are met here; the adsorbate has to be located in a liquid form in some part of the apparatus and also, because of eventual condensation of the adsorbate on some cooler parts of the system, the entire apparatus need to be temperature controlled.

7. Practical applications of water sorption measurements.

The importance of water sorption isotherms in the food field and some related areas such as pharmaceutical is now well accepted. From a practical point of view, the main field of application of sorption isotherms in food is in food processing where economic considerations are concerned. However, the fundamental aspect of the water sorption phenomenon and the study of the state of water in biological materials have never ceased to intrigue scientists around the world (Duckworth,1983). Table 1 adapted from Gal (1983), summarizes the theoretical and practical uses of water sorption isotherms.

7.1 Food processing

The drying of foodstuffs is one of the most used unit operations in the food industry. With the recent increase of the cost of energy and the constant preocupation for best quality products, emphasis has been placed on the optimization of this operation and finding the so-called "end point" of the drying process, that is the optimum moisture content of the final product for quality, stability and cost. The knowledge of the sorption isotherm is essential to define the final moisture content and the corresponding a_w which will ensure the stability of the product. From a theoretical point of view, if one

TABLE 1

Theoretical and practical use of sorption isotherms
(adapted from Gal, 1983)

THERMODYNAMICS

Sorption - desorption enthalpies

Bound water

STRUCTURE INVESTIGATIONS

Specific surface area

Pore volume / size distribution

Crystallinity

FOOD PROCESSING

Drying

Mixing

Packaging

Storage / air conditionning

knows the moisture content and the surface temperature of a product, the isotherm will give the corresponding a_w then the partial water vapour pressure at the surface and the drying curve can be calculated (Daudin,1983).

Following the drying operation, it is important to know under which conditions the final product has to be stored to prevent any moisture uptake or loss, thus preserving its quality. Laumoro et al (1985) equated the moisture transfer properties of dry and semimoist foods from sorption measurements.

Several authors have compiled water sorption isotherms of foodstuffs; the most recent work is due to Iglesias and Chirife (1982).

The knowledge of the influence of temperature on water sorption isotherms is particularly important in packaging when a foodstuff is sealed in an impermeable material (constant moisture). For most biological materials, a decrease of the hygroscopicity with increased temperatures (Bandyopadhyay et al,1980) is observed. In a sealed package, this will result in an increase in the water activity and eventually a decrease of the shelf life of the product. Some sugars however, present a different behavior and become more hygroscopic at higher temperatures and a_w because of the dissolution of sugar in water.

Mixing operations are common practice in the food industry. When several ingredients are mixed, each ingredient gains or loses moisture to the other in order to attain a state of equilibrium. In food formulation, the achievement of a desired level of water activity is generally a trial and error process. Lang & Steinberg (1980) developed a model for the calculation of moisture content of a formulated food system at any given water activity; they hypothesized that the total moisture content is equal to the weighted average of the moisture bound by each component at a specified a_w. Chuang & Toledo (1976) and Lang & Steinberg (1981) predicted the water activity of multicomponent system of known composition at a given moisture content from water sorption isotherms of individual components. They found a good agreement between the calculated and experimentally determined water activity for binary and ternary systems; however, those calculations assume no interactions between the different components.

7.2.Structure investigation

The specific surface area of solids can be evaluated from sorption measurements. This surface area is directly proportional to the monolayer capacity of the adsorbent.

The determination of specific surface areas from nitrogen adsorption gives a good image of the structure of the product; this is confirmed on starch by the good agreement with the photomicrographic determination

(Hellman & Melvin,1950).

The surface areas calculated from water sorption isotherms are always significantly lower than the nitrogen ones thus confirming that the adsorption of water is not a surface condensation phenomenon but a "localized adsorption" on polar sites as defined by Van den Berg & Bruin (1981) in reference to primary bound water.

The structure of biological products is strongly affected by the level of hydration. In a study on starch, Bizot et al (1981) showed that the crystallinity of the starch granule revealed by X-ray diffraction patterns is significantly dependent on the water content and is modified by the adsorption-desorption state. In addition, the specific gravity decreases at higher water content, accounting for a swelling effect of the lattice.

The majority of biological materials have no rigid pore structure for water which renders any structure investigation difficult. However, useful information can be drawn from water sorption isotherms regarding the accessibility of water molecules to the substrate.

7.3.Thermodynamics

The investigation of the thermodynamics of water-food interactions through sorption measurements represents an important approach in understanding the effects of water on foods (Le Maguer,1985).

Because thermodynamic laws are based on true equilibrium conditions which are rarely satisfied in food systems (heterogeneity of structure, hysteresis phenomenon), some care has to be taken concerning the validity of some calculated thermodynamic functions from sorption data. However, many authors determined the isosteric heat of sorption using the Clausius Clapeyron equation. The use of the Clausius Clapeyron equation requires the determination of sorption isotherms at different temperatures (Weisser,1985).

The knowledge of the enthalpy of sorption gives some indications on the strength of the binding of water molecules to the solid; the binding energy being defined as the difference between the heat of adsorption of water by the solid and the heat of condensation (or evaporation) of pure water at the same temperature.

Soekarto & Steinberg (1981) in an attempt to determine the binding energy of the three fractions of sorbed water, hypothesized that each fraction of water is characterized by a constant binding energy. They calculated the isosteric heat of sorption for the primary bound water from the BET constant despite the recommandations of Iglesias and Chirife (1976) who advised not to use the BET equation for this purpose. The BET heats are systematically lower than the "initial heat" (i.e. very low moisture content) as well as the heats averaged over values of moisture content required for one monolayer. The use of the Clausius-Clapeyron equation is then recommanded for the entire isotherm. However, at low a_w level (0-0.5), there is a lack of experimental data in the literature, and the temperature dependence of the sorption phenomenon is hardly detectable with classic sorption methods.

The calorimetric methods (Simatos & al,1975) which are alternative methods for the study of the state of water in biological materials are not precise at very low moisture level (i.e. small quantities of heat involved). Hence, there is a need for sorption data at low a_w level (0-0.15), with a good precision and accuracy.

III. VOLUMETRIC APPARATUS FOR THE DETERMINATION OF WATER SORPTION ISOTHERMS

The following chapter will discuss the design and calibration of a volumetric apparatus for the determination of water adsorption isotherms on biological materials.

1. Introduction and design considerations

1.1 Essential components

Figure 2 shows a simplified representation of a volumetric apparatus for the determination of water sorption isotherms. It includes four essential components, namely, the sample holder, the dosing volume, the water vapour pressure saturator and the vapour pressure measuring device (manometer). This apparatus can work in both adsorption and desorption and the dosing device can be used respectively to admit or remove measured increments of water vapour from the sample.

As mentionned earlier, the use of the volumetric technique for the determination of sorption isotherms of condensable vapours requires special attention. This concerns the design of the different components of the apparatus, but also the control of the parameters temperature, volume and pressure used to determine the

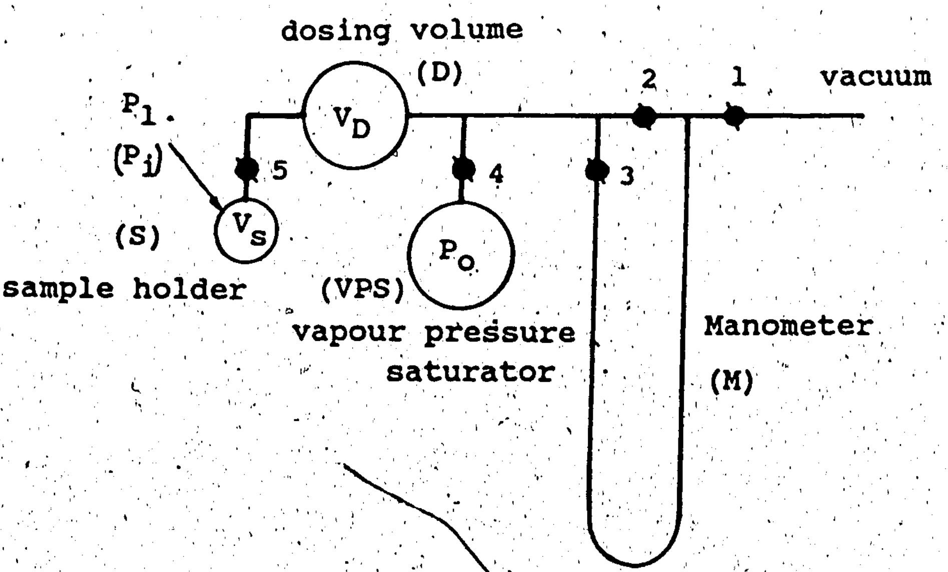


Figure 2: Shematic representation of a volumetric adsorption apparatus for the determination of water adsorption isotherms.

moisture uptake from the sample and the corresponding equilibrium relative humidity.

1.2 Temperature requirements

In ambient conditions, water vapour can condense on any cooler part of the apparatus. Hence the temperature of the entire system needs to be controlled, especially the dead space (sample flask volume) as the sample temperature is used as the reference for the determination of the isotherm. Some apparatus reported in the literature (Gregg & Sing,1967) are totally immersed in a bath with a temperature control better than ±0.05°.

1.3 Vacuum requirements

The determination of water sorption isotherm using the volumetric technique relies on precise measurements of water vapour pressures. Thus the system needs to be free of any volatile compounds which would interfere in the calculation of the amount of moisture adsorbed and the equilibrium relative humidity. A good initial vacuum is then required and as the water supply is located in liquid form in some part of the apparatus, special attention has to be paid to the removal of dissolved air. At low vapour pressure, a very small proportion of dissolved air may give rise to a large partial pressure of air in the vapour phase. Hence, the water to be used is generally pure and degassed by repeated distillation in vacuum. In addition dissolved air and other volatile matter present in the manometric fluid must be pumped out of the instrument before use. A vacuum of $1x10^{-3}$ torr or lower is generally sufficient for such equipments.

1.4 Volume requirements

In estimating the sources of error in the volumetric technique, two points should be emphasized: first the errors in the measure of the quantities of water adsorbed are cummulative, and second, the amount of water remaining in the dead space becomes more important as the pressure increases. For these reasons, special care in the determination of the dosing volume and the dead space volume is required for best accuracy and precision. In this connection , the recommendations of Ross & Olivier (1964) apply; they showed that the reference volume, used for the determination of the dosing volume, should have nearly the same size as the dosing volume V_D. In addition, the dead space volume (sample flask volume V_S) must be kept as small as possible, consistent with the use of tubes wide enough to permit a reasonable rate of pumping.

1.5 Pressure requirements

The determination of water adsorption isotherms at room temperature in the low a_w range (0-0.35) requires precise measurements of pressures often less than 10 mm

Hg. Hence a mercury manometer is not suitable for good precision and it is recommended to substitute an oil of low vapour pressure for mercury. The advantage derived is due not only to the lower density of the fluid, which confers a magnification of about 15, but also to its lower surface tension and low contact angle against glass, which increase the inherent precision of readings of the meniscus (Ross & Olivier, 1964). Some oil manometer are capable of a sensitivity of $1x10^{-3}$ Torr (Gregg & Sing,1967). Because the density of the oil varies with the temperature, a correlation of the oil density with temperature has to be determined.

Since the amount adsorbed represents the difference between the amount admitted to the dead space (sample flask volume) and the amount remaining there at equilibrium, it can be evaluated with confidence, only if these two quantities (measured pressures) are of unlike magnitude. The most favorable situation is then obtained under conditions where virtually all the gas admitted is adsorbed (e.g. at low relative pressure) and the amount not adsorbed is either negligeable or represents a minor correction term (Young & Crowell, 1962). In most cases, with biological materials, as the relative pressure increases, the adsorption isotherm flattens out (type II isotherm) and the addition of further increments of water does not contribute to an increase of the amount adsorbed.

In consequence, the amount unadsorbed becomes commensurate with the total amount of gas admitted. For these reasons, this method is much less sensitive at intermediate relative pressures, especially with adsorbents having low surface/volume ratios (low specific surfaces; less than 5 m^2/g of material).

Following these recommendations, a volumetric apparatus for the determination of water adsorption isotherms has been designed. The final version of the apparatus presented in figure 3 is the achievement of a design evolution which was found the most suitable for later applications.

2. Equipment description

Figure 3 illustrates the experimental set-up used in this study.

The glass portion of the apparatus was built at the University of Alberta (Glass Shop, Technical Services).

The manometer (M) is made from 3 mm inner diameter thick wall glass tubing and is connected to the vacuum line, water vapour pressure saturator (VPS), dosing flask (D), sample flask (S) and a Pirani vacuum gauge (PG) G9 from EDWARDS (EDWARDS Vacuum Components Ltd., England). All the stopcocks used are high vacuum proof manufactured.

The entire equipment is enclosed in a thermally

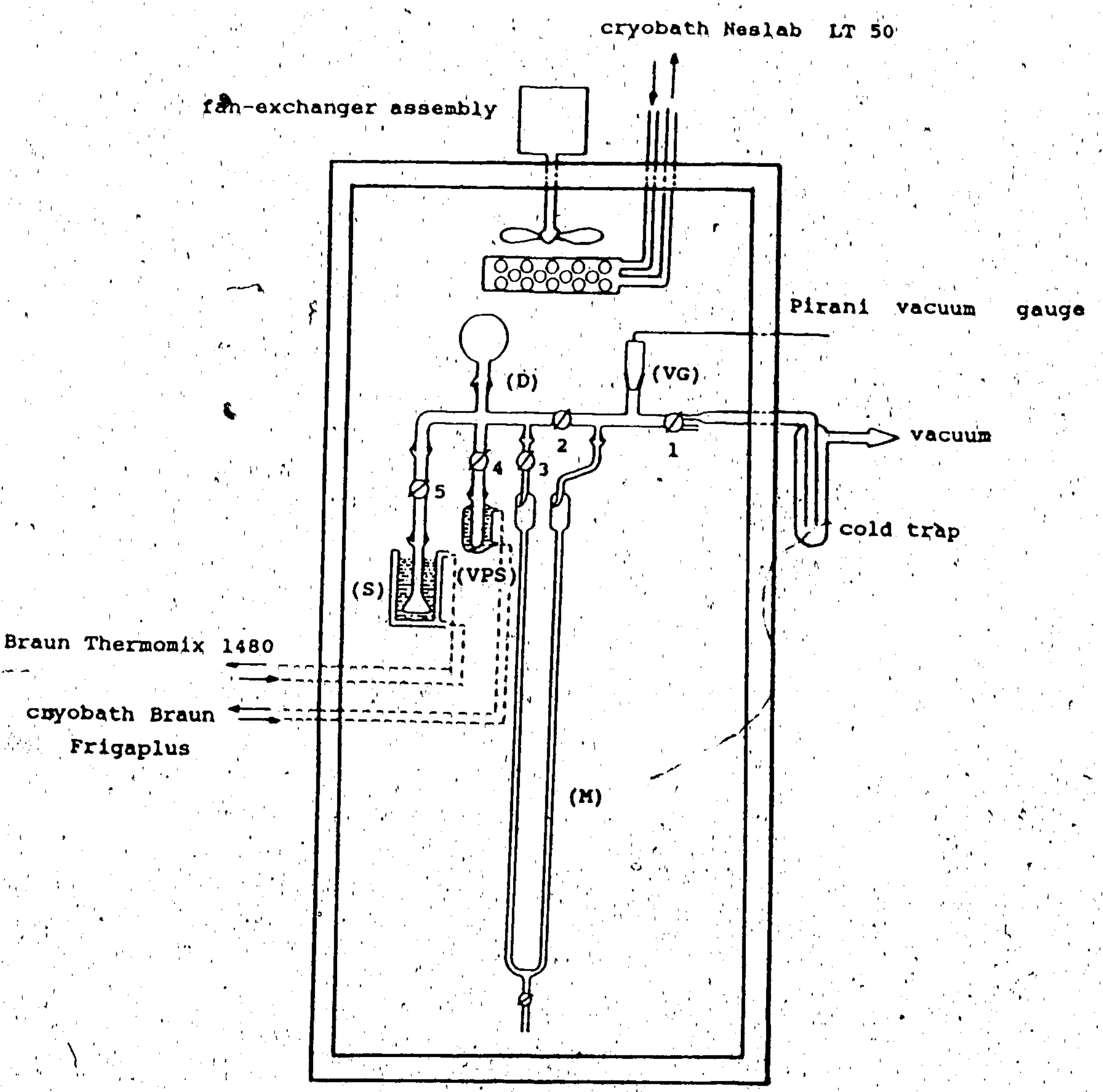


Figure 3: Experimental volumetric adsorption apparatus in the thermostated enclosure

insulated thermostatic enclosure. The thermostatic condition is maintained by an external cryobath NESLAB LT 50 (NESLAB instruments Inc., N.H., U.S.A.) connected to a fan-exchanger assembly in the chamber. The temperatures of the enclosure and the sample are constantly monitored by type T (copper-constantan) thermocouples hooked to a digital datalogger FLUKE 2240 B (FLUKE MFG. Co. Inc., U.S.A.). The temperature is controlled at ± 0.2°C in the enclosure while the sample temperature, separately regulated by an external bath THERMOMIX 1480 (BRAUN, Melsungen AG, W. Germany) is controlled at better than ± 0.1°C.

In normal operation, the entire system is evacuated and a $1x10^{-3}$ torr vacuum is achieved using a mechanical-diffusion combination pump WELCH 1392 (WELCH Scientific, Illinois, U.S.A.). The ultimate vacuum is monitored by the Pirani vacuum gauge (PG).

All the connections and stopcocks on the equipment were assembled and greased with Dow Corning high vacuum grease (Dow Corning corp., USA).

Apiezon B oil (APIEZON products Ltd., London, England), a low density and low vapour pressure oil was used as the manometric fluid.

Pure degassed water was used in the vapour pressure saturator (VPS); the temperature being controlled by an external cryobath FRIGAPLUS (B.BRAUN Mesungen AG, W. Germany).

This apparatus can work in both adsorption and desorption and the entire set-up has been designed to work at temperatures other than room temperature ranging from 4 to 40°C.

Despite the fact that an increase in the number of connections and valves may cause more leaks to occur in the evacuated system, the apparatus has been voluntarily 'broken' in different sections for more flexibility. In addition, the dosing volume can be interchangeable for a smaller or bigger size flask; this can have some applications when studying samples of different moisture capacities (variation of the amount of water admitted to the sample) and also in desorption, for a better control of the amount of water removed.

3. Principle of operation

The following typical experimental procedure concerns a case of adsorption isotherm determination used in this study:

After the sample has been oven dried in the sample holder (S), it is weighed (dry mass M_0), mounted on the apparatus and evacuated to ± $1x10^{-3}$ torr.

When starting an experiment, the whole system (figure 2) is evacuated and stopcock 4 is closed. A typical procedure for an adsorption isotherm determination is as follows:

i. stopcocks 2, 4 and 5 are closed. The pressure reading on the manometer (M) is 0 cm of oil.

ii. stopcock 4 is opened; the temperature of the water in the vapour pressure saturator (VPS) defines a water vapour pressure (P_0) read on the manometer (M). Stopcock 4 is then closed. The amount of water (n_0) in the dosing flask volume (V_D) is determined using the gas law

iii. Stopcock 5 is opened. This results in a pressure drop due to the increase in volume from V_D to V_D+V_S (V_S = sample flask volume). At that time, the sample adsorbs water resulting in an additional pressure drop. When the pressure is stable (P_1 read on the manometer (M)), the sample is in equilibrium with the surroundings.

The moisture uptake is determined from the pressure drop using the gas law. The equilibrium vapour pressure P_i defines the equilibrium relative humidity for the corresponding moisture uptake.

For the determination of subsequent points, stopcock 5 is closed (the residual pressure in V_S is P_i) and the procedure is repeated again beginning at step ii.

4. Calibration procedure

The volumetric technique is based on the ideal gas law; hence, temperatures, pressures and volumes must be determined with great care to achieve good accuracy on the moisture and water activity calculations. In addition, the initial dry mass of the sample must be accurately determined.

The determination of a single point on the isotherm using the volumetric technique is directly related to the following experimental constraints:

- sample size and adsorptive properties.
- size of the dosing volume (V_D).
- the initial vapour pressure in the saturator (VPS).

Hence, if the dosing volume is fixed, only the sample size and the water vapour pressure in the saturator can be varied. Accordingly, the determination of the first desired point on the adsorption isotherm (a_w and moisture levels) is a trial and error process for an unknown sample.

4.1 Volume determination

The dosing volume (V_D) has been determined volumetrically using a reference volume and water vapour as the working gas. The reference volume, as well as the sample flask volume (V_S) were determined by the method of filling the unknown volume with a liquid of known density and weighing. Water was used for this determination.

The dead space (sample flask volume) was determined at ±0.01% (18.39 ±0.02 cm^3) while the dosing volume was determined at ± 0.2% (295.42 ±0.68 cm^3) (Appendix 1).

The variation of the dosing volume due to the oil displacement in the U manometer has been included in the determination of the dosing volume. All further calculations take in consideration the volume variation equal to $P_i.S_c/2$ where P_i is the equilibrium vapour pressure and S_c, the cross sectional area of the manometer tubing (0.0706 cm^2).

4.2 Pressure measurements

The accuracy of the pressure measurements relies only on the cathetometer readings.

The characteristics of the cathetometer are the following:

- span 0 - 40 cm
- 1 division = 1 mm
- 1 division on vernier = 0.1 mm

The manometer and the lens were levelled regularly with a level.

Under these experimental conditions, the absolute error on the pressure measurements (2 readings on the cathetometer) is ±0.02 cm of oil.

The equilibrium vapour pressure measured for each point of the isotherm is used for the calculation of the water activity or equilibrium relative humidity (ERH):

$$a_w(i) = P_i \,/\, P^*_w\,(T) = \%\ \mathrm{ERH} \,/\, 100 \qquad (3.1)$$

Where P_i is the equilibrium vapour pressure at point i and P^*_w is the saturation water vapour pressure at the reference temperature (T).

The saturation water vapour pressure is determined at the temperature of the sample at equilibrium using the correlation of Lowe & Ficke (1974) (appendix 2). This saturation vapour pressure is expressed in pascals; hence, for the determination of $a_w(i)$, the equilibrium pressure P_i must be expressed in pascals and the density of the oil manometer as a function of the temperature is used.

The density of the oil has been determined pichnometrically at different temperatures in the temperature range 4 - 40°C. The linear correlation of the oil density as a function of temperature is:

$$\rho_{oil}(g/cm^3) = -5.683\ 10^{-4} T(^{o}C) + 0.8865 \qquad (3.2)$$

with an absolute error on the oil density decreasing from ± 0.0635 g/cm^3 at 4°C to ± 0.0415 g/cm^3 at 40°C.

4.3 Calculations

With full knowledge of the characteristics of the system and the precision at which each of the parameters is controlled, a FORTRAN 77 program has been written for the calculation of the moisture uptake and the corresponding a_w from the experimental data (pressure and temperature readings).

A listing of the program with detailed explanations is presented in appendix 3.

4.4 Test adsorption on microcrystalline cellulose

In order to test the accuracy and precision of the apparatus, a water adsorption isotherm was performed on microcrystalline cellulose (MCC). The MCC was recently adopted as the reference material for equipment calibration purposes within the COST project 90 and 90 bis (Wolf et al,1984).

4.4.1 Material, preparation and method

The MCC used for this study is the Avicel pH 101 from FMC (FMC corporation, Philadelphia, USA).

Approximately 400 mg of dry MCC were used and accurately weighed on an analytical balance with 4 decimal places precision (±0.0001 g).
The experimental procedure outlined in this chapter (section 3) was followed.

The sample was dried at 100°C for 3 hours (Spiess

& Wolf,1983) in the sample flask, cooled over phosphorus pentoxide (5 hours) and weighed to determine the initial dry mass M_0.

The sample flask was then mounted on the apparatus and evacuated to $1x10^{-3}$ torr at 50°C (degassing of the sample). The adsorption isotherm determination was performed at 25°C ±0.1°C.

Three determinations using the same product were performed at the same temperature (experimental replications).

The water adsorption isotherm of MCC Avicel pH 101 taken from Wolf et al(1984)(COST 90 project) was used as the reference isotherm for comparison purposes.

4.4.2 Equilibrium moisture content criteria

The equilibrium criteria for each point of the isotherm was chosen with reference to Spiess & Wolf (1983). They studied the kinetics of water adsorption on MCC for different thicknesses of the material layer and different absolute pressures in the sorption container. They concluded that the equilibration times are drastically reduced at low pressure (less than 130 mbar = $1.3x10^4$ Pa). In addition, under atmospheric conditions, after 48 hours of exposure time, the sorption process is essentially completed if the material thickness does not exceed 4 mm.

In this apparatus, the sample flask offers a large surface of exposure (about 5 cm^2) and the thickness of the material does not exceed 1 mm. The equipment works under high vacuum and the only pressure present is the water vapour pressure which is much lower than $1.3x10^4$ Pa. At 40^oC, the saturator water vapour pressure is 7377.22 Pa.

A kinetic study on MCC at 25^oC was conducted. The sample was brought from a moisture content of 4.906 $\%H_2O/d.b.$ (a_w=0.443) to a moisture content of (5.379 $\%H_2O/d.b.$ (a_w=0.497) (Figure 4). It can be seen that after 45 minutes, 90% of the adsorption is completed. Above 95% adsorption, the inset plot (Figure 4) confirms that the adsorption kinetic is exponential in nature. It is then possible from this plot to estimate a minimum time required to reach an acceptable state of equilibrium.

The 99% level of adsorption is achieved after about 35 hours. Experimentally, the last 1% of water adsorption correspond to a 1 mm oil water vapour pressure drop over 24 hours (hence about 0.04 mm oil/hour).

With respect to these results, the equilibrium criteria was set at a point when no pressure variation is observed over a 5 hours period time in the limit of precision of the pressure reading (0.2 mm oil). The final estimated equilibrium state for each point is then expected to lie between 98 to 99% level of adsorption. It has to be noted that the correction of the moisture content for a 100% level of adsorption has not been

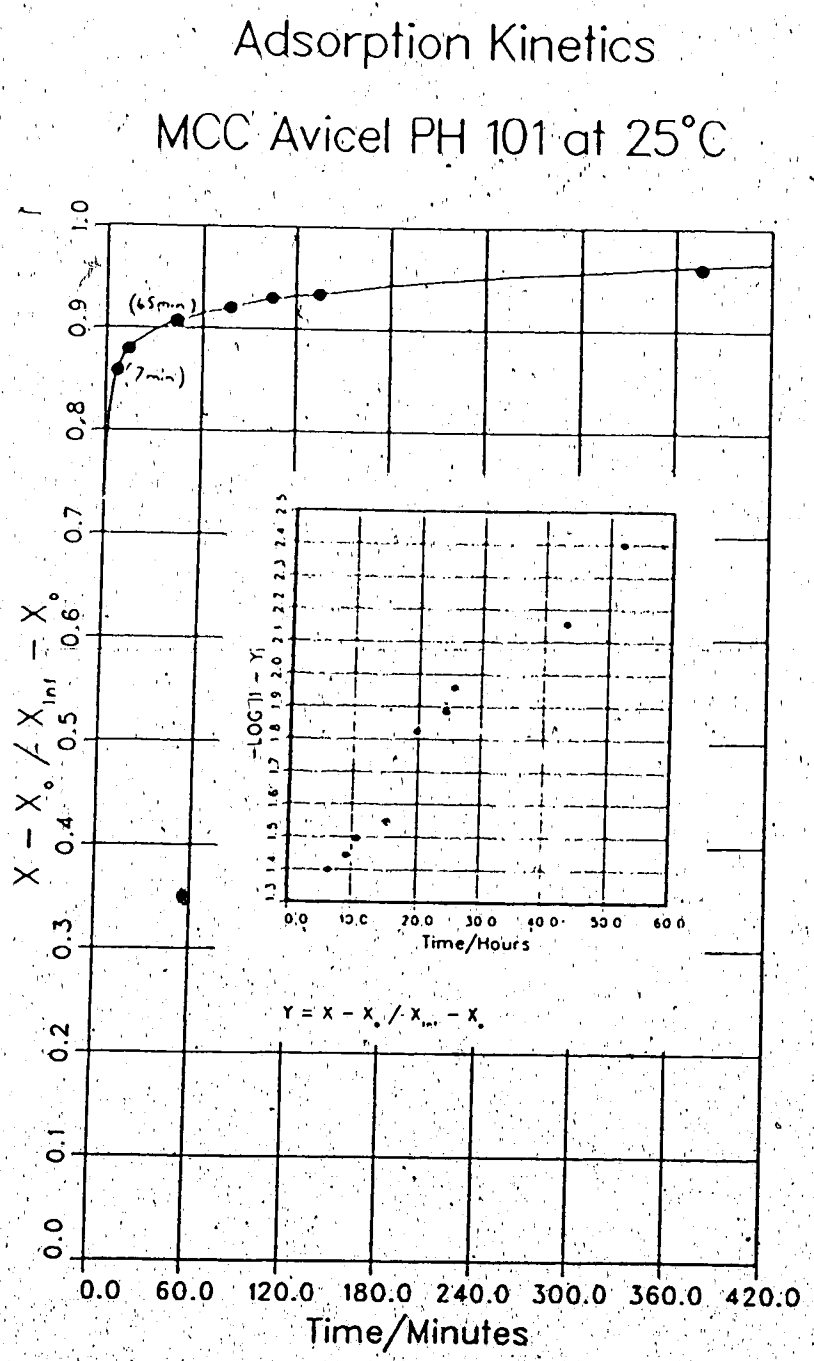


Figure 4: Adsorption kinetics of MCC at 25°C
a_w step: 0.443 - 0.497
moisture step: 4.906 - 5.379 %H_2O/d.b.
with X_1, water content (dry matter); X_0, initial water content; X_{inf}, equilibrium water content.

considered.

4.4.3 Results and Discussion

The water adsorption isotherms of MCC are presented in Figure 5 along with the COST isotherm. The experimental data and the COST data are presented in Appendices 4 and 5.

The good agreement of the experimental isotherm with the COST isotherm validates the calibration procedure. However, we can see that the experimental isotherm is slightly shifted at higher water activity levels; this can be attributed to the method itself and the fact that the errors on the calculated moisture content are cumulative. In addition, the equilibrium condition was considered to be achieved at 98 to 99% level of adsorption without corrections; this may have caused an increase of the error propagation on the calculated moisture contents.

4.4.3.1 Accuracy

In order to test the accuracy of the equipment, all the experimental data (three runs combined) have been compared to the corresponding data of the COST isotherm. The COST isotherm was fitted using the GAB model (from Guggenheim, Anderson and De Boer)(Van den Berg,1981; Bizot,1983):

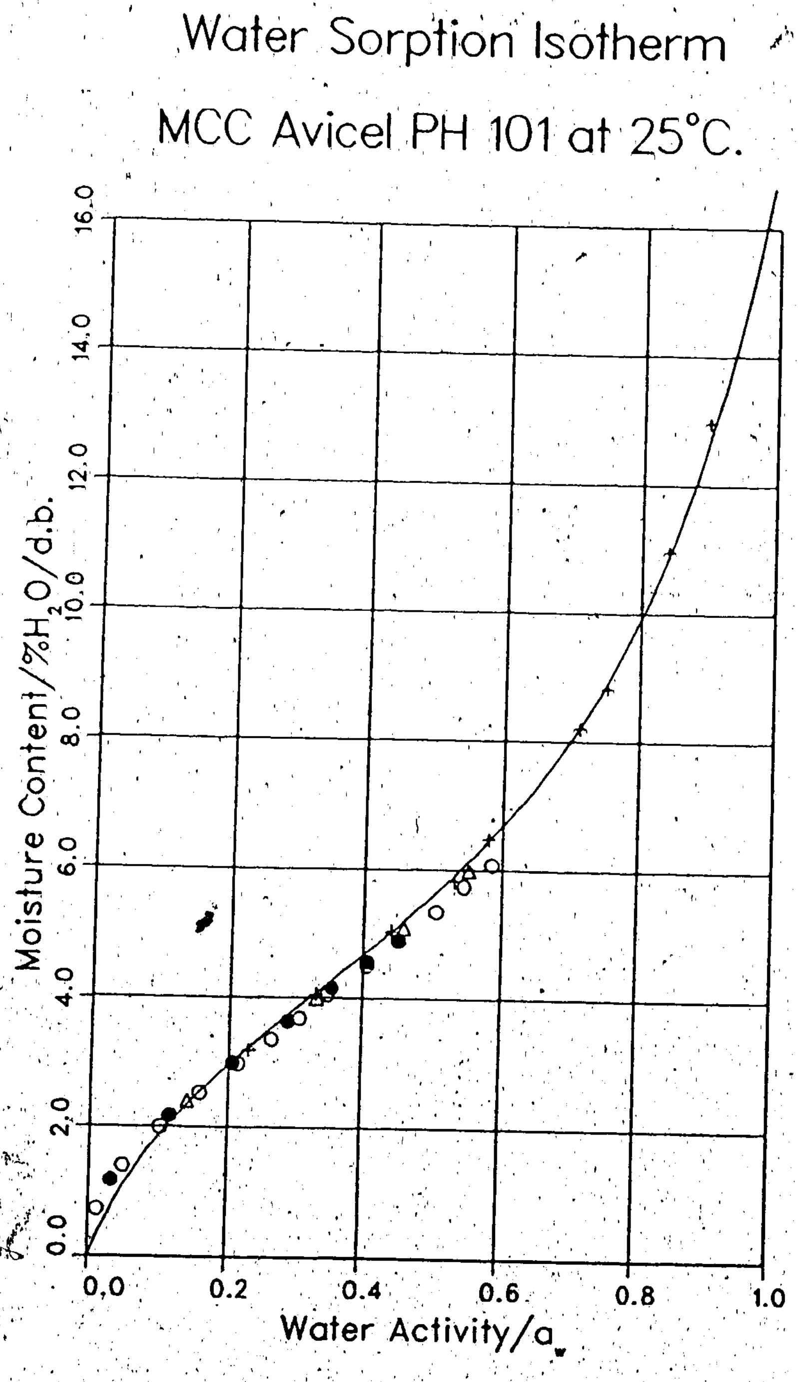


Figure 5: Water adsorption isotherm of MCC at 25°C
Experimental data: ● RUN #1; ○ RUN #2; △ RUN #3; + COST data fitted with the GAB model

$X / X_m = C.k.a_w / (1-a_w).(1-k.a_w+C.k.a_w)$ (3.3)

where a_w = water activity

C = GAB constant = $C' \exp [(H_1-H_m) / R.T]$

H_1 = total heat of sorption of the first layer in primary sites

H_e = heat of condensation of pure water

H_m = total heat of sorption of the multilayer which differs from the heat of condensation of pure water

k = factor correcting properties of the multilayer molecules relative to the bulk liquid

$(k = k' \exp(H_m-H_e) / R.T)$ (3.4)

X = water content on a dry basis

X_m = monolayer water content on a dry basis

The coefficients C, k and X_m of the GAB equation for MCC at 25°C are given by Wolf et al (1984): X_m=4.064 %H_2O/d.b., C=8.776, k=0.772.

The overall experimental data and the corresponding estimated moisture contents from the experimental a_w using the GAB model and the given constants (C, K and X_m for MCC) are presented in Table 2. The experimental values below 0.1a_w have been voluntarily discarded as the GAB model has been fitted to the cost data (starting at 0.11a_w). The maximum absolute deviation of the

TABLE 2

Water adsorption data for MCC at 25^{o}C
(combined data - appendix 3.)

Comparison of experimental moisture contents to estimated ones from the experimental a_w using the G.A.B. model and the given constants (C, k, X_m) for MCC (appendix 4).

a_W	X experimental	X_r from GAB	ΔX deviation
0.0125	0.725	0.324	0.402
0.0319	1.175	0.756	0.419
0.0487	1.399	1.078	0.321
0.1014	2.000	1.883	0.117
0.1143	2.176	2.047	0.129
0.1402	2.402	2.351	0.051
0.1585	2.525	2.548	0.023
0.2062	2.989	3.017	0.028
0.2139	2.975	3.089	0.114
0.2639	3.358	3.532	0.174
0.2881	3.638	3.737	0.099
0.3066	3.686	3.894	0.208
0.3307	4.006	4.095	0.089
0.3464	4.052	4.228	0.176
0.3530	4.156	4.283	0.127
0.4051	4.570	4.729	0.159
0.4054	4.507	4.731	0.224
0.4492	4.894	5.122	0.228
0.4526	4.898	5.153	0.255
0.4590	5.092	5.212	0.120
0.5040	5.350	5.643	0.293
0.5448	5.743	6.062	0.319
0.5519	5.989	6.139	0.150

a_w = water activity
X = moisture content (%H_2O/d.b.)
X_r = reference moisture content (%H_2O/d.b.)
ΔX = absolute deviation from reference

experimental moisture content from the estimated ones is 0.32 %H_2O/d.b.

An acceptable estimate of the accuracy of the equipment over the water activity range concerned (0.1 to 0.55 a_w) is to calculate the percentage root mean standard deviation (%RMSD) defined as (Bizot,1983):

$$\%RMSD = \sqrt{\sum_{i}^{N}((X_i - X_r) / X_r)^2 / N} \cdot 100 \qquad (3.5)$$

where X_i = experimental moisture content
$X_r(i)$ = estimated moisture content (reference)
N = number of experimental points

For the given set of data (20 experimental points): %RMSD = 4.08 . It can be noted that, because of a systematic error on the determination of X_i, the real value of %RMSD is probably smaller.

4.4.3.2 Precision

The overall precision of the equipment has been estimated in terms of the reproducibility of the experimental data.

The experimental data of the three MCC runs have been fitted by a fourth order polynomial equation constrained to pass through zero. This polynomial regression was adjusted using the three set of data from the plot X/a_w versus a_w.

The best fit equation along with the predicted moisture contents (replicates) at fixed water activities are presented in Table 3. We can see that for RUN #3, because the number of experimental points is reduced to 4, the standard deviations of the coefficients are not relevant; the polynomial passes exactly by the given points.

For each a_w, the calculated standard deviation of the moisture content replicates is considered as an acceptable estimate of the precision of the equipment. The standard deviation varies from 0.03 to 0.19% H_2O/dry solid in the a_w range 0.11 - 0.55; the mean standard deviation being 0.14% H_2O/dry solid.

5. Conclusions

It can be seen that the volumetric technique is suitable for the determination of water sorption isotherms. The experimental results presented in appendix 4 illustrate the possibility of the method to achieve very low level of water activity (as low as 0.01) with a good relative error decreasing at higher a_w from about 4% to less than 1% at 0.5 a_w.

It is clear that the absolute probable error on the moisture content is cummulative when using a unique sample for the isotherm determination. However, the relative error remains low (less than 0.1%) on the range 0-0.5 a_w for MCC. The number of experimental points to be

TABLE 3

Water adsorption data for MCC at 25°C

Best fit polynomials for the three sets of of data (appendix 4) and predicted moisture content at fixed a_w in the range 0.11 - 0.53 a_w

---

General equation

$$X = b_o.a_w + b_1.a_w^2 + b_2.a_w^3 + b_3.a_w^4$$

RUN #1
$b_o = 45.76 \pm 2.17$
$b_1 = -326.78 \pm 41.10$
$b_2 = 1048.48 \pm 198.74$
$b_3 = -1105.70 \pm 270.81$
correlation coefficient = 0.995

RUN #2
$b_o = 35.37 \pm 1.44$
$b_1 = -180.01 \pm 19.02$
$b_2 = 442.76 \pm 68.69$
$b_3 = -358.62 \pm 71.55$
correlation coefficient = 0.990

RUN #3
$b_o = 25.24 \pm 4.32\text{x}10^{-6}$
$b_1 = -74.96 \pm 4.85\text{x}10^{-5}$
$b_2 = 133.64 \pm 1.53\text{x}10^{-4}$
$b_3 = -81.65 \pm 1.45\text{x}10^{-4}$
correlation coefficient = 1

COST data[a]

a_w	X	X_1	X_2	X_3	$\overline{X}$	S
0.11	2.02	2.32	2.25	2.04	2.20	0.15
0.23	3.19	2.90	2.94	3.24	3.03	0.19
0.33	4.06	4.08	3.73	4.00	3.94	0.18
0.44	5.04	4.74	4.99	4.92	4.88	0.13
0.53	5.82	---[b]	5.80	5.76	5.78	0.03

[a] (see appendix 5)
[b] missing data

$\overline{X}$ = mean moisture (%H_2O/d.b.)

$$\overline{X} = (\sum_{i}^{N} X_i)/N$$

S = standard deviation

$$S = \sqrt{(\sum_{i}^{N} (X_i - X)^2/N-1)}$$

determined is then a limiting factor for best accuracy and precision; it is suggested to not exceed 5 to 10 experimental points in the coverage of a given a_w range.

The absolute probable error figures are in good agreement with the values proposed by Gal (1981) for research and routine sorption equipments (respectively ±0.001 a_w and ±0.01% H_2O/d.b. and ±0.01 a_w and ±0.1% H_2O/d.b.).

The overall precision and accuracy of the apparatus respectively ±0.14% H_2O/d.b. and 4.08% On the a_w range 0-0.5 are similar to the values found with other techniques (e.g. gravimetric techniques).

APPLICATION OF THE VOLUMETRIC TECHNIQUE

As part of this work, the volumetric technique was applied to two areas where a reliable sorption methodology is lacking:

i. Determination of water sorption isotherms of low moisture capacity materials (less than 0.2% moisture uptake (on dry basis) on the a_w range (0 - 0.5).

ii. Estimation of the heat of water binding of biological materials in the low a_w range (0 - 0.15).

1. Determination of the surface properties of biologically active immobilized molecules from water sorption measurements.

The volumetric technique is particularly suitable for the determination of water sorption isotherms of low moisture capacity products. The major advantage of the technique over gravimetric methods is the possibility of using a relatively small amount of sample without loosing the precision on the calculated moisture content.

Because the amount of moisture uptake is small (less than 0.1% H_2O/d.b., most gravimetric techniques fail due to a lack of precision on the mass measurements, unless a great amount of sample is used (which is not possible with analytical balances and microbalances).

An other alternative would be the use of a chromatographic technique (Inverse gas chromatography, I.G.C.) where the sample is used as the stationnary phase. Although applied successfully for the determination of low levels of water adsorption on sucrose and glucose (Smith et al, 1981), IGC requires an expensive setting-up (gas chromatograph) and a relatively important amount of sample is needed to fill the chromatographic column. In addition, the method requires the analysis of several sample for the determination of a single isotherm (integral method).

1.1. Experimental

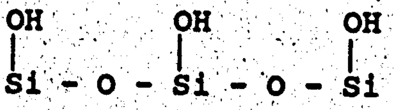
1.1.1. Material

The samples were prepared by CHEMBIOMED Ltd.(Edmonton, Alberta, CANADA) and stored over phosphorus pentoxide (P_2O_5) in an evacuated dessicator prior to use.

Four different samples were provided; a chromatographic support (diatomite support, Chromosorb P_R from Johns Manville products corp.,N.Y.) and three different materials, resulting from three successive surface treatments of the diatomite support (immobilization of the biologically active molecules).

Chromosorb P (or pink Chromosorb) is made from diatomite (also called diatomaceous earth) which has been crushed, blended then calcined or burned above 900°C. Diatomite is composed of the skeletons of diatoms, single-celled algae. The skeletons consist primarily of hydrated microamorphous silica and some minor impurities, mainly metallic oxides. Diatomite has a nitrogen specific surface area of approximately 20 m^2/g. During the calcination of the material, the diatomite particles fuse and a portion of the microamorphous silica is converted to crystalline form, cristobalite. Much of the fine structure is then destroyed, dropping the surface area of the starting material from approximately 20 m^2/g to 3 to 5 m^2/g. Also during the calcination, the mineral impurities present in the diatomite form complex oxides or silicates. The oxide iron is thought to impart the characteristic pink colour (Ottenstein, 1966).

Diatomite supports are siliceous material with about 10% mineral impurities. It is well established in the literature that the surface of siliceous materials is covered with the silanol (Si-OH) and siloxane (-Si-O-Si-) groups. The surface of the diatomite support can then be represented as:



$$\overset{\overset{OH}{|}}{Si} - O - \overset{\overset{OH}{|}}{Si} - O - \overset{\overset{OH}{|}}{Si}$$

(surface)

The presence of mineral impurities give the diatomite a heterogeneous rather than a homogeneous surface usually found in pure silica. Hence the surface properties of the chromosorb P are expected to differ slightly from those of silica.

The preparation sequence of the different products along with their molecular structure is presented in Figure 6 and table 4. A more detailed description of the preparation of similar immunoadsorbents can be found in the literature (Boullanger et al, 1978). Table 5 presents some of the physical properties of the different materials. The density measurements were performed pichnometrically using hexane.

1.1.2. Method

Approximately 1g of sample was weighed accurately on an analytical balance (±0.0001 g), loaded in the sample flask, dried (90 minutes at 130°C) (Ottenstein,1966), cooled over P_2O_5 (2 hours), weighed again (dry mass) and mounted on the apparatus. The entire apparatus was then evacuated to $\pm 1x10^{-3}$ Torr and the sample heated overnight at 50°C (degassing).

No leaks were detected in the equipment over a 15 hours period time.

The determination of the water adsorption isotherm was performed following the experimental procedure outline

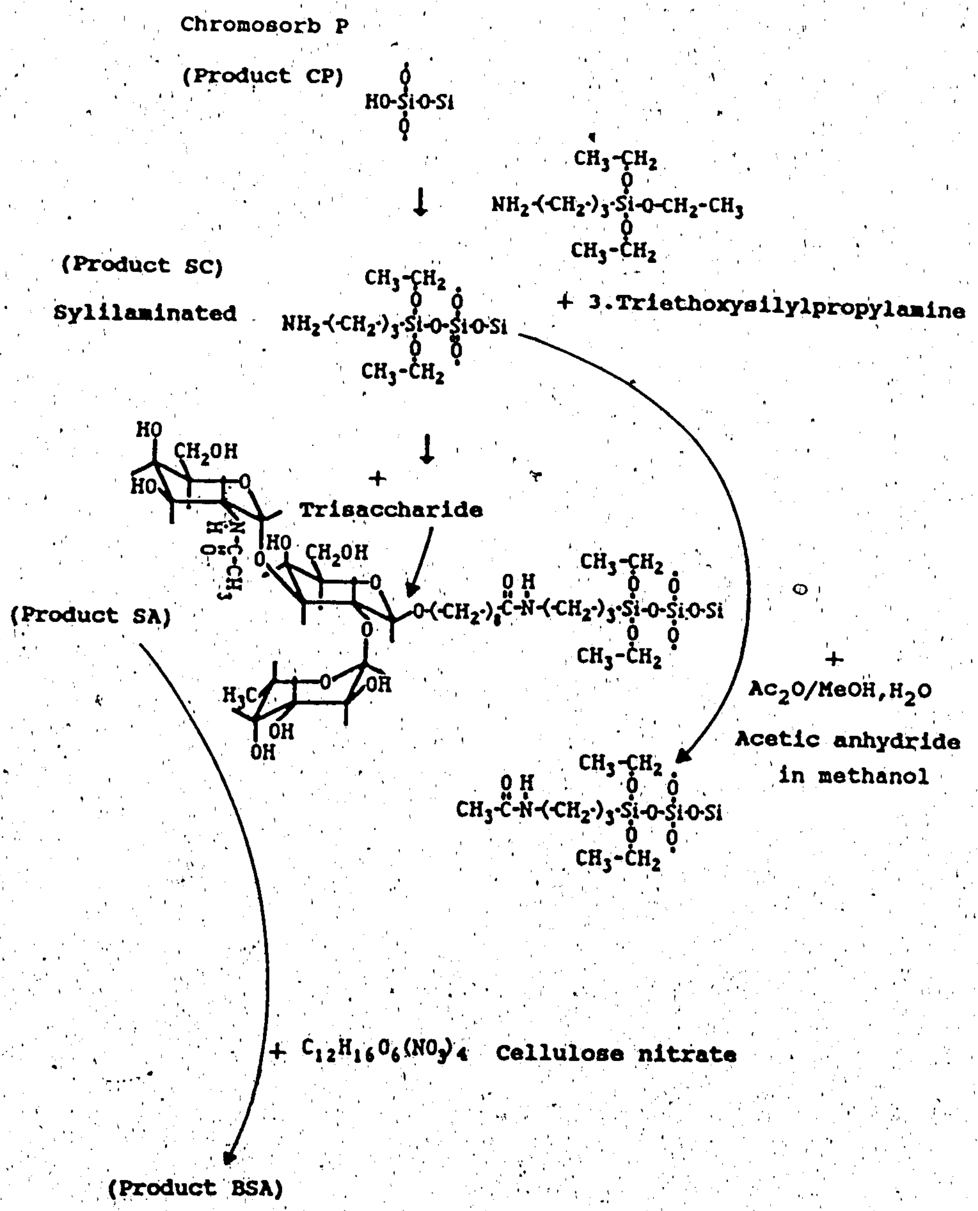


Figure 6: Chemical structures and chemical reaction sequences for the preparation of products CP, SC, SA and BSA.

Table 4

Incorporation rates of the coated materials for products SC, SA and BSA

<u>SC</u> Incorporation of 3.triethoxysilylpropylamine:

SC I : 4.45 µmoles/g support

SC III : 11 µmoles/g support

<u>SA</u> Incorporation of 3.triethoxysilylpropylamine:
(5 to 10 µmoles/g support)

Incorporation of blood group trisaccharide:

SA I : 0.43 µmoles/g support

SAII : 0.44 µmoles/g support

<u>BSA</u> Incorporation of 3.triethoxysilylpropylamine:
(5 to 10 µmoles/g support)

Incorporation of blood group trisaccharide:

$\left.\begin{array}{l}\text{BSA I}\\ \text{BSA II}\end{array}\right\}$ 1 µmole/g support

Nitrocellulose coating:
(2.37x10^{-4} ml/g support)

TABLE 5

Physical properties and constants for the different products CP, SC, SA, and BSA.

true density (g/cm^3)	particule size (mesh range)	apparent surface area (m^2/g)	average surface area
CP			
2.42 ± 0.28	100/120	0.017/0.021	(0.019)
SC			
2.39 ± 0.25	100/120	0.017/0.021	(0.019)
SA			
2.41 ± 0.26	100/120	0.017/0.021	(0.019)
BSA			
2.17 ± 0.22	30/60	0.005/0.011	(0.008)

Specific surface area of CP (BET determination from nitrogen adsorption): 4.64 m^2.

Porosity determination from Mercury Porosimetry Technique:

Most probable pore radius: 2.55 nm
Average pore radius : 28.95 nm

Size relationship

mesh	microns
30	595
40	420
60	250
80	177
100	149
120	125

chapter 3 (section 3) and using the calculation program (Appendix 2).

For a better knowledge of the surface characteristics of the diatomite support, a nitrogen adsorption was performed for the determination of the specific surface area. In addition, a pore size distribution estimation was conducted using the mercury porosimetry technique (Gregg & Sing,1967). Both determination were done in the chemical engineering department of the University.

To obtain relevant data on the monolayer capacity and the specific surface area regarding the adsorbate, we used the BET equation:

$$X/X_m = (a/(1-a)).(C_B/((C_B-1).a + 1)) \qquad (4.1)$$

where a = activity of the adsorbate

C_B = BET constant = $K.\exp(Q_n{}^{st}/R.T)$ (4.2)
related to the net isosteric heat of sorption $Q_n{}^{st}$ for the first adsorbed layer

X = equilibrium adsorbate uptake (moles of adsorbate per gram of dry solid)

X_m = monolayer adsorbate capacity (same units as X)

The BET constants C_B and X_m were estimated from a weighed non-linear least squares' procedure on the experimental data using a BMDP non-linear regression program (BMDP (a),1983) and equation 4.1. Toupin et al (1983) compared this procedure with the usual methods using the linearized forms of equation 4.1; they concluded that the non-linear least squares' procedure was the most reliable technique and that this approach should be used in preference to corrected linear relations.

The specific surface area exposed to the adsorbate was determined using the relation:

$$S = X_m.N.A \qquad (4.3)$$

where s = specific surface area (m^2/g)

X_m = monolayer adsorbate capacity (μmoles/g)

N = avogadro number = $6.023x10^{23}$ molecules/mole

A = adsorbate molecular cross sectional area (m^2)

If we assume the apparent molecular cross sectional area of a water molecule to be $A_{H2O} = 10.6x10^{-20}\ m^2$ (Labuza,1968), equation 4.3 reduces to:

$$S_{H2O} = 6.384x10^{-2}\ X_m \qquad (4.4)$$

for nitrogen $A_{N2} = 16x10^{-20}\ m^2$ (Gregg & Sing,1967)

$$S_{N2} = 9.634x10^{-2}\ X_m \qquad (4.5)$$

1.2. Results and Discussion

1.2.1. Nitrogen adsorption isotherm

The nitrogen adsorption isotherm on chromosorb P (product CP) (Figure 7) is of a type III in the BET classification (Gregg & Sing, 1967). The isotherm is characterized by its convexity to the relative pressure axis and is typical of a non-specific adsorption (condensation phenomenon).

The specific surface area (S_{N2}) of CP determined from the experimental isotherm using the BET method is 4.64 m^2/g of material and is well in accordance with the specific surface area given by the manufacturer (4-6 m^2/g). Gregg & Sing (1967) analyzed the validity of the BET procedure for the determination of specific surface areas from type III isotherms; they concluded that the BET analysis appeared to be correct within 20% or better. However, despite this lack of precision and being unaware of any better existing method, the calculated specific surface area of CP from nitrogen adsorption was used as a reference for this study.

Since the total surface area and pore volume of the catalyst are small, the pore size distribution analysis is not fully reliable. However, the most probable pore radius is 2.55 nm (25.5 Å); the average pore radius being 28.95 nm (289.5 Å).

The apparent surface area S_a of the diatomite

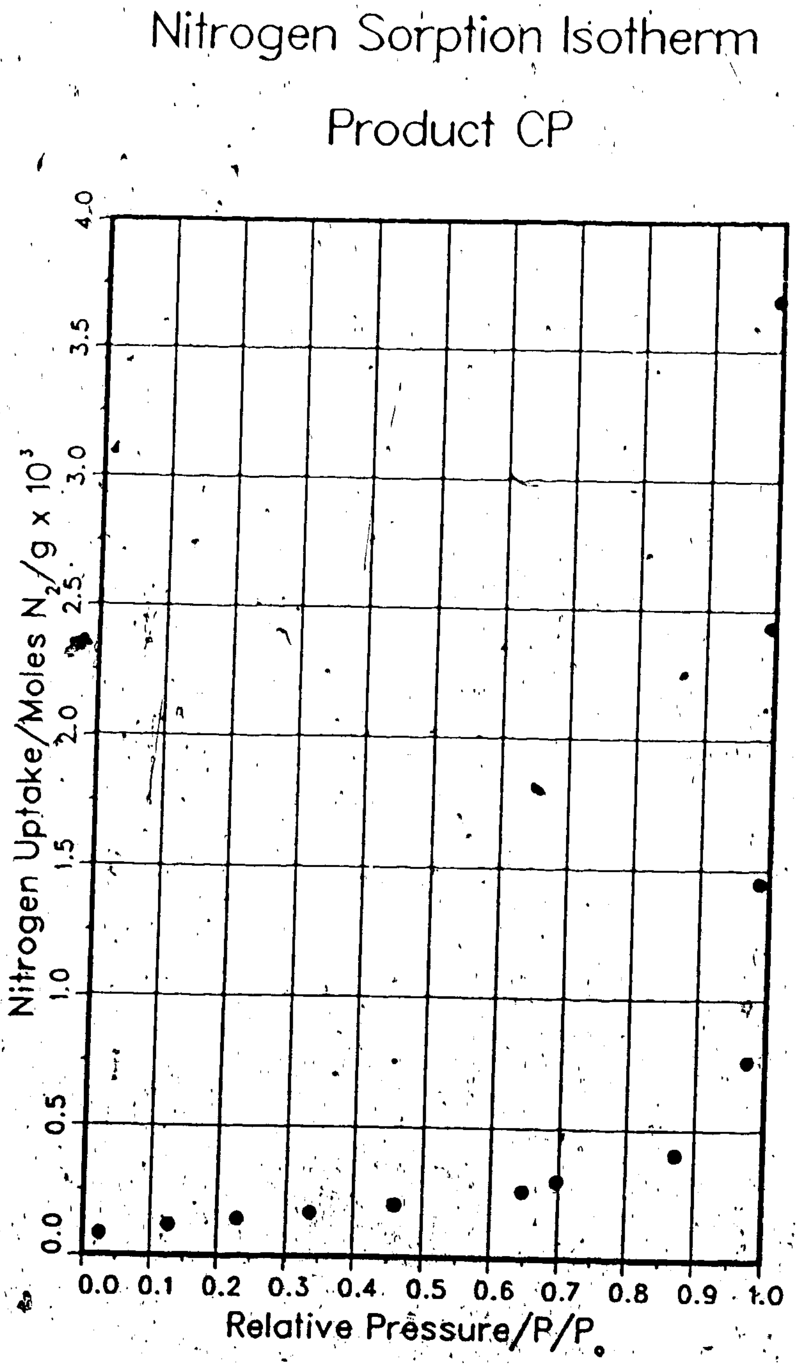


Figure 7: Nitrogen adsorption isotherm on product CP.

support CP calculated from the particule diameter (D in m), the density (about $\rho = 2400 kg/m^3$) and the equation (4.6):

$$S_a = 0.006/D.\rho \qquad (4.6)$$

is about 0.019 m^2 per gram of material.

In comparison with the nitrogen surface area (S_{N2}), it can be seen that the diatomite support is porous with about 99.6% of its active surface on the inside of the particle.

1.2.2. Water adsorption isotherm

The partial water adsorption isotherm (up to 0.5 a_w) of the materials CP, SC, SA and BSA at 25°C are presented in figure 8 to 11 (the experimental data are presented in Appendix 6). With the exception of the support CP, the two isotherms determined for each sample are not replicates; the samples coming from different batches of the same preparation.

It can be seen that due to the low level of water adsorbed, the relative error on the moisture content is high and cummulates, ranging from about 2% to more than 10% in the 0-0.5 a_w range. However, because the water activity corresponds to an exact measure of the equilibrium pressure, the absolute probable error remains very low to the third a_w decimal (±0.001). The BET

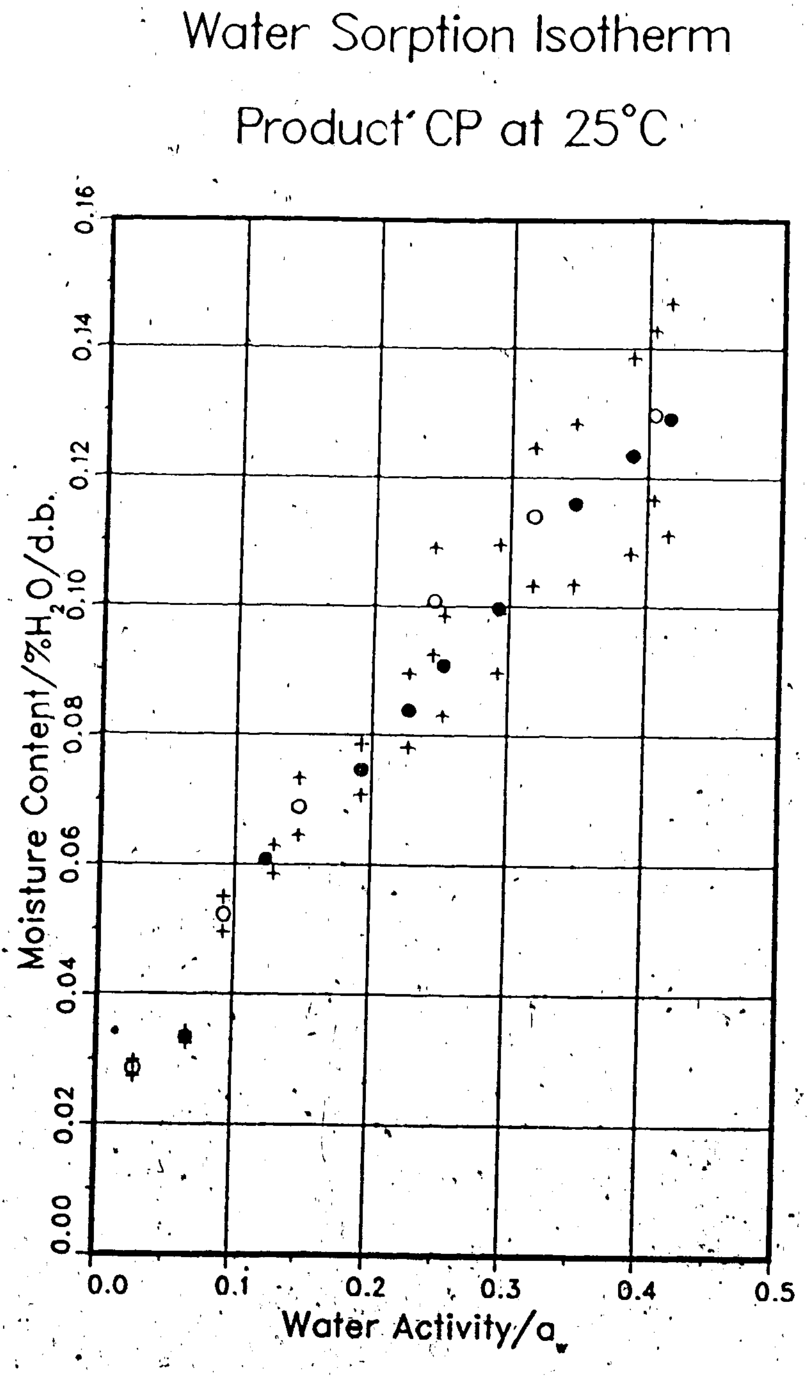


Figure 8: Water adsorption isotherm of product CP at 25°C; ● Run#1; ○ RUN#2; + calculated probable error on the moisture content for each point.

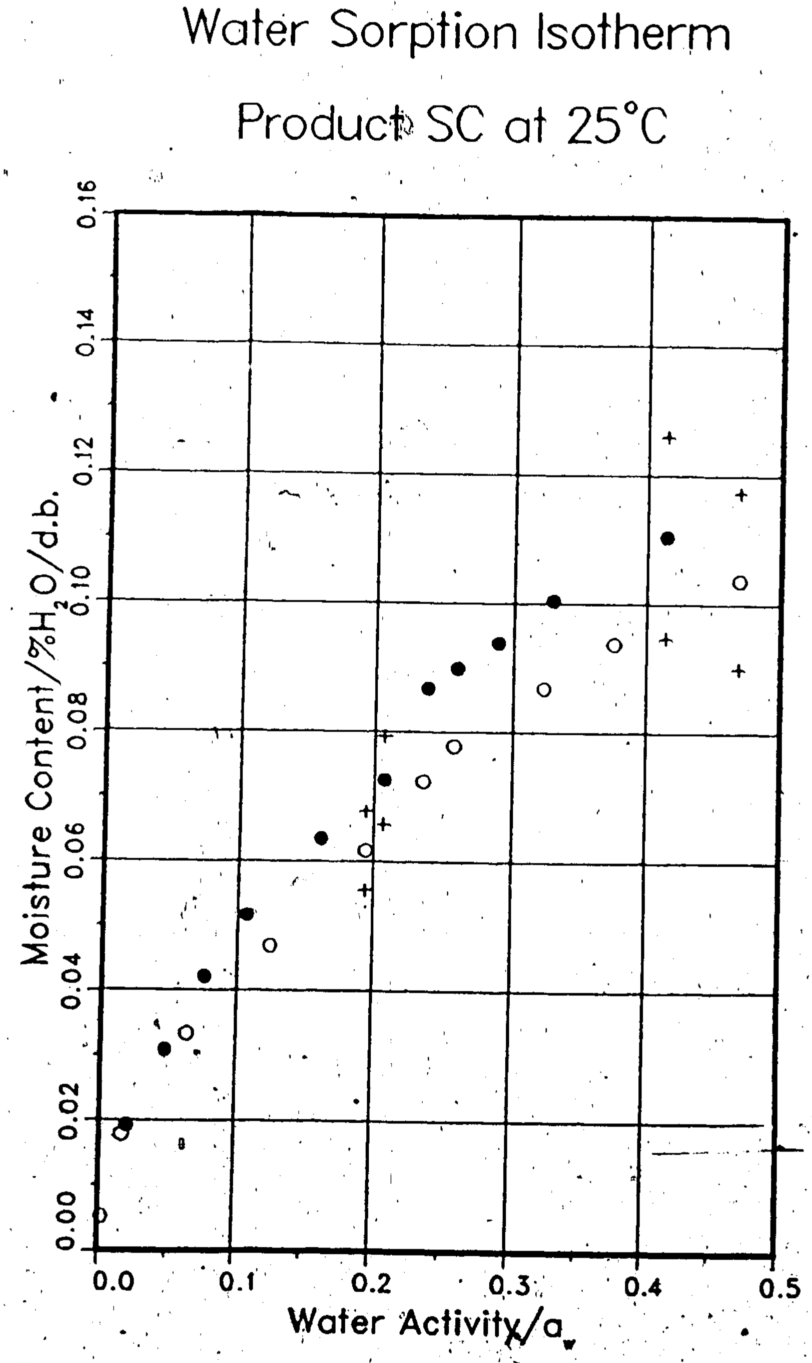


Figure 9: Water adsorption isotherm of product SC at $25^\circ C$; ● product SCI; ○ product SCIII; + calculated probable error on the moisture content

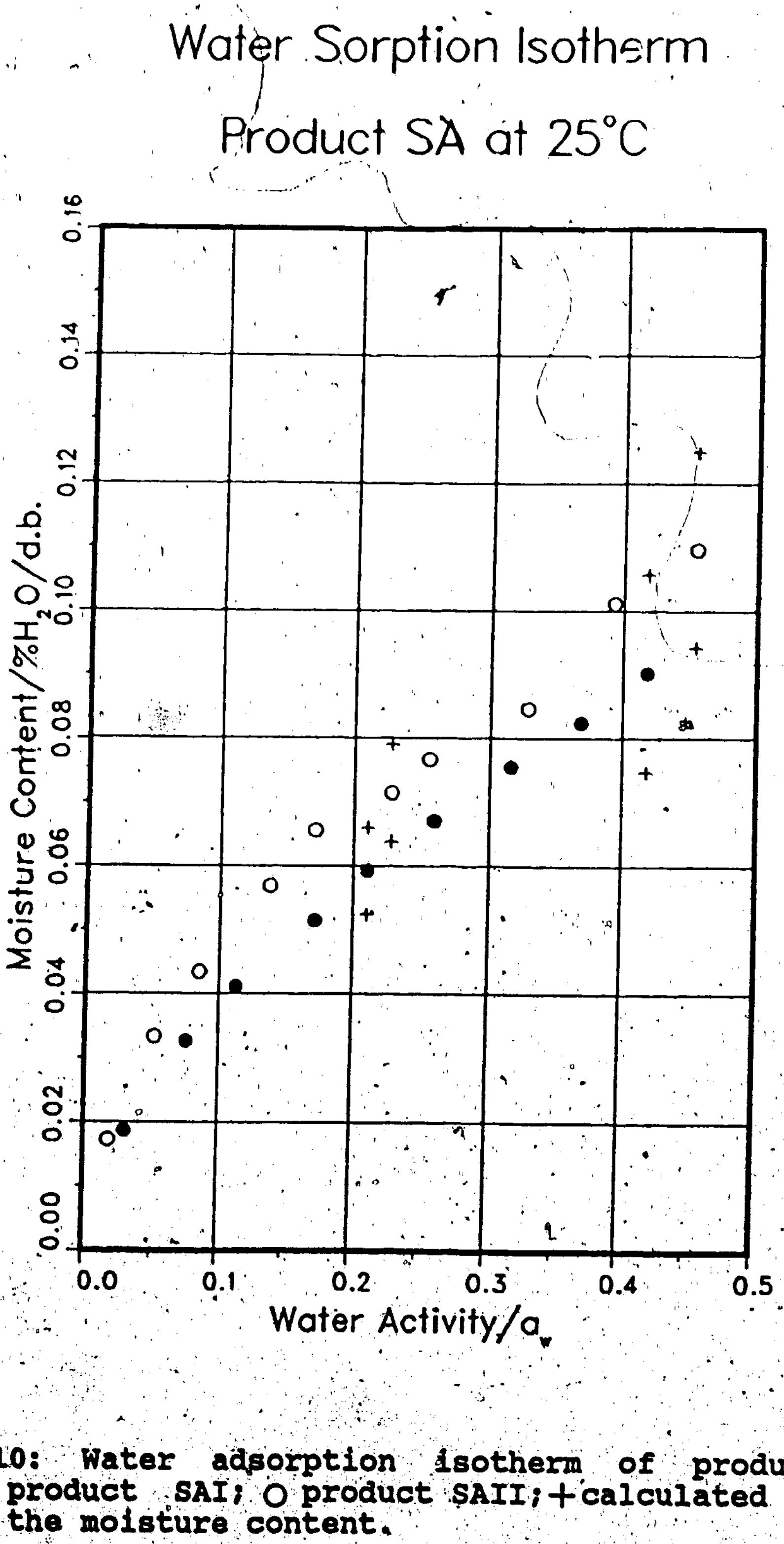


Figure 10: Water adsorption isotherm of product SA at 25°C; ● product SAI; ○ product SAII; + calculated probable error on the moisture content.

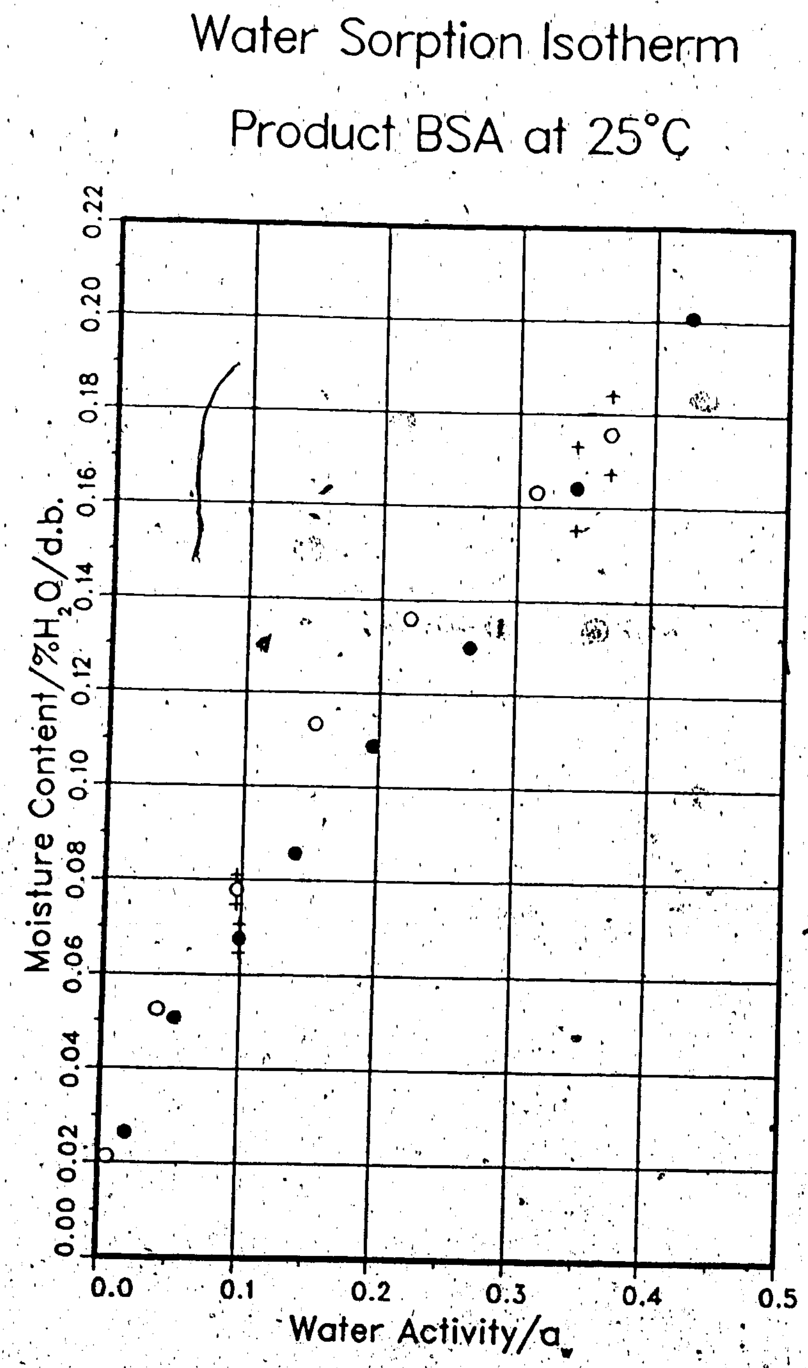
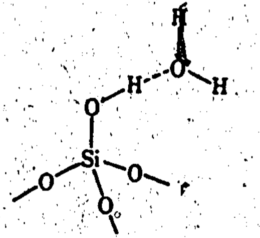


Figure 11: Water adsorption isotherm of product BSA at 25 °C; ● product BSAI; ○ product BSAII; + calculated probable error on the moisture content.

analysis (estimation of X_m and C_B) for each isotherm is presented in Table 6. The experimental data out of the range 0.1-0.35 a_w were discarded for the BET analysis because of the non suitability of the method outside this a_w range.

The water adsorption isotherm of CP (Figure 8) compared to the nitrogen one, corresponds to a drastic change in the adsorbent-adsorbate interactions. The water sorption isotherm is reminiscent of a type II isotherm in the BET classification.

The predominent active centers for water adsorption on silicas type materials are the surface hydroxyl groups (OH). It has been clearly demonstrated in the literature (Klier et al,1973; Naono et al,1980) that the water molecule is initially hydrogen bonded to one hydroxyl group :



SiOH is donor, H_2O is acceptor

The hydroxyl groups are generally donors and the water molecules acceptors of the hydrogen bond. Naono et al (1980) showed that the ratio of physisorbed water to surface hydroxyl groups varies with the surface density of the hydroxyl groups; the ratio going from 1:1 to 1:2 with increasing density. In a first approximation the ratio 1:1

TABLE 6

BET analysis from water adsorption isotherms data of products CP, SC, SA, BSA at 25°C (Appendix 6)

	Monolayer capacity (µmoles/g)	BET constant C_B
CP		
	53.85 ± 3.26	9.25 ± 2.19
	50.42 ± 1.53	8.86 ± 1.13
SC		
SC I	45.53 ± 2.38	10.69 ± 2.55
SC III	40.46 ± 2.13	9.68 ± 2.15
SA		
SA I	33.23 ± 0.66	12.88 ± 1.46
SA II	34.18 ± 1.04	29.99 ± 9.22
BSA		
BSA I	74.86 ± 2.37	7.36 ± 0.83
BSA II	69.69 ± 2.75	16.03 ± 4.28

was considered.

The monolayer capacity of CP is about 52 µmoles of water per gram of material (dry basis) which correspond to 3.3 m^2 of a monolayer of water molecules at most. Considering the size of the pores in the material, the entire surface (S_{N2}) of CP is accessible to water. However, the surface coverage by water represents only about 70 % of that entire surface. An acceptable representation of the CP surface would show patches of hydroxyl groups (active centers)-water complexes. Klier et al (1973) showed that the water monolayer does not exhaust all surface OH groups available for adsorption, and the BET monolayer corresponds to an occupation of about 50% of the sylanols available. The water molecules tend to form clusters long before all adsorption centers are occupied. Perrett & Purnell (1962) found that there may be two types of active sites for adsorption on the Chromosorb surface (low, and high-activity sites). The high-activity site was found to cover 23 per cent of the surface. In their study on quantitative relations between infrared spectra and the concentrations of water and surface OH groups on silica and silicate, Klier et al (1973) suspected the presence of different kinds of OH groups on the surface of silicas; some of them only (about 50%) being the primary active sites for water adsorption.

The incorporation of the amine groups on CP to produce SC is accompanied by a reduction of the monolayer moisture uptake (about -6.5 µmoles H_2O/g for SCI and -11.5 µmoles/g for SCIII).

If the amine incorporation occurs only on the active OH groups, we can see that the reduction of the water adsorption correspond about exactly to this incorporation. The incorporation of the aminopropyl group increase the hydrophobicity of product SC probably due to the methyl groups.

The incorporation of the blood group trisaccharide to produce product SA follows an incorporation of 5 to 10 µmoles NH_2/g. Only 0.4 µmole of trisaccharide is incorporated; the remaining NH_2 groups are blocked by addition of acetic anhydride. The trisaccharide is of a branched type (length = 17-18 Å and apparent molecular cross sectional area about 70 $Å^2$). The trisaccharide is attached to a long hydrocarbon chain, hydrophobic in nature, but very flexible. Hence the trisaccharide complex is expected to swing over the surface of the support.

The monolayer capacity of product SA is about 34 µmoles H_2O/g dry solid. Each trisaccharide contains 8 hydroxyl groups ; however, it has been stated (Franks, 1975) that only the equatorial hydroxyl groups of the sugar molecule adsorb water preferably, without distorsion of the sugar molecule.

On a nitrogen specific surface area basis, the surface density of the hydroxyl groups (100%, considering 23 to 50 per cent of active sites) is:

(52/0.50 to 52/0.23 µmoles OH) / 4.64 = 22.4 to 48.7 µmoles OH / m^2

The apparent molecular cross sectionnal area of the trisaccharide is 70 Å^2 corresponding to a 2.4 µmoles/m^2 coverage.

We can see that 1 µmole of trisaccharide could cover about 9.5 to 20.6 µmoles of surface OH groups/m^2 (about 15 OH µmoles/m^2 average). From these OH groups, only 23 to 50 per cent are active adsorption sites; hence 3.5 to 7.5 µmole/m^2 (16 to 34.8 µmoles/g). If we consider these OH groups to be blocked that way to water adsorption, the level of hydration for one gram of product SA should equal:

52 µmoles of active hydroxyl groups

-(5/10) µmoles of amine incorporation

-(0.4x(16 to 34.8)) µmoles of active OH groups covered by the trisaccharide

+(0.4x5) µmoles of equatorial OH groups on the trisaccharide molecule

= 30.1 / 42.6 µmoles of OH groups, corresponding to 30.1 / 42.6 µmoles of water adsorbed in our convention.

We can see that the experimental level of adsorption (34 µmoles H_2O/g) lies in the range of water adsorption calculated; hence confirming our hypothesis. In addition, the trisaccharide is 'mobile' and the experimental level of adsorption should correspond to an average value.

The product BSA, unlike the products CP, SC and SA, is constituted of bigger mesh size particules (30-60 mesh instead of 100-120 mesh). This difference affects the apparent surface area of BSA but does not affect significantly the other physical characteristics (true density, porosity, specific surface area). The product BSA is equivalent to product SA but with a coating of nitrocellulose. The nitrocellulose is a straight 1-4 cellobiose chain. The NO_3 groups are most probably attached to the carbons 2 and 6. The nitrocellulose comes from a collodion solution made of 4g of pyroxylin (chiefly nitrocellulose) in 100 ml of a mixture of alcohol and ether (MERCK index, 1983). The solution exposed in thin layer evaporates leaving a tough, colorless film.

The monolayer capacity of BSA is about 72 µmoles H_2O/g dry solid and correspond to an incorporation of 1 µmole of trisaccharide per gram.

At present no reliable explanations permits the explaination of the increase of the adsorption capacity for BSA; we know very little about the interactions of the nitrocellulose in solution with BSA. Soluble in organic solvents, the nitrocellulose is thought to be very hydrophobic. When in contact with BSA in solution, it is possible to picture each active hydroxyl group excluded from the coating action and still available for water adsorption. However, only the difference in the amount of trisaccharide incorporated could provoke an increase in the adsorption capacity. Compared with product SA, there is a difference of only 0.6 µmoles of trisaccharide incorporated; this cannot account for a double adsorption capacity of product BSA.

2. Net isosteric heat of water sorption on potato starch at low water activity (0-0.25).

Starch is the form of carbohydrate reserve in nearly all green plants; it is the major carbohydrate in food for man and, moreover, in isolated forms, it has numerous applications. Generally, native and modified starches are applied to improve physical and physico-chemical properties of food products, such as viscosity, gelation, surfaces, freeze thaw stability and strengh. Further applications are as glue, carriers, binder, filler, emulsifier, moisture buffer and shaping powder etc. Because starch is very common and widely used and water is ubiquitous, studies on water interactions in biological materials have been chiefly conducted on starch products.

The net isosteric heat of sorption is a useful quantity because it yields informations on the energetics of water sorption processes in biological materials and their interpretation.

Two methods are usually available for the determination of the net isosteric of sorption. One is the application of the Clausius-Clapeyron equation on the isosteric equilibrium pressures at different temperatures (the isosteric heat of sorption) and the second is the direct calorimetric measurement of the heat evolved in the sorption process. Sorption calorimetry is considered as the most accurate method for the determination of heats of sorption. However, the method looses its precision at low moisture content because of low quantities of heat involved (Simatos et al, 1975).

The determination of the net heat of sorption from sorption isotherms is generally limited to the a_w range 0.05-0.80 as no sorption methods are fully reliable at either very high and very low water activity levels.

In the present study, water adsorption isotherms of starch in the low a_w range (0-0.15) using the volumetric technique have been determined. The Clausius-Clapeyron equation was used for the determination of the net isosteric heat of water sorption from the sorption data.

2.1. Thermodynamics

The isosteric heat of sorption Q^{st} is a differential molar quantity derived from the temperature dependence of the isotherm:

$$(\delta \ln P/\delta(1/T))_X = -Q^{st}/R \qquad (4.1)$$

provided the amount of water sorbed is held constant. For pure water, the Clausius-Clapeyron equation is:

$$(\delta \ln P_o/\delta(1/T)) = -L/R \qquad (4.2)$$

where L is the latent heat of vaporization of pure water.

Substracting equations 4.2 from 4.1 gives:

$$(\delta \ln(P/P_o)/\delta(1/T))_X = Q_n^{st}/R \qquad (4.3)$$

where P/P_o is the water activity, Q_n^{st} the net isosteric heat of sorption ($Q_n^{st} = L-Q^{st}$).

The determination of the net isosteric heat of sorption using the Clausius-Clapeyron equation requires the determination of water sorption isotherms at different temperatures.

The net isosteric heat of sorption, at any moisture X, may be calculated from equation 4.3 by plotting the sorption isostere as $\ln a_w$ versus 1/T and determining the slope, which equals Q_n^{st}/R. The application of this method assumes that Q_n^{st} is invariant with temperature.

The net isosteric heat of sorption may also be calculated directly from the derivation of equation 4.4 used to fit the isotherms data (all temperatures combined).

$$\ln(a_w/X) = b_o + b_1.X + b_2.X^2 \qquad (4.4)$$

where b_o, b_1 and b_2 are constants linearly related to the inverse of the temperature:

$$b_o = b_o' + b_o''/T \qquad (4.5)$$

$$b_1 = b_1' + b_1''/T \qquad (4.6)$$

$$b_2 = b_2' + b_2''/T \qquad (4.7)$$

combining equations 4.5, 4.6, 4.7 and 4.4 gives:

$$\ln(a_w/X) = b_o' + b_o''/T + b_1'.X + b_1''.X/T + b_2'.X^2 + b_2''.X^2/T \qquad (4.8)$$

The coefficients b_o', b_o'', b_1', b_1'', b_2', b_2'' can be estimated by a multiple linear least sqares' procedure using the experimental data.

The derivation in 1/T of equation 4.8 gives:

$$d\ln a_w = (b_o'' + b_1''.X + b_2''.X^2).d(1/T) \quad (4.9)$$

hence from equation 4.3:

$$Q_n^{st} = R.(b_o'' + b_1''.X + b_2''.X^2) \qquad (4.10)$$

This procedure seems more reliable since the determination is performed numerically and no assumption is made concerning the variation of Q_n^{st} with temperature..

the calculation program (Appendix 2). The temperature of the sample and the chamber was then decreased to lower temperatures (25.5 and 6.5°C) at constant volume (V_D+V_s). At each temperature, the equilibrium moisture content was achieved in accordance with the equilibrium criteria defined chapter 3 (subsection 4.4.2.).

As the hygroscopicity of most biological material increases with decreasing temperatures, it is more probable that adsorption on the sample will result when changing the temperature from a high to a low level. Because of an important sorption/desorption hysteresis cycle observed on starch products (Bizot et al,1981), a sample temperature change from the cold to the initial temperature setting (39.5°C) would cause a desorption process to occur; therefore, only one sample was used at a time and no sample was brought back to the initial temperature (39.5°C) for an other run.

Practically, when changing the temperature of the sample and surroundings from T1 to T2 (T1 > T2), the kinetics of the sorption process may follow different pathways:

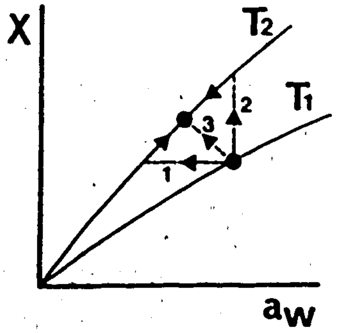


Figure 12: Different pathways for the sorption process; temperature change from a high to a low level

2.2. Experimental

2.2.1. Material

The material used for this study is native potato starch granule. Potato starch is a complex substance constituted (99.5%) of chains of 1-4 linked D-glucopyranose units, either linear (23% amylose) or branched (amylopectine) with about one 1-6 bond per 20-26 monomere units. The chains are arranged in a radial and concentric symetry architecture having the shape of an ellipsoid of revolution: the granule. The average diameter of the potato starch granule is 40 um.

The present starch granule also called "green" was provided as is; it was originally extracted in solution, decanted, washed, sieved and fluidized bed dried at a moderate temperature (50°C) up to a moisture content of 8% (dry matter) (Bizot et al,1981). The starch was stored in a sealed container prior to use.

2.1.2. Method

The experimental isotherms were determined at the three temperatures; 39.5, 25.5 and 6.5 ± 0.1°C using an integral procedure.

Each point of the isotherm is a result of water adsorption on individual samples. Each sample was brought to a given moisture content at 39.5 ± 0.1°C using the experimental procedure outline _ chapter 3 (section 3) and

<u>case 1</u>: the moisture content X of the sample stays constant and a_w decreases until it crosses the isotherm at T2. Then adsorption occurs to reach the final equilibrium moisture content.

<u>case 2</u>: a_w stays constant and the moisture content X of the sample increases to cross the isotherm at T2. Then desorption occurs to reach the final equilibrium moisture content.

Experimentally, both a_w and X vary as the temperature decreases and a description of the real phenomenon would be an intermediate situation between case 1 and case 2 (idealized case 3). This phenomenon cannot be analysed through the volumetric technique as the calculated moisture content is a function of the pressure. However, the specific heats and mass differences between the gas phase and the sample should favor case 1 where adsorption only occurs.

Approximately 300 mg of potato starch were used and accurately weighed on an analytical balance with four decimal places precision (±0.0001g). The sample, loaded in the sample flask was oven dried at 130°C for 90 minutes (Bizot et al,1981), cooled over P_2O_5, weighed again (dry mass) and mounted on the apparatus. The entire apparatus was then evacuated at $1x10^{-3}$ Torr at 50°C (degassing). The working temperature was then set at 39.5 ± 0.1°C to start the run.

2.3 Results and Discussion

The water adsorption isotherms for potato starch at 39.5, 25.5 and 6.5°C are shown in Figure 13. The experimental data are presented in Appendix 7.

It can be seen that although very close in value, the calculated moisture contents are significantly different. Because the slopes of the isotherms are very steep at low water activity (type II isotherm), the separation of isotherms obtained at different temperatures requires precise determination of moisture content and water activities. It is clear that the use of the volumetric technique for the determination of adsorption isotherm in the low a_w range achieves this goal.

Equation 4.8 was used to fit the experimental data. The expression of $\ln(a_w/X)$ enables not only the direct determination of the net isosteric heat of sorption but also ensures the treatment of experimental data extrapolated to zero isotherm intercept ($X=0$, $a_w=0$).

The coefficient b_o, b_o', b_1', b_1'', b_2', b_2'' of equation 4.8 were estimated by a multiple linear least squares' procedure on the experimental data (BMDP(b),1983) (correlation coefficient > 0.98). Each coefficient determined through the least squares' procedure was tested against a zero value by the stastitical student test (t-test). Only the significant coefficients were retained for the expression of the regression equation 4.9:-

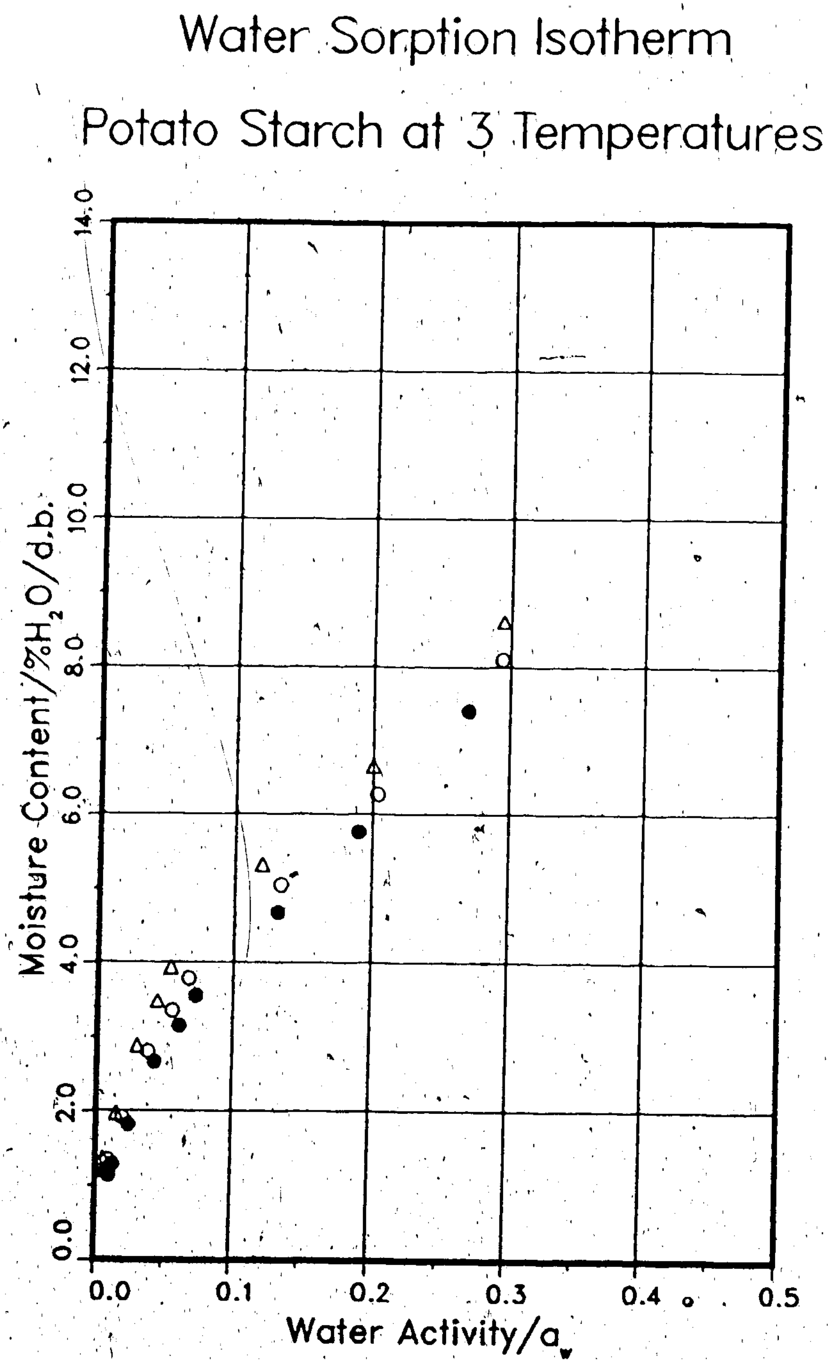


Figure 13: Water adsorption isotherms of native potato starch at three temperatures: ● 39.5°C; ○ 25.5°C △ 6.5°C.

$b_o' = -1.39$

$b_o'' = -1257 \pm 139$

$b_1' = 0.54 \pm 0.04$

$b_2' = -0.031 \pm 0.005$

$$\ln(a_w/X) = -1.39 - 1257/T + 0.54.X - 0.031.X^2 \quad (4.9)$$

The net isosteric heat of sorption is directly determined from the Clausius-Clapeyron equation 4.3 and the derivative in 1/T of equation 4.9. Hence:

$Q_n^{st} = R.\ 1257 = 10462$ J/mole H_2O

$Q_n^{st} = 581 \pm 64$ kJ/kg H_2O

We can see that for the moisture content range considered (below the monolayer coverage; about 10% H_2O/d.b. for native starch)(Wolf et al,1984), the net isosteric heat of sorption is constant. This result is confirmed by studies of Nara (1979) and Van den Berg (1975) who observed a constant net heat of sorption between 2 and 10% moisture content corresponding to an homogeneous process of adsorption (constant heat of adsorption). However, Van den Berg (1981) derived the enthalpy of sorption from measured heats of immersion and found that for the first 2% of water adsorbed, the net isosteric of sorption falls exponentially. Although the present experimental data cover partially this moisture range, it was not possible to reveal this phenomenon.

It is clear, however that the magnitude of the net isosteric heat of adsorption (581.2 ± 64.2 kJ/kg water) up to the monolayer coverage is in good agreement with already published values (Nara,1979; Van den Berg,1981; Le Maguer,1985); respectively 638, 650 and 650 kJ/kg water.

The determination of the net isosteric heat of sorption from precise sorption measurements using the volumetric technique should then be considered as a valuable alternative to calorimetric techniques. However, some experimental work is still needed to investigate the very low portion of the isotherm (moisture content of less than 2% H_2O/d.b.).

V. CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

1. Conclusions.

The suitability of the volumetric technique for the determination of water adsorption isotherms was studied for biological materials.

The water adsorption isotherm determination of Microcrystalline Cellulose (M.C.C.; reference material) and the good agreement of these data with published values confirms the suitability of the method for the determination of water adsorption isotherms with good accuracy and precision. However, because the errors on the calculated moisture contents are cumulative throughout the isotherm determination, the volumetric technique is more suitable for studies at the low a_w range (0 - 0.5) and with a limited number of experimental points (5 to 10).

The volumetric technique has been shown to be very reliable for the determination of water adsorption isotherm of low moisture capacity products. Levels of water adsorption of less than 0.1%/d.b. on the 0-0.5 a_w range have been successfully measured. However, the use of these sorption data for the characterisation of active adsorption sites on biological materials is insufficient and a combination of the method with other techniques (NMR, IR spectroscopy) should produce a real understanding of the sorption process for the primary bound water.

Within the limit of precision of the pressure readings, innumerable water activity levels can be obtained with the volumetric technique and the temperature does not constitute a limiting factor.

The use of an integral adsorption method for the determination of adsorption isotherms at different temperatures constitutes a new approach to the separation of isotherms at low a_w and renders the determination of the net isosteric of sorption possible for the primary bound water using the Clausius-Clapeyron equation.

2. Suggestions for future work

Although the apparatus and the methodology are at present well established, some future work is definitely needed in the design of ideal fully automatized equipment. This includes:

- the installation of reliable and precise pressure sensors to measure different ranges of pressure on the sample and the dosing flask. The replacement of the oil manometer would ensure a fixed dosing volume. In addicion, the constant monitoring of the pressure (difficult with the oil manometer) would permit the study of adsorption kinetics.

- The installation of high vacuum proof electronic valves would allow the use of a microcomputer to control the entire sorption isotherm determination.

This volumetric technique has been tested on only a few materials. A systematic determination of adsorption isotherms on other materials would probably hasten the recognition of the volumetric as a valuable alternative to study the water relations in biological materials.

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APPENDIX 1.

Experimental volume determination

The dosing volume V_D was determined using a reference flask of similar size mounted on the sample flask socket.

The reference flask volume V_o was determined gravimetrically using water at 23°C (density=997.4 kg/m^3) and was found to be 207.78 ± 0.02 cm^3.

The volumetric determination of the dosing volume was conducted has follows:

Water vapour at different pressures was produced by the saturator (VPS).

i. The entire apparatus being evacuated at 1.10^{-3} Torr, stopcocks 2 and 5 were closed (figure 3).

ii. For a given temperature of the water in the saturator, the vapour pressure P_o in volume V_D is read on the manometer.

iii. Stopcock 4 is then closed and 5 opened. This results to a pressure drop P_1 due to the volume expension from V_D to V_D+V_o.

The number of moles of water being unchanged in the closed system, it follows the relation:

$$P_o(V_o+(P_o.S_c/2))/R.T_o = P_1(V_D+V_o+(P_1.S_c/2))/R.T_o \qquad (A.1)$$

where $P_o.S_c/2$ and $P_1.S_c/2$ are the correction terms for the oil displacement in the manometer.

T_o = working temperature (^oK)
R = gas constant (8.32 j/mole.K)
S_c = cross sectional area of the manometer tubing (0.0706 cm^2)

It follows the expression of V_D:

$$V_D = (V_o+(P_1.S_c/2)-(P_o^2.S_c/2.P_1))(P_1/(P_o-P_1)) \qquad (A.2)$$

The volume V_D was estimated from the stastitical mean of the 15 following experimental points:

V_{Di} (cm^3)	
289.05	298.15
296.86	300.23
299.14	289.85
300.03	295.60
296.13	290.02
292.32	299.15
297.08	294.17
293.56	

$V_D = 295.42\ \mathrm{cm}^2 \qquad S_{V_D} = 0.98\ \mathrm{cm}^3$

where

$$V_D = \sum_{i}^{N} V_{Di}/N \tag{A.3}$$

$$S_{V_D} = \sqrt{\left(\sum_{i}^{N} (V_{Di}-V_D)^2/N-1\right)/N} \tag{A.4}$$

The 50% confidence interval for the volume V_D is then:

$V_D = 295.42 \pm t.S_{V_D} = 295.42 \pm 0.68\ \mathrm{cm}^3$

$t_{14,0.5} = 0.691$

APPENDIX 2.

Convenient formulation for determining the saturation vapour pressure over water (Lowe & Ficke, 1974)

$$e_{sat}=(a_0+T(a_1+T(a_2+T(a_3+T(a_4+T(a_5+a_6T)))))).100 \qquad (A.1)$$

$$de_{sat}/dT=(b_0+T(b_1+T(b_2+T(b_3+T(b_4+T(b_5+b_6T)))))).100 \qquad (A.2)$$

with T(^{o}C) and e_{sat} in Pa.

$a_0=6.107799961$

$a_1=4.436518521\text{x}10^{-1}$

$a_2=1.428945805\text{x}10^{-2}$

$a_3=2.650648471\text{x}10^{-4}$

$a_4=3.031240396\text{x}10^{-6}$

$a_5=2.034080948\text{x}10^{-8}$

$a_6=6.136820929\text{x}10^{-11}$

$b_0=4.438099984\text{x}10^{-1}$

$b_1=2.857002636\text{x}10^{-2}$

$b_2=7.938054040\text{x}10^{-4}$

$b_3=1.215215065\text{x}10^{-5}$

$b_4=1.036561403\text{x}10^{-7}$

$b_5=3.532421810\text{x}10^{-10}$

$b_6=7.090244804\text{x}10^{-13}$

Range of validity: -50^{o}C to $+50^{o}$C

APPENDIX 3.

Basic routine for moisture content and water activity calculations from experimental measurements

Basic routine

i. Amount of water (moles N_0) admitted over the sample at t=0:

$$N_0=P_0.V_D/R.T_0 \qquad \text{(A 3.1)}$$

where P_0 is the pressure read and V_D the dosing flask volume.

ii. Amount of water (moles N_1) remaining over the sample at equilibrium:

$$P_1.(V_D+V_S)/R.T_0 \qquad \text{(A 3.2)}$$

where P_1 is the equilibrium pressure read and V_S is the sample flask volume

iii. Amount of water (moles N_1) adsorbed by the sample (first point of the isotherm)

$$N_1=N_0-P_1.(V_D+V_S)/R.T_0 \qquad \text{(A 3.3)}$$

iv. For the second point of the isotherm, the amount of water (moles N_2) adsorbed is:

$$N_2 = N_1 + N_0 + (P_1.V_S/R.T_0) - (P_2.(V_S+V_D)/R.T_0) \qquad \text{(A 3.4)}$$

$N_2 = 2.N_0 - (P_1.V_D/R.T_0) - (P_2.(V_S+V_D)/R.T_0)$ (A 3.5)

. . . .

v. For i points, the amount of water (moles N_i) adsorbed by the sample is:

$N_i = i.N_0 - (P_{i-1}.V_D/R.T_0) - (P_i.(V_S+V_D)/R.T_0)$ (A 3.6)

The moisture content X_i (%H_2O/d.b.) is calculated from N_i and the dry mass (M_0) of the sample:

$X_i = ((N_i.18)/M_0).100$ (A 3.7)

The water activity a_w is calculated from the equilibrium vapour pressure P_i and the saturation water vapour pressure P^*_w at the reference temperature T_0:

$a_w = P_i/P^*_w . (T_o)$ (A 3.8)

Error calculations

The errors on the water activity and the moisture content are the absolute probable errors calculated from the uncertainty at the 50 per cent confidence level (±0.68) on the temperature and pressure readings.

It can be seen, from equation A 3.4 that the error on the calculated moisture content propagates because the calculated number of moles of water adsorbed N_i for the

i^{th} point of the isotherm is function of the amount adsorbed N_{i-1} for the $i-1^{th}$ point.

Hence:

$$\Delta N_i = \Delta N_{i-1} + \Delta N_o + \Delta(P_{i-1} \cdot V_s/R \cdot T_o) + \Delta(P_i \cdot (V_s+V_D)/R \cdot T_o \qquad (A\ 3.9)$$

It appears that a limitation on the number of isotherm points in a given a_w range is recommended.

Any expression of a number of moles N is determined from the perfect gas law equation:

$$P.V = N.R.T \qquad (A\ 3.10)$$

$$N = P.V/R.T \qquad (A\ 3.11)$$

Then

$$\Delta N/N = \Delta P/P + \Delta V/V + \Delta T/T \qquad (A\ 3.12)$$

As an example; for a given isotherm determination at 25°C (298°K) ($P_w^* = 37.01$ cm oil), the relative error on the volumes and temperatures are constant.

$$\Delta T/T \simeq 0.068/298 = 0.00023$$

$$\Delta V/V \simeq 0.68/295.42 = 0.002$$

If the water vapour pressures to be measured range from 0.37 to 18.51 cm of oil (corresponding to the a_w range 0.01 - 0.5), we can see that at low pressures, the precision on the pressure readings (±0.02cm oil) is the limiting factor ($\Delta P/P$ = .054) for good precision on the calculated number of moles of water while at high pressure ($\Delta P/P$ = 0.001) the limiting factor is the precision on the volume determination.

Calculation program

The following program, written in FORTRAN 77 , uses this basic routine and calculates the probable absolute error on the moisture content and water activity.

```
*******************************************************************
*   february 1986                                                 *
*   program EC.ISO8.S                                             *
*                                                                 *
*   Etienne CORTET                                                *
*   Food science department                                       *
*   The University of Alberta                                     *
*   Edmonton - Alberta                                            *
*                                                                 *
*   Calculation program for the determination of water sorption   *
*   isotherm using a volumetric technique.                        *
*                                                                 *
*   The program includes 4 subroutines:                           *
*                                                                 *
*   SUBROUTINE INPUT : READ THE DATA FILE                         *
*                                                                 *
*   SUBROUTINE MOIST : CALCULATE, FOR EACH POINT, THE MOISTURE    *
*                      UPTAKE X (%H2O/D.B.)                       *
*   SUBROUTINE WATACT : CALCULATE, FOR EACH POINT, THE WATER      *
*                       ACTIVITY (AW)                             *
*                                                                 *
*   SUBROUTINE PSAT : CALCULATE THE SATURATION VAPOUR PRESSURE    *
*                     AT THE EQUILIBRIUM SAMPLE TEMPERATURE       *
*                     USING THE CORRELATION OF LOWE & FICKE (1974)*
*                                                                 *
*   THE INPUT FILE IS FORMATTED                                   *
*                                                                 *
*******************************************************************

*  main program

*  Declaration statements


*  Aw, dAw  : water activity & error
*  H0       : vapor pressure (cm oil) in VD (t=0)
*  H1       : vapor pressure (cm oil) in VD+VS (equilibrium)
*  K0, K1 : temperatures (kelvin) at t=0 & equilibrium
*  M0, dM0  : dry sample mass & error
*  NPOINT   : number of experimental points
*  N0, DN0  : moles of water in VD & error(t=0)
*  N1, DN1  : moles of water in VD+VS (equilibrium) & error
*  N2, DN2  : moles of water in VS (equilibrium) & error
*  NFIX, DNFIX : moles of water adsorbed & error
*  PS, DPS  : saturation vapor pressure at T2 & error
*  P0, dP0  : vapor pressure from H0 & error(Pa)
*  P1, DP1  : vapor pressure from H1 & error(Pa)
*  PRODT    : product name
*  R        : gas constant
*  RTEMP    : reference temperature(celcius)
*  T0, T1, DT : temperatures at t=0 & equilibrium & error
*  T2, DT   : reference temperature& error
*  VD,VS,DVD,DVS : dosing volume, sample volume & errors
*  X, DX    : moisture (%H2O/D.B.) & error
*  RO, DRO  : density of the manometer oil=f(T1) & error


      IMPLICIT LOGICAL*1 (A-Z)
      INTEGER NPOINT,I
      REAL T0(50), T1(50), T2(50)
```

```
      REAL M0, H0(50), H1(50), P0(50), P1(50), DP1(50),
      REAL AW(50),DAW(50),X(50),DX(50)
      INTEGER RTEMP
      CHARACTER*20 DATE, PRODT

      CALL INPUT(DATE,PRODT,NPOINT,M0,RTEMP,H0,H1,T0,T1,T2)
      CALL MOIST(NPOINT,M0,H0,H1,T0,T1,T2,X,DX,P0,P1,DP1)
      CALL WATACT(NPOINT,P1,DP1,T2,AW,DAW)


*     Create the output file

      WRITE(6,*) DATE
      WRITE(6,*) PRODT
      WRITE(6,*) 'Temperature: ',RTEMP
      WRITE(6,1) 'AW','DAW','X(%H2O/D.B.)','DX'
    1 FORMAT(//T4,A,T15,A,T24,A,T39,A)

      DO 100 I=1,NPOINT
         WRITE(6,2) AW(I),DAW(I),X(I),DX(I)
    2    FORMAT(T2,F7.5,T13,F7.5,T25,F8.4,T37,F8.4)
  100 CONTINUE

      END

************************************************************************
      SUBROUTINE INPUT(DATE,PRODT,NPOINT,M0,RTEMP,H0,H1,T0,T1,T2)


      INTEGER NPOINT,I,MAXPT,RTEMP
      REAL T0(50), T1(50), T2(50)
      REAL H0(50), H1(50), M0
      CHARACTER*20 DATE, PRODT

      MAXPT=50

      READ(5,1) DATE
      READ(5,1) PRODT
    1 FORMAT(A20)
      READ(5,3) NPOINT
    3 FORMAT(I2)
         IF(NPOINT.GT.MAXPT) THEN
           WRITE(6,4) 'ERROR IN INPUT DATA, NPOINT IS: ',NPOINT
    4      FORMAT(T2,A,I2)
           STOP
         ENDIF

      READ(5,5) M0
    5 FORMAT(F6.4)

      READ(5,6) RTEMP
    6 FORMAT(I2)

      DO 100 I=1,NPOINT
         READ(5,7) H0(I),T0(I),H1(I),T1(I),T2(I)
    7    FORMAT(F5.2,1X,F4.1,1X,F5.2,1X,F4.1,1X,F4.1)
  100 CONTINUE

      RETURN
      END
```

```
****************************************************************
      SUBROUTINE MOIST(NPOINT, M0,H0,H1,T0,T1,T2,X,DX,P0,P1,DP1)


      INTEGER NPOINT, I
      REAL T0(50), T1(50), T2(50), K0(50), K1(50)
      REAL H0(50), H1(50), P0(50), P1(50)
      REAL M0, N0, N1, N2, NFIX, DN0, DN1, DN2, DNFIX, X(50), DX(5(
      REAL DM0,DT,DP1(50),DP0(50),R,RO(50),DRO(50),MV1(50),MV2(50)
      REAL VD, VS, DVD, DVS,DMV
      DATA VD,VS/ 295.42E-06, 18.39E-06/
      DATA DVD,DVS/ 2.10E-06, 0.010E-06/
      DATA DM0,DT,R/ 1.0E-04, 1.0E-01, 8.32/

      DO 100 I=1, NPOINT

* temperatures transformed in kelvins
          K0(I)=T0(I)+273.16
          K1(I)=T1(I)+273.16
* density of oil = f(T1) and error
          RO(I)=(-5.68339E-04*T1(I)+8.865235E-01)*1.0E+03
          DRO(I)=(2.172697E-5/5.68339E-4)+(0.1/T1(I))
          DRO(I)=(DRO(I)*(+5.68339E-4*T1(I))+5.092723E-5)*1.0E+03
* calculation of P in Pascals and error
          P0(I)=H0(I)*0.01*9.81*RO(I)
          P1(I)=H1(I)*0.01*9.81*RO(I)
          DP0(I)=(2E-2/H0(I)+DRO(I)/RO(I))*P0(I)
          DP1(I)=(2E-2/H1(I)+DRO(I)/RO(I))*P1(I)
* volume corrections in the manometer and error
          MVD(I)=H0(I)*3.534E-8
          MVS(I)=H1(I)*3.534E-8
          DMV=0.02E-2*3.534E-8

  100 CONTINUE

* Variables initialisation

      NFIX=0
      DNFIX=0
      N2=0
      DN2=0

* For each point, amount of water admitted at t=0
  and error.

      DO 200 I=1, NPOINT
       N0 = (P0(I)*(VD+MVD(I)))/(K0(I)*R)
       DN0=(DP0(I)/P0(I)+DVD/VD+DT/K0(I))*P0(I)*VD/(K0(I)*R)
       DN0=(DP0(I)/P0(I)+DMV/MVD(I)+DT/K0(I))*P0(I)*MVD(I)/(K0(I)*R

* Amount of water remaining in the gas phase at equilibrium
  and error.

       N1 = (P1(I)*(VD+VS+MVS(I)))/(K1(I)*R)
       DN1 = (DP1(I)/P1(I)+DVD/VD+DT/K1(I))*P1(I)*VD/(K1(I)*R)
       DN1 = DN1+(DP1(I)/P1(I)+DVS/VS+DT/K1(I))*P1(I)*VS/(K1(I)*R)
       DN1=DN1+(DP1(I)/P1(I)+DMV/MVS(I)+DT/K1(I))*P1(I)*MVS(I)/
     +   (K1(I)*R)
```

```
*  Amount of water adsorbed and error

        NFIX = NFIX+N0+N2-N1
        DNFIX = DNFIX+DN0+DN2+DN1

*  Amount of water remaining in the sample flask at equilibrium
       and error

        N2 = (P1(I)*VS)/(K1(I)*R)
        DN2 = (DP1(I)/P1(I)+DVS/VS+DT/K1(I))*P1(I)*VS/(K1(I)*R)

*  Moisture content calculation (%H20/d.b..) and error

        X(I) = ((NFIX*18)/M0)*100
        DX(I)=((DNFIX/NFIX)+(DM0/M0))*X(I)

  200 CONTINUE

      RETURN
      END
**************************************************************
      SUBROUTINE WATACT(NPOINT.P1.DP1.T2.AW.DAW)


      INTEGER NPOINT
      REAL AW(50).PS(50).DAW(50).DP1(50).P1(50).T2(50).DPS(50)

      DO 100 I=1, NPOINT
         CALL PSAT(T2, I, PS, DPS)
         AW(I) = P1(I)/PS(I)
         DAW(I) = (DP1(I)/P1(I)+DPS(I)/PS(I))*AW(I)
  100 CONTINUE

      RETURN
      END

**************************************************************
      SUBROUTINE PSAT(T2.I.PS.DPS)

* Convenient formulation for the saturation vapor pressure over
* water (Lowe and Ficke, 1974)
* The saturation vapor pressure is computed at T2 (sample
* temperature) at equilibrium.

      INTEGER I
      REAL T2(50).DT/.1/.PS(30).DPS(30)
      REAL A0.A1.A2.A3.A4.A5.A6
      REAL B0.B1.B2.B3.B4.B5.B6
      DATA A0.A1.A2.A3.A4.A5.A6/6.10779996.4.43651852E-1.
     +     1.42894580E-2.2.65064847E-4.3.03124039E-6.
     +     2.03408094E-8.6.13682092E-11/
      DATA B0.B1.B2.B3.B4.B5.B6/4.43809998E-1.2.85700264E-2.
     +     7.93805404E-4.1.21521506E-5.1.03656140E-7.
     +     3.53242181E-10.-7.090244804E-13/

      PS(I) = (A0+T2(I)*(A1+T2(I)*(A2+T2(I)*(A3+T2(I)*(A4+T2(I)*(
     +       A5+T2(I)*A6))))))*100

      DPS(I)=DT*(B0+T2(I)*(B1+T2(I)*(B2+T2(I)*(B3+T2(I)*(B4+T2(I)*(

     +         B5+T2(I)*B6))))))*100

      RETURN
      END
End of file
```

APPENDIX 4.

Water adsorption isotherm data on MCC at 25^{o}C (Experimental data - 3 runs)

	a_w	Δa_w	X	ΔX
RUN #1	0.0319	0.0008	1.175	0.002
	0.1143	0.0013	2.176	0.006
	0.2062	0.0019	2.989	0.011
	0.2881	0.0025	3.638	0.017
	0.3530	0.0029	4.156	0.024
	0.4051	0.0033	4.570	0.033
	0.4492	0.0036	4.894	0.042
RUN #2	0.0125	0.0006	0.725	0.001
	0.0487	0.0009	1.399	0.003
	0.1014	0.0012	2.000	0.005
	0.1585	0.0016	2.525	0.007
	0.2139	0.0020	2.975	0.011
	0.2639	0.0023	3.358	0.015
	0.3066	0.0026	3.686	0.019
	0.3464	0.0029	4.052	0.024
	0.4054	0.0033	4.507	0.029
	0.4526	0.0036	4.898	0.035
	0.5040	0.0039	5.350	0.042
	0.5448	0.0042	5.743	0.049
	0.5847	0.0045	6.084	0.057
RUN #3	0.1402	0.0015	2.402	0.009
	0.3307	0.0028	4.006	0.024
	0.4590	0.0036	5.092	0.043
	0.5519	0.0042	5.989	0.065

a_w = water activity
Δa_w = calculated absolute probable error on a_w
X = moisture content (%H_2O/d.b.)
ΔX = calculated absolute probable error on X

APPENDIX 5.

Water adsorption isotherm data for MCC at 25°C
Repeatability and reproducibility values from a
collaborative study. (Wolf et al,1984)

a_w	$\overline{X}$	(s)	(r)	(R)
0.11	2.02	0.25	0.13	0.72
0.23	3.19	0.21	0.11	0.63
0.33	4.06	0.25	0.11	0.73
0.44	5.04	0.19	0.14	0.54
0.53	5.82	0.27	0.22	0.79
0.58	6.48	0.21	0.07	0.61
0.71	8.21	0.20	0.22	0.57
0.75	8.83	0.31	0.21	0.89
0.84	10.95	0.29	0.19	0.85
0.90	12.96	0.30	0.26	0.88

a_w = water activity
$\overline{X}$ = mean water content (%H_2O/d.b.)
s = standard deviation
r = repeatability (as defined by
R = reproducibility Wolf &al, 1984)

(G.A.B. constants: $X_m = 4.064$; $C = 8.776$; $k = 0.772$.)

APPENDIX 6.

Water adsorption isotherm data for products CP, SC, SA and BSA at 25°C

	a_w	Δa_w	X	ΔX
CP				
RUN #1	0.0664	0.0046	0.0334	0.0009
	0.1229	0.0080	0.0608	0.0022
	0.1925	0.0122	0.0747	0.0039
	0.2272	0.0143	0.0839	0.0057
	0.2522	0.0158	0.0909	0.0077
	0.2924	0.0182	0.0997	0.0099
	0.3470	0.0215	0.1159	0.0124
	0.3884	0.0240	0.1234	0.0151
	0.4162	0.0257	0.1291	0.0180
RUN #2	0.0282	0.0009	0.0286	0.0012
	0.0924	0.0013	0.0523	0.0027
	0.1478	0.0017	0.0689	0.0044
	0.2442	0.0023	0.1008	0.0083
	0.3168	0.0027	0.1139	0.0106
	0.4044	0.0032	0.1297	0.0131
SC I	0.0200	0.0006	0.0193	0.0008
	0.0474	0.0008	0.0309	0.0018
	0.0756	0.0010	0.0422	0.0028
	0.1065	0.0012	0.0517	0.0040
	0.1611	0.0015	0.0635	0.0053
	0.2071	0.0018	0.0726	0.0068
	0.2370	0.0020	0.0868	0.0083
	0.2589	0.0021	0.0899	0.0100
	0.2888	0.0023	0.0939	0.0117
	0.3293	0.0025	0.1005	0.0135
	0.4146	0.0030	0.1104	0.0156
SC III	0.0024	0.0006	0.0052	0.0009
	0.0168	0.0007	0.0179	0.0021
	0.0632	0.0010	0.0334	0.0034
	0.1246	0.0013	0.0470	0.0047
	0.1938	0.0017	0.0617	0.0061
	0.2344	0.0020	0.0724	0.0075
	0.2570	0.0025	0.0869	0.0104
	0.3755	0.0028	0.0938	0.0120
	0.4691	0.0034	0.1037	0.0136

	a_w	Δa_w	X	ΔX
SA I	0.0300	0.0007	0.0187	0.0010
	0.0755	0.0010	0.0327	0.0022
	0.1134	0.0012	0.0412	0.0035
	0.1723	0.0016	0.0515	0.0051
	0.2102	0.0018	0.0593	0.0068
	0.2592	0.0021	0.0671	0.0087
	0.3155	0.0024	0.0756	0.0108
	0.3680	0.0027	0.0825	0.0131
	0.4174	0.0030	0.0903	0.0155
SA II	0.0183	0.0007	0.0173	0.0009
	0.0517	0.0009	0.0334	0.0022
	0.0849	0.0011	0.0435	0.0035
	0.1382	0.0014	0.0569	0.0048
	0.1720	0.0016	0.0656	0.0062
	0.2276	0.0019	0.0715	0.0076
	0.2555	0.0021	0.0767	0.0090
	0.3276	0.0026	0.0847	0.0105
	0.3930	0.0029	0.1012	0.0136
	0.4540	0.0032	0.1096	0.0153
BSA I	0.0187	0.0009	0.0264	0.0009
	0.0532	0.0011	0.0507	0.0019
	0.0990	0.0014	0.0676	0.0030
	0.1390	0.0016	0.0858	0.0043
	0.1954	0.0019	0.1088	0.0057
	0.2662	0.0024	0.1300	0.0072
	0.3428	0.0029	0.1642	0.0089
	0.4261	0.0034	0.2008	0.0109
BSA II	0.0060	0.0006	0.0213	0.0008
	0.0404	0.0009	0.0526	0.0019
	0.0965	0.0012	0.0780	0.0031
	0.1518	0.0015	0.1134	0.0043
	0.2215	0.0019	0.1360	0.0055
	0.3134	0.0024	0.1632	0.0069
	0.3672	0.0027	0.1757	0.0083

APPENDIX 7.

Water adsorption isotherm data for native potato starch at three temperatures

	a_w	Δa_w	X	ΔX
Temperature				
39.5°C	0.0096	0.0003	1.1444	0.0017
	0.0123	0.0003	1.2899	0.0019
	0.0237	0.0004	1.8225	0.0028
	0.0427	0.0005	2.6641	0.0064
	0.0609	0.0006	3.1511	0.0073
	0.0729	0.0007	3.5554	0.0080
	0.1321	0.0011	4.6785	0.0153
	0.1902	0.0015	5.7665	0.0251
	0.2694	0.0020	7.3979	0.0492
25.5°C	0.0076	0.0006	1.1814	0.0016
	0.0092	0.0006	1.3373	0.0016
	0.0193	0.0006	1.9134	0.0022
	0.0378	0.0008	2.8006	0.0055
	0.0556	0.0009	3.3519	0.0059
	0.0677	0.0010	3.7873	0.0063
	0.1342	0.0014	5.0519	0.0125
	0.2035	0.0019	6.2744	0.0212
	0.2939	0.0025	8.0975	0.0437
6.5°C	0.0055	0.0019	1.2082	0.0016
	0.0064	0.0019	1.3698	0.0016
	0.0144	0.0019	1.9741	0.0021
	0.0297	0.0021	2.8881	0.0051
	0.0445	0.0021	3.4801	0.0052
	0.0541	0.0022	3.9387	0.0055
	0.1198	0.0027	5.3206	0.0107
	0.2002	0.0033	6.6512	0.0186
	0.2948	0.0039	8.6115	0.0400