STUDIES ON THE CONDITION OF WATER IN PLANT SYSTEMS AND OF THE INFLUENCE OF THE MAJOR SOLID CONSTITUENTS

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PART 1

I N T R O D U C T I O N

1.0 WATER AND ITS INTER-RELATIONS WITH SYSTEMS OF BIOLOGICAL ORIGIN.

In all biological material, both living and non-living, water plays a major role in the complex of biochemical processes. Its removal, or effective removal, has far reaching effects on the reaction rates of these processes, usually acting to cause a shift toward complete cessation, as well as inducing structural changes in certain cases.

Techniques which cause such a reduction in the amount of liquid water, in particular dehydration and freezing, have historically been used in food preservation, and ever since the first attempts to investigate the scientific basis behind their action interest has been expressed in the condition of water both in the fresh and the processed material.

Detailed knowledge of the behaviour of water in complex food systems will, therefore, provide the necessary background to the understanding and control of water dependent deteriorative processes.

1.1 WATER AND ICE

A study of the basic structural unit of water provides the common starting point from which its behaviour in different environments can be deduced.

Early analytical work in the seventeenth and eighteenth centuries established the molecular composition of water, although with the later discovery of the several isotopes of oxygen and hydrogen it became apparent that naturally occurring water is actually a mixture of several species. The nuclei of a water molecule form an isoceles triangle, with a slightly obtuse angle of 104.52° at the oxygen atom and a bond length between the oxygen and hydrogen atoms of 0.95718 Å (Fig. 1).

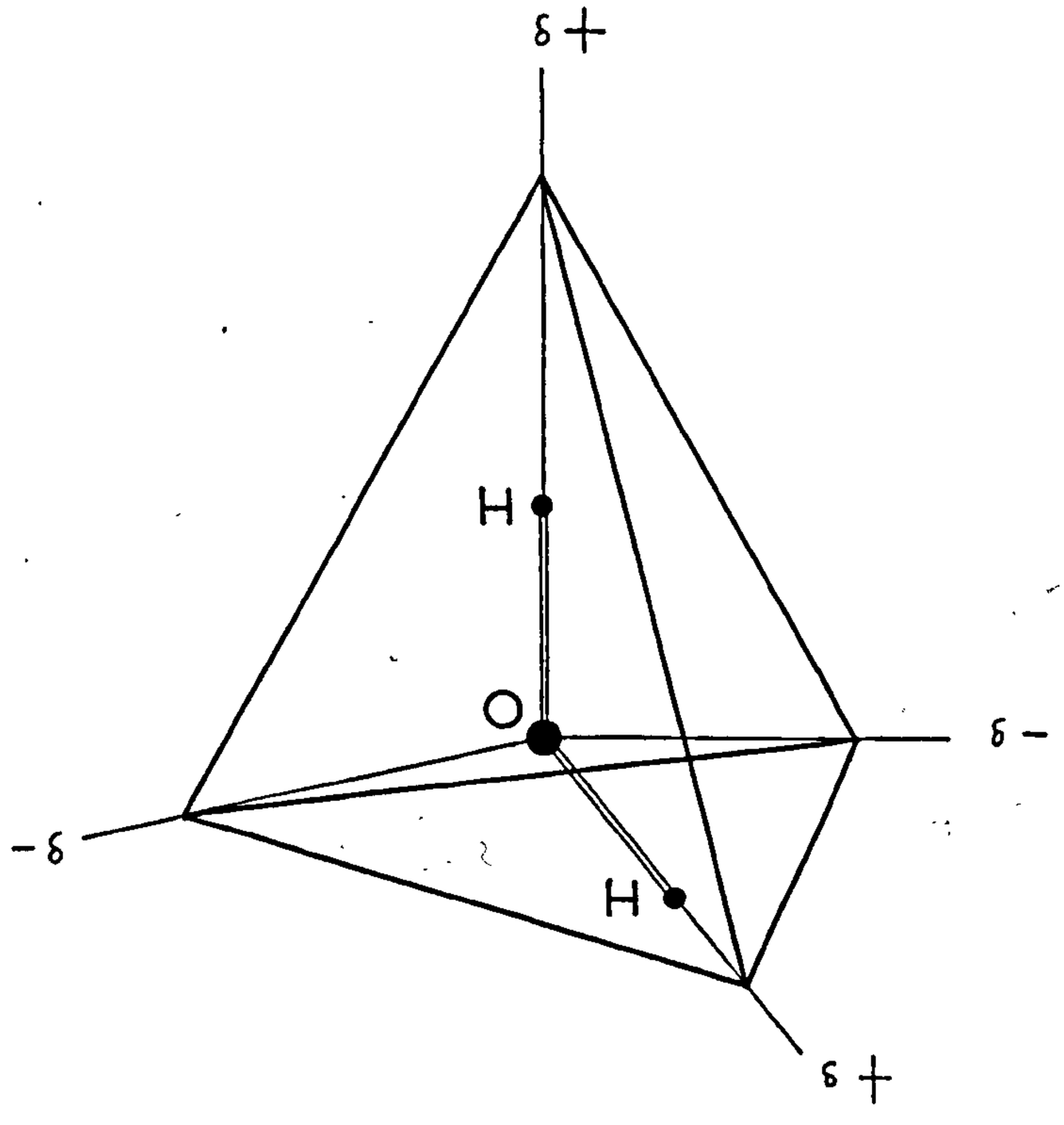

Fig. 1. Diagrammatic representation of a water molecule.

These values apply to the equilibrium state of the molecule with the actual dimensions depending on the energy state at any one moment.

The water molecule has an energy of formation of -219.337 Kcal mole^{-1} at 0^{o}K with a zero point vibrational energy of $+13.25$ Kcal mole^{-1}. The oxygen-hydrogen bond energy at 0^{o}K is -109.7 Kcal mole^{-1}, although the dissociation energy of the O-H bond is lower than that of the H-OH bond, the dissociation of the H-OH bond allowing the oxygen atom to undergo a favourable electronic rearrangement, thereby reducing the dissociation energy of the remaining bond.

Each of these O-H bonds of the water molecule consists of a molecular orbital formed from one of the 2p orbitals of the oxygen atom and the 1s orbital of the hydrogen atom (Fig. 2).

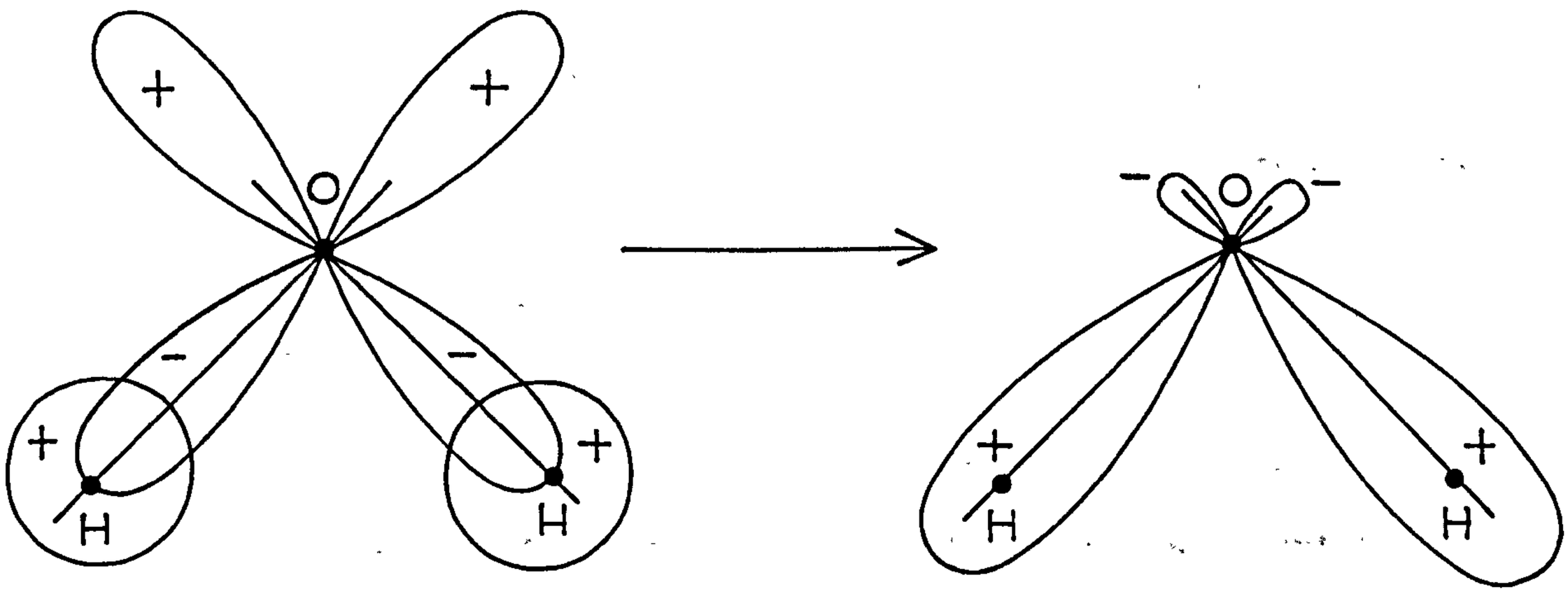

Fig. 2. Diagrammatic representation of the molecular orbital picture of a water molecule.

The resultant orbital is slightly electropositive. This leaves two electronegative lone pairs of electrons on the two unbonded p orbitals of the oxygen atom and results in the molecule as a whole having a considerable dipole moment. This dipole moment facilitates the formation of hydrogen bonds between water molecules, a phenomenon which explains many of the unique properties of water.

All theories on the structure of water have common to them this basic characteristic of the molecule, and the resultant ability to form a hydrogen-bonded network of molecules. In most other liquids composed of small, simple molecules there is close packing, and each molecule has nearly the maximum number of nearest neighbours that spatial considerations allow, with no spatial order beyond the nearest neighbour. In water, however, the average number of nearest neighbours is low, both in the liquid and in the solid, and ordering of the molecules extends over at least several molecular dimensions due to the formation of intermolecular hydrogen bonds. This basic structure is best observed in the low energy, low temperature, solid form of water.

1.1.1 Ice Structure

Unlike the structure of water, the basic structure of ice has been elucidated beyond reasonable doubt and is accepted by all workers in the field.[1-5] Each water molecule can participate in up to four hydrogen-bonds, acting in two as a donor of the electrostatic charge involved, and in two as the acceptor of the charge. In the form of ice found at normal pressures, ice I, each oxygen atom is at the centre of a tetrahedron

formed by another four oxygen atoms, each at a distance of 2.76 Å. Thus every water molecule is hydrogen-bonded to its four nearest neighbours. This arrangement leads to an open, strain-free, lattice in which intermolecular cohesion is large and the density is about half that if the molecules were as closely packed as spatially possible (Fig. 3).

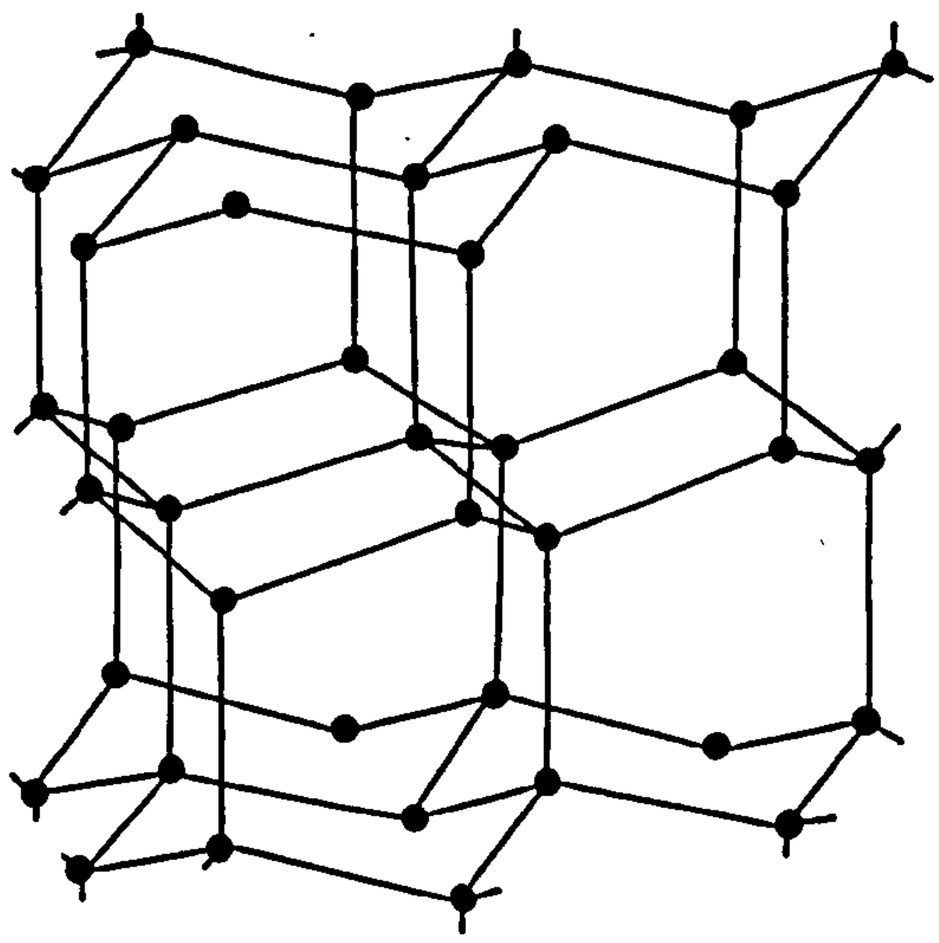

Fig. 3. Diagrammatic representation of the arrangement of hydrogen-bonded water molecules in ice I^2.

Solid circles represent oxygen atoms. Hydrogen atoms are not shown.

Also of interest within the context of this study is the existence of cubic ice. This is a metastable form found at temperatures below -140°C but only being formed under special conditions and changing to the hexagonal ice I structure on warming. The six high-pressure forms of ice, which are also known to exist, have no significance in the study of foodstuffs at low temperatures.

1.1.2 Water Structure

During consideration of the modern theories on water structure it should be borne in mind that water is a dynamic system, much more so than its truly crystalline form, ice. In the latter, at $0^{o}C$, there are 10^{5} to 10^{6} thermally induced movements per second, compared to 10^{11} to 10^{12} such movements in liquid water at the same temperature. These movements may be regarded as being of two types; rapid oscillations about temporary equilibrium positions, and the slower displacements of these positions. Theories on water structure are best taken as applying to the mutual arrangement of relative molecular positions during the time of 'equilibrium', ignoring the short-lived movements. It must be realised, however, that this state of 'equilibrium' is also constantly changing, but at a slower rate.

Over the last few years several theories describing the structure of water have been put forward. These have been well reviewed by Némethy[2, 7], Eisenberg and Kauzman[1] and Frank[3]. Essential to all of them is the existence of a hydrogen bonded network of molecules as proposed by Bernal and Fowler[8] when they rejected the small aggregate model which was in vogue at the time. The theories disagree on the details of structure, particularly when dealing with the breakage of molecular hydrogen-bonds.

Two main schools of thought have arisen, their hypotheses based on experimental and theoretical evidence; one favouring a distorted hydrogen-bond model and the other supporting a mixture model.

a) Distorted hydrogen-bond model:

This model, supported by Haggis, Hasted and Buchanan[9],

Woessner[10], and Pople[11], assumes that the surroundings of any water molecule in the liquid must always be the same as that of any other, suggesting some kind of distorted hydrogen-bonded structure persisting throughout the liquid. As an extension of this idea, the random network model[12] postulates that instead of forming an ordered lattice, as in ice, an irregular network of rings is present. Many of these rings contain five molecules, thus correlating the 104.5° H-O-H bond angle of a water molecule with the 108° angle of a five membered ring. The theory also allows for other rings containing 4, 6, 7 and more molecules. Eisenberg and Kauzman[1] expressed some support for this theory and believe that it merits further investigation.

b) Mixture model:

Support for this model can be traced back to 1892 when Roentgen[6], basing his assumptions on experimental results, considered liquid water as a solution of ice in a denser, genuinely fluid, water species. Much more recently, considerably more attention[2,3,5,7,13,14] has been given to this idea. The basis of the hypothesis for a mixture model is the assumption that the forming and breaking of hydrogen-bonds in liquid water is a co-operative process and can be explained in terms of mutual polarization of two water molecules as they interact. This makes one molecule more basic and the other more acidic, which in turn stimulates interaction with a further two molecules, etc. etc.. Conversely, when one bond breaks as a result of thermal fluctuation, a corresponding "unzipping" is imaginable leading to the practically simultaneous breaking of a whole group of bonds. This argument has been opposed, however, by

Haggis, Hasted and Buchanan[9] and Woessner[10] who postulated that a water molecule will enter into the formation of a hydrogen bond independently of the number of bonds in which it may already be a partner.

This mixture model presents a system in which, at any instant, there are two distinct states of water molecules. Those present in a quasi-crystalline state, in which each molecule is capable of taking part in four tetrahedrally arranged hydrogen-bonds, and which has a low density, compared to the second, dense state of non-hydrogen-bonded molecules. The properties of this latter species are not well defined at present, although it has been realised that they are still close enough in the liquid to interact to some extent by dipole forces[5]. It has been estimated that only about a quarter of the heat of sublimation of ice comes from the breaking of hydrogen bonds, the rest being due to non-hydrogen-bonding interactions in the liquid. The molecules present in these two states are, however, continually changing their role with the continuous breakdown and construction of the structured form, so no molecule is permanently less reactive or less mobile than any other, with each having the same average environment. Frank[3] suggested that the average lifetime of a cluster is about one hundred times as long as the time involved in a molecular vibration.

Within this theory there have been two suggestions for the forms of these ordered areas. The first is that a structure comparable to that of a gas hydrate exists, with the non-bonded molecules present in cavities occurring within the hydrogen-bonded lattice which has a more open structure than ice I, in much the same way as non polar gas molecules are trapped in a surrounding ordered network of water molecules. The second idea

emphasises the limited spatial extent of the hydrogen-bonded network in a matrix of non-bonded molecules, and suggests the existence of flickering clusters with an ice I type of structure. These two theories can be paralleled to an emulsion of non-bonded molecules in an ordered bulk, and to an emulsion of bonded networks in a non-bonded bulk. It would be quite plausible, however, to accept both of these ideas and to postulate a temperature dependent scale passing from the totally ordered structure of ice, through the gas hydrate model existing at low temperatures which would then pass to the cluster model with increasing temperature, and finally to a totally non-bonded state at high temperatures when the behaviour of water approaches that of other polar liquids. Némethy and Sheraga[14] have given values for cluster sizes of 90 molecules at 0°C, 50 at 25°C and 25 at 70°C.

At the present time the flickering cluster idea seems to enjoy the greatest support[2,3,5,7], with the suggestion that these clusters are nearly spherical in shape, their size varying with temperature. Inside the clusters will be tetra-bonded molecules, while on the surface there are tri, di and some mono-bonded species. An energy level can be assigned to each species depending on the number of its hydrogen-bonds; the fewer the bonds, the higher the energy state, with the numbers of the various species being dependent upon the size of the cluster.

Némethy[2], however, has warned against taking a narrow point of view about possible cluster structure and restricting them to analogues of crystals. This is because the hydrogen-bond arrangement does not have to extend indefinitely in all directions, as in crystals, and thus irregular networks of clusters are possible, although they could not be extended

beyond a few dozen molecules without the disruption of many hydrogen-bonds. Indeed, Eisenberg and Kazyman[1] said, "Even if some distinctly different molecular species do exist in water, the common verbal descriptions of them are inaccurate. 'Ice-like' clusters cannot possibly be ice-like on experimental grounds."

At the present time the state of opinion about the structure of liquid water can be summed up as being predominately in favour of the flickering cluster theory, although there are certain workers favouring the distorted hydrogen-bond idea.

Having discussed current theories on the structure of water in the bulk liquid, it is now necessary to see how this may be related to the state of water as it is present in complex materials of biological origin. This involves:

a) that water which is in intimate contact with the macromolecular species present, or which is influenced by the forces exerted by them;

b) that which is influenced by the microscopic structure;

c) that remaining which is not affected by these two factors, but which acts as a solvent system with possible resultant effects on structure.

1.2 WATER IN LIVING SYSTEMS

A brief consideration of the ideas on the state of water in living systems, as at their present state of development, provides a good starting point for the study of water as it occurs in foodstuffs. Most foodstuffs were originally living systems or are primary derivatives of living systems and, indeed, some foodstuffs are still living at the time of consumption. Such considerations can thus lead onto a more detailed examination of the relationships of water with food materials, and, in fact, could be argued to be inseparable from them.

Gortner[15] recounts the story of a jellyfish which was found to suffer over a thousand times reduction in weight upon drying, and yet in which, in the living state, the water present must be sharply differentiated from that in its surrounding environment. While this is an extreme case, water is nevertheless the major integral component of most biological systems. Living protoplasm is not simply a dilute salt solution, but a three dimensional lattice of protein, water and salts, in which the charge bearing macromolecules constitute a fixed charge system. The problem of the exact state of water in biological systems and of its special role in such systems has been discussed for many years. While it is likely that at least some of the water is present in essentially the same state as bulk water it is equally certain that some of it, due to its close proximity with the protein and other biologically important macromolecules appears to exist in a physical state different from that of normal water[15-22].

The first postulations along these lines came from three independent sources around about 1920[23-25]. Following this, at

the end of the subsequent decade, Gortner[26] reviewed the results of a host of workers, who, using a variety of different techniques, had confirmed the presence of a fraction of water with the apparently unusual physical properties of low vapour pressure, depressed freezing point or complete non-freezability and the inability to act as a solvent, and who had attempted to quantify this "bound" water, as it was then rather sweepingly called.

In spite of all this evidence in support of the presence of a distinct water fraction with different physical properties, views held by cell physiologists on the physical state of water in living cells are still divergent. This conflict arises when the role of water in the cell is considered, with attempts being made to explain the asymmetry of ion and non-electrolyte distribution between the cell and its environment. A detailed consideration of this field of study is outside the present terms of reference, but it is pertinent to mention the Association-Induction hypothesis put forward by Ling in 1951-2.[27,28] This was presented as an alternative to the classical membrane theory and its associated ideas of "Na pumps", "permeases" and pore size restriction[29], to explain the asymmetrical distribution of solutes. The basis of Ling's hypothesis, which developed the earlier ideas of Fischer and More[30], Neuschloss[31], Ernst and Morocz[32], and Troschin[33] is a belief that all, or nearly all, water molecules in a living cell can be considered to exist in polarised multilayers oriented on the surface of cell proteins, these constituting an important part of the cellular lipo-protein membranes. This orientation, Ling has suggested, results in a reduction of rotational freedom, the effect decreasing with increasing distance from the macromolecular surface, and the introduction of ions into this polarized water is, in many cases,

an energetically unfavourable process. This is attributable to an increase in order, or loss of entropy, within the system which results from the ions ability to strongly polarize water within their own immediate vicinity.

In a series of papers[29,34,35] Ling has explained the significance of his multilayer concept in terms of membrane diffusion and cell organization and function, and he mentions the evidence of both freezing studies[36] and of Bradley's mathematical approach to the water relations of macromolecules[37] (see Section 2.3) which apparently support his multilayer concept. While propounding his hypothesis however, Ling also stressed that living protoplasm, and hence protoplasmic water, can vary in physical state from cell to cell, between different regions within the cell, and also in the same region of the same cell at different times.

The idea that water in the cell probably has a continuous spectrum of activation energies has been supported by Fernandez-Moran[38] and Hechter[39], the latter postulating five different energy states through which it might move. These, in order of decreasing organization, were:-

1) Water within the membrane structure.
2) One or more layers of water directly in contact with extended, relatively immobile, macromolecular surfaces.
3) Water between closely paired systems of unit membranes.
4) Water within interior chambers of an organelle.
5) Water in the hyaloplasm, between the various organelles in the cell.

While not dismissing completely that some of the points of

the classical membrane theory may still be of importance, Hechter probably carries the concensus of modern opinion on cell water with him when he says that a sizeable fraction of all the water in the typical cell is intimately associated with the membrane system and as such must be sharply differentiated from the bulk water.

1.3 ASSOCIATION BETWEEN FOOD CONSTITUENTS AND WATER

1.3.1 General Considerations

Just as workers studying living systems have come to the conclusion that a portion of the water present has a special association with the basic structure of macromolecules, so have those studying the related problem of water in foodstuffs. Indeed it is perhaps obvious that all the water present must be restricted in some way, as witnessed by the virtual absence of water freely dripping from both intact and divided foods in their normal state of hydration. The question which has occupied the minds of many over the last 150 years, and particularly the last 50 years, and which is still not satisfactorily answered, lies in the elucidation and description of the state of this water, with, as yet, no theoretical approach which can accurately cover the problem over the entire moisture content range.[40]

Study of water sorption by biopolymers, that is the process of interaction over the complete moisture content range between vapour molecules and the solid, has resulted in knowledge of the now undisputed fact that a small amount of water (16g-50g/100g dry matter in animal tissue and from 4g/100g dry matter upward in

plant tissue[41]) is so intimately connected with the macromolecules present as to have a typical physical properties. However, there have been only a few specific experiments[21,42] which have yielded clear cut evidence on water structure near macromolecules. Almost from the first, workers have attempted to differentiate between "bound" water, which appeared to have unusual physical properties, and "free" water. This has resulted in as many definitions of "bound" water as there are methods for measuring it[43,44], although that water which does not act as a solvent[45-47] or that which does not freeze[48-54] were two of the most favoured early definitions. In the light of more recent work[41], however, even these two descriptions have been shown to define distinctly different quantities of water. Gortner[15] believed that there could be no sharp line of demarcation between the states of water present, a view later supported by other investigators.[55,56] This lead him to propose an insensible gradation between water molecules having the normal activity of pure water and those whose activity is so reduced that such molecules become, to all intents and purposes, a part of the solid upon which they are absorbed. Kuprianoff[57] preferred to describe "bound" water as that part of the water in a product which remains in an unchanged state after the application of the usual drying procedures.

The term "bound" water is, however, unsatisfactory in view of the multiplicity of possible and incomparable methods for its determination. Instead, it might be more useful to talk of immobilized water or unfreezable water, perhaps defining it in terms of that water which does not freeze at, say, -30°C after a specified time period. There would still be some possible disagreement about this definition in the light of the contention

concerning how much water can be frozen out, at what temperature, and over what time period, as reviewed by Love.[58] Within this quantity of unfreezable water recognition should then be given to a further subdivision consisting of the quasi-chemically combined water, that which is chemically bound to the macro-molecules, as suggested by Riedel.[59] Even recognition of this latter water is at the mercy of mathematical approaches to water relations, based on the sorption isotherm*, with no universally agreed approach; or is dependent upon analytical techniques whose results are open to a variety of interpretations.

Little positive attention has yet been given to that water in excess of the truly immobilized fraction; that which acts as bulk water in a physico-chemical sense. Hamm[55] has suggested that this water is present enmeshed by a more or less flexible network of filaments and membranes and probably even by cross linkages and electrostatic forces between molecular chains[60] while Szent-Gyorgi[18] mentioned that around surfaces there is the possibility of the establishment of long range regularity, reaching hundreds of molecules deep into the fluid, an idea which has been refuted, however, by Ward.[61]

Foote and Saxton[48] considered that water in certain moist hydrogels contained three types of water:-

a) Free water which freezes sharply just below $0^{o}C$.

b) Water which freezes gradually as the temperature is lowered and which is considered to be under the influence of fine capillaries.

* A sorption isotherm is a plot of moisture content against the equilibrium relative humidity above a material.

c) Water which does not freeze and is considered to be combined water.

Gortner[15], who showed that even at -72°C there is a definite amount of water not frozen in a concentrated gelatine gel, has suggested that this unfreezable water can exist in two possible states, both of which can be present at any one time.

1) Oriented molecules at the surface of the polymer.

2) Selected adsorbed hydrogen and hydroxyl ions forming a shell surrounding the polymer.

While the situation concerning definitions is obviously unsatifactory, it must be emphasised that indeed it is unlikely to be anything else. Foodstuffs are dynamic biological systems presenting a complex environment dependent upon chemical composition and physical structure. Different types of water immobilization are found in one and the same product[57], and are quantitatively dependent upon external conditions such as temperature.[59] Perhaps the salient point to emerge from this is the importance of stating exact methods used when considering quantitative aspects of water relations of foodstuffs and to avoid the danger of drawing untenable conclusions about complex systems from simpler model systems.

1.3.2. Interactions Between Water and Proteins in Simple Systems and in High Protein Foods

Although proteins are minor constituents in the majority of plant materials, investigations concerning their association with water in other systems, or in isolation, have been numerous and thorough. The basic concepts of protein-water interactions are very relevant to similar interactions involving the macromolecular polysaccharide constituents of plant materials, which have received

less attention in the past. Thus a review of the state of water in proteins can be justified, and indeed is necessary, in considering the water relations of foodstuffs in general and plant products in particular.

The study of the interaction between the various constituents of foodstuffs and water has progressed naturally from earlier work carried out on inorganic systems and on simple models of biological origin.[62,63] Although there is still considerable argument[64] about the correctness of the various possible basic approaches to sorption, the Brunauer, Emmett and Teller (BET) theory[65] has perhaps enjoyed the most support over the years, if only for describing sorption up to an equilibrium relative humidity of about 50%.

These authors took a middle course between the idea of Zsigmondy[66], that absorption is based entirely on capillary condensation phenomena with no additional energy relation between the surface and the absorbent; and that of De Boer and Zwicker[67], supported by Keyes and Marshall[68], who postulated that the extensive polarization forces of the surface would hold multilayers of vapour molecules with a force greater than their energy of condensation. The BET theory developed the kinetic approach used by Langmuir[69], and later Freundlich[70], who had earlier suggested that only a monomolecular layer of vapour molecules could be absorbed with an energy greater than the heat of condensation of the sorbate.

While this theory has undergone subsequent critical examination, the introduction of the concept of monomolecular absorption provided the starting point for more detailed considerations of the state of water in biopolymers, and particularly in proteins.

Pure Protein Systems:

Bull first applied the BET theory to proteins and other natural polymers, although Briggs[44] had earlier shown that these substances exhibit sigmoid water sorption isotherms. Sponsler, Bath and Ellis[72] had also suggested that proteins contained two types of hydrophilic groups which were capable of binding water through hydrogen-bond formation. These were described as the polar side chains of amino acids, for example those of threonine, tyrosine, hydroxyproline, tryptophan, histidine, lysine, arginine, aspartic acid, glutamic acid and hydroxyglutamic acid; and the imino and carbonyl groups of the peptide bond.

Shaw[73] and Bull[71] found that the area covered by the BET monomolecular layer was only a fraction of the calculated total surface area of the protein when spread in a thin film, but that it was also several orders of magnitude greater than the surface area when measured by nitrogen gas absorption.[74,75]

This evidence has been interpreted[40] to mean that sorption must take place on specific sites, but that these are distributed throughout the polymer and that the process therefore involves penetration of the molecule as well as a mere surface association. Further evidence that sorption only occurs at specific sites was provided from a study of casein[76], when it was found that 24% to 33% of the total water sorbed was due to the amino groups, which contribute less than 1% of the total weight of the protein.

Although Cardew and Eley[77] suggested that in the monolayer condition only 73% of the total available side chains in haemoglobin are occupied, and that water fails to penetrate within the molecule itself, studies of a range of other pure protein and

peptide systems have lent support to the idea that, although sorption initially takes place only at the surface, it is quickly followed by a fast diffusion of water molecules into the interior of the absorbent.[78,79] This process may result in configurational changes being induced in the macromolecule.[78,79]

There is some argument in the literature over the role of the various potential binding sites of proteins. Pauling[80] has suggested that the different polar groups present vary in their affinity for water, and that the backbone peptide groups play little or no part in the immobilization of water. On theoretical grounds, however, Sponsler, Bath and Ellis[72] calculated that each peptide group could associate with four molecules of water, although they suggested that steric and interaction factors are likely to reduce this by at least half. Later experimental results[81,82] have shown that the sorptive capacity of the entire CONH group in polyglycine esters is only 0.7 mole of water per mole CONH, and in polyglycine D L alanine is 1.0 mole per mole, while Mellon, Korn and Hoover[82] calculated that at 60% relative humidity the peptide groups in casein appear to be responsible for 45% of the vapour absorption, and for 70% in the case of zein. Frey and More[83] also have examined the ability of the peptide group to associate with water, as well as indicating the possible importance of any carboxyl groups.

Olcott and Frankel-Conrat[84] emphasized the importance of polar side chain groups, while McLaren and Katchman[81], studying vinyl polymers, found the ratio of water to binding sites to be about unity for hydroxyl, carbonyl and peptide groups, but very much less than unity for the other polar groups.

In casein, it has been suggested that one water molecule may initially be shared by two amino groups,[76] but, as can be

seen from Table 1, the maximum possible number of water molecules which could become associated with the different polar groups in proteins is considerably greater.

TABLE 1

RELATION BETWEEN PROTEIN POLAR GROUPS AND COORDINATED WATER MOLECULES

GROUP	MAXIMUM POSSIBLE NUMBER OF COORDINATED WATER MOLECULES
H_2O	4
- OH	3
- COOH	4-5
= O	2
- NH_2	3
- NH	2
= N -	1
- SH	slight electrostatic type bonding[102].

However, the affinity of each potential binding site for water is likely to depend not only on its nature, but also on steric factors,[77,85] and molecular complexity may well be important in determining the role of each group. Mellon, Korn and Hoover[86], however, did not subscribe to this view except in the case of groups which are very highly coordinated in a crystal structure.[87]

Water uptake in pure proteins in excess of the hydration of the polar groups has been attributed to capillary condensation.[71,72,80,83]

Gortner,[26] being more specific about the water itself, has mentioned that heats of hydration and dielectric constant measurements suggest that the water molecules in the immobilized fraction are closely associated and probably specifically oriented in relation to each other, possibly in a more or less true lattice which is more densely packed than the ice crystal lattice. It has been suggested by Moran,[52] however, that the forces involved in this ordering would nevertheless be of the same nature as those which cause the association of water molecules in the ordinary ice lattice and in the bulk liquid.

Arising from interest in the fine structure of proteins, it has emerged that in addition to the polar groups of these molecules, the non-polar groups also play an important part in determining the state of water in their hydration shells. At one time inter-peptide hydrogen-bonding was thought to be a major factor in maintaining protein integrity, but present thought emphasizes the role of the apolar, or hydrophobic, bond. It is perhaps likely, however, that all types of bonds, individually weak as they are, will act cooperatively to stabilize the folded polypeptide chain.

Considerations about the role of apolar groups, which are present to an extent of about 40% of the total amino acid side-chains in most proteins; for example the methyl group of leucine, the benzyl group of phenylalanine; are based on observations of the behaviour of small hydrocarbon molecules in aqueous solution. There is a large, unfavourable, entropy loss when an apolar group

dissolves in water due to a change in the structure of the liquid surrounding it. Frank and Evens[88] envisage an "ice-berg" of ordered water building up around the group, such a formation disrupting the overall flickering cluster structure of bulk water.

When considering the consequences of this entropy loss in the specific case of apolar protein groups, two main schools of thought have arisen. Perhaps the most favoured idea is that of the hydrophobic bond. This was first postulated and developed by Kauzman,[89,90] and later supported by Némethy and Scheraga[91], experimental supporting evidence having been supplied by the X-ray diffraction studies of Kendrew.[92] The first two groups of workers concluded that "ice-like" lattices around polar groups cannot serve as stabilizing structures in proteins and that the system would follow the universal law of Clausius, "Die Energie der Welt ist konstant; die Entropie der Welt strebt einen Maximum zu", "The energy of the universe is constant; the entropy tends toward a maximum". In order to achieve the maximum entropy in a protein-water system there would be a tendency for the apolar groups to come together within the sphere of their own van der Waals radii. This would reduce the quantity of water with which they were in contact and cause a proportional reduction in the amount of "ice-like" water, thus minimizing the entropy loss. Were it not for the unique physical properties of water and the tendency for water molecules to exist in hydrogen bonded clusters, however, the interaction of non-polar groups would be so weak as to be insignificant as a stabilizing force for the folded configuration of proteins. Thus it has been envisaged that where sterically possible,

non-polar side chains would be in contact with each other in the interior of a protein molecule, where there is relatively little water.

Klotz,[19,93,94] while he admits the reasonableness of the apolar bond theory, has favoured instead the idea that the apolar side chains protrude from the protein molecule causing the formation of clathrate-like water structures around them and creating a degree of long range cooperative order in the aqueous medium, especially if the local concentration of apolar groups is high. In support of his theory, he has cited the ability of water to form stable clathrates in the presence of certain small apolar molecules[95] and has mentioned that such ideas on water-protein interaction fit in well with the temperature dependence of protein integrity, the melting of the ordered water with increasing temperature introducing instability to the fine protein structure.

Obviously, the ideas of Klotz[94] will have considerable implication when considering the mobility of water in association with proteins, although it is perhaps likely that such ordered water may nevertheless act to all intents and purposes as bulk water in physico-chemical terms.

The careful application of modern analytical tools has, more recently, allowed attempts to be made at a more precise, quantitative approach to the problem of elucidating the nature and extent of protein-water interactions.

Berendsen[21] and Berendsen and Migchelson[96] have used nuclear magnetic resonance (NMR) to study the water relations of collagen. An earlier report[97], that at intermediate relative humidities the water present in this structured

protein suffers rotational restriction consistent with the water molecules forming chains in the fibre direction, was confirmed by these authors. Their findings were also later substantiated by X-ray studies.[98,99] Berendsen[21] points out that one repeat distance of the threefold collagen helix (28.6 Å) is almost exactly six times the repeat distance of a water molecule chain (4.74 Å). This suggests that the helix can stabilize the water chain in the fibre direction and the hydrogen-bonding sites not occupied in intermolecular bonds may be oriented perpendicularly to the fibre direction. The lifetime of a single chain is expected to be fairly short, of the order of microseconds, but this is still about five times longer than the residence time of a molecule in any particular configuration in liquid water. Thus, the water associated with the protein can be said to be in a state between that of the solid and the liquid, a view also propounded by Schwan[100] and Grant[101] on the basis of dielectric evidence.

A similar (one to seven) relationship of repeat distances has been reported to occur in the DNA helix,[102] and from low-angle X-ray studies[103] a value for 'bound' water of 70% of the wet weight of the molecules has been given. This value does not compare favourably with the values presented by Riltand, Keesburg and Beeman[104] who, following X-ray studies of several pure proteins, have suggested that a single hydration layer for a small molecule is achieved at a moisture content of 0.5g water per g dry protein, and for a large molecule at a moisture content of 0.3g per g dry protein. This latter figure agrees well with other results for horse metmyoglobin and for serum and egg albumins obtained

respectively by Boyes-Wilson, Davidson and Perutz[105], and Perutz.[42]

From an NMR study of ovalbumin, a globular protein, Daziewicz et al[106] have concluded that the water protons present in a solution of the protein could be differentiated into two states:- 1) those which do not belong to the solvation layer of any protein molecule, or, if they do, are bound in such a way as to enable them to react in the same way as pure water, and 2) those bound to the protein molecule in a rigid, irrotational way, being indistinguishable by NMR from the protein protons themselves. They suggested, however, that there would be a rapid exchange between molecules in these two states.

Blears and Danyluk[107] extended the temperature range used in the earlier NMR examination of albumin, by examining the signal obtained from hydrated bovine serum albumin over the range from -140°C to 180°C. They reported that the liquid water signal disappears below -60°C, the temperature also quoted by Duckworth[108] for the disappearance, or virtual disappearance, of the liquid water signal in gelatin when studied by low resolution NMR. The possible significance of these results will be discussed, together with those obtained for whole, high-protein, foodstuffs, later in this section.

Examination of the dielectric properties of protein-water systems[109,110] has lead to the suggestion that in certain cases a fraction of the water, more highly immobilized than the solid-liquid fraction previously mentioned, can exist. It has been postulated that this irrotationally bound water is present if available polar

groups lie within the protein molecule, or are set back a little into the surface. This fraction might be comparable to the second class of water in ovalbumin as defined by Daziewicz et al.[106] Water molecules adjacent to charged groups sticking out from the macromolecule, as in collagen and haemoglobin, are immobilized, but not irrotationally. Water in excess of this immobilized fraction will act, in dielectric terms, as bulk water, although it may be influenced to some extent by its proximity to the protein.

Attempts to use infra-red spectroscopy to study protein hydration have not resulted in meaningful results.[111,112]

Rockland[286], applying his Local Isotherm Theory (Section 2.4) has divided the sorption isotherm of gelatin into three distinctive sections. Each of these local isotherms, he has claimed, represents a different stage in the accumulation of water, each dominated by a different mechanism. Marked changes in respect of several other attributes of the gelatin-water system were also found to occur at the transition points between the local isotherms mentioned above, thus lending support to his theory. These results are summarized in Table 2.

Table 2/...

TABLE 2

MOISTURE CONTENTS OF GELATIN AT APPARENT INTERCEPTS OF LOCALIZED SORPTION STAGES

	Moisture Content (g water/100g gelatin) at intercepts of	
Method of Analysis	Localized Stages I-II	Localized Stages II-III
Moisture Sorption	12	29
NMR line width at half peak height	14	29
Electron spin resonance saturation peak height of irradiated gelatin	13	28
Phosphorescence decay time	11	22
Differential free energy of sorption	9	29

Another much used approach when considering the state of water in hydrated biopolymers has been the thermodynamic and calorimetric one. Application of the Clausius-Clapeyron equation (Section 2.6) to sorption data obtained at different temperatures allows the derivation of values, as a function of moisture content, for the total heat of association of water molecules to the macromolecular sorbent. Such values indicate, in quantitative terms, the affinity of the solid for the vapour, although the accuracy

of such calculations is of a relatively low order.[71,77] At very low moisture levels, the heat of association is greater than the heat of condensation of water, indicating an energy-involving interaction between the two components. With increasing moisture content, a decrease in the total heat of association is found, until finally the value reaches a level very close to, or equal to, the heat of condensation of water (Fig.4).

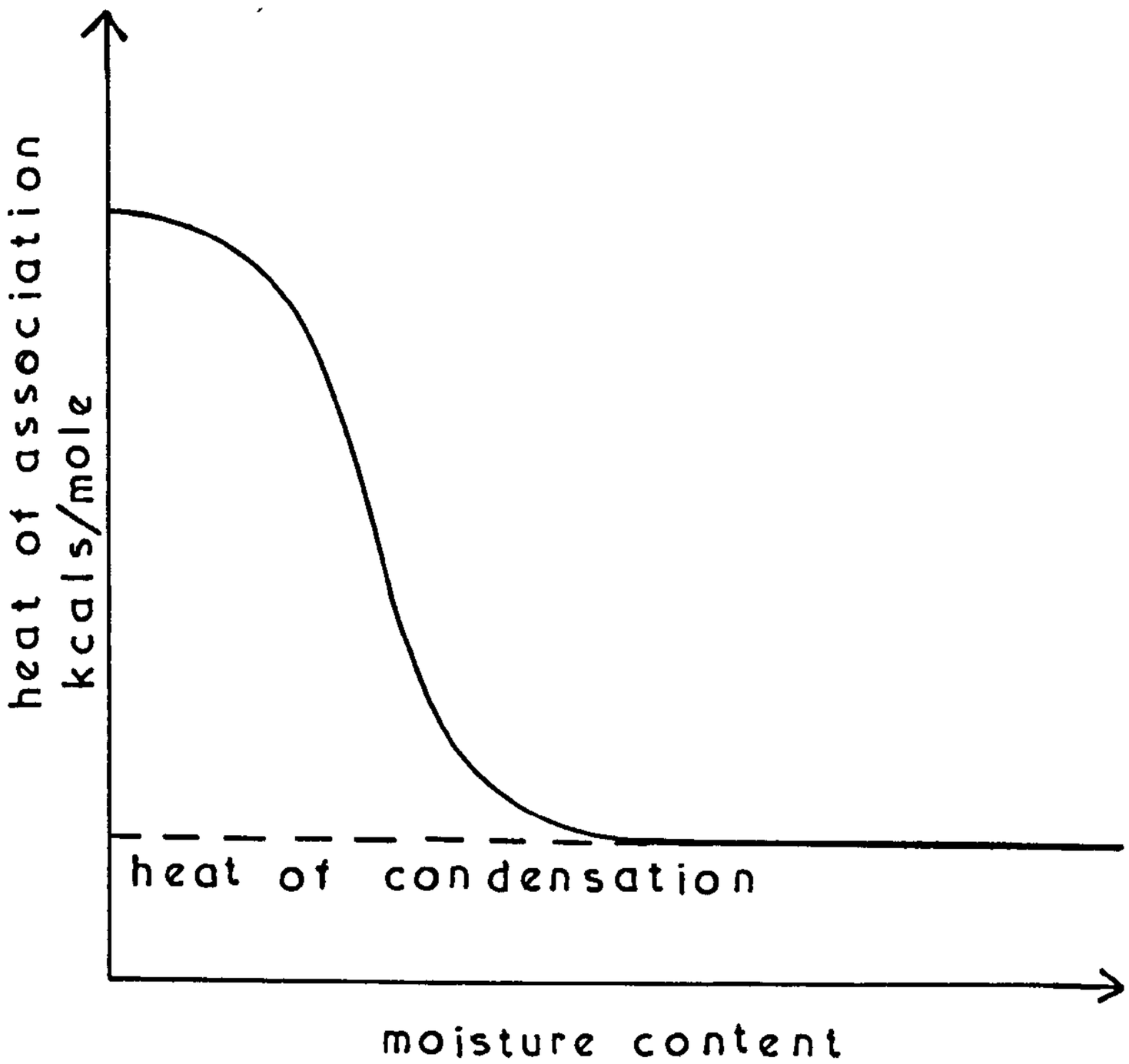

Fig. 4. Change in total heat of association of water with increasing moisture content for a macromolecular system.

The results of such thermodynamic examinations are of greatest interest when attempts are made to correlate them with other results pertinent to water-protein systems.

Bull[71] has compared the change in the enthalpy curve of gelatin with the increase in the spacings of the peptide chains as determined by X-ray diffraction. He concludes that up to a moisture content of 20% on a wet weight basis (wwb) most of the change in enthalpy can be accounted for by the water molecule forcing the peptide chains apart, while it has also been found[113] that in the same system the heat of association approaches the heat of condensation of water above a moisture content of 20%. Cardew and Eley[77] have shown that an inflection point in the differential entropy curve for haemoglobin corresponded to the value for the calculated BET monomolecular layer, this water having a lower entropy than the rest. Hnojewyj and Reyerson[114] have shown that in lyzozyme a differential heat of binding is observable up to a moisture content of 19% (wwb), while, were each nitrogen atom to associate with one water molecule, the corresponding moisture content would be 18.3% (wwb).

A further method which has been applied to the study of the partial immobilization of water by proteins, among other materials, is differential thermal analysis (DTA). By this means a fraction of water remaining unfrozen at low temperature in hydrated gelatin has been recognized and found to be appreciably greater than that showing a differential heat of binding, as determined by sorption studies. Values obtained by DTA of 48g - 50g water per 100g dry matter in gelatin[108] agree quite closely with the results of earlier calorimetric studies showing amounts of unfreezable water of 54g[115] and between 48g and 58g/100g dry matter[116] respectively.

Duckworth[108] has presented further supporting evidence for the existence of this relatively large unfreezable water

fraction from his low temperature studies of hydrated gelatin using NMR. At moisture contents of approximately 37g/100g dry matter and below, no instantaneous reduction in signal size occurs as the temperature is lowered below $0^{o}C$, such as would be expected if any appreciable freezing of the constituent water had occurred.

Recently, Ladbrooke and Chapman[117] have reviewed the results of other work on proteins and polypeptides which used DTA and differential scanning calorimetry (DSC). These techniques have confirmed the earlier findings of Blears and Danyluk[107] who, using NMR, detected the loss of tightly bound water from proteins when heated to $100^{o}C$ to $150^{o}C$.

DSC has also been used at low temperatures in an attempt to follow changes in ice structure upon the freezing and rewarming of gelatin gels. Ladbrooke and Chapman[117] correlated these DSC results with those from low temperature X-ray studies on similar systems and suggest that very rapid cooling results in the formation of vitreous ice in a 60% gelatin gel, whereas, under similar conditions, a mixture of vitreous and cubic ice is formed in a gel containing 40% gelatin. Observed thermal phenomena upon rewarming have been attributed to recrystallization as the ice passes through the cubic and hexagonal forms before finally melting.[117]

High Protein Foods.

The work of Nemitz[118] on egg white provides a useful link between the above discussions of the water relations of pure proteins and those of more complex, high-protein food materials. Although dialysis of egg white produced a

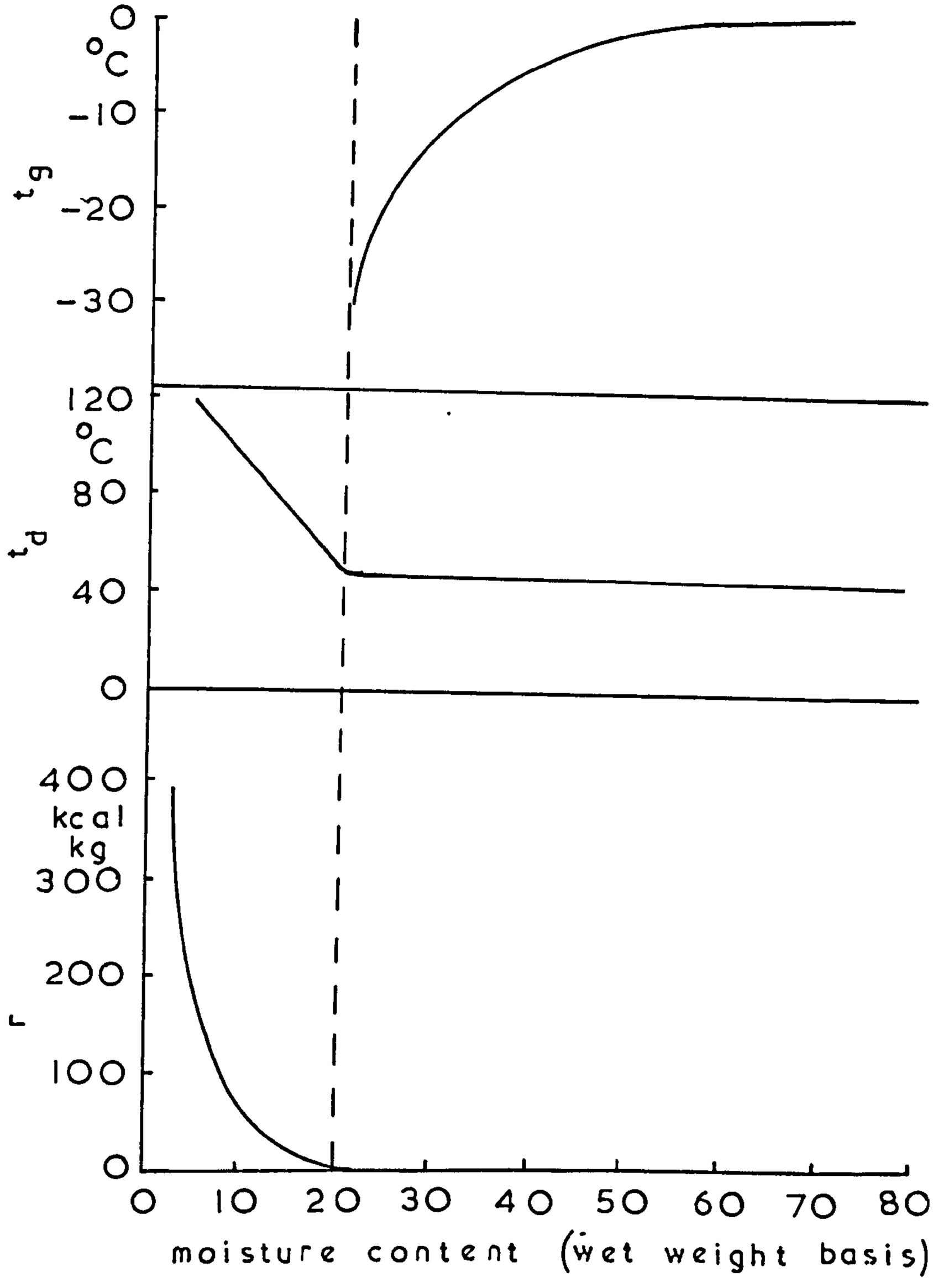

Fig. 5 The Relationship between moisture content and temperature of onset of freezing, t_g, the temperature of onset of denaturation, t_d, and the heat of binding, r, for salt free egg white. From Nemitz, 1967[118].

reduction in size of the bound water fraction determined in this work, the overall qualitative behaviour of both whole and desalted egg white was similar.

Nemitz[118] has interpreted the process of sorption as taking place in three stages, 1) a positioning of the first sorbed molecules on the polar side chain groups of proteins, 2) subsequent molecules penetrating into the protein molecule where they become associated through hydrogen-bonding with the peptide groups, and 3) capillary condensation occurring as more water becomes available. For the pure protein he has claimed the presence of a stoichiometric relationship between the maximum number of relatively tightly immobilized, unfreezable, water molecules, and the number of nitrogen atoms in the molecule.

Nemitz[118] has also shown an interesting relationship in desalted egg white between the point of disappearance of the heat of binding, the lowest freezing temperature, and the heat sensitivity to denaturation, all as functions of moisture content (Fig. 5). This suggests that below the indicated moisture content of 20% (wwb), the water does have some distinct degree of association with the protein, and that its presence has an influence on the properties of the protein itself.

For air dried beef, Kapsalis et al[119] have related the disappearance of the heat of binding at 12.0g water/100g dry matter with a value of twice the calculated BET monomolecular layer, or, rather, the summation of the BET and Harkins-Jura monolayer values. Evidence that only one of these mono-molecular layers, in muscle tissue, is likely to be restricted

TABLE 3

QUANTITATIVE ASPECTS OF THE WATER BINDING RELATIONS OF PROTEIN SYSTEMS

MATERIAL	BET MONOLAYER WATER	DISAPPEARANCE OF DIFFERENTIAL HEAT OF BINDING	UNFROZEN WATER DETERMINED BY: CALORIMETRY	DTA
Gelatine	8.73[133]	25[71]	54 (-20)[115]	48.50 (-30)[108]
		30[113]	49-58 (-20)[116]	
Egg White	6.78[136]	25[118]	28.6 (-70)[134]	
			25.5 (-30)[118]	
Cooked Beef	5.8[132]	12[132], 18-20[108], 20[59]	25.5 (-180)[59]	24.3-24.7 (-60)[108]
Beef			43.0 (-40)[115]	
			26.6-35(-40)[134]	
Cod			39 (-70)[135]	25.7-27.0 (-60)[108]
Haddock	4.92[41]		39 (-70)[71]	

The temperature at which determinations of unfrozen water were conducted are given in brackets.

to the extent of not possessing solvent properties has been presented by Duckworth and Smith.[120] Using an autoradiographic technique they showed that ^{14}C labelled glucose would only diffuse through haddock muscle at moisture contents greater than that corresponding to the calculated BET monomolecular layer.

Values for the calculated BET monomolecular layer, the unfreezable water content, and the moisture content at which the differential heat of binding reaches zero, are presented in Table 3 for a variety of principally protein materials.

Recent freezing studies conducted on muscle tissue from a variety of sources have also contributed to our knowledge of protein-water interactions. Excellent reviews by Fennema and Powrie[121] and Merryman[56] have covered the extensive literature of cryobiology, the former authors with special reference to food materials. It is, however, pertinent to the present review to give special attention to the work of a small number of outstanding contributors.

Within the context of recent discussions regarding the extent and reversability of ice formation in muscle tissue[58,59,122,123] Riedel[59] has developed a theoretical approach based on experimental calorimetric studies on cooked beef. He postulates a temperature dependent variation in the amount of combined water, this latter fraction becoming progressively thermally dissociated with increasing temperature to yield free "capillary" water having solvent properties. Riedel suggests, however, that some "capillary" water will persist at temperatures down to about $-100^{o}C$ in a fully hydrated material.

Riedel's theory is based upon the derivation from basic principles of an isotherm equation (presented in detail in Section 2.5) which related the proportions of combined and "capillary" water. The equilibrium constant for the dissociation of combined water to the free "capillary" state, once established for a given temperature, can be calculated for other temperatures by using van't Hoff's equation. In this way Riedel was able, on the basis of experimental data obtained at 20°C, to calculate theoretical isotherms for temperatures down to -180°C. These are illustrated in Fig. 6.

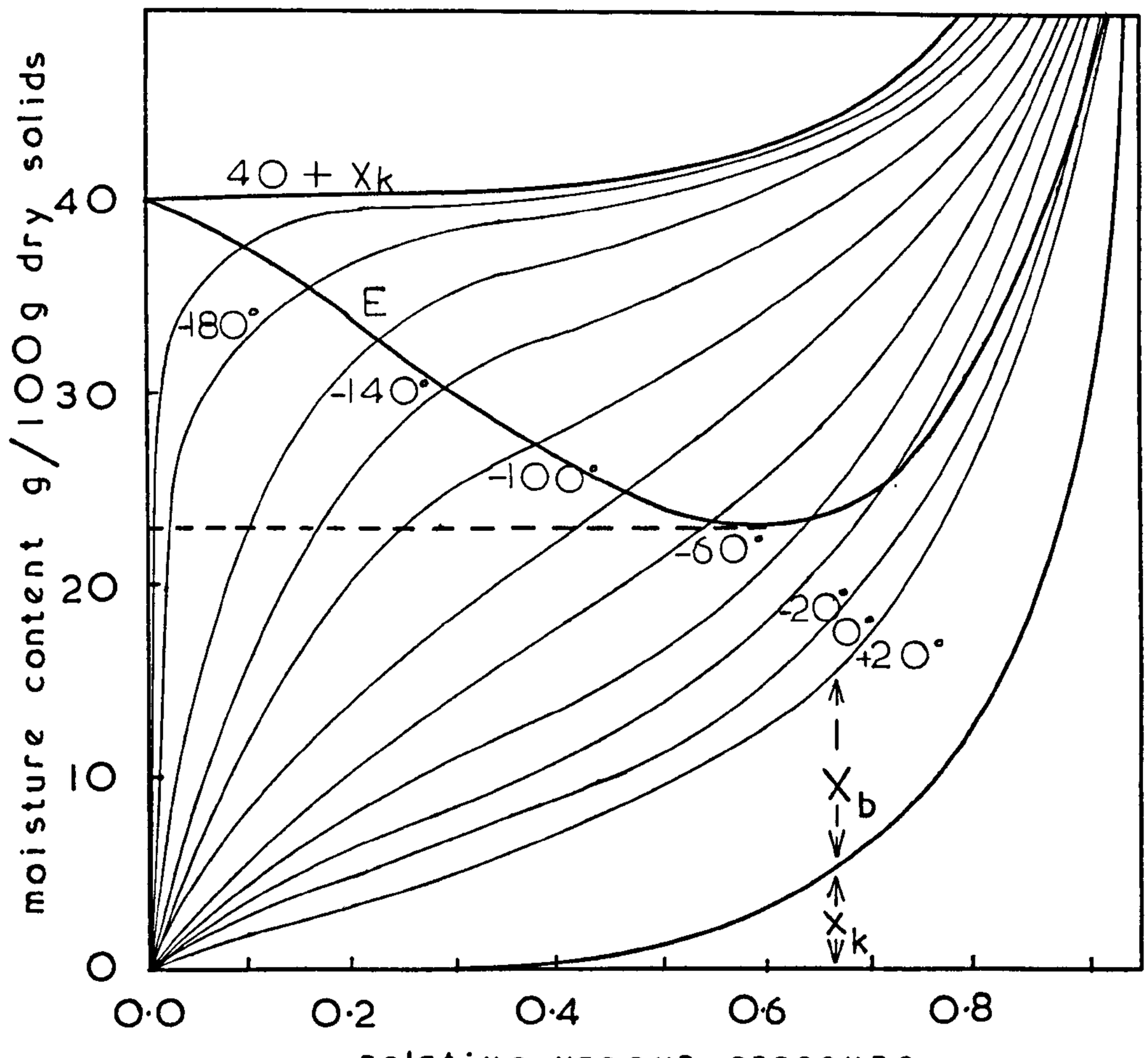

Fig. 6. Theoretical isotherms for cooked beef as calculated by Riedel, 1961[59].

E = ice curve

X_k = capillary solution water

X_b = combined water

Calculation of values for the relative vapour pressure of ice at each of these temperatures allowed the superimposition of the ice curve (E) also shown in Fig. 6. Points on this curve at any given temperature represent the equilibrium condition when the partial pressure of the water vapour over the meat sample would be equal to the vapour pressure of ice at that temperature. It can be seen that the ice curve passes through a minimum in terms of moisture at about 22.5g water per 100g dry matter and in the temperature range between $-50^{o}C$ and $-60^{o}C$. The water below this moisture content, Riedel suggests, will be permanently unfrozen, while, with further lowering of temperature, there will be a reversible increase in the amount of combined water at the expense of either "capillary" water or ice, depending on the overall moisture content of the sample.

A recently reported study by Storey and Stainsby[124] has experimentally confirmed that frozen muscle tissue will exert a relative vapour pressure equivalent to that exerted by ice at the same temperature.

Riedel developed his theory from observations made during the calorimetric examination of lean beef and from the resulting calculated changes in specific heat with changes in moisture content and in temperature.

As illustrated in Fig. 7, a linear increase in specific heat with increasing temperature was observed in the dry material.

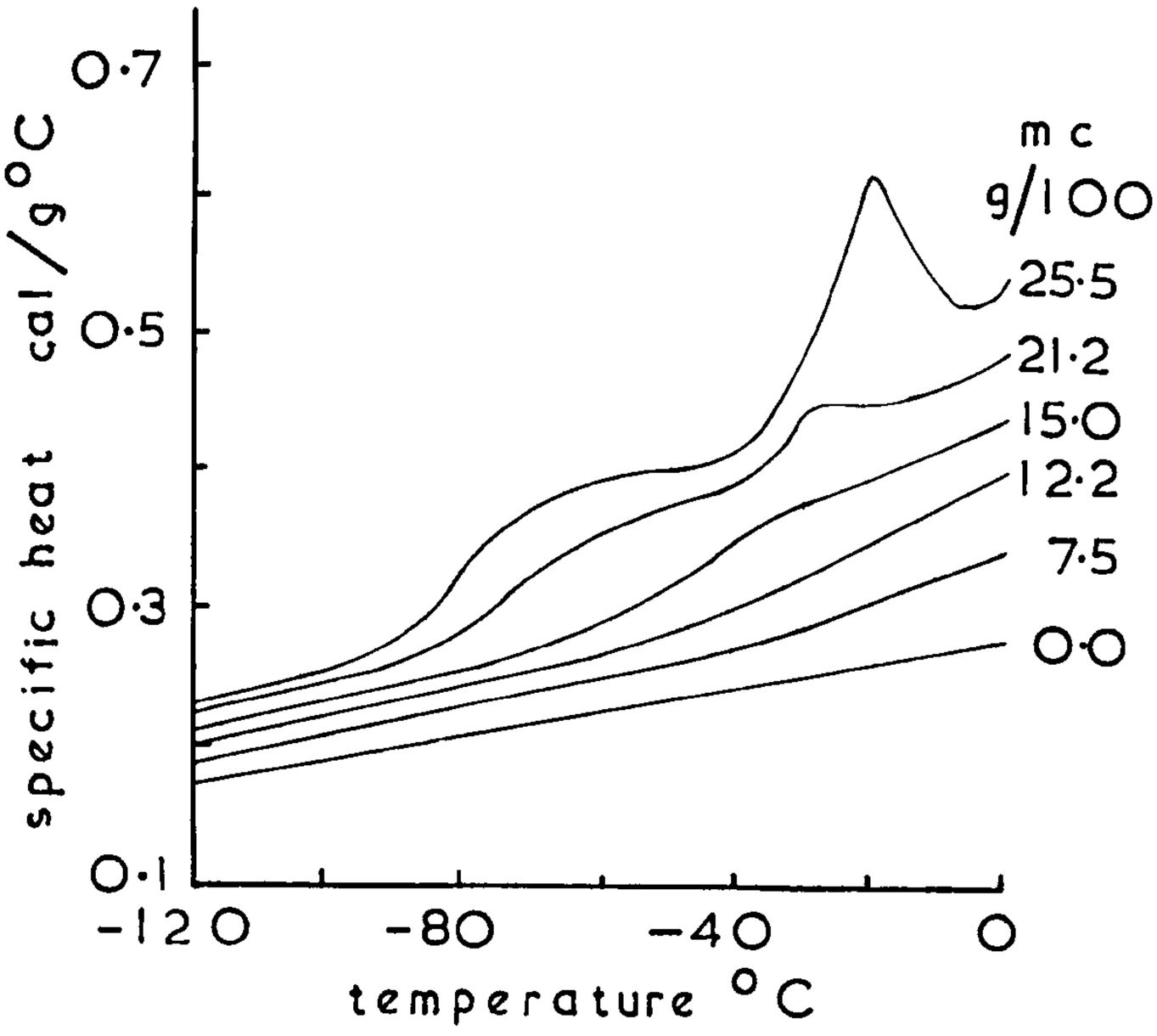

Fig. 7. Specific Heat curves for beef hydrated to different moisture contents(mc), as illustrated by Riedel[120].

However, in samples containing water an inflection point appeared and this occurred at progressively lower temperatures, the higher the moisture content of the sample. The increases in slope above these inflection points are accounted for by Riedel in terms of the dissociation of combined water, which he considers will result in an increased heat demand in the material as a whole. As mentioned previously, however, Ladbrooke and Chapman[117] have explained thermal changes occurring during the freezing and thawing of gelatin gels in terms of crystalline rearrangements occurring in the ice which is present.

Love and his co-workers[58,123,125-127], while agreeing that there is a fraction of water present in animal tissue which will not freeze however low the temperature is taken, have mentioned, nevertheless, that water in excess of this fraction, but which still has some degree of specific association with the solids, can be irreversibly removed as ice. Basing their beliefs upon the results of various investigations of cod muscle they have suggested that the water in muscle cells is attached to the proteins with a whole range of bond energies. An observed increasing amount of protein damage as the temperature of freezing is reduced has been explained by them both in terms of a direct effect of water removal by freezing and, alternatively, as a result of the well documented[58,121,128,129] damaging effects of increased salt concentration obtained upon freezing and subsequent storage at temperatures above the eutectic zone of the salts concerned.

Apparent supporting evidence for the ability of nearly all of the water present in protein systems to freeze, has come from the low temperature NMR investigations of Sussman and Chin[130] and of Toledo, Steinberg and Nelson.[131] The former workers, examining cod muscle, down to -20°C suggest, by extrapolation, that the liquid water signal would,at -70°C, disappear. They also found that a reduction in signal size below the main freezing zone is accompanied by an associated increase in the line width of the signal. Toledo, Steinberg and Nelson[131] studying wheat flour dough, a partially protein system, down to -60°C, also found a progressive reduction in signal size with a reduction in temperature.

An alternative explanation of this phenomenon can, however, be expressed in terms of a loss of mobility of that water still giving a signal below the main freezing zone. Such a loss of mobility would result in a broadening of the signal, as found by Sussman and Chin.[130] Further evidence in support of this explanation has also been provided by the low temperature NMR investigation of Duckworth.[108] Examination of beef and cod over a complete cooling and rewarming cycle down to -60°C has failed to show any evidence of a hysteresis effect which would be expected if ice were indeed formed from the unfreezeable water present.

All low temperature NMR studies[108,130,131] have shown that with high-moisture-content-samples there is a rapid fall in signal size just below 0°C as the truly mobile water freezes, but that irrespective of initial moisture content above a certain limit, the reading falls to the same level. This suggests that all samples contain the same amount of restricted water, expressed on a unit dry weight basis, thus providing further evidence for the existence of an immobilized fraction.

Recently, high-resolution NMR studies at ambient temperatures have provided yet more evidence in favour of the existence of an immobilized water fraction in muscle tissue.[132,133] It has been suggested, however, that a smaller fraction, put at 27% and 10% water respectively, by Cope,[132] and Hazelwood, Nichols and Chamberlin[133] will be present in rat muscle, with, in addition, a major, less restricted fraction, as well as totally free water.

Even when presented with this mass of factual and theoretical information on the association between water and protein, it is perhaps still only possible to give an overall qualitative picture of the system with any certainty. This is as follows:

1) There would appear to be a definite fraction of water which is immobilized to the extent where it does not freeze.

2) Within this fraction there is a smaller quantity of water much more closely associated with the macromolecule and showing an appreciable differential heat of binding.

3) The remainder of the fraction is immobilized to differing, lesser degrees, some of it still showing a measureable, but relatively small, differential heat of binding.

4) In complex systems with observable structures there is also likely to be a fraction of unfreezeable capillary water present.

5) The relative amounts of these fractions are likely to be temperature dependent, as well as varying greatly throughout the spectrum of protein species.

6) The whole system is in a state of dynamic equilibrium with a constant exchange between water molecules in the highly immobilized state, those immobilized to a lesser degree and the truly free species; the degree of immobilization being reflected in the differences in mean residence times in any one position.[95]

1.3.3 Polysaccharide-Water Interactions

Many of the general conclusions drawn about protein-water interactions can also be applied to polysaccharide-water interactions, and the six considerations enumerated in the last paragraph will apply here as well.

Together with the poorly defined hemicelluloses, cellulose and pectic substances are nearly always present as constituents of plant materials. Their role in the structure of the cell wall has been the subject of extensive reviews, for example Preston 1952,[137] Sterling 1963[138] and Roelefson 1959.[139] An additional polysaccharide constituent frequently present in that botanical material used as a food source, is starch.

The history of the water relations of cellulose, pectic substances and starch will be reviewed in this section, the behaviour of the remaining minor polysaccharides being similar to these materials, at least in general principle.[140]

While many practical investigations have been carried out to provide information about the hydration characteristics of cellulose as it occurs in textiles; starch as it occurs in dough and pastes; and pectin as it occurs in gums, less attention has been given to the actual mechanism of water sorption. These materials are, however, much simpler than the proteins with regard to the variety of potential water binding sites. In cellulose and starch, the only polar group involved is the hydroxyl group, while in pectic substances the carboxyl group of carbon atom 6 may also be important.

Sorption of water in these polysaccharides involves penetration by the sorbate through closely associated chains of the sorbent, this generally resulting in a certain amount of swelling. A degree of complication is introduced, however, by the presence of truly crystalline regions which influence sorptive properties, both with regard to the extent and to the strength of water immobilization, and reflects the availability of binding sites. Cellulose is the most highly crystalline, pectin the least.

In a crystalline region each group capable of forming covalent bonds or hydrogen-bonds plays an important part in the stabilization of the regular structure of the material and is involved in intrachain binding with neighbouring molecules, rendering them less available for association with water. The crystalline regions of cellulose are completely impervious to water.[141-144] The starch crystallite, however, requires from 10% to 17% water (wwb) to produce a crystalline type of X-ray interference pattern[146,147] although it will not absorb more water above this level, at least in its native form. In the amorphous regions, there are free potential binding sites which are responsible for the immobilization of water and for the swelling of the gel. Processing, particularly dehydration, can cause an increase in crystallinity[64,143,148], as can extended storage[143,150-152], an acceleration of this effect occurring at temperatures in the region of 0°C[152], although blanching appears to minimize these changes.[153]

The water relations of each major polysaccharide will now be dealt with in turn.

Cellulose:

While the literature on the absorbtion of water vapour by cellulose is extensive, much of it does not have direct application to cellulose in foodstuffs,due to its origin in textile or packaging studies. Here, interest is often in molecularly modified celluloseswith a greater sorbtive capacity than native cellulose, due to a decrease in crystallinity.[154]

Cellulose is composed of a long chain of β-D-Glucose residues connected by 1:4 linkages, which protrude at a 20° angle to the plane of the polymer[155] and produce a relatively straight chain molecule. Primary and secondary hydroxyl groups project in close order permitting the easy formation of hydrogen-bonds between neighbouring molecules. Examples of reported values for the degree of crystallinity of cellulose are 70% for the case of cotton, and 65% for wood pulp.[156]

The earliest demonstration that water absorption was intimately associated with the chemical nature of the cellulose molecule was given by Sheppard and Newsome[157,158], when it was shown that acetylation of the hydroxyl groups decreased sorbtive capacity. Stam and Loughborough[159] suggest from thermodynamic considerations that the most tightly associated water molecules are bound in a more or less oriented way.

Hermans[154] has suggested that the first stage of sorption will be the formation of hydrates by some of the molecules in the amorphous region. These are hydrates I and II with $\frac{1}{3}$ and $1\frac{1}{3}$ moles of water per mole of ~~polymer~~ monomer, respectively. Initially, hydrate I will form, and, as more water is absorbed, hydrate II will come into existence.

Hermans suggests that all the hydrate form possible will be present at a moisture content of 5.9% (wwb), assuming a degree of crystallinity of 60%. All this water will have a heat of combination considerably greater than the heat of condensation of water. In addition to this absorbtion, he has suggested that further limited monomolecular absorption can take place, to give a final "monolayer" value of 10% to 12% (wwb). Additional water uptake will be due to capillary condensation.

This value of 10% to 12% of initially absorbed water corresponds to 2.5 molecules of water per glucosidic group, although description of this water as monolayer water does not agree with the present day conception of this value. Indeed, Hermans has suggested that only the formation of hydrate I corresponds to the first steep part of the sigmoid isotherm, the completion of which is now considered to represent the end of monolayer formation.

Later workers have postulated different quantitative relationships between water and monomer units at the monolayer level. McLaren and Rowen[40] gave a value of 0.58 moles of water per 100g dry matter (0.62 moles per glucose residue) corresponding to a monolayer value of 10.44g/100g. Babbitt's[160] figure is 3.43g/100g for cotton and 6.58g/100g for wood, and that of Stamm[161] 2.82g/100g for cotton. In a recent NMR study of native cellulose[162], it has been found that water below 5% moisture content (wwb) is irrotationally bound and cannot be detected at the liquid-water frequency, while that between 5% and 20% (wwb), although mobile to some extent, can be differentiated from the truly mobile water above 20%.

Giles[163] has postulated that each anhydroglucose residue in the amorphous portion of cellulose could absorb

five to six molecules of "bound" water, although Stamm[161] favours a value of only four molecules per residue. Equating this "bound" water to unfreezeable water it considerably surpasses a value obtained using DTA for whole cellulose of between 13.1g and 15.9g/100g dry matter.[108] Nevertheless the point is made that a fraction of water, greater than calculated monolayer values, is associated to the macro-molecule to the extent of losing its ability to freeze. The fractionation of the water present into three groups has also been suggested by Magne, Portas and Wakenham.[164]

As was the case in protein systems, thermodynamic examinations have shown a gradual reduction in the heat of binding with increasing moisture content, to a level where the degree of overall association is equivalent to the heat of condensation of water.[161,154]

Although the various quantitative examinations of sorption by native cellulose have yielded a spectrum of results this may be almost totally ascribed to differences in degree of crystallinity. From the shape of the sorption isotherm, thermodynamic considerations and other analytical examinations, it is clear that the process is comparable to that occurring in a protein.

Starch:

As in cellulose, the starch polymer is basically composed of a chain of D-glucose monomers, with the difference that these have 1:4 α-glucosidic linkages. Starch is not a simple substance, however, but is composed of two types of molecular species which differ in the length and arrangement of the monomer chains. Amylose, which constitutes 20% to 30%

of starch, is a totally straight-chain macromolecule; amylopectin which makes up the remaining 70% to 80% consists of short, 20 to 30 monomer, chains of 1:4 linked reisudes, randomly joined through 1:6 glucosidic bonds. In the starch granule, these two forms are present associated by hydrogen-bonds, either directly or through water hydrate bridges, to form radially oriented micelles, or crystalline areas, of varying degrees of order. Thus, a three dimensional lattice is built up by the participation of different segments of individual chains in several micellar regions. This ordering of the native starch is responsible for the limiting of water vapour sorption to a level of between 10% and 17% (wwb), depending on conditions and variety[165,166], although Leach[165] has found that in a saturated atmosphere, corn, tapioca, potato and waxy-corn starches can absorb a maximum of 39.9, 42.9, 50.9 and 151.4g water per 100g dry matter respectively. The absorption of water from the completely dry state is accompanied by limited intra-micellar swelling, and Volman et al[147] have postulated that various crystalline configurations may be encountered as transitory lattices during this process. The granules will increase in size by 10% to 30% of their dry dimensions, depending on variety.[167]

Several studies of native granular starch and separated amylose and amylopectin have allowed attempts to be made to follow the process of sorption in a quantitative manner.

Hellman and Melvin[166] and Winkler and Geddes[168] have shown that water sorption by starch is not merely a surface phenomenon but involves penetration of the crystallite, while work by Taylor et al[169] suggests that the free hydroxyl groups

are easily accessible.

McLaren and Rowen[40] have reported a monolayer value of 0.71 moles of water per mole of glucose residue, that is, 7.92 g water per 100g dry matter. Leach[165], however, suggests that at this point a monohydrate condition would exist with a monolayer value of between 8% to 11% (wwb). This he has called water of crystallization or water of constitution. Volman et al[147], studying amylose and amylopectin, favoured a 2:3 water to monomer ratio, this being close to the value of 2.3 moles of water per 3.0 moles of monomer obtained by Mcsazawa and Sterling[170] with maize starch, and also the previously mentioned value cited by McLaren and Rowen.[40] In this arrangement it has been envisaged that one water molecule would join two adjacent glucose residues through a single functional group on each residue. This would leave one additional water molecule per six residues, the suggestion having been made that this is involved in the stabilization of a helical type of structure, similar to that found in the starch-iodine complex.[147]

It would therefore appear that of the three hydroxyl groups per monomer in starch, only one is initially available for association with water. It has been suggested that the other two groups participate in intramolecular or non-crystalline intermolecular hydrogen-bonding, or are sterically unavailable.[175]

Studies of the change of refractive index of wheat starch granules during sorption[171] indicate that moisture up to 10% (wwb) is tightly associated with the polymer, a similar value being given by absorbtion spectra studies at radio frequencies.[172] Values of about 24g per 100g dry matter

have been given by most workers for the moisture content at which, during sorption by both granular and gelatinized starch, the heat of binding becomes equivalent to the heat of condensation of water.[147,168,170,173,174].

Fish[146], examining gelatinized starch, in which a loss of the crystallite structure will have occurred, favours a 1:1, water to monomer relationship to exist at the monolayer level. He also mentions the possibility of a 3:2 ratio, noting, however, the absence of direct X-ray evidence at that time which would allow irrefutable placement of water molecules.

NMR studies of gelatinized starch[175] have indicated that water below a moisture content of 15g/100g dry matter has reduced rotational freedom, while that below 4.5g/100g dry matter may not register any observable signal at the resolution used.

Sorption studies on gelatinized starches from a variety of sources, pertinent to considerations of the water relations of starch as it occurs in cooked foods, have also been reported by a number of authors.[113,146,168,173,176,177]

Pectic Substances:

The pectic substances are a composite group of polymers of 1:4-α-linked galacturonic acid monomers of various degrees of esterification and neutralization, and with varying amounts of associated minor low molecular weight constituents.[178] The chains have a contracted, threefold screw-like configuration due to the carbon-oxygen bond

involved in the glucosidic linkage projecting perpendicularly to the plane of the pyranose ring.[155] Inter-chain hydrogen-bonding of hydroxyl and carbonyl groups is far less tenacious than it is in cellulose, although inorganic ions are known to contribute to the stability of the pectinates, calcium pectinate being the most abundant natural salt.

Sorption studies are relatively few for this group of compounds[179-184], most interest having been focussed on X-ray examinations during swelling as sorption takes place. There has been one reported NMR examination of pectin[175] which found that at the resolution used a moisture content below 5g/100g dry matter gave a signal virtually indistinguishable from the pectin signal, indicating a high degree of immobility in this water.

Palmer and his co-workers[179,180] have argued that in addition to the number of polar groups, the packing of the chains in the crystalline regions of pectic substances will play an important part in determining their sorption characteristics. These regions in sodium pectate sorbed water only up to a moisture content of 24% (wwb) with an associated increase in interchain spacings, although the amorphous regions became fully hydrated. They also determined that sorption is independent of methyl ester content up to a relative humidity of 88%, suggesting that any unesterified carboxyl groups are totally occupied in strong interchain bonding. Speculation on the effect of inorganic ions on the type of bonding involved, and their possible roles as dehydrating agents or as participants in salt linkages, has been reviewed by Doesburg.[185] In pectinic

acids it appears that water molecules become associated with the hydroxyl groups of the polymer and increasing moisture content results in an increase in interchain spacings and in crystallinity. Pectic acid was found to behave in a similar way upon hydration although in highly esterified pectic substances no chain separation was observed.

Some of the findings quoted above were contradicted in a later series of papers by Bettelheim and his co-workers.[181-183] They found that in all representatives of the pectic substances, interchain swelling occurs with an increase in moisture content, approximately mirroring the shape of the sorption isotherm, although at high moisture contents, with capillary condensation occurring, the relationship is no longer linear. In all but pectic acid, a chain elongation was also observed, ceasing when five water molecules had been absorped by three galacturonic acid residues. The associated increase in crystallinity observed in all but calcium pectate justified the experimentally calculated entropy decrease.

Thermodynamic considerations, as illustrated in Fig.8, led them to postulate that for pure pectic acid initial absorption only occurs at one carboxyl group in every three monomers, but that the water molecule involved is simultaneously associated with a further polymer chain, thus linking the two together. Swelling is accompanied by the uptake of a further water molecule by the two adjacent hydroxyl groups of carbon atoms 2 and 3 of a single monomer, and finally the two remaining carboxyl groups per three monomer unit each become associated through a hydrogen bond with a further water molecule.

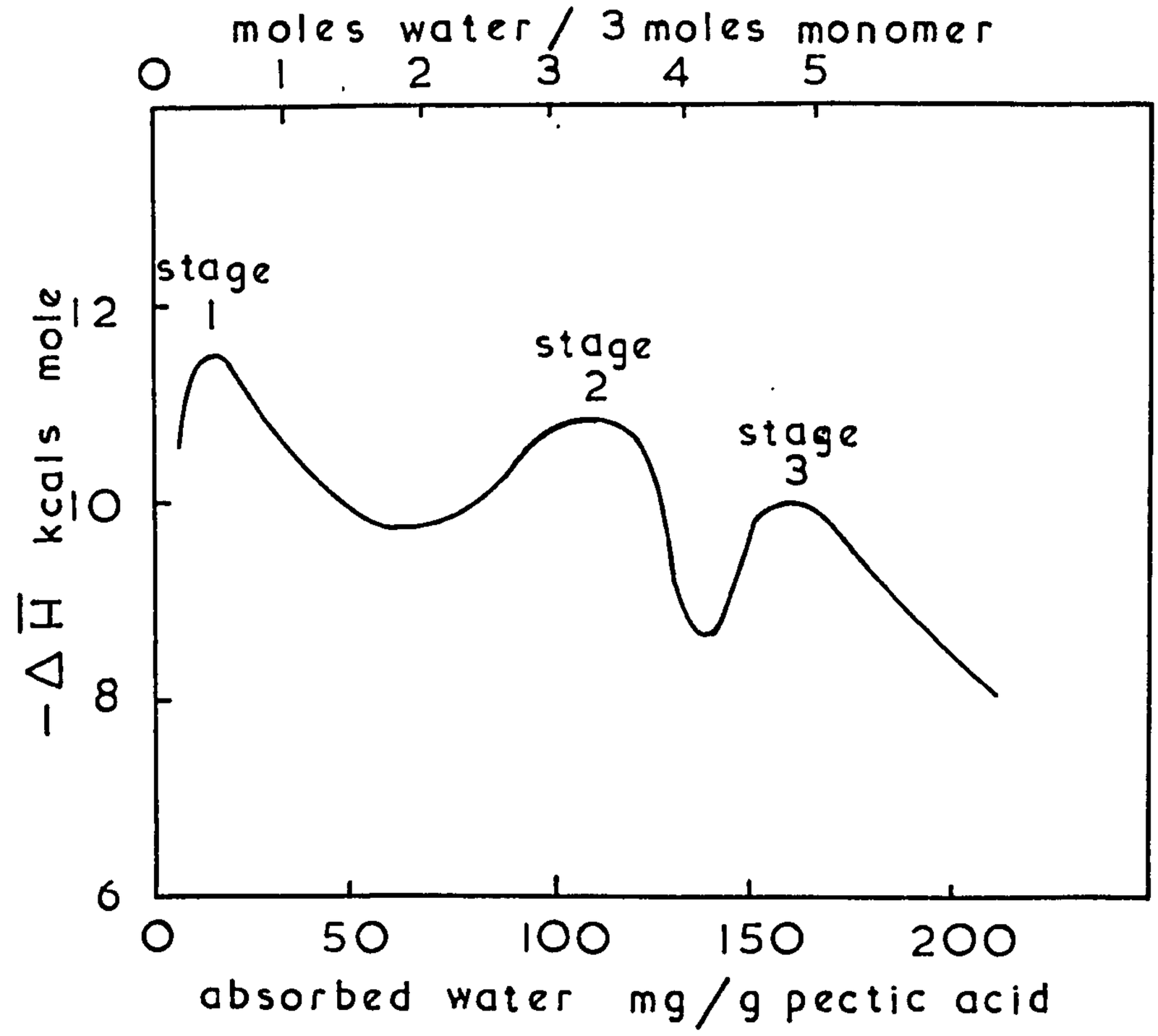

Fig. 8. Standard differential enthalpy function for absorption of water vapour on pure pectic acid at 29.0°C.

Calcium pectate gel appeared to swell to a smaller degree due to the greater effect on gel strength of the presence of the calcium ion. In contrast to the other polygalacturonide chains it was shown that in native pectin the swelling is not associated with the exposure of more polar sites.

It would appear, therefore, that the extent of water immobilization in pectic substances will be determined to some extent by the degree of esterification and also by the

TABLE 4

QUANTITATIVE ASPECTS OF THE WATER BINDING RELATIONS OF POLYSACCHARIDE SYSTEMS

MATERIAL	BET MONOLAYER WATER	UNFROZEN WATER	DISAPPEARANCE OF DIFFERENTIAL HEAT OF BINDING
STARCH:			
Unspecified	7.5[170]		≃ 25[113]
Wheat			35.0[168], 24.0[173]
Rice			33.25[168]
Potato	12.02[166]	38-40[187] (-40)	37[168]
	5.68[186]		29.5[146]
	6.85[41]		
	7.92[40]		
Corn	9.15[166]		≃ 31.5[174]
amylose	6.97[147]		> 16.0[147]
amylopectin	7.27[147]		> 16.0[147]
Tapioca	9.90[166]		
Soluble		22.1-30.2[108] (-70)	
CELLULOSE	10.44[40]	13.1-15.9[108] (-60)	
PECTIC SUBSTANCES:			
Pectin, Citrus	10.8[182]		≃ 20[182]
Sodium Pectate	12.8[182]		> 25[182]
	13.6[179]		
Calcium Pectate	10.0[183]		
Pectic Acid	7.3[182]		≃ 22[182]

number of interchain bridges.

One of the over-riding influences on the sorption of water by these polysaccharides is evidently the degree of crystallinity, and variation in the values for fractions of immobilized water may be attributed to this. Hydroxyl and, where present, carboxyl groups are the major polar groups responsible for the immobilization of the water. Table 4 shows various values obtained for these fractions.

1.3.4 Lipid-Water Interactions

The outstanding characteristic of the lipids is their hydrophobic nature and in foodstuffs they generally sorb negligible amounts of water. While some lipids contain polar groups (phospholipids-glycolipids), which may have some form of association with water, the hydrophobic alkyl residues by far outnumber them.

The only reported sorption study of lipid present in foodstuffs has been Loncin, Bimbernet and Lenge's[176] examination of peanut oil and oleic acid. At 30°C and 75% relative humidity the peanut oil had a moisture content of only 0.2g per 100g of lipid and that of the oleic acid was only 0.7g per 100g of lipid. Hygroscopicity increased with increasing temperature due, they have suggested, to the greater solubility of water in these substances at a higher temperature.

1.3.5 Role of Solutes in Water Relations

The role of solutes in the water relations of biological materials is complex and, as yet, not well defined. The general field, however, is of great interest and embraces the wider concepts of drug action mechanisms, winter hardiness and drought resistance, and even theories on the origin of life on Earth.[95]

The action of solutes in water relations is, perhaps, best thought of in terms of a) their effect on water structure, a subject which has been reviewed recently by Némethy[2] and Franks[188], and b) their effect on the water relations of macromolecules as a result of their mutual interaction.

The subject will be dealt with here in terms of the action of the four different classes of solutes.

Ionic Solutes:

Although it has been realized for some time that ions exist in a hydrated state in aqueous solutions[8], the modern concept of this hydration[2,3,95,188] has been based on the work of Frank and Wen[13]. Ions must be envisaged as charged spheres which, in the presence of dipole oriented water molecules, will induce an ordered structure in their immediate vicinity. This structure is alien to that of normal liquid water and consists of oriented molecules radiating from the sperical charge sphere of the ion. These molecules have a residence time greater or less than their fellows in the remaining liquid[2,189], the number of such ordered molecules and the strength of their immobilization

being determined by the ionic surface charge density. It has been postulated that the force involved in the immobilization of water in this primary hydration sphere must be of the same order of magnitude as those in the bulk liquid, as no increase in the latent heat of vaporization is observed in aqueous solutions, over that of the pure solvent, except in the cases when chemical combination is involved.[190]

The presence of these oriented hydration spheres in liquid water results in a further disordering of the water molecules adjacent to them, but only to the extent where the molecules exhibit randomness in their interactions, being influenced by the opposing forces of the bulk water, and the hydration spheres. Thus the situation is envisaged as in Fig.9, although Davies[189] has reported that in certain cases two separate regions may be distinguishable within the primary hydration sphere.

In this sense the introduction of any ion induces a local structure breaking effect in pure water. It has become customary, however, to differentiate between the intensity of this effect in terms of the overall degree of structure making or breaking in the entire system. The larger the primary ionic hydration sphere the greater the structure making effect, with small, polyvalent ions having the greatest influence and large, monovalent ions the least, as shown in Table 5.

Estimates of the number of water molecules in the inner hydration shell have varied depending on the method used.[189] Nemethy has reported that for alkali metal and halide ions, it is in the range of five to twelve, with the

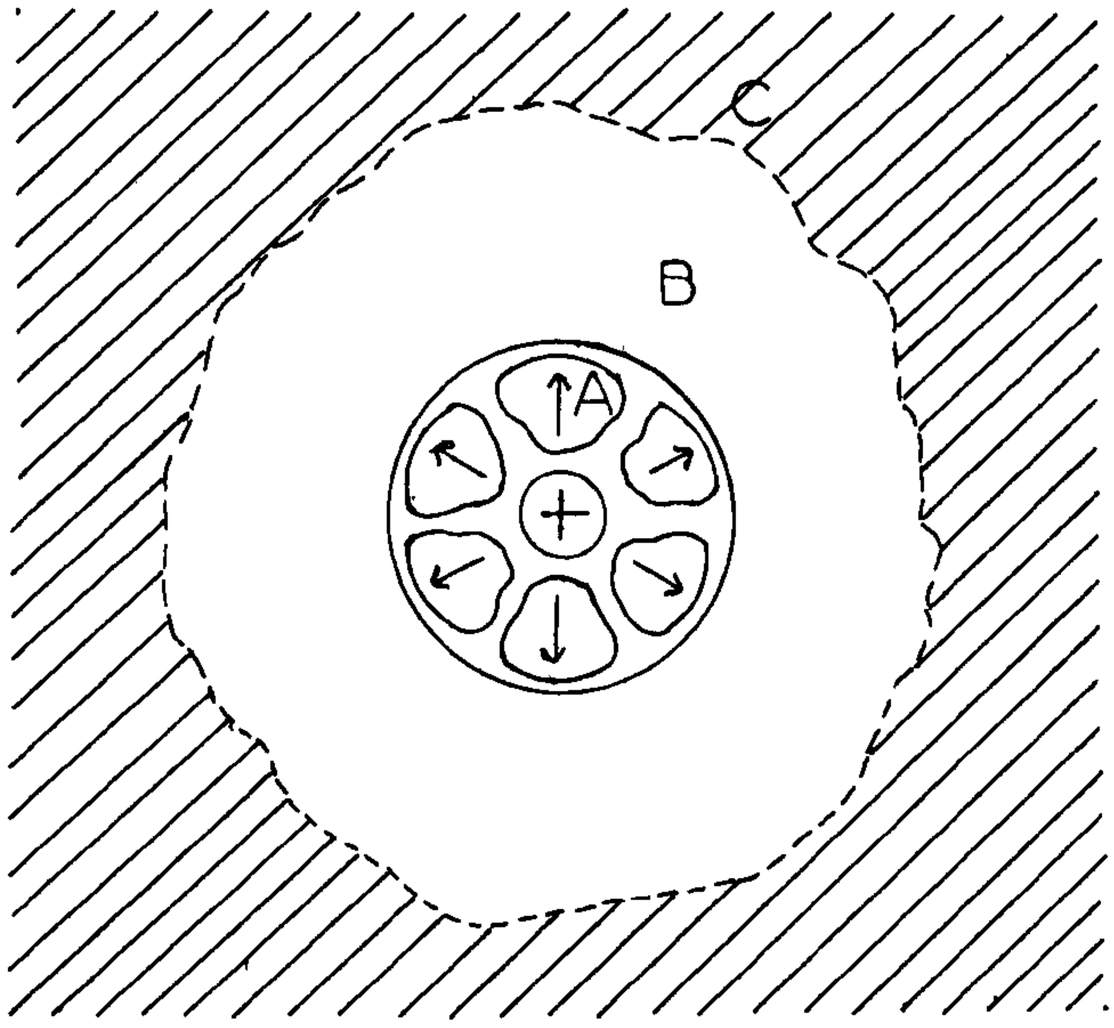

Fig. 9. Schematic representation of the various regions of water structure around an ion, from Nemethy, 1968[2].

Region A, radially oriented water molecules

Region B, disoriented water molecules

Region C, normal water

number of randomly oriented molecules in the outer region being four to five times greater. This restriction and disorientation of single water molecules will also presumably result in a reduction in the size and numbers of clusters of hydrogen-bonded molecules in the remaining water.

TABLE 5

APPROXIMATE CLASSIFICATION OF THE EFFECT

OF SOME IONS ON THE STRUCTURE OF WATER[2]

STRUCTURE MAKERS	STRUCTURE BREAKERS
Al^{+++} Mg^{++} Ca^{++} Ba^{++} Li^{+} Na^{+} K^{+} Rb^{+} Cs^{+} NH_4^{+}	
F^{-}	Cl^{-} Br^{-} I^{-} SCN^{-} NO_3^{-} ClO_4^{-}
CH_3COO^{-}	

In addition to the solute-solvent interaction described above, attention has recently been focussed on ion-macromolecule, and in particular ion-protein, interactions and the resulting effect that these have on the water relations of the polymer. Jenks[191] has reasoned that as water plays an important part in protein stabilization, any change induced in water structure by the interaction of ionic solute may be important in causing denaturation. However, following an experimental investigation of this hypothesis, he concluded that the major part of the effect of salts and urea on denaturation occurs by a mechanism other than a simple effect on water structure. He found that while protein showed an intense sensitivity to the nature of the anion, it was insensitive to the type of cation.

Gál and his co-workers[192-199], studying a system with more relevance to foodstuffs, have found that in the presence

of NaCl, the magnitude of sorption of water vapour by casein is considerably raised. In this case, however, the effect appeared to be cation dependent, with the chloride ion in isolation, using HCl, having, if anything, a slight depressing effect.[192] Subsequent experiment showed that up to 120 ± 20 moles of NaCl per 10^5g of dry casein can be bound, although this binding ability appeared to bear no stoichiometric relation to the number of ionic groups of the protein when a range of other alkali salts were examined. Gál suggests[197] that this binding occurs only in the presence of water, and that initially chloride is the principal ion bound. Upon drying, however, the sodium ions will also be deposited, the bound salt existing in the form of single absorbed ion pairs. The rise in sorptive capacity for water has been attributed to the stronger hydration of the sodium ions over that of the ionic protein group. The presence of an apparent saturation point with respect of the ability of casein to bind different salts has been related by Gál to the solubility differences of different ions in the water adjacent to the protein, as influenced by the size of their hydration spheres.

Additional mention of the existence of ion-macromolecule interaction has been made by Ling[29], who has reported that over 90% of the intercellular ions are absorbed onto cell proteins, and also by Sterling[138] and Love.[58] Tracey[95], however, has suggested that ionic solutes may compete for water in their own right in a water-poor macromolecular system, rather than interacting with the macromolecule itself. He has presented experimental evidence showing that the presence of salts in a flour-water dough

decreases the amount of immobilized water, or at least increases the amount available to affect the extensibility of the dough, explaining the effect in terms of a salt induced disruption of the ordered protein water.

Nemitz[118] also explains the effect of salts solely in terms of their partial hydrate formation, this increasing the amount of non-freezing water in intact egg white, when compared to the same water in desalted egg white. He also found that upon desalting there is an increase from 50% to 81% of the amount of water frozen out at -1°C.

The work of Hamm[55,200], for instance, allows yet another explanation of the effect of salts on the water relations of macromolecules. In extensive studies on meat he found that the addition of NaCl at the normal pH of meat increases the water holding capacity, but decreases it at low pH's. This was attributed to the effect of the anion on the inter-molecular salt cross-linkages and electrostatic repulsion forces, these being responsible for determining the fine structure of muscle protein. On the basic side of the iso-electric point, NaCl caused a weakening of the salt cross-linkages, while on the acidic side the anion weakened the repulsive electrostatic forces. Binding of cations can also take place, but with an opposite result to anion binding.[55]

Polar Hydrophilic Solutes:

Any interaction between water and solutes in this group will be through specific hydrogen-bonding effects[2,188]. In theory, any compound possessing an atom capable of acting as a proton acceptor can interact with water in this manner.

Some solute molecules, for instance alcohols and amines, can also act as proton donors, enabling cooperative hydrogen-bonding to occur with water. As a result of steric size incompatability, these solutes alter water structure comparatively little, although there is a tendency to cause a slight shift to a greater proportion of the dense water species, the interaction occurring only with single molecules and not hydrogen bonded clusters. An extreme example of this is the water structure breaking effect of urea.[3]

Representatives of this group most common in foodstuffs, and in particular those of plant origin, the mono and disaccharides, have well defined relations with water. They exhibit a high degree of hygroscopicity in their amorphous, glassy state at low relative humidities, but form crystalline hydrates or anhydrates at higher relative humidities, before finally passing into solution. One of the more extensively studied of the disaccharides is lactose as it occurs in dairy products.[201-204] In spray dried milk powders lactose exists in the amorphous form and will absorb water in this state. At a relative humidity between 39% and 50%, crystallization occurs with transformation into the non-hygroscopic α-monohydrate or β-anyhdride. This crystallization results in the release of some, or all, of the absorbed water, and the latter may be responsible for the stickiness found in milk powders exposed to the atmosphere. Other sugars behave in a similar manner.[205-210] At a very high relative humidity, characteristic of the material, these crystalline sugars will go into solution and absorb a large amount of water.

Sorption isotherms for foods containing sugars will be influenced by this typical behaviour.[113,211]

Non-Polar Solutes:

These solutes, many containing alkyl residues, have a low solubility in water due to a high negative entropy of solution caused by the increased order of water in their vicinity. This increased order is comparable to clathrate formation around small, non-polar gas molecules. Although these solutes favour the formation of highly hydrogen-bonded water structures around them, it is likely that, at the most, only partial clathrates or cages will form, and the associated increase in structural order will be small.[2,3,5,188]

The increase in hydrogen-bonding per mole of water has been reported to be less than 0.1% in a saturated solution of hydrocarbon[95] and so the partial cages are only slightly more permanent than a cluster in liquid water.

Tracey[95], however, found quite marked symptoms of water removal when certain central nervous system depressants were introduced into a flour-water dough. He attributes this to their tendency to form clathrate-like structures which effectively remove the water concerned from the system.

The idea of possible application of the knowledge concerning true clathrate formers, for example CCl_3F, CH_3Br and ethylene oxide, to foodstuffs has been developed in a series of papers by Fennema and his co-workers.[212-216]

These compounds, in an aqueous environment, are physically entrapped by the host water molecules in an ice-like structure which forms around them. This also effectively removes the water molecules involved, from the system. Knowledge of the property of gas hydrates, some of which are stable up to temperatures between 5°C and 10°C at normal pressures, has led to the patenting of a commercial process for water purification and to the suggestion that use could be made of them in the concentration of fruit juices or in the 'freezing' of whole tissue at temperatures above 0°C.

It has been shown[212] that gas hydrate formation can occur in aqueous systems containing sizeable quantities of protein, carbohydrate or lipid and that about 80% water removal can be obtained from apple, orange and tomato juices by the use of CH_3Br and CCl_3F.[213] Gas hydrate formation has also been shown to be possible in polyacrylamide gel[214] and, recently, in intact plant tissue[215], providing sufficient time is allowed for diffusion of the hydrate former into the tissue prior to the initiation of crystallization. The ability of some gas hydrates to inhibit ascorbic acid oxidation has also been recently shown.[216]

Although it would appear that the effect of gas hydrates on the water relations of food materials may have some practical applications there are also considerable problems of alteration of flavour and colour, cost, and general aesthetic considerations.[213]

Polyfunctional Solutes.

Different chemical groupings according to the

classification into ionic, polar-hydrophilic or non-polar groups are found, in certain cases, in the same solute molecule. Each group will affect the water adjacent to it in the way outlined above, although the induced changes may be incompatible. The total effect, however, is probably additive[2,3], although there is little information in the literature on this point.

In addition to the effect on sorption behaviour, the presence of solutes may also be expected to have an effect on the freezing pattern of foodstuffs in view of the well known eutectic phenomena exhibited by simple solutions of many soluble substances. Fennema and Powrie[119] and Merryman[56] both present a table of the eutectic points of various salts.

In nearly all foodstuffs, due to the relatively low concentrations involved and the effects on the solubility characteristics of the presence of other salts, sharp eutectic freezing effects are not normally observed.[58,119,217,218] Various authors have quoted from -50°C to -60°C as the lowest temperature at which all the solutes present will have been removed from solution due to eutectic phenomena.[118,119,217,219]

Sugars do not exhibit a distinct eutectic phenomenon but at a critical concentration, reached due to the freezing out of pure water, and on further temperature reduction, the remaining solution slowly solidifies into a glass-like structure.[56,218] The presence of such solutes in relatively small amounts may prevent the eutectic freezing of salts,

the latter undergoing a glassy solidification along with the sugar. Certain hydroxycompounds, and especially glycerol, are well known to act in a protective capacity during freezing, limiting tissue damage. This subject has been well covered by several reviewers.[56,119,220]

Thus it can be seen that the effect of solutes on the water relations of foodstuffs is a complex one, with a divergence of opinion existing over certain aspects of their action.

1.3.6 Role of Structure in Water Relations

The condition of the bulk of the water present in fully hydrated biological materials; that is the fraction in excess of the non-freezing water; and the effect of structure on its distribution and properties, has received little detailed attention in the past. From the results of numerous sorption studies, it can be seen that a large proportion of the water present in foods does not exert the same vapour pressure as bulk water at a given temperature. What is not yet clear, however, is the relative importance of long range effects of those macromolecules capable of chemically associating with water; the precise quantitative aspects of the effect of solutes on the colligative properties of water; and the physical effects of the micro and macro structure of the material.

In this section the state of knowledge concerning this last mentioned factor will be dealt with.

Nearly all non-manufactured foodstuffs, and particularly those of plant origin, at least in their native states, may be thought of as containing networks of pores and fine capillaries. Theories derived from results of physical studies on the state of water in model, inorganic, capillary systems will therefore have some application to their biological counterparts.

The nature of the behaviour of water within a chemically inert capillary of macroscopic size is a well known physical phenomenon, with the attractive forces of the capillary walls acting against the counter forms in the bulk liquid. It is not until sub-microscopic dimensions are reached, however, that significant changes in the physical properties are noted,[221] although the water still exhibits the same basic properties as the bulk liquid.

Parker[50] has reported that the magnitude of the freezing point depression of water in an inorganic capillary system depends partly on the relative surface area of the solid, although Jones and Gortner[51] have stated that all the water will freeze if a low enough temperature is attained. In a more recent study along similar lines, Hori[223] found a steadily decreasing temperature of spontaneous freezing as the thickness of a water film between glass or quartz plates was reduced from 1.00mm. to 0.01mm. At this latter thickness the water froze at $-30^{o}C$, but with even thinner films no crystallization was observed at $-100^{o}C$, while no vapour pressure was detectable at a film thickness of about 0.09μ in the case of quartz and 0.14μ in the case of glass.

Within the last five years there have been reports of the formation of a polymeric form of water of unusual properties which can condense from unsaturated water vapour into fine quartz capillaries, and which maintains its anomalous characteristics when transferred into a bulk container.[224] The postulated existence of this "ortho-water", the name proposed by its first discoverer, was the result of experimental investigations conducted by Russian workers and first widely reported by Derjyagin.[224] Confirmatory reports of its preparation have subsequently been published,[225,226] and its anomalous properties of low freezing point, high boiling point, high viscosity, greater density and changed thermal expansion characteristics, confirmed.

This water appears to be formed as a result of the interaction between the water molecules and a silicate surface,[227,228] the template surface structure inducing a corresponding spacing of bonded water molecules which can extend through a film of up to 800 Å in thickness.

Some infra-red[226,229], Raman spectra[226], mass-spectroscopy[229] and NMR[229] studies have shown it to be identical to normal water in respect of the measured properties, although there is some disagreement about this.[225] Workers have postulated a variety of strongly-bonded, polymeric structures for it[225-227,230], but there is still some dispute over whether it does indeed have a polymeric form.[231]

The possible existence of anomalous water in biological materials must be questioned, however, as it seems unlikely that the critical conditions needed for its formation would

ever exist. The presence of the correct template of groupings is also unlikely.

While it is recognised that inorganic capillaries can thus alter the physical and energetic state of water to some extent, the situation in foodstuffs must be very much more complex. The molecular nature of the limiting surfaces will be different and there will be a wide range of pore sizes present.

In animal products the biochemical state of the tissue, which influences the spatial molecular structure and degree of interchain binding by proteins, will control the total water holding capacity.[200,232] Cooking and other processing operations will alter the orientation and quantity of pores in all foods,[138,323] as well as influencing the degree of crystallinity of certain plant constituents.

It must be admitted, however, that to a great extent the precise role of structure is somewhat of an undefined factor in water relations of foodstuffs.

1.3.7 Hysteresis

Hysteresis, the lag phenomenon observed between desorption and the lower magnitude of absorption at any given relative vapour pressure, has been observed in many inorganic and biological systems (for example see Mellon, Korn and Hoover, 1948[234], McBain, 1935[235] and Chung and Pfost, 1967[236]). This difference can amount to 2g water per 100g dry matter in proteins at a relative humidity of

51% and to half the total sorbed water at 6% relative humidity.[234] The study of this phenomenon, and theories explaining it, developed initially from the examination of inelastic inorganic systems where capillary condensation theory was used to account for the effect. Attempts to extend the various hypotheses to cellular food materials can account for some aspects of hysteresis, but in these more complex systems with chemical as well as physical interactions involved during a desorption-absorption cycle, other influencing factors have been suggested and no widely accepted explanation yet exists.

Hysteresis signifies the absence of thermodynamic equilibrium or the existence of a meta-stable state,[237] the water molecule apparently having a greater degree of association in the desorption stage than during absorption.[236] Benson and Richardson[238] have suggested that hysteresis is due to a failure to reach equilibrium on desorption, the meta-stable state having a half-time too long to observe under reasonable laboratory conditions.

The Zsigmondy theory[66,239] was one of the first theories proposed to explain the absorption of vapours by solids and this allowed a possible explanation of the hysteresis effect. The theory was based on the Kelvin equation (Section 2.4) which relates the radius of a capillary to the pressure of a vapour in equilibrium with the liquid condensed in it. One of the variables in the Kelvin equation is the angle of contact, θ; the explanation of hyteresis being based on a proposed difference in θ during desorption and absorption. During desorption the capillary is full of liquid, allowing

intimate contact between capillary wall and liquid, so there is complete wetting and an angle of contact of zero. During absorption the liquid has to wet the dry capillary wall, the Zsigmondy theory postulating that initially it is unable to do so completely due to the presence of impurities, particularly gases and air, which are only displaced at high pressure. This results in an angle of contact greater than zero.

The shortcomings of this theory are, however, obvious when explanation has to be made of observed hysteresis in the low relative humidity regions where capillary condensation may not occur. Similarly, there are difficulties in application to observed cases of continuously reversible hysteresis[238,240], if one assumes that all impurities are removed during the first few sorption cycles and should, therefore, not cause interference in subsequent cycles.

An alternative theory, which was also based on the Kelvin equation and which has found considerable support[235,241,242], is the so-called "Bottle Neck Theory". This assumes that capillaries or parts of capillaries within a material are not all of the same diameter, and that the sorption process is controlled by the narrower parts. On absorption, water will condense in the narrow sections at a relatively low vapour pressure in accordance with the Kelvin equation. Condensation will only spread to the wider parts as the pressure is raised, dependent upon their particular radii. Thus, continuous condensation is occurring over the complete relative humidity range. On desorption, however, although the water in the wider

capillaries could evaporate at relatively high vapour pressures, if open to the atmosphere, the water has to first pass through the narrow section where evaporation will not occur until a much lower pressure is reached. Thus, more water is held in the material and hysteresis is observed.

These two theories still do not fully explain hysteresis[240], which has been observed in non-porous bodies such as soap, plastic-clay and graphite.[236] In an attempt to derive a more general approach, Pearce and Smith[243] have extended the "Open Pore Theory" of Foster[244] and Cohan[245]. In this the latter authors postulate that on absorption molecular layers of water build up on capillary walls, but that meniscus formation will not occur until the films on opposite walls meet. This delay in meniscus formation, it has been suggested, accounts for the lower moisture content at a given relative humidity an absorption. They suggest that open cylindrical capillaries would need to have a radius greater than twice one film thickness to exhibit hysteresis. Pearce and Smith[243] extended this idea to cover any situation where water uptake proceeds by the mechanism of molecular absorption at specific active sites. They argue that clumps of molecules form at these sites, which subsequently merge as more water is absorbed. In this merged state the forces are stronger, with each molecule coming under the influence of more than one binding site; vapour pressure is therefore reduced and the water is more difficult to remove on desorption. The most common case of this, they have suggested, is the meeting of multilayer films held on capillary walls. After the films meet the

molecules are under the influence of both walls.

While all these theories are certainly plausible explanations when related to inorganic materials, and will have a certain applicability to biological systems, events occurring during a sorption cycle in the latter materials are likely to be more complex. Several investigators have attempted to explain hysteresis in these materials in terms of molecular rearrangement during the cycle, and particularly the bonding between potential water immobilizing sites during desorption as water is removed from them.[146,238]

This rearrangement could be a temporary and reversible one, in which case hysteresis may be exhibited, or a permanent one, as in the case of the increasing crystallinity of cellulose upon dehydration. In casein, however, Mellon, Korn and Hoover[234] have claimed to have proved that the hysteresis effect is independent of amino group content showing that no new sorptive sites are exposed on swelling; that is, they were all available at low moisture contents during rehydration. The assumption that their method of blocking amino groups prevented all sorption at these points has been questioned however.[78]

Chung and Pfost[236], have suggested that in wheat a molecular reorientation occurs. They found that hysteresis disappeared after two successive sorption-desorption cycles, an occurrence also observed by other workers in certain systems and after different numbers of sorption cycles.[242,246,247] The proposed mechanism in this case is that a molecular shrinkage occurs during the initial wetting of the grain prior to the first desorption cycle. This results in the

appearance of cracks in the grain, which allows the absorption of more water and increased moisture levels. The hypothesis appears to have been based on the assumption that in wheat grains the sorption process is purely a surface effect, an assumption which must be questioned in the light of the behaviour of other materials.

Several workers have commented that hysteresis will be dependent on the pre-treatment of a foodstuff and its degree of denaturation[205,238], and one attempt has been made to use the magnitude of the hysteresis effect as a quality index for dried beef.[248]

It is quite credible, therefore, to postulate the involvement of both physical and chemical effects in the hysteresis phenomenon in foodstuffs, but assertations about the precise mechanisms are difficult to make in such complex systems.

1.3.8 Water Relations of Whole Foods and the Effect of Composition

Reported examinations of the water relations of whole foods are legion. These have been conducted in the main to provide basic information on dehydration parameters and to allow predictions about likely behaviour during storage in the dried state. Theoretical studies into the state of water in these materials are less numerous.

Only studies conducted on foods of botanical origin will be described in this review.

TABLE 6

QUANTITATIVE ASPECTS OF THE WATER BINDING

RELATIONS OF PLANT MATERIAL FOODSTUFFS

MATERIAL	BET MONOLAYER WATER	UNFROZEN WATER	DISAPPEARANCE OF DIFFERENTIAL HEAT OF BINDING
Potato	5.4[41]	23.1-23.7(-60)[108]	>20[113]
	5.46[186]		
	7.2-9.6[269]		
	7.7[255]		
freeze dried	7.8[249]		
puff dried	5.8[249]		
air dried	5.5[249]		
Carrot		22.7-24.5(-60)[108]	
Peas	3.64[133]	4.1-32.0(-20)[270]	
		19.1-19.7(-60)[108]	
Cabbage		18.2-22.2(-60)[108]	
Beans green		11.0-68.5(-20)[270]	
		22.5-22.9(-60)[108]	
lima	5.37[186]	4.6 (-20)[270]	
Asparagus		35.0 (-20)[270]	
Brussels Sprouts		23.3-25.1(-60)[108]	
Wheat	6.0-7.8[257]		30[257]
Flour	6.62[173]	33.0 (-40)[271]	19[272]
		29.0 (-18)[129] by NMR	22[173]
		25.6 non[273] solvent	
		42.1-45.4 water [274]	
Bread white		25-30 (-40)[276]	
Corn		220 (-20)[270]	≃ 20[174]
Rice	7.2[262]		≃ 24[262]
Raspberry	2.14[186]	32.0 (-20)[270]	
Strawberry	4.78[133]	17.1-49.0(-20)[270]	
Rhubarb	5.71[133]	4-18 (-20)[270]	

The major materials upon which sorption studies have been conducted are potato[113,133,201,249-255], carrot[201,250-252,256], peas[251,252], cabbage[201,250-252,254], leek[201], beans[201,251,256], asparagus[201,251], wheat[174,247,257,258], flour[173,259], corn[174,247,260], rice[201,252,261-265], nuts[266,267], peach[113], raspberry[251], apple[249,251,252], and rhubarb[251]. Table 6 represents a collection of values derived from various studies which are pertinent to the state of water in whole plant foodstuffs. In addition Speiss, Solé and Pritzwald-Stegmann[268] have presented the two constants derived from the application of the BET theory to isotherms for over seventy-five different examinations of over forty-five different foodstuffs of plant origin, from which monolayer values may be calculated. Rockland[267] has presented the constants for his Local Isotherm Theory for a range of plant materials.

As for the major polymeric constituents, there is a definite fraction of water present in whole foods which may be differentiated from the remainder by virtue of its inability to freeze, or its greater energy of association with the constituents of the food. A small fraction of the water will be chemi-sorbed to these main constituents, a further fraction having a lesser degree of association.

Low resolution NMR examinations of foodstuffs of plant origin have shown that in the case of wheat flour dough the moisture content - signal size calibration curve crossed the zero signal axis at a moisture content of approximately 4g/100g dry matter.[129] In another examination using wheat flour it has been found that

below about 6% moisture (wwb) the calibration curve deviated from the linear, and convexly to the moisture content axis, before passing through the origin.[275] These results could be interpreted to indicate a degree of restricted rotation of the water at very low moisture contents, although not all materials examined have supported these findings.[276-278] In such examinations of whole foodstuffs, however, a signal will also be derived from any non-water hydrogen protons present, such as those of lipids in the mobile state.

Low temperature NMR examinations of wheat flour dough have indicated that a considerable portion of the water present did not freeze at -18°C, and that there was still a distinct signal at -50°C.[129] Calorimetric freezing studies[119,217] have indicated that in no case does 100% of the water present in plant material freeze at -30°C and Riedel[279] places the unfreezeable water content of white bread in the range of 25g to 30g per 100g dry matter.

When considering the practical applications of water removal from foodstuffs and the ability of the remaining water to support deteriorative chemical or microbiological processes, the mere presence of water is not necessarily the over-riding factor. It is instead the availability of the water to act as a support medium; an indication of its freedom from restriction. This availability is best expressed in terms of water activity, that is the ratio of the vapour pressure exerted by the water in the material, to that of bulk water at the same temperature. This ratio, expressed as a percentage gives the relative humidity of water vapour in equilibrium with a material. A sorption

isotherm relates this equilibrium relative humidity with moisture content, and thus gives a direct indication of the water activity of a foodstuff at any given moisture content. It has often been demonstrated that deteriorative reactions can occur at relatively low moisture contents, and indeed that certain oxidative reactions are favoured by such conditions, and while freezing temperatures generally reduce reaction rates, they do not stop all deteriorative processes. The mass of information concerning storage deterioration of all kinds is best covered by consideration of the many reviews and major studies.[44,64,119,133,136,176,201,280-286]

Salwin[141] has suggested that dehydration to a moisture content equivalent to the BET monomolecular layer will result in the greatest storage stability, a concept given support by the diffusion studies of Duckworth and Smith[41], who have shown that glucose movement through haddock and scalded potato tissue ceased at moisture levels only just above those representing the monolayer condition. Heiss[201] has presented a table of optimum moisture contents for storage for a variety of dried vegetables, the values ranging from 4.5% to 8.5% (wwb). In addition Rockland[286] has defined the region of optimum storage stability to lie within the second of the local isotherms as developed using his Local Isotherm Theory, the lower limit of which coincides with the calculated BET monomolecular moisture content.

Fig. 10 illustrates a typical sigmoid isotherm, such as exhibited by most foodstuffs.

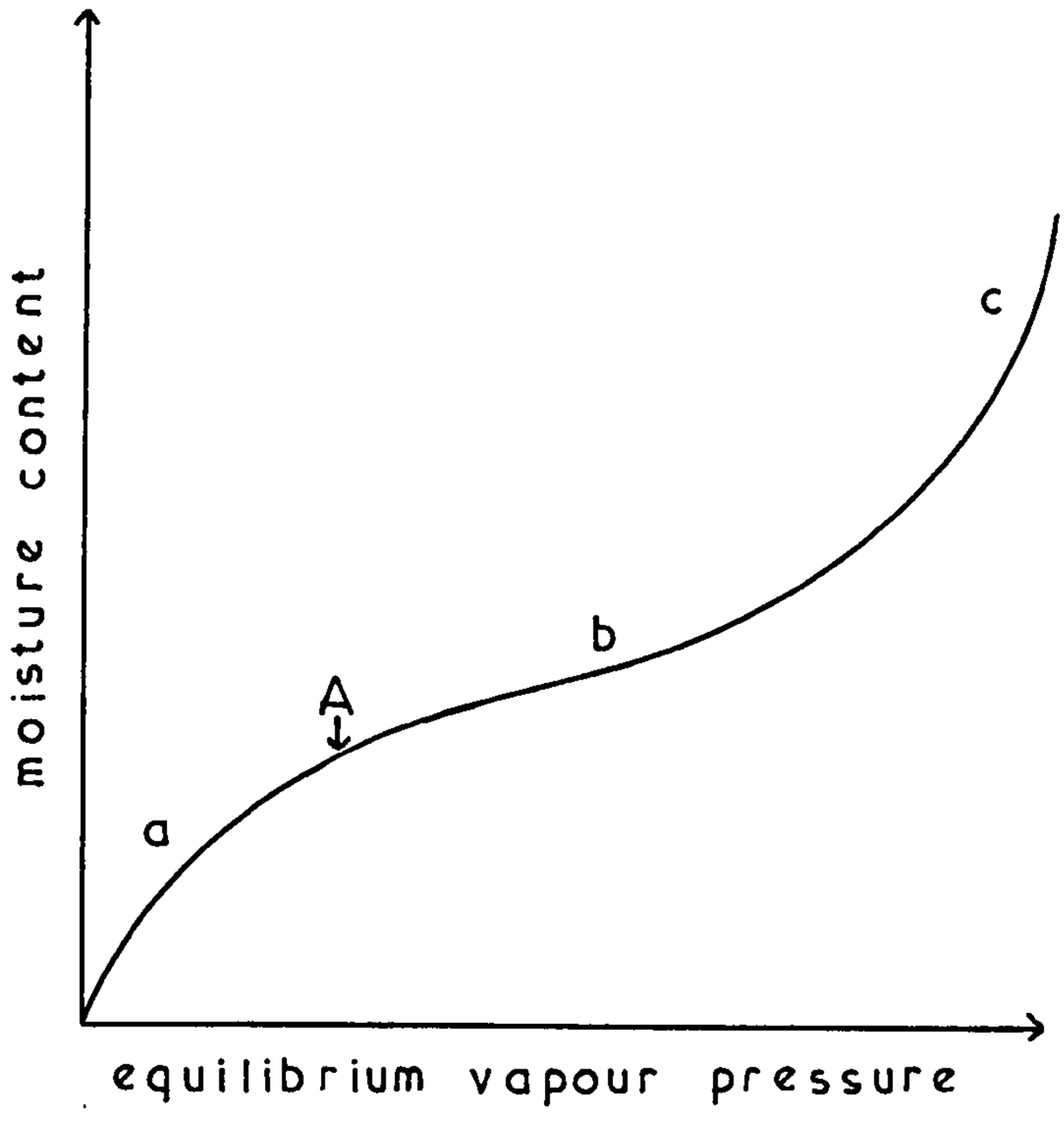

Fig. 10. Typical sigmoid isotherm.

Section (a) represents the build-up of a mono-molecular layer of water molecules, which is complete at point A. As sorption proceeds additional layers of molecules are built up, as represented by section (b), before capillary condensation occurs at high pressures in section (c).

The effect of temperature on isotherm shape is shown in Fig. 11, as well as the effect that changes in temperature and in equilibrium relative humidity above a foodstuff during storage will have on the condition of water within the material. This emphasises the need for defining

dehydration and storage parameters if control of deteriorative changes is to be achieved.

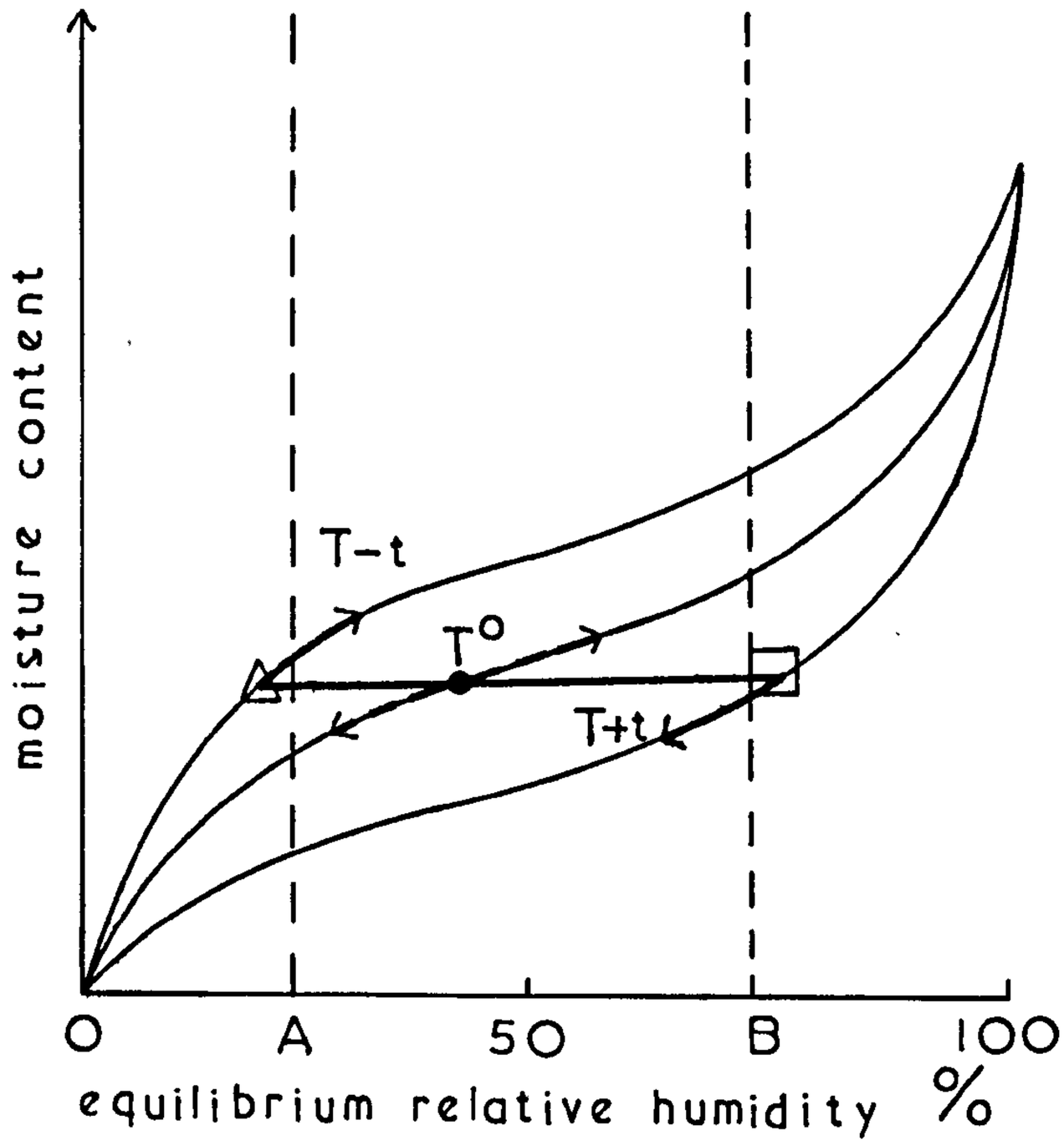

Fig. 11. Diagrammatic representation of the effect of changes in ambient relative humidity and temperature in enclosed and non-enclosed products after Rockland, 1969[286].

A — Suggested Monolayer

B — Suggested Relative Humidity equivalent to Unfreezeable Water Level

● — Initial Moisture/Equilibrium Relative Humidity condition

- - → — Direction of change induced by alteration of Relative Humidity

⟶ — Direction of change induced by alteration of temperature in non-enclosed products

Δ , □ — Equilibrium Relative Humidity attained by alteration of Temperature in enclosed products

Moisture sorption isotherms of whole materials represent the relative roles and interactions of their constituents, and one should be able to interpret the isotherms on the basis of the components present. The dependence on composition was shown by Salwin[284] in his diagram of isotherms for four representative foodstuffs with markedly differing compositions (Fig. 12).

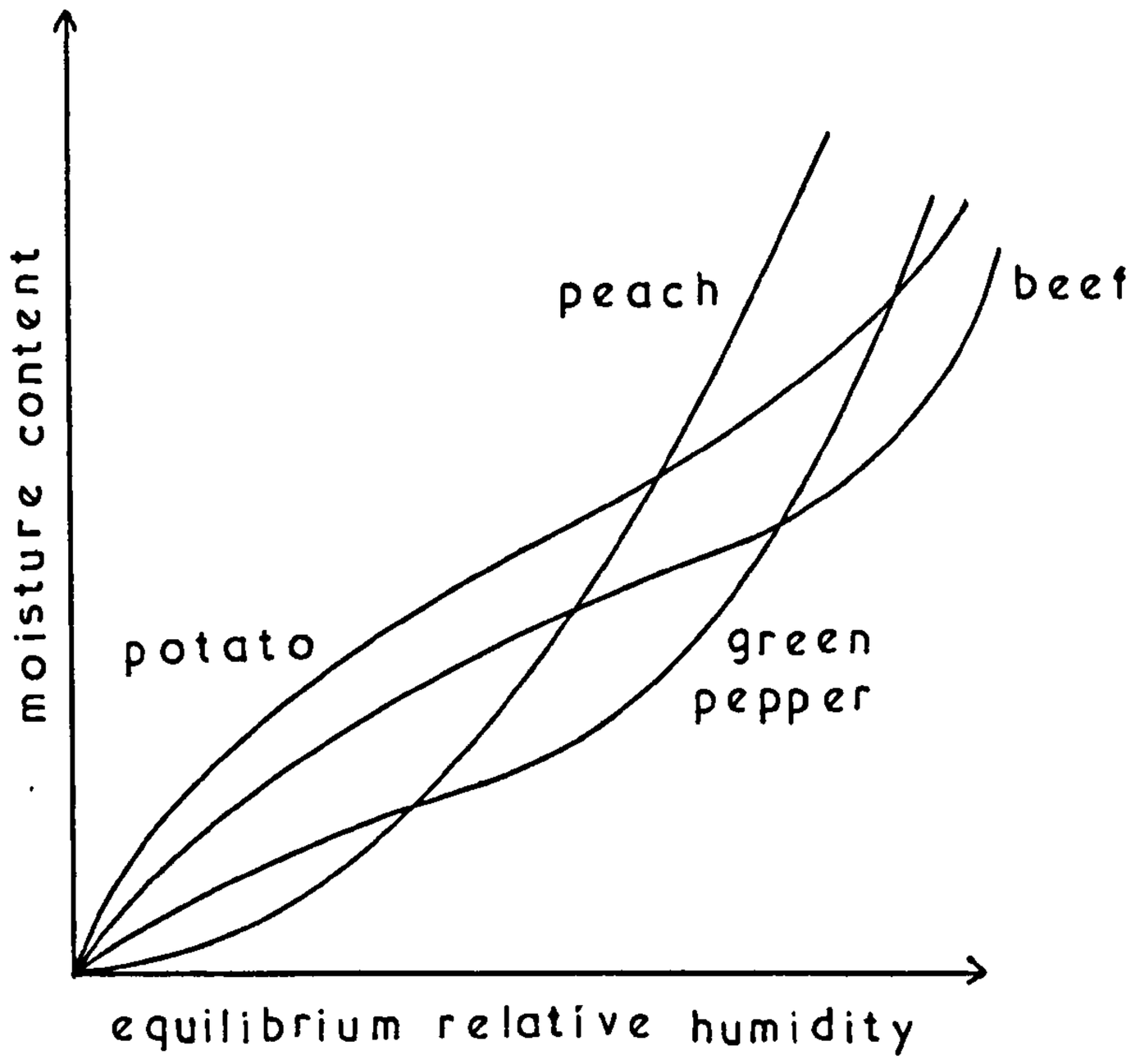

Fig. 12. Effect of composition on water relations of foodstuffs from Salwin, 1963[284].

At low relative humidities, starchy foods have the greatest water binding capacity, followed by high-protein

foods. In green peppers with a composition of 30% sugars, 40% other carbohydrates and 12% protein, the high molecular weight components dominate the behaviour of the material at low vapour pressures. The low molecular weight components have a relatively larger influence at higher vapour pressures, and over a wider range at high temperatures. Peach, with 67% sugars, 25% other carbohydrates and only 4% protein, is almost totally dominated by the behaviour of the sugars. Obviously, at any one vapour pressure or any one moisture content, the value of the other parameter for each class of materials is quite widely different. The significance of this is clearest when several foodstuffs are packaged together, as in a dry soup mix or dried convenience meal.

While each constituent may be dried to its own particular optimum moisture content, they will normally not exert the same vapour pressure. After mixing, all the constituents will, at equilibrium, exert the same vapour pressure, involving an exchange of water between them while achieving this equilibrium. This may result in a diversion away from their optimum moisture contents, thus creating an increased spoilage potential.

In an effort to control and quantify these changes Salwin and Slawson[285] have developed an equation, which should allow prediction of the final relative humidity, knowing the initial moisture contents of the ingredients of the mixture. This provides a possible tool for control and manipulation of the conditions so as to achieve maximum stability in the most sensitive component.

With just two ingredients in the package, the direction of moisture transfer is obvious, as shown in Fig. 13, and the equilibrium point can be estimated with reasonable accuracy. However, neither direction nor extent of transfer is obvious when more ingredients are present. Salwin and Slawson claim that the final equilibrium relative humidity can be computed from the dry weights of the components; their initial relative humidities; and the estimated slopes of the isotherms between initial and final humidity points.

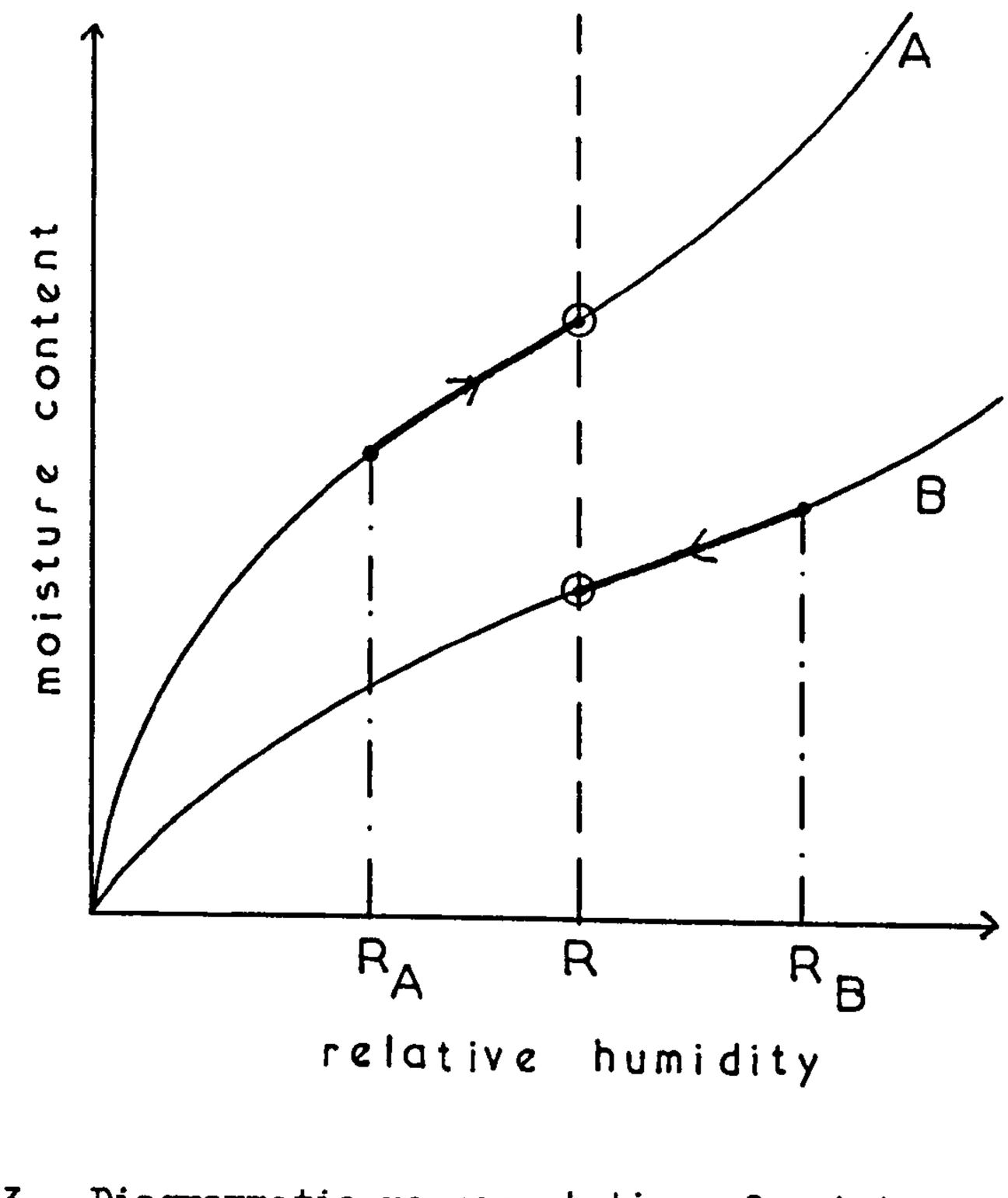

Fig. 13. Diagrammatic representation of moisture transfer between materials when mixed from Salwin and Lawson, 1959[285].

- ● Initial moisture content/relative humidity condition
- ◉ Equilibrium moisture content/ relative humidity after mixing

TABLE 7

COMPARISON OF SORBTION CHARACTERISTICS OF FOOD CONSTITUENTS

CONSTITUENT	MOISTURE CONTENT AT 40% RELATIVE HUMIDITY	% RELATIVE HUMIDITY AT MOISTURE CONTENT OF 10g / 100g DRY MATTER
Pectin	15.5g/100g	17.0%
Potato Starch	11.0g/100g	32.0%
Egg Albumin	9.5g/100g	41.0%
Amorphous Sucrose	7.5g/100g	48.0%
Cellulose	5.5g/100g	76.0%

They claim a high degree of success with this method, although its value is questionable, as assumptions must be made about the final equilibrium condition before calculation is possible.

The concept of moisture transfer between whole foods packaged together may also have application in considerations of the water relations between constituents of a single foodstuff. As is shown in Table 7, the variation in water sorption behaviour between the major constituents is considerable and at any one relative humidity each constituent has a different moisture content.

The condition of water in each will depend principally upon its number of binding sites and its fine structure. Thus one may have solvent water present in some constituents but not in others, with all that this infers in terms of chemical stability.

Duckworth and Smith[41] have illustrated this point during their glucose diffusion studies in scalded potato tissue. It was found that at low moisture contents solute diffusion occurred predominantly along the cell-wall network in preference to the starch gel, indicating the presence of more solvent water in the cell wall tissue at these moisture contents.

Several authors, from the time of Briggs[44] in 1932, have suggested that the amount of water absorbed by a foodstuff at any particular relative humidity can be found by summation of the weight percentage of each constituent, multiplied by the amount of water with which it would itself associate.[64,113,205,267,286] This statement may, however, be a gross simplification as it does not take into account any interactions between constituents, the effect of structure;

nor the ill-defined role of solutes. Indeed Duckworth and Smith[41] state: "It is in fact unlikely that the water remaining in a freshly dried product is distributed according to the theoretical picture ... . Films of water may persist in capillary spaces .... The distribution of water to a state of equilibrium is likely to be slow and free water may persist in dehydrated foods for considerable periods of time ." Thus a uniform activity in terms of relative vapour pressure does not necessarily imply a homogenous distribution of water at the molecular level and the significance of values calculated from sorption data as indicating the state of water in complete foods is therefore to be questioned.

Nevertheless for the more homogenous foodstuffs without an intricate structure, the original premise may hold true. Gane[136] found that the behaviour of dried whole egg is directly proportional to, and can be calculated from, the relative amounts of white and yolk present. Saravacos and Stinchfield[113] found a significant change in isotherms shape when glucose was added to starch gel in a 1:1 ratio. Coulter, Jenness and Geddes[202] claimed that the isotherm for whole dried milk represents a weighted average of the isotherms for the lactose, protein and salts which it contains. However, at a relative vapour pressure of 0.1, they estimate that the salts are responsible for absorption to a level of 1.25g water per 100g dry matter, compared to 1.75g per 100g dry matter for the other two constituents. This value for the salts would seem to be rather high.

Obviously, resolution of this complex situation will benefit from further study.

2.0 MATHEMATICAL TREATMENTS OF WATER SORPTION

Expression of the water relations of inorganic and biological materials has historically been through the medium of the sorption isotherm. This allows a quantitative description to be presented of the interaction between the two phases as a result of the adsorption or absorption of the vapour by the solid, and may be thought of as indirectly reflecting the free energy change of the vapour as a function of the amount absorbed.

In the majority of systems, experimental results long ago showed that such interactions did not obey the ideal situation represented by Henry's Law, according to which the amount of vapour absorbed varies directly with the equilibrium gas pressure. Such a situation has never been shown in the case where water vapour is the gaseous phase.

This observed non-ideal behaviour has led to many attempts to explain the nature of the process over the complete vapour pressure range and to the derivations of mathematical models, which would be able to describe the complete sorption process. Basically, these mathematical treatments are divisible between the following fundamental hypotheses on the nature of the sorption process: a) the kinetic approach b) the potential approach c) the capillary condensation approach. More recent attempts involving greater sophistication of approach in order to achieve a comprehensive explanation of sorption cannot be so easily compartmentalized.[287]

In spite of the relative profusion of such isotherm equations and of the claims made for each of them, no one

equation can yet, when tested against observed data, be successfully applied to cover the complete vapour pressure range, and to simultaneously accurately describe the various energetic and thermodynamic changes which accompany sorption.

Many of these equations, and particularly the early ones, have been developed from the study of inorganic systems, presenting additional problems when attempts are made to apply them to foodstuffs, in view of the unhomogenity and complexity of these biological materials.

2.1 ISOTHERM TYPES

Absorption occurs on the surface of a solid because of the attractive forces exerted by the atoms or molecules at the surface. The absorbed molecule may keep some, or lose all, of its translational freedom, but both the partial molal free energy change of the sorbate and the net entropy change are usually negative, resulting in the evolution of heat. Differences in the extent of the interaction and the course of sorption were represented by Brunauer, Demming, Demming and Teller[288] in the form of the five different isotherm types shown in Fig.14.

The type 1 isotherm represents unimolecular absorption, where there is a definite limit to the quantity of vapour absorbed. Langmuir[69] derived an equation which is applicable in this situation.

Fig.14/...

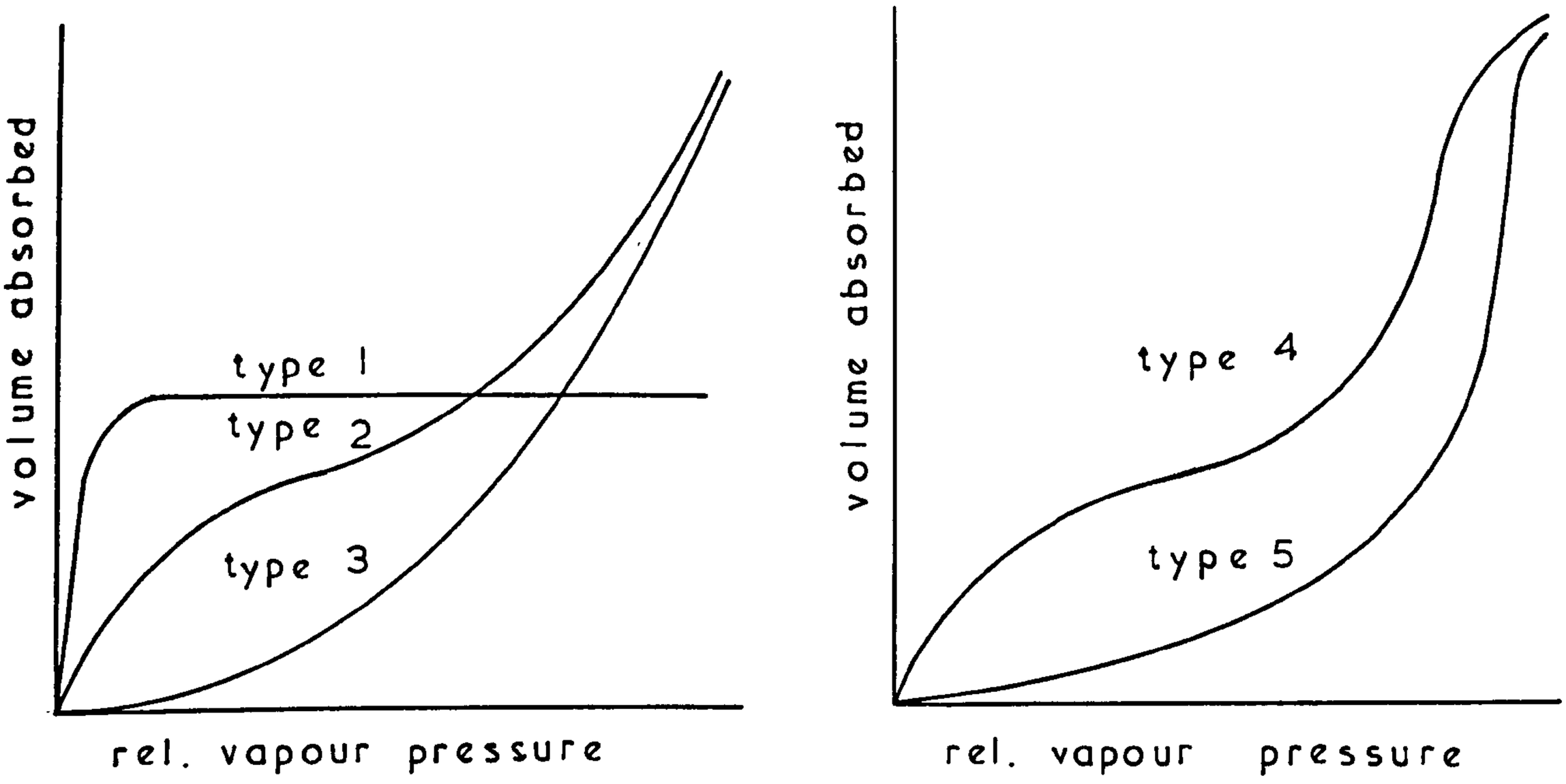

Fig. 14. Diagrammatic representation of five isotherm types from Brunauer et al, 1942[288].

The type 2, sigmoid isotherm, is that found in the case of absorption by macromolecular species and materials containing them as major constituents, and represents multimolecular absorption occurring on top of monomolecular absorption, before capillary condensation occurs at higher pressures.

The type 3 isotherm, convex to the vapour pressure axis over the complete pressure range, also represents a sorption process in which there is no limit to the amount of vapour absorbed.

Types 4 and 5, in contrast, while being related to types 2 and 3, describe processes where there is a definite

limit to vapour sorption due to a restriction of the available space for accommodation of the sorbate.

In the case of foodstuffs, most interest centres around the type 2 isotherm, although the type 3 isotherm is exhibited by materials rich in low molecular weight compounds which pass into solution at high relative humidities.

2.2 THE KINETIC APPROACH

This approach was initially based on the concept that random collisions between vapour and solid are inelastic and that the vapour molecule stays in contact with the surface, at specific sites, for a certain length of time, dependent on the nature of the material, before returning to the gas phase. The forces active in absorption are short range forces. Langmuir[69] was the first to develop this approach and derived an isotherm equation based on the following considerations.

a) That the surface of the sorbent is homogenous and that the heat of absorbtion is identical for vapour molecules absorbed at any site.

b) That there is no interaction between adjacent absorbed vapour molecules.

c) That the attractive forces of the surface are strong enough to hold only one molecular layer of sorbate.

d) That the absorbed gas molecules are localized and do not move around the surface.

The isotherm equation can be represented as:

$$V = Vm\left(\frac{ba}{K + ba}\right) \qquad (1)$$

where V = volume of gas absorbed in g/g of solid

Vm = value for monomolecular layer

$b = \frac{k_o}{RT_o} \exp\left(\frac{Qs}{RT}\right)$ = a constant

k_o = Boltzman constant = 1.38×10^{-16} erg /°C

R = universal gas constant

Qs = heat of absorption

a = activity of vapour expressed as a fraction of the activity of the pure vapour

k = $\frac{1}{p_o}$, where p_o = vapour pressure of water at T^o

T^o = absolute temperature ^oK

This may be rearranged to give:

$$\frac{a}{V} = \left(\frac{k}{b\ Vm}\right) + \frac{a}{Vm} \qquad (2)$$

and plotting $\frac{a}{V}$ versus a should give a straight line the slope of which is $\frac{1}{Vm}$. From this a value for the monomolecular layer can be easily calculated.

At infinite dilution Langmuir's equation reduces to Henry's Law.[289]

In foodstuffs, however, all heterogenous in nature, none of the four initial assumptions hold. In an effort to extend Langmuir's treatment, Freundlich[70] presented an equation which allowed for more than one monolayer to be absorbed, envisaging a series of monomolecular layers each with its unique heat of absorbtion. A plot of log of vapour pressure versus log of the volume absorbed would yield a straight line if this situation held. This is most likely to be applicable in the linear middle portion of a type II isotherm and Gál and Signer[197] claimed that it represents the absorption of water on casein at low vapour pressures in preference to the Langmuir or BET model, but it certainly does not describe the situation over the complete range.

The systems to which these two models are applicable and a detailed description of their derivation can be obtained from standard texts on surface chemistry.[240,289]

These two empirical equations provided the basis from which Brunauer and co-workers[65] developed their ideas on

multilayer absorption and upon which they based their well-known and widely-used sorption equation (the BET equation).

This equation has perhaps contributed more than any other of the mathematical rationalisations of sorption processes, and though not applicable to all systems, has stimulated many attempts at modification to overcome its limitations.

Initially developed to apply to non-polar gases, the BET theory embodies the following basic assumptions:

a) That the absorbent surface is homogenous with localized sites of absorption, all having the same heat of absorption potential.

b) That there is no interaction between neighbouring absorbed molecules.

c)* That more than one layer of absorbate molecules can be present but that only absorption of the first layer results in a heat of absorbtion greater than that of the heat of condensation.

d) That the surface area of the nth. layer is equal to the coverage of the (n-1)th. layer, although the nth. layer can become partially filled before the (n-1)th. is complete.

The equation is insensitive to any difference between chemisorption and physical sorption.[40]

*Brunauer, Emmett and Teller[65] do, however, say that for polar gases there is the possibility of mutual polarization occurring between the absorbent and absorbed layer, including those above the monolayer, as is postulated in the potential approach of Bradley.[37]

Thus they developed the equation in the form:

$$\frac{p}{V(p_o-p)} = \frac{1}{Vm\ C} + \frac{C-1}{Vm\ C} \cdot \frac{p}{p_o} \qquad (3)$$

where V = total volume/weight of vapour absorbed at pressure p

Vm = volume/weight of vapour represented by the monomolecular layer.

p_o = saturation pressure of the vapour

C = constant related to the heat of absorption

A plot of $\frac{p}{V(p_o-p)}$ versus $\frac{p}{p_o}$ should give a straight line of slope $\frac{(c-1)}{Vm\ C}$ and ordinate intercept $\frac{1}{Vm\ C}$

Thus from the graph the two constants C and Vm can be found, and from C, an approximate average heat of binding for the monomolecular layer can be calculated.[240,289]

On application of their equation Brunauer, Emmett and Teller[65] found it gave a linear plot for their experimentally determined isotherm only between relative vapour pressures of 0.05 and 0.35, the equation predicting too little absorbtion at low vapour pressures and too much at high vapour pressures. This limited range of applicability has subsequently been confirmed by all who have used it, although claims that it describes sorption above 0.35[113,174] and even up to 0.50[40,71] relative vapour pressure have been made.

One of the main failings of the equation is that it does not recognise the presence of binding sites of different energies. As a result of this one of the many modifications of the equation has been to attempt to take account of two different types of sites, although the

resultant equation in the event was only applicable over an even shorter range.[290] Other modifications and statistical and thermodynamic derivations of the equation have been made in attempts to account for the swelling of the absorbent; variable heats of absorbtion in different layers; a treatment of the system as a solid solution involving the formation of a monohydrate followed by a simple solution of water in the solid;[291] among other things,[237,288,292-296] without greatly extending the usefulness of the original equation. In the practical field of water relations of foodstuffs none of these has proved to be generally widely applicable. Most real systems of interest are obviously more complex than the model from which the BET equation was derived. One modification which has perhaps received more attention than the others is that of Smith[297], who has considered sorbed water to be present in two states: a) that which is in some way bound to the surface by forces in excess of the normal forces responsible for condensation of water, and b) that which is condensed within the absorbent. He also attempted to account for any swelling during absorption. Becker and Sallans[257] found that for wheat the Smith equation applied to their data at high relative humidities. They used this, together with the BET equation which was applicable at low relative humidities, to describe sorption over the complete range, a straight line joining the two from the first BET inflection point. Chung and Pfost[174] have found the equation to apply between 50% and 90% relative humidity.

Salwin[186] made a useful modification of the form of the original equation to allow easier calculation of monolayer values of water sorbed on foodstuffs. This is:

$$\frac{R}{a(100-R)} = I + SR \qquad (4)$$

where R = percentage relative humidity

I = y axis intercept of a plot of $\frac{R}{a(100-R)}$ versus R

S = slope of the same plot

a = g water adsorbed per 100g dry matter

From this the value of the monolayer, a_m, can be calculated as follows:

$$a_m = \frac{1}{I + 100s} \qquad (5)$$

In spite of the inadequacies of the BET equation, it still has its usefulness in determination of monolayer values, and, because of this, has remained in favour as a useful component of the range of techniques which can be used to build up an overall picture of the water relations of foods.

2.3 THE POTENTIAL APPROACH

This approach, as first developed by Polyani[239], embodies an assumption that absorption occurs as a result of the potential attractive field at the surface of a solid into which absorbate molecules are pulled; that is, work is done by the absorption forces in bringing a molecule from the

gas phase to a point just above the surface. This attraction results in a greater density of vapour molecules at the surface producing a compression effect which, at lower temperatures, is responsible for a condensation effect, the sorbate molecules coming to lie on the surface.

De Boer and Zwicker[67] widened this approach and introduced the concept of induced polarization of non-polar gases absorbed on polar surfaces. The orientation of the dipoles in the first layer in turn influence the second layer, and so on, changing the normal liquid structure. These induced dipole moments and binding energies decrease exponentially as the number of adsorbed layers increases. This approach was severely critized by Brunauer[240] on the grounds that the effect could not be great enough to be responsible for considerable multilayer absorption, although recently the idea of polarization forces being involved is coming back into favour.[298]

A situation in which polarization may be of more importance was treated by Bradley[37] considering the specific case of the absorption of polar molecules, which already have considerable dipole moments. It is envisaged that a considerable film of vapour molecules could be built up, with a gradually decreasing polarization effect as the distance from the surface increases. The theory has gathered considerable support.[29,170,174,299]

The Bradley equation is written as:/

$$\text{In } \frac{p_o}{p} = k_1 \, k_2^{\,a} \qquad (6)$$

Where a = amount of vapour absorbed at pressure p

p_o = saturation pressure of the vapour

k_1 = a temperature dependent constant being a function of the dipole moment of the vapour

k_2 = a temperature dependent constant being a function of the sorptive polar groups.

An alternate form of expression is:

$$\log (\log \frac{p_o}{p}) = \log k_2 + a \log k_1 \qquad (7)$$

A plot of $\log (\log \frac{p_o}{p})$ versus a should give a straight line of slope $\log k_1$ and ordinate intercept of $\log k_2$.

Recently Chung and Pfost[300] developed an equation very similar to that of Bradley by using a modified potential approach from a thermodynamic angle. They assumed that in the system under study (cereal grains) the free energy function of absorption decreased exponentially with increasing film thickness, and that this function was temperature variant.

Their equation can be written as:

$$\text{In } \frac{p}{p_o} = -\frac{A}{RT} \exp (-Bm) \qquad (8)$$

where p_o = saturation pressure of water at temperature T

m = moisture content at pressure p

A and B = constants characteristic of temperature and the nature of the absorbent

R = universal gas constant

Chung and Pfost[300] claimed applicability for this equation between relative humidities of 8.9% and 88.9% .

Another major isotherm equation was developed by Harkins and Jura[301] using the potential approach, their equation being based on the hypothesis that the surface force distribution results in the condensation of a film of liquid. This is:

$$\log \frac{p}{p_o} = B - \frac{A}{V^2} \qquad (9)$$

where V = g of water absorbed per g of dry matter at pressure p

p_o = saturation pressure of the vapour

A and B = constants

A plot of $\log \frac{p}{p_o}$ versus $\frac{1}{V^2}$ should be a straight line of slope A.

There seems to be some argument over the range of applicability of the Harkins-Jura equation. Several authors say that the plot ceases to be linear below a water activity of 0.5 or 0.6.[119,262,302] Adamson[298] claims applicability in the range 0.2 to 0.8, while Labuza[64] suggests that it does not apply above an activity of 0.4 to 0.5. However a much better straight line could be drawn through points 2,3,4 and 5, numbering from the top, of Fig. 6 in Labuza's paper. This would give applicability in the range above a water activity of 0.35.

Liang[303] showed that the slope of the line produced is equal to the square root of the monolayer value, and thus the equation allows derivation of the amount of water in the monolayer. Several people have shown the excellent agreement

that the Harkins-Jura monolayer has with the BET monolayer value,[132,262,302] and that the Harkins-Jura equation becomes applicable at a moisture content of almost exactly double this monolayer value. The implication of this would be, therefore, that condensation of vapour in films only occurs after two layers, identical in quantity, have been absorbed. Thus it might be envisaged that the binding forces of the absorbent are capable of holding two absorbed layers with a heat of absorption greater than the heat of condensation, or perhaps that a polarization mechanism may be present in these two layers. This idea of two more highly immobilized layers being present before normal condensation occurs agrees with Bull's [71] suggestion concerning the path of sorption at low vapour pressures, which he derived from a quantitative study of isotherm inflection points.

2.4 THE CAPILLARY CONDENSATION APPROACH

As the title suggests, this approach to sorption is based totally on capillary condensation phenomena and it has suffered considerable criticism because of this narrowness in approach. Zsigmondy[66] was among the first to examine sorption in this way, basing his calculations of the amount absorbed on the Kelvin equation:-

$$\ln \frac{p}{p_o} = - \frac{2 \sigma V \cos \theta}{r RT} \qquad (10)$$

where p_o = vapour pressure above a plane surface in dyne cm^{-2}, or saturation pressure of the vapour phase.

p = vapour pressure in the capillary in dyne cm^{-2}

σ = surface tension of the liquid in dyne cm^{-2}.

V = molar volume of gas in cm^3 $mole^{-1}$ or molar volume of water vapour.

r = radius of the capillary in cm.

R = universal gas constant in cal^{o} k^{-1} $mole^{-1}$.

T = absolute temperature.

Θ = angle of wetting

While the capillary condensation theory is able to account for hysteresis as previously explained (Section 1.3.7), it is criticised because of its inapplicability at low vapour pressures and low moisture contents. Observed absorption, over the lower range of moisture content, results in calculated capillary radii smaller than those of water molecules. Attempts to explain this anomaly have been made in terms of a postulated change in the physical constants of the absorbate at low pressure.[245] McBain[235] claims that the Kelvin equation is only applicable to pores of 20 Å and upward. Brunauer[240] has pointed out that this approach cannot explain unimolecular absorption, while his potential theory is more comprehensive in its applicability. Indeed, the fact that true condensation can occur at all in very fine capillaries has itself been questioned.

Görling[255], utilized the Kelvin equation to estimate the mean pore size of capillaries in potato. He conceded, however, that true capillary condensation does not occur

until a film of water two molecules thick is built up on the surface.

Henderson[304] derived an empirical isotherm equation based on capillary condensation. This can be given in the form:

$$\text{In}\ \left(1-\frac{p}{p_o}\right) = -kTM^n \qquad (11)$$

where p_o = saturation pressure of the vapour

M = moisture content on a dry basis at pressure p

n and k = constants characteristic of the material

T = absolute temperature

This can be written as:

$$\log\ \left[-\log\ \left(1-\frac{p}{p_o}\right)\right] = \log k + \log T + n \log M \qquad (12)$$

when a plot of $\log\ [-\log\ (1-\frac{p}{p_o})]$ versus log M should give a straight line of slope n and intercept of log k.

Henderson obtained values for the two constants n and k on the strength of two arbitrarily chosen experimental points at 20% and 75% relative humidity. Applying the equation to eighteen miscellaneous products, he claims satisfactory agreement between observed and experimental results, except at extremes of relative humidity. Such agreement, however, has not been found by other investigators applying the equation to foodstuffs.[252,267]

Rockland[267] has modified the form of the equation and eliminated the temperature function to give:

$$\log\ \log\ \left(\frac{1}{1-rh}\right) = n \log M + K \qquad (12a)$$

Applying this equation to sorption data he found that the resultant points could be joined to give two or three intersecting straight lines. He reasoned, therefore, that the sorption process is not uniform but that it can be characterized by two or three stages, each indicating the accumulation of water in a different condition as sorption proceeds, and each of which can be described by Henderson's equation using separately evaluated constants for each section. This he has described as the Local Isotherm Theory. Constructing such local isotherms for a variety of materials, using both his own experimental results and those from the literature, he claims good agreement in all cases when the relevant parts of these local isotherms are joined to construct a curve covering the complete relative humidity range. Equations have been presented for the estimation of the point of intercept of the isotherms.[267]

2.5 OTHER APPROACHES

In an attempt to introduce all the various approaches discussed above, Kühn[287] has developed an isotherm equation based initially on a capillary condensation approach, but also incorporating the idea of absorption potential as well as a kinetic factor. Kühn considered that each one of these approaches correctly described some specific aspect of sorption, the kinetic concept holding at low vapour pressure and yielding to potential absorption and capillary condensation at higher vapour pressures.

Young[258] has derived an equation which attempts to describe sorption in wheat grains and which is based on the hypothesis that the surface area remains constant during sorption and that the area covered by the second and subsequent layers is equivalent to the coverage of the first layer. The first assumption is perhaps contradicted by Chung and Pfost's[236] observations of cracks developing during initial wetting, which might increase the available surface area and certainly increase accessibility to water penetration. Young's approach to the problem must be further doubted in view of his rather naive concept of the state of water in the grain. He characterises three states.

"a) Bound water held on the surface of the cells by energy in excess of the heat of condensation.

b) Normally condensed water.

c) Absorbed water which has actually entered into the protoplasm of the cell."

In view of the history of the study of water relations in biological material it might be safe to assume that water in class c) will be more highly immobilized than that in class a). Nevertheless Young claims good agreement between his theoretical and observed results.

Perhaps the most revolutionary and interesting theoretical approach to water sorption was made by Riedel[59] in 1961 from studies on cooked beef. Stressing that the problem of water immobilization is probably far too complex to be accurately described by means of a few formulae based on relatively simple premises, he rejects the BET approach as obscuring the true situation. Within

the context of his admittedly theoretical hypothesis concerning the temperature dependent equilibrium between free and combined water, he derived the following isotherm equation, based on a fundamentally kinetic approach.

$$x = x_b + x_k = \alpha + \frac{\varepsilon\varphi}{k+\varphi} + \frac{\lambda\varphi^3}{1+\varphi} \qquad (13)$$

where x = amount of water absorbed in kg/kg dry matter

x_b = combined water

x_k = capillary solution water

φ = water activity

ε = the maximum possible amount of combined water

k = an equilibrium constant for the dissociation of bound water to capillary water

λ = a constant for a given material dependent on the concentration of dissolved salts.

α = a constant representing the Langmuir terms for a very large binding energy and a very low k value.

The value of k is temperature dependent and this in turn controls the various proportions of combined and capillary water, while ε will be characteristic of the system and its number of potential water immobilization sites.

Subsequent to this paper, however, there have been no further attempts to confirm Riedel's initial assumptions, or to apply his equation to other materials.

2.6 THERMODYNAMIC CONSIDERATIONS

As is the case with any interaction between two components, the changes occurring may be considered in terms of the thermodynamics of the process. The temperature dependence of the sorption process, which has been shown in many cases (for example see Saravacos and Stinchfield[113]), allows calculation of these thermodynamic functions.

The absorption of a gas by a solid is a spontaneous and therefore exothermic process, usually accompanied by a decrease in the free energy of the system, although there may be either a positive or negative entropy change. The heat of absorption or desorption gives an indication of the combination energy between the vapour and the surface of the absorbent, water being immobilized by different classes of polar groups with different heats of sorption. The presence of a heat of sorption above that of the heat of condensation of the vapour indicates some type of definite interaction with the surface and provides useful information about the state of the sorbed vapour in the material.

The classical method of deriving values for the heats of absorption or desorption is by application of the Clausius-Clapeyron equation to results from isotherm studies at different temperatures. Simple systems show the presence of a smaller quantity of adsorbed vapour, at a given relative humidity, as the temperature increases. There are several ways of expressing this equation and various modifications of it[240,289,298,305], but in calculations of total heat of absorption, or heat of association,either a direct or a graphical method can be

used, both of which assume that over a short temperature interval between T_1 and T_2 the heat of absorption is constant for any given moisture content.

Expressing the equation in the form

$$\frac{d \ \ln p}{d \ (\frac{1}{T})} = - \frac{q}{R} \qquad (14)$$

where p = relative vapour pressure at temperature $T^{o}k$ and a constant moisture content

q = heat of absorption at that moisture content, that is the isosteric heat of absorption, in ~~cal per g or~~ kcal per mole.

R = universal gas constant

and assuming then that over the small temperature range the term $\frac{q}{R}$ is constant, a plot of ln p versus $\frac{1}{T}$ should give a straight line of slope $-\frac{q}{R}$. The slope increases as moisture content decreases below a certain level. Values of isosteric heats of absorption at different moisture contents can then be plotted against moisture contents.

Alternatively,[305] the equation can be expressed as

$$-q = 4.574 \ \frac{T_1 \ T_2}{T_2 - T_1} \ \log \ (\frac{p1}{p_2}) \qquad (15)$$

where p_1 = vapour pressure at temperature T_1

p_2 = vapour pressure at temperature T_2

The factor of 4.574 is obtained as a result of the multiplication of the gas constant, R, by 2.303, ($\log_e$ 10).

The Clausius-Clapeyron equation and values substituted in it have various inherent errors and generally a more accurate value of heat of absorption can be obtained calorimetrically.[77]

As mentioned earlier, the free energy of a vapour-solid system decreases as sorption occurs. This free energy change, $\triangle F$, is the sum of the partial molal free energy changes of the constituents.

Where pure water is the vapour source:

$$\triangle \bar{F} = \frac{d\ F}{d\ n} = RT \ln x$$

where x = relative vapour pressure of water at temperature T.

n = number of moles of component involved.

R = universal gas constant

$\triangle F$ = partial molal free energy change

A reduction in the free energy of a condensed phase occurs when it is present in a capillary,[222] as well as when it is chemisorbed.

Differential entropy changes, $\triangle S$, during sorption are related to $\triangle F$ and to $\triangle H$ (the differential heat of absorption), by the following relationship:

$$\triangle \bar{S} = \frac{\triangle \bar{H} - \triangle \bar{F}}{T}$$

Numerous authors, for example Fish,[146] Bettelheim and Volman[182], Babbitt[160], and Bull[71], have calculated these

three thermodynamic functions and their dependence on changing moisture content during the sorption of water vapour by foodstuffs and their constituents. In all cases there is an observed fall in the heat of absorption present at very low moisture contents, to a value equal, or very close, to the heat of condensation of water, at higher moisture contents. Moisture contents at which this differential heat reaches zero for different materials are presented in tables3, 4 and 6.

Simultaneously with this reduction in heat of absorption, which indicates that the first of the absorbed water is associated more firmly with the material than the remainder, there is usually a fall in differential entropy values as well. An initial high negative entropy indicates greater order in the system after absorption, suggesting a loss of rotational freedom of the sorbate molecules.[40,71,85,146,205,306], or increasing crystallinity during the first stages of sorption.[182] In a few systems, however, a positive net entropy change has been observed and explained in terms of disordering configurational changes occurring in the material as a result of absorption, possibly exposing more sites which could interact with water.[71,79,170]. Observed minima in differential heat of absorption and entropy curves have been related in some cases to the completion of the monomolecular layer.[182]

Thus thermodynamic data yield valuable information about the state of water in foodstuffs and provide a useful means of evaluating results obtained from isotherm equations. Values derived from such thermodynamic treatments can help in interpretation of results obtained by various directly analytical methods such as NMR, DTA, dielectric constants etc.[71,182,286]

3.0 METHODS OF STUDYING WATER RELATIONS

In the last thirty years many advances and diversifications have been made in methods used in the study of water relations. Sophisticated analytical techniques now allow approaches to be made from several different angles, although some of the older methods are still widely used and yield valuable information.

All methods have in common the fact that the results they give must ultimately be related to known moisture contents of the samples examined. The accurate and reproducible determination of moisture content is therefore a most important factor, about which there has been considerable discussion as reviewed, for example, by Stitt[205] and Rockland[267]. A detailed comparison of methods will not be made here, however.

In addition, temperature is also an important factor which should be stated when any result is presented.

3.1 SORPTION STUDIES

An important consideration in isotherm preparation is the time necessary to achieve an equilibrium water distribution throughout the sample. Equilibrium is particularly slow at high relative humidities and in the absence of a vacuum.

There is some difference of opinion concerning the length of time which should be allowed for this, but it seems correct to say that the selection of an equilibrium period must be arbitrary. It has been shown[40] that attainment of

equilibrium is exponential and indeed that if a very accurate means of following weight on vapour pressure changes is used, it is likely that constancy may never be achieved. A point must therefore be chosen after which the great extent of the change has occurred. Removal of air from a system will greatly shorten equilibrium times.

Statement of temperature used and whether a desorption or absorption process was utilized will allow more accurate evaluation of results. In order to detect any discontinuity in the sorption process as a result of, for instance, the crystallization of sugars from the amorphous state, a stepwise adsorption procedure would have to be used.[203,250]

In spite of more recent advances tending to remove distinctions between methods of isotherm preparation, clarity is still achieved by division into a) those methods in which relative humidity is basically the varied factor, and b) those in which moisture content is varied.

3.1.1. Variation of Relative Humidity

The earliest and subsequently the most widely used method for isotherm preparation (for example see Gane, 1950[251] Makower and Dehority, 1943[250] Wink, 1946[310]) incorporated equilibration of samples over salt solutions or sulphuric acid solutions giving different equilibrium relative humidities, followed by moisture content determinations. This method's simplicity is much in its favour, although it has been criticized for the length of time needed for equilibration, and for reported lack of sensitivity at low relative humidities.

There have been several extensive tables published of the relative humidities over different saturated salt solutions[310-316] and over sulphuric acid solutions of different density.[317,318] Temperature will have an important influence on the relative vapour pressure of a salt solution and care should be taken to ensure that equilibration at all relative humidities takes place at the same fixed temperature, both to avoid changes in vapour pressure during equilibration and to allow comparable results to be obtained for the different samples of the same material. Obviously equilibration must be carried out in airtight vessels, but the time necessary can be much reduced if these are evacuated to allow faster diffusion of water molecules within the enclosed atmosphere. Reduction of particle size and initial freeze-drying of the sample both shorten equilibration time, which, however, may still be of the order of weeks or even months, involving problems of microbiological spoilage at the higher relative humidities.

In an effort to eliminate the need for such extended periods of equilibration, Landrock and Procter[318] developed a graphical interpolation method of obtaining humidity equilibration data based on the principle that at any given initial moisture content and under any given atmospheric relative humidity, the rate of gain or loss of moisture content of a material is a factor of the difference between the relative humidity of the product and that of the atmosphere. Using a series of identical samples, the weight loss or gain of a sample is determined after exposure for one hour at a given relative humidity; a range of relative humidities

being used to examine different samples. Plotting this weight change per unit time against relative humidity, the equilibrium relative humidity equivalent to the initial moisture content of the sample is found at the poiht of intersection at the baseline representing no weight change (Fig. 14). Determining the equilibrium relative humidity for a range of moisture contents an isotherm can then be constructed, the authors claiming that the whole procedure could be completed within two days.

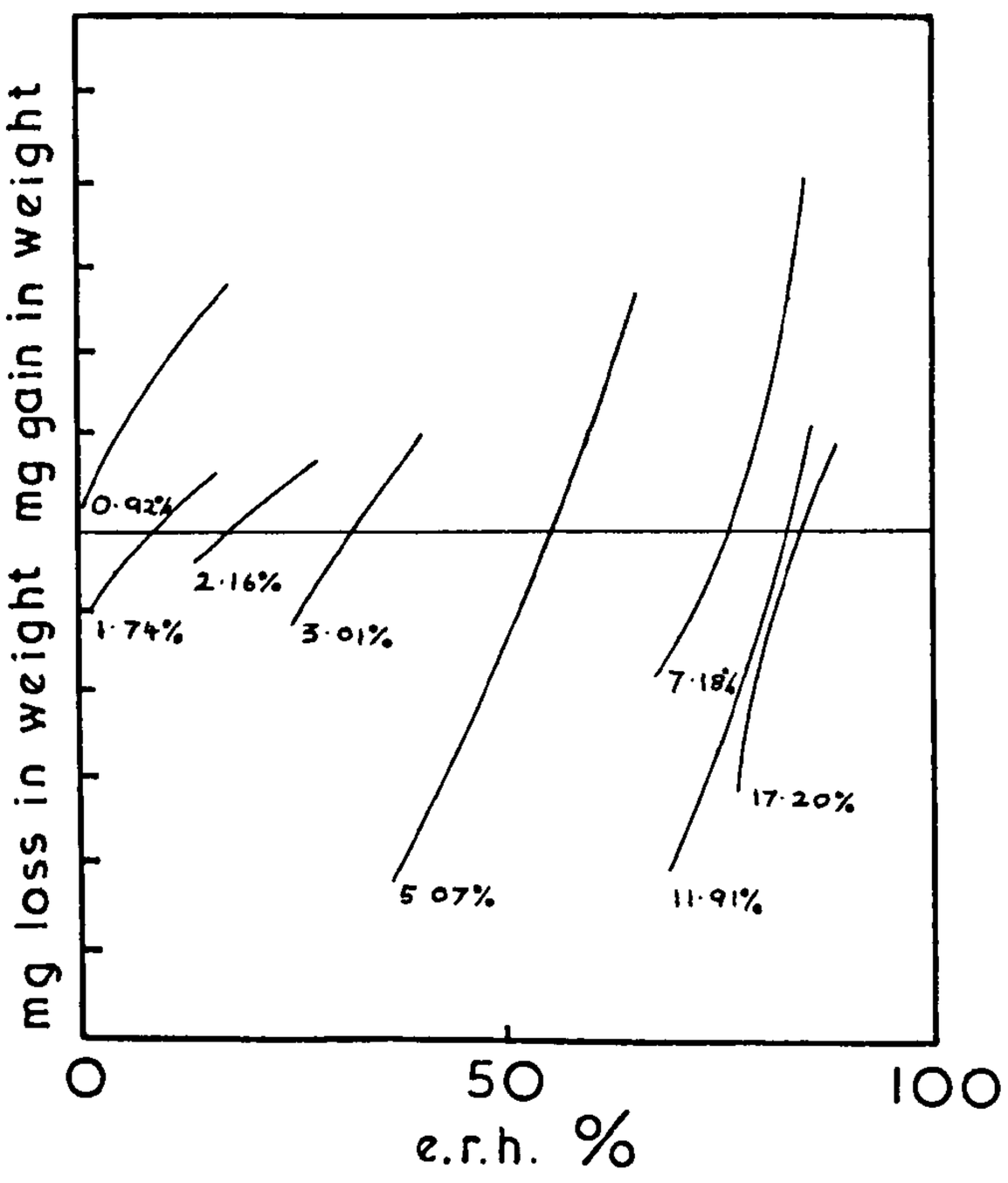

Fig. 14. Graphical interpolation isotherms for a dried chocolate syrup product. Initial moisture contents shown at bottom of each curve. From Landrock and Procter, 1951.[281]

Loncin, Bimbernet and Longes[176] have developed a method of isotherm preparation, which only requires the use of one saturated salt solution, (NaCl). Exact values of atmospheric water activity below the maximum of 0.75 are obtained by temperature adjustment of the thermostated water baths surrounding the salt solution jar and sample container, the air being pre-saturated by initially bubbling through water. Upon attainment of equilibrium; one hour at 60°C to 80°C and three hours at 30°c for a 200 mg sample; the sample moisture content is determined thus allowing isotherm construction. They claim that their method has the advantage of eliminating condensation problems, which have occurred when attempts have been made to use water at different temperatures to provide variable vapour pressures.[74]

The effect of applied pressure on water vapour pressure is another extension of the relative humidity variation method, which Gur-Arieh, Nelson, Steinberg and Weir[319] developed after having presented a more conventional rapid method for isotherm preparation based on the use of sulphuric acid solutions.[320]

The use of water or ice at different controlled temperatures has also been incorporated into an extension of the static weighing methods described above. Saravacos[249] and later Saravacos and Stinchfield[113] used water and ice as their humidity control media for a method incorporating a quartz spring balance as developed by McBain and Bakr.[321] This involves a sensitive calibrated helical spring made of silica suspended in an all glass thermostated jacket which can be evacuated and at the bottom of which is a thin glass

bulb containing a suitable salt solution. After spring calibration and evacuation, the bulb is broken by a magnetically controlled iron bar enclosed in glass. Equilibrium is then allowed, and spring extension measured. Adjustment of the temperature allows a series of determinations to be made at different vapour pressures with the same sample, over either absorbtion or desorption cycles. Frequent spring breakages tend to slow the method down, although more robust materials have been tried.[40] In the case of cotton, most of the weight change occurred in the first eighteen minutes and an isotherm prepared by plotting readings obtained at 45 minute equilibration intervals was found to be in relatively good agreement with one using 72 hour intervals.

This spring balance method has also been used by several other investigators working with biological materials,[173,207] although Cardew and Eley[77] discarded it because they claimed it was impossible to reach equilibrium within one week, and used equilibration over salt solutions in desiccators instead. In direct opposition to this, Karel and Nickerson[207] rejected the salt solution - weighing bottle method as being too slow and used a McBain-Bakr sorption balance incorporating provision for manometric measurement as an alternative!

Modern electronic continuous recording balances allow greater accuracy to be attained using the same principle of monitoring weight changes while equilibration is reached at a constant relative humidity, and allowing only one sample to be used for a complete isotherm.[203,322]

Both these methods have the disadvantage of the necessity of using small sample sizes to achieve high sensitivity, a consideration important when studying heterogenous materials. Gregg[323] has asserted that he has overcome this by the development of his electrical sorption balance in which weight changes are electromagnetically counterbalanced. He claims an accuracy in weighing of 0.0003g irrespective of sample size, which was usually between 10g to 20g.

3.1.2 Variation of Moisture Content

In this approach the equilibrium vapour pressure is measured over samples of known, but different, moisture content. High vacuum manometric systems were used to determine vapour pressure by Urquart and Williams[324] in 1926 and later by Frey and More[83] in 1948. Makower and Meyers[325] also developed a manometric apparatus, which has been improved by Taylor[252] to allow a series of desorption determinations to be made using the same sample. After an initial reading of a sample of a high, known, moisture content, a subsequently weighed amount of water is removed by freeze drying, and after equilibration at this new moisture content another vapour pressure determination is made. Thus a stepwise removal of water is effected, although Taylor does not mention what equilibration time is needed after water removal and before the next reading can be taken. This could clearly be monitored by observing

changes in vapour pressure, however. Taylor also obtained increased sensitivity by the incorporation of a low vapour pressure oil, Apiezon B, in the manometer to replace mercury. A deflection of 1mm of mercury is equivalent to a deflection of 15.7mm of Apiezon B. Taylor claims that it is possible to dry a sample to 0.5% moisture content by this method, intermediate moisture contents being calculable from the initial value.

Fish[146] also used a similar type of manometer system, his "tensimeter", which allowed vapour pressure determinations, at each of a range of moisture contents to be made on a single sample.

As an alternative to methods involving a determination of vapour pressure, others involving a direct reading of equilibrium relative humidity above a sample of known moisture content have also been utilized. The early hair hygrometers were discarded because of their inaccuracy, the first reliable apparatus for use with foodstuffs having been reported by Mossel and van Kuijk[326] as an adaptation of the lithium chloride cell developed by Brastad and Borchardt.[327] Rockland[267] has used a direct reading electric hygrometer and Ayerst[328] developed a dew point determination method for use with foodstuffs, although recently more sophisticated direct reading humidity meters have increased the potential of this approach still further.

Isotherm data has also been obtained from the use of electrical conductivity measurements at different moisture contents.[133,146,309] Relationships between these two factors have been described by Ward[61] and by Eley and

Leslie[309], maximum conductivity being obtained at moisture contents corresponding to four BET monomolecular layers and above. The rise in resistance with falling temperature has also been reported by Duckworth and Smith[120], a value similar to that obtained from a sample at ambient temperature and with a moisture content corresponding to the monolayer value being given by a fully hydrated material between -50°C and-60°C.

3.2 OTHER ANALYTICAL STUDIES AT AMBIENT TEMPERATURES

Nuclear Magnetic Resonance:

The use of NMR in the study of water relations relies upon the characteristic magnetic properties of mobile hydrogen nuclei (protons). When placed in a strong magnetic field these protons will absorb energy in the presence of a radio frequency field applied perpendicularly to the magnetic field, over a characteristic narrow frequency band. This absorbtion of energy causes the protons to enter a high energy, metastable state, antiparallel to the direction of the magnetic field. Measurement of the energy absorbtion, which is at a maximum at the point of this resonance, is utilized by the NMR technique.

General aspects of the application of NMR to foodstuffs has been given in a report from the British Scientific Instrument Research Association[329] but more specific use may also be made of the technique to provide information concerning the state of water in foodstuffs, although results obtained must be qualified by the resolution

used.

Using high resolution instruments, observation of changes in signal line width with varying moisture contents allows conclusions to be drawn about the rotational freedom of the water present at low moisture contents. Its use has been reported with a variety of biological materials.[96,97,106,133,162,175].

The use of low resolution instruments for routine moisture content analysis has shown in some cases[131,278,330] that the moisture content - signal size calibration curve did not pass through the origin,or that a distinct convex curve was obtained at low moisture contents, suggesting a reduced or absent energy absorbtion by the hydrogen protons of this water fraction.

Measurement of Dielectric Constants:

Dielectric constants of materials in effect give an indication of the force needed to orient the material between two opposite, electrically induced poles in a capacitor, compared to the force exerted in a vacuum, with no material present. Materials with a permanent dipole moment have a greater dielectric constant than those without, and such measurements are indicative of the degree of freedom of molecules to rotate. Measurement of the dielectric constants of hydrated biological materials have been used by several authors to study the degree of freedom of water in the systems. This has been done both at ambient temperatures[101,109,110,331,332], examining either the relaxation regions of the macromolecular material or the water molecules themselves, and at sub-zero temperatures as

a method of determining the proportion of ice present in a system.[333] The dielectric constant of water falls from about 80 to less than 4 on freezing.

The lack of any empirical or theoretical standard for converting dielectric measurements into water contents, and therefore the need for calibration against some other method of measurement, introduces a limiting factor into the method.[56]

Infra Red Spectroscopy:

There are no reports of the use of infra-red spectroscopy for the examination of water in foodstuffs although other biological materials have been examined in this way.[97,111,112,140]

X-ray Spectroscopy:

X-ray analysis of structured biological material has been conducted simultaneously with sorption studies in order to examine configurational changes occurring during sorption[147,179-183] and the role of water in the structure of biopolymers.[72,98,104]

Diffusion Studies:

Duckworth[269], and Duckworth and Smith[41,120] have studied the diffusion of simple solutes through biological materials at different moisture contents. As was expected no diffusion was found at levels below the calculated monolayer value although it was observed, albeit slowly, at moisture levels only one or two percent above the monolayer, thus suggesting the presence of free solvent water. Radioactive tracer techniques using ^{14}C labelled

glucose were used to follow the movement of the solutes; autoradiography or counting methods being used to record the extent and nature of the diffusion after different time intervals. Tracer techniques have also been used to show that during drying of pieces of vegetable material there is an overall centripetal movement of the solutes.[269,336] Low temperature diffusion studies have also been conducted.[120]

Change in Colligative Properties:

Methods involving examination of the amount of combined water as defined by determining the amount of solvent water present were once frequently used, but have not found recent application. Such methods depended upon analysis of the difference between the observed and theoretical freezing point depression[44,46,337], vapour pressure reduction[45,338] or change in refractometric properties[339] following the addition of a known quantity of a low molecular weight solute, usually sucrose, to a biological system. The presence of non-solvent water increased the solutes concentration in the remaining water with a resultant greater change in colligative properties.

Chemical Methods:

In 1936, Hatschek[340] developed a method for determining the amount of solvent water present. This was dependent upon the colour change between the anhydrous and hydrated form of cobaltus chloride. A material containing the chemical was dried slowly and its moisture content was determined at the point where a change from pink to blue

was observed, the water remaining at this stage being unavailable to act as a solvent. Copper sulphate can also be used in a similar way.[57] Only one reported use of this method has appeared recently.[341]

3.3. FREEZING STUDIES

Initially, dilatometric methods were widely used to examine the amount of water immobilized against freezing, but the use of calorimetry superseded this method, later to be supported by the more recent application of DTA ahd low temperature NMR studies.

Dilatometric Investigations:

The dilatometer takes advantage of the approximately 10% volume difference between water and ice. The total volume change produced when a frozen sample, immersed in a non-freezing fluid (for example paraffin), is allowed to thaw, is related to the known moisture content of the sample and the calculated theoretical volume change which would be obtained if all the water present froze and subsequently thawed. Any temperature dependent volume changes in the dilatometer fluid, the solids of the sample and the apparatus must be taken into account, and inaccuracies can arise due to the presence of dissolved gases in the material, which may be displaced upon freezing.[56,58] Foote and Saxton[48,49], Jones and Gortner[51] and Heiss[54] all used this method, certain aspects of which were criticized by Fisher.[47]

A more recent method of analysing the amount of ice present in a system, which depends on the specific gravity changes occurring as a result of the transformation of water to ice has been developed by Scholander et al[342] and utilized by Love and Elerian[127] in a study of the freezing of cod muscle. This is a flotation technique, which involves matching the specific gravity of the frozen material with that of a mixture of bromobenzene and kerosene, whose relative proportions are altered until the material just floats off the bottom of the container or sinks from the top. The specific gravity of the fluid mixture can be determined, and this must necessarily be the same as that of the material at the point where it just floats or sinks. Knowing the moisture content of the material and the specific gravity of ice and water, the proportions of these two forms in the material may be found. Considerable precautions must be taken to remove all the air and dissolved gases from the materials prior to freezing[127,342]. While the method itself would appear to be perfectly viable, contradictory results have been observed by different authors.[127,343]

Calorimetric Investigations:

As a method for the quantitative determination of unfrozen water, calorimetry was first used by Theones[53] in 1925. The total amount of thermal energy involved in the ice-water phase change in a sample is measured upon thawing, and differences between the observed and a theoretical value, calculated from the known water content of the sample, can be used to determine the quantity of unfrozen water.

In early calorimeters the frozen sample, at a known temperature, was placed in an insulated vessel containing warm water of known heat capacity and temperature. The specimen was allowed to thaw and, after equilibration, the change in bath temperature determined, thus allowing calculation of the amount of ice present in the sample, knowing its specific heat in the dry state. This method, and modifications of it, have been used by several workers to examine food materials.[270,343]

More sophisticated devices now allow continuous monitoring of energy changes with temperature variation to be made. After attainment of initial equilibrium, samples are electrically heated in an insulated vessel at a known, constant rate, and in this way Riedel[59] has conducted calorimetric examinations down to -180°C. Several other workers have also used calorimetry to study the freezing pattern of foods[118,335], although there are several potential sources of error.[335]

Differential Thermal Analysis:

DTA is an extension of the calorimetric approach and is used to study the thermal behaviour of materials as they undergo physical and chemical changes during heating or cooling. Although the principle was put to use in the nineteenth century the technique has only relatively recently been applied to the study of biological materials, its application previously being limited to observation of inorganic systems.

In DTA, the sample and an inert reference material are

heated or cooled at the same rate, and the difference in temperature between them is recorded. The differential temperature remains zero, or constant, until a thermal reaction occurs in the sample, such as the freezing or thawing of ice, when the differential temperature increases until the transitiion is completed, whereupon it decreases again. Thus a peak is obtained on the curve for differential temperature against temperature or time, and the direction of the peak indicates whether the transition is endothermic or exothermic.

There are many difficulties in the truly quantitative application of DTA, but a better founded basis for quantitative studies is provided by the alternative technique of differential scanning calorimetry (DSC). In this method the temperature of sample and reference are maintained at an equal level, or fixed differential, throughout the analysis and the variation in heatflow to the sample, required to maintain this level during a transition, is measured.

Ladbrooke and Chapman[117] have recently reviewed the applications of DTA and DSC in the study of water in proteins, and a recent report of the use of DSC in the study of water in wheat doughs has also been reported.[271] Several investigators have used DTA in an examination of the unfreezeable water in a variety of foodstuffs and model systems.[108,341,343,345,346]

Low Temperature Nuclear Magnetic Resonance:

The changes occurring in the mobility of water over the

main freezing zone and below can be followed by high-resolution NMR[107,130] by investigation of changing peak width. Low-resolution NMR studies on foodstuffs[131,108] have shown a gradual reduction in signal size due to the water remaining unfrozen below the main freezing zone. At this resolution, however, it is not possible to definitely attribute this reduction to either a continuing freezing out of water or to an increasing degree of immobility of the unfrozen water with reducing temperature.

PART 2

E X P E R I M E N T A L

4.0 MATERIALS

4.1 INDIVIDUAL PLANT CONSTITUENTS

Commercial preparations of Potato Starch, Apple Pectin, 250 grade (British Drug Houses Ltd., Poole, Dorset) and Cotton Cellulose, in the form of a high purity, coarse, fibrous powder with a high degree of crystallinity (Whatman Column Chromedia, CF 1) were used.

The apple pectin and cotton cellulose were used directly.

The potato starch was used in a dried form after gelatinization. A suspension of starch in three times its weight of water was slowly heated, with continuous stirring, until gelatinization occurred. Subsequent heating in an electric oven at 90°C ensured completion of this process, this being indicated by the attainment of a semi-transparent appearance. After division of the gel into small, evenly sized cubes, it was dried in a forced-draught, hot air oven at 70°C-75°C to an approximate moisture content of 10g/100g dry matter. It was finally ground (Glen Creston C.580 Micro Hammer Mill) to pass an 0.7mm mesh diameter screen and placed in an air-tight storage container to allow equilibration of moisture throughout the material.

An identical drying, grinding and storage procedure was used in the preparation of all materials subsequently described, except where an alternative method is mentioned.

4.2 SIMPLE, MULTI-COMPONENT, PLANT SYSTEM.

Citrus albedo was examined in order to study the effect of two of the individual plant constituents under examination on the water properties of an intact system in which they are the major constituents.

Orange Albedo was prepared from large, Valencia type oranges, all from the same Spanish shipment. The peel was removed in quarters from the fruit and the albedo was further separated from the flavedo by dissection. The albedo was dried to an approximate moisture content of 9g/100g dry matter, then ground and stored.

Orange Pectin was prepared from fresh orange albedo. The albedo (30g quantities) was blended (M.S.E. Ato-Mix Blender) with 500 ml. of distilled water to a smooth, cream-like, consistency. Cold-water-soluble pectins were assumed to have passed into solution as a result of this homogenization. Following the method described by Williams,[347] the remaining pectic substances were solubilized by three successive one hour treatments at 90°C and pH 6.0, with the tetra-substituted sodium salt of ethylenediaminetetra-acetic acid (EDTA) (Sequestrene N A 4 powder, Giegy (U.K.) Ltd., Manchester) added to give a 2% (w/w) concentration. pH adjustment was made after the addition of EDTA. The temperature was maintained by the use of a constant temperature water bath and the material was stirred mechanically during the extraction period.

Following each EDTA treatment, the aqueous fraction was removed by filtration through a Buchner funnel, and the residue washed twice with 500 ml. aliquots of hot distilled water.

The extracts and washings were collected and the pectin removed from them by precipitation with two volumes of 70% ethanol and, having allowed the solution to stand for 24 hours, by subsequent filtration.

The completeness of extraction was checked by a fourth, small scale, EDTA treatment similar to the previous three. The absence of flocculation upon the addition of 70% ethanol to the filtrate was taken to signify the removal of all pectic substances in the previous extractions.

The semi-dry pectic material remaining after precipitation and filtration was allowed to dry further at room temperature before a short final drying in a hot air oven at 44°C to a moisture content of approximately 12g/100g dry matter. The material was then ground and stored.

Orange Cellulose was taken as that fraction remaining after the EDTA extraction of fresh orange albedo, as described in the method for the preparation of orange pectin. The material was dried, ground and stored following the method described for orange pectin.

This fraction, which composed approximately 37% (dry weight basis) of the whole albedo, as determined by weight loss on extraction, is unlikely to be pure cellulose. It will also contain hemicellulose, small quantities of water in-soluble nitrogenous materials, and uronide material. No detailed analysis was attempted.

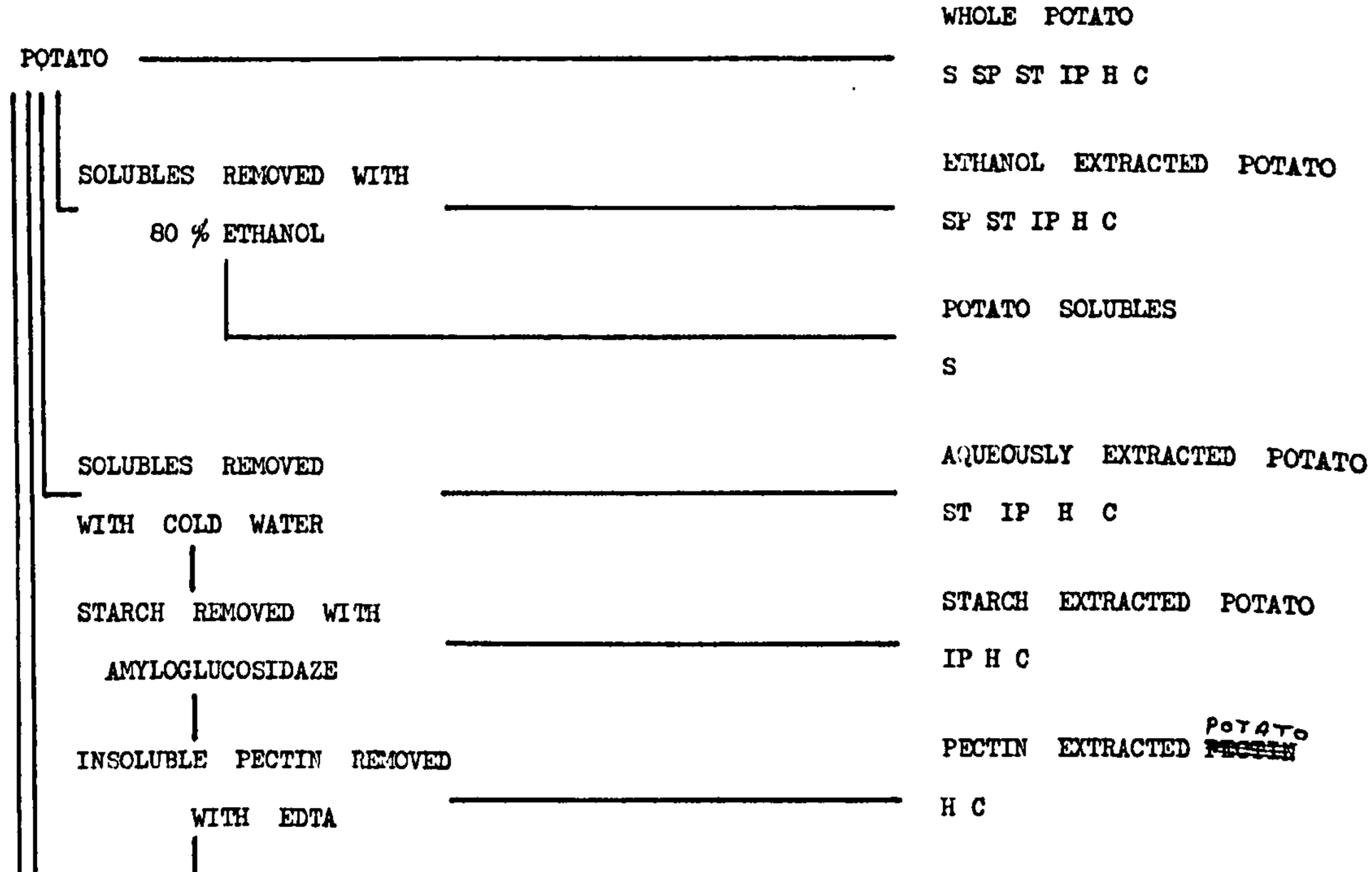

Fig. 15. Diagram of extraction steps undertaken in the examination of potato.

4.3 COMPLEX, MULTI-COMPONENT, PLANT SYSTEM

In this study the potato was taken as an example of a foodstuff of botanical origin and was used to examine the influence of the major constituents, and the interaction between these, on the hydration characteristics of this type of material.

Two varieties, Pentland Dell and Red Craigs Royal were examined. These were large size, grade A, seed potatoes obtained from local sources. Their previous history was unknown. After receipt they were stored at 4°C until used. This temperature was chosen as a compromise between the higher optimum temperature needed to minimise the accumulation of sugars at the expense of starch, and the lower optimum temperature needed to delay the onset of sprouting and the occurrence of microbial rots. Treatment with a sprout supressant ("Fusarex", Plant Protection, Yalding, Kent) at the rate of $\frac{1}{2}$lb/lcwt and storage at 10°C was rejected because of the continuing appearance of sprouts after two to three weeks.

Whole potato was prepared for subsequent study as follows. The tuber was peeled and, using a sharp knife to minimise cell breakage and resultant loss of granular starch, was cut into cubes of approximately 1 x 1 x 0.2 cm. in dimension.

The cubes were immediately blanched for a one minute actual boiling time in an 0.5% (w/v) solution of sodium sulphite, drained, and dried in a forced draught, hot air oven at 70°C-75°C to a moisture content of approximately 10g/100g dry matter. The material was then ground and stored.

Fig. 15 illustrates the range of fractions obtained from whole potato and the basic procedures used in their preparation.

Only the major constituents were isolated from Red Craigs Royal, but both these, and the fractions obtained from the stepwise degradation illustrated were prepared from Pentland Dell.

Water-Extracted Pentland Dell was prepared by subjecting blanched cubes of whole potato to successive extractions in cold distilled water. A potato to water ratio of 1:3 was used, with a total extraction time of 96 hours. This involved four extractions, each of 24 hours, the material being washed twice with cold distilled water between extractions.

The completeness of extraction in terms of removal of reducing sugars was confirmed by the absence of a red precipitate of cuprous oxide.when a small aliquot of the water used in the final extraction was heated with an equal volume of Fehling's solution (Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th Ed., 1965, p.494).

It was assumed that complete removal of reducing sugars would also indicate the complete removal of other water soluble materials.

The extracted potato was finally dried, ground and stored.

Ethanol-Extracted Pentland Dell was prepared by subjecting blanched cubes of whole potato to successive extractions in boiling 80% ethanol. Initially, the material was refluxed for 30 minutes after the addition of 95% ethanol in the ratio of 426.5ml per 100g potato. This took into account the initial dilution effect of the water present in the potato, assuming a moisture content of 80% (wwb), and resulted in a potato:ethanol ratio of approximately 1:5. Following this extraction, the material was washed twice with fresh aliquots of 80% ethanol. Three, 60

minute extractions using a 1:3, potato:80% ethanol ratio were then carried out under reflux, the material being washed twice between each extraction.

The extracted potato was finally dried, ground and stored.

A small quantity of Ethanol Soluble Material extracted from Pentland Dell was recovered from the bulked extracts and washings of one batch of potato. The extracted potato and recovered extracted material were kept separate from the other fractions for subsequent study.

The ethanol was removed from the bulked extracts and washings by distillation. The predominantly aqueous fraction which remained was then freeze dried (Edwards High Vacuum Shelf Freeze Drying Plant, Model 30 P 1 T).

Starch-Extracted Pentland Dell was prepared by the enzymic removal of starch from water-extracted potato. Water-Extracted Pentland Dell (1 kg), taken after the completion of the fourth aqueous extraction, was added to a 500 ml quantity of distilled water. In order to avoid microbiological spoilage the material was bought to the boil and maintained at 100°C for one minute. It was then rapidly cooled in a cold water bath. The potato was either treated in the cube form or, following the heat treatment, was reduced to a pulp in an Ato-Mix Blender. Following adjustment of the mixture to pH 4.5, 5g of Amyloglucosidase (Grade II, prepared from a species of Rhizopus and obtained from Sigma, London) was added and thoroughly distributed by a mechanical stirrer. The material was then left at 45°C, maintained by use of a constant temperature water bath, until no characteristic

blue-black colour development, indicative of the presence of starch, was observed upon the addition of a solution of iodine in potassium iodide. The material was then repeatedly washed in a Buchner funnel with hot distilled water until the filtrate gave a negative Fehling's test. At this point it was assumed that all the starch breakdown products had been removed from the potato.

The material was then dried, ground and stored.

Pectin-Extracted Pentland Dell was prepared by treatment of starch-extracted material with a sequestering agent. If not already in a pulp form, the potato (1 kg) was blended (Ato-Mix Blender) to a smooth cream in 500 ml of distilled water following the final washing stage described in the previous section.

The extraction of insoluble pectic substances was effected by the method described for the removal of orange pectin, and the same test for completeness of extraction was conducted. The material remaining after the final EDTA treatment and subsequent washings was dried, ground and stored following the method described for orange cellulose.

No attempt was made to recover the pectic substances removed by the extraction procedure.

Pure fractions of starch, pectin and cellulose were prepared from both Pentland Dell and Red Craigs Royal by the methods described below.

Potato Starch was obtained by the displacement of granular starch from the ruptured cells of the tuber. Thin slices of tissue were removed from the peeled tuber with a hand potato peeler, and allowed to fall into cold distilled water. A handful of slices were repeatedly squeezed under water to displace the

starch. The remaining cellular material was then removed. Although the method does not result in a complete removal of all the starch present, a high yield was obtained by the use of very thin slices. Inevitably, a small amount of cell wall material was also present after squeezing. By allowing repeated sedimentation of the starch suspension the slower-sedimenting cell-wall material was removed with forceps.

The pure granular starch was then gelatinized, dried, ground and stored following the method previously described for commercially obtained potato starch.

Potato Pectin was obtained following the method described for the preparation of orange pectin. Preceding the first treatment with EDTA, the potato was peeled, diced and blanched following the method described for the preparation of whole potato. The potato (500g quantities) was then reduced to a pulp in distilled water (500 ml) in an Ato-Mix Blender, and the extraction continued as previously described.

Potato Cellulose was prepared by treatment with hot sodium hydroxide solution of material from which the solubles, starch and pectic substances had been previously removed. Blanched potato, prepared following the method described for whole potato, was reduced to a pulp with an equal weight of distilled water in an Ato-Mix Blender. Starch removal was effected following the method described for the preparation of Starch-Extracted Pentland Dell. It was assumed that soluble constituents would also be removed during this extraction stage and the subsequent washing treatments. Insoluble pectic substances were removed following the method described for the preparation of Pectin-Extracted Pentland Dell. The semi-dry material remaining after the final EDTA treatment

and subsequent washings was suspended in ten times its weight of a 1% (w/v) sodium hydroxide solution. The suspension was maintained, while being mechanically stirred, at 60°C for 48 hours in a constant temperature water bath. The remaining solid material was then thoroughly washed with hot distilled water in a Buchner funnel.

This fraction was called 'Potato Cellulose', although it will also contain the more resistant hemicelluloses present in potato in addition to the cellulose itself.

The material was finally dried, ground and stored following the method previously described for orange cellulose.

4.4 INVESTIGATION INTO THE EFFECT OF SOLUBLE ADDITIVES

Caesium Iodide, Sodium Chloride and Tetra-Hydro-Furan (British Drug Houses Ltd., Poole, Dorset) were used in a study of the effect of soluble materials on the water relations of model systems derived from plant materials.

NaCl, CsI, and tetra-hydro-furan were separately added to either potato starch or cotton cellulose to achieve a concentration of 100 moles of additive per 10^5g of plant constituent.

In the case of starch, 30g, in the granular form, was suspended in 90g of distilled water containing the correct weight of the additive under investigation. Gelatinization and subsequent drying, grinding and storage was carried out as described for pure potato starch in Section 4.1

A similar suspension was made in the case of cellulose, and, following this, the mixture was gently agitated for 24 hours. The material was then freeze dried, powdered and stored.

The dried ethanol soluble material recovered from the ethanol extraction of Pentland Dell was also added in a similar way to potato starch and cotton cellulose to give a final concentration of 15% on a dry weight basis.

5.0 METHODS

5.1 DETERMINATION OF MOISTURE CONTENT

Throughout this study the following standard procedure for the determination of moisture was used.

Duplicate samples of approximately 2g in weight were accurately weighed into aluminium moisture dishes of known weight. The open dishes were heated in a vacuum oven at 70°C for exactly 24 hours under a constant high vacuum. Air was then gradually admitted to the oven over a period of three to five minutes.

The dishes were then closed, removed to a desiccator containing phosphorous pentoxide, and allowed to cool. After reweighing the moisture content was calculated from the loss in weight.

All moisture contents quoted in this study are expressed on a dry weight basis as g water per 100g dry solids.

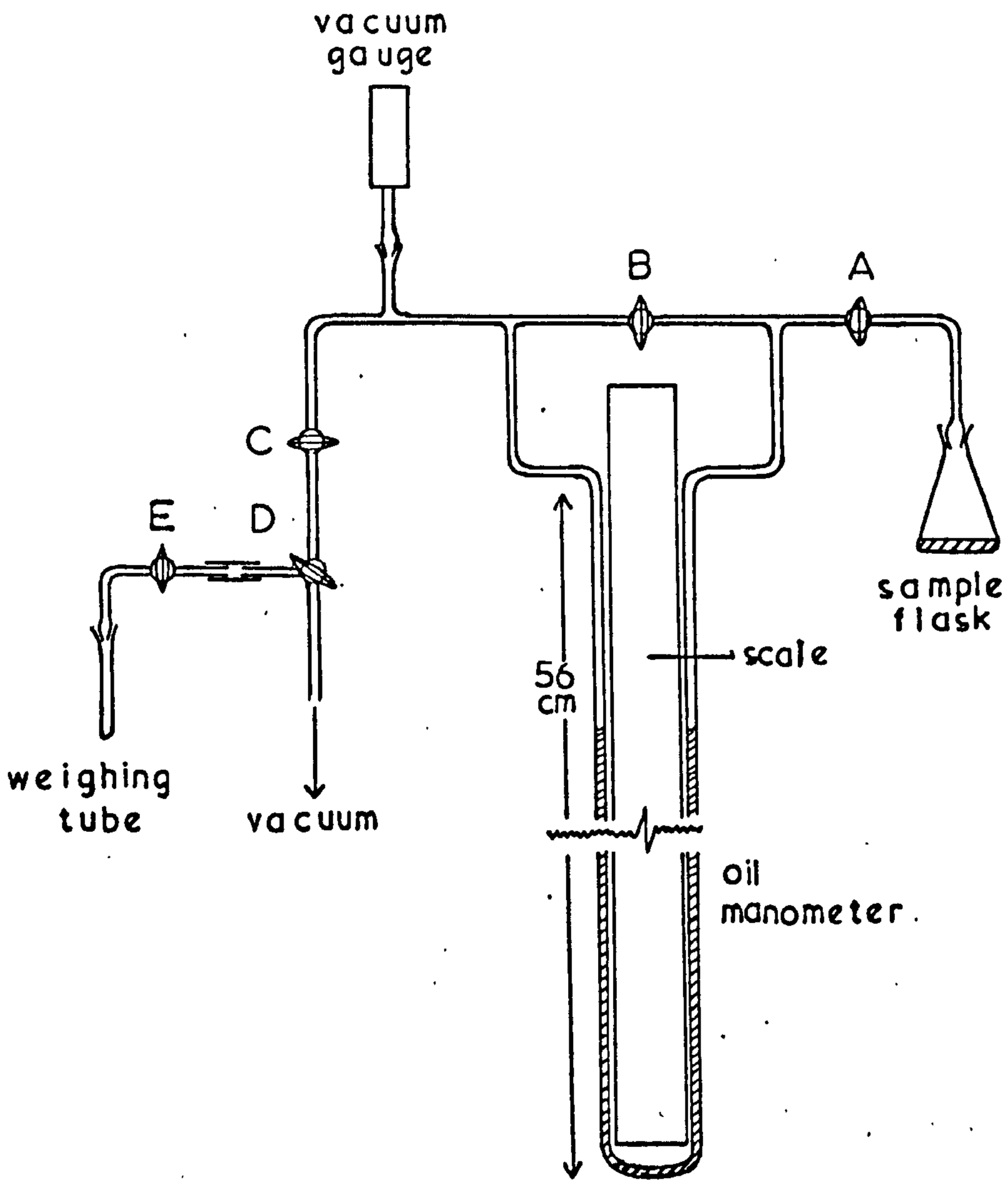

Fig. 16. Diagram of manometric apparatus used to determine desorption isotherms of plant materials.

5.2 SORPTION STUDIES

Two different methods for the preparation of sorption isotherms have been used in this investigation. The majority of isotherms were obtained by measurement of the vapour pressure above a sample using a manometric technique similar to that described by Taylor.[252] From these measurements, values for the relative humidity, above a sample at a given moisture content could be calculated.

In addition, isotherms at different temperatures were obtained for a limited range of materials using a method involving equilibration of samples over saturated salt solutions or sulphuric acid solutions of different densities.

Manometric Method

The apparatus as finally developed is shown in Fig. 16.

A low vapour pressure oil, Apiezon B (Edwards High Vacuum, Crawley, Sussex), was used as the manometric fluid (a pressure of 1mm mercury is equivalent to a pressure of 15.7mm Apiezon B). High vacuum stopcocks were used in all cases and 1.5 mm. bore glass tubing used throughout, except for that part of the apparatus below tap C. A direct reading Pirani type vacuum gauge (Edwards High Vacuum "Speedivac" Gauge, Model B5 used in conjunction with an Edwards High Vacuum Pirani Gauge Head, Model G 6 A), was used.

Following initial degasing of the manometric fluid whenever this was changed, the procedure described below was used to obtain vapour pressure data at progressively lower moisture contents on the same sample.

Initial humidification of the sample was achieved by thinly spreading approximately 7g of material in a petri dish and allowing it to attain a moisture content in the range of 40g/100g to 60g/100g in a desiccator over water during a period of three to four days. On removal from the desiccator the material was thoroughly mixed by shaking in the closed petri dish. Approximately 2.0g of the material was accurately weighed into a 25 ml stoppered conical sample flask of known weight. The flask was then placed in an acetone-solid carbon dioxide (Drikold) freezing mixture until attachment to the manometer was made.

Duplicate 2g samples were taken for moisture content determination at this stage.

Following attachment of the sample flask, which was maintained in the freezing mixture to avoid removal of water during the subsequent evacuation of the system, taps A, B, C and D were opened and a steady vacuum of approximately 10^{-2}mm mercury was obtained. Tap D, a three way tap, was set in a position which did not allow connection between the weighing tube and the rest of the system. Tap B was then closed and the freezing mixture removed from around the sample flask. When the sample thawed, the vapour pressure exerted by the water in the sample caused a deflection of the oil levels in the manometer. As soon as no further change in the oil levels was observed, usually after one to three hours, the magnitude of the deflection was measured to the nearest 0.5mm. Any part of the deflection due to air trapped in the sample during the initial freezing was recorded by refreezing the sample and determining any residual deflection.

Water was then removed from the sample in stages using the principle of freeze drying.

Following the determination of the residual deflection as previously described, and with the sample maintained in the frozen state, tap A was closed and tap B simultaneously opened. The weighing tube, with tap E closed, was removed from the apparatus. The tube was weighed, reconnected to the apparatus and a flask containing an acetone-solid carbon dioxide freezing mixture placed around it. Having allowed five minutes for temperature equilibration, tap E was opened and tap D set so that the weighing tube was in contact with the rest of the apparatus. As soon as a constant vacuum had been re-established, the apparatus was isolated from the vacuum source by re-setting tap D; tap A was opened and the freezing mixture removed from the sample flask. Water was then able to distil from the sample to the weighing flask. After a satisfactory amount of water had been removed from the sample, tap A was closed and the sample refrozen. Tap E was closed, the weighing tube isolated from the apparatus at tap D, and subsequently removed. While still in the freezing mixture the tube was rapidly vented to atmospheric pressure by the almost instantaneous opening and closing of tap E by moving it through half a full revolution. This ensured that all weighings were conducted under the same internal conditions. The tube was allowed to attain ambient temperature, was thoroughly dried externally and weighed. By reference to the initial known moisture content, the weight of water removed by the drying process was used to derive a value for the new moisture content of the sample.

The vapour pressure above the sample was then determined at this new moisture content. It was assumed that an equilibrium condition, following removal of the water, had been achieved within the sample when no change in deflection could be observed. This

was usually after one to three hours.

In this way the vapour pressures above the same sample were determined for a full range of moisture contents, downwards from the initial level. As, by definition, equilibrium relative humidity is the ratio of the vapour pressure above a material to the vapour pressure exerted by water at the same temperature, knowing the vapour pressure of water it was possible, from the vapour pressure measurements, to derive values for the equilibrium relative humidity above the sample at each moisture content.

Vapour pressure is, however, highly temperature dependent. As constant temperature room facilities were not available when the apparatus was first used, determinations were conducted at ambient temperature, (22.0°C-26.0°C) and all readings were corrected to 25°C. Tables were used to obtain values for the temperature dependent change in vapour pressure of pure water, which were used in the correction.

Desiccator Method

A series of pairs of duplicate samples, each individual sample being of approximately 1g, were placed in small plastic dishes as supplied for use with a Sina continuous-reading hygrometer (Sina equi-hygro-scope sensor assembly, type ZFBA/ PP , used in conjunction with a Sina Scope recorder, type SCT-B, Sina A.G., Zurich, Switzerland). The open dishes were placed in vacuum desiccators, each desiccator containing a quantity of one of the saturated salt solutions or sulphuric acid solutions shown in Table 8.

TABLE 8

SATURATED SALT SOLUTIONS AND SULPHURIC ACID SOLUTIONS USED TO MAINTAIN DIFFERENT EQUILIBRIUM RELATIVE HUMIDITIES FOR ISOTHERM PREPARATION

Salt/Sulphuric Acid Solution	Nominal ERH	Salt/Sulphuric Acid Solution	Nominal ERH
H_2SO_4 S.G. 1.128	91.0%	$K_2CO_3.2H_2O$	44.0%
H_2SO_4 S.G. 1.142	88.0%	H_2SO_4 S.G. 1.492	20.0%
H_2SO_4 S.G. 1.188	83.0%	$LiCl_2.H_2O$	15.0%
H_2SO_4 S.G. 1.218	77.0%	H_2SO_4 S.G. 1.580	10.0%
$NaHSO_4.H_2O$	52.0%	H_2SO_4 S.G. 1.658	5.0%

The samples were allowed to reach equilibrium, under vacuum, over a three week period at 4°C. They were then removed from the desiccator and the dishes immediately closed and sealed with transparent adhesive tape.

For preparation of isotherms at 4°C, 12°C, 25°C and 44°C the sealed samples, in duplicate, were allowed to equilibrate at the selected temperature for 24 hours before the equilibrium relative humidity above the sample was determined with the Sina instrument. The sensor assembly of the hygrometer was placed in the constant temperature cabinet so that readings could be made without removal of samples. The equilibrium relative humidity was read as soon as a constant value was obtained and the samples were then removed for moisture determination, thus allowing isotherm preparation.

An instrument calibration unit was provided, which allowed periodic checks to be made on instrument accuracy. In addition, the facility was provided to allow readings to be taken over a range of either 5% to 20% or 15% to 95% equilibrium relative humidity.

5.3 NUCLEAR MAGNETIC RESONANCE

A Newport Nuclear Magnetic Resonance Quantity Analyser (Newport Instruments, Newport Pagnell, Bucks.) was used in conjunction with a CDV 200 Digital Voltmeter (Coutant Electronics Ltd., Reading, Berks.). In this low resolution NMR instrument, a fixed frequency of approximately 2.7 MHz is maintained by a radio frequency (RF) coil, into which the sample is inserted, situated in the centre of a stable permanent magnet (Fig.17).

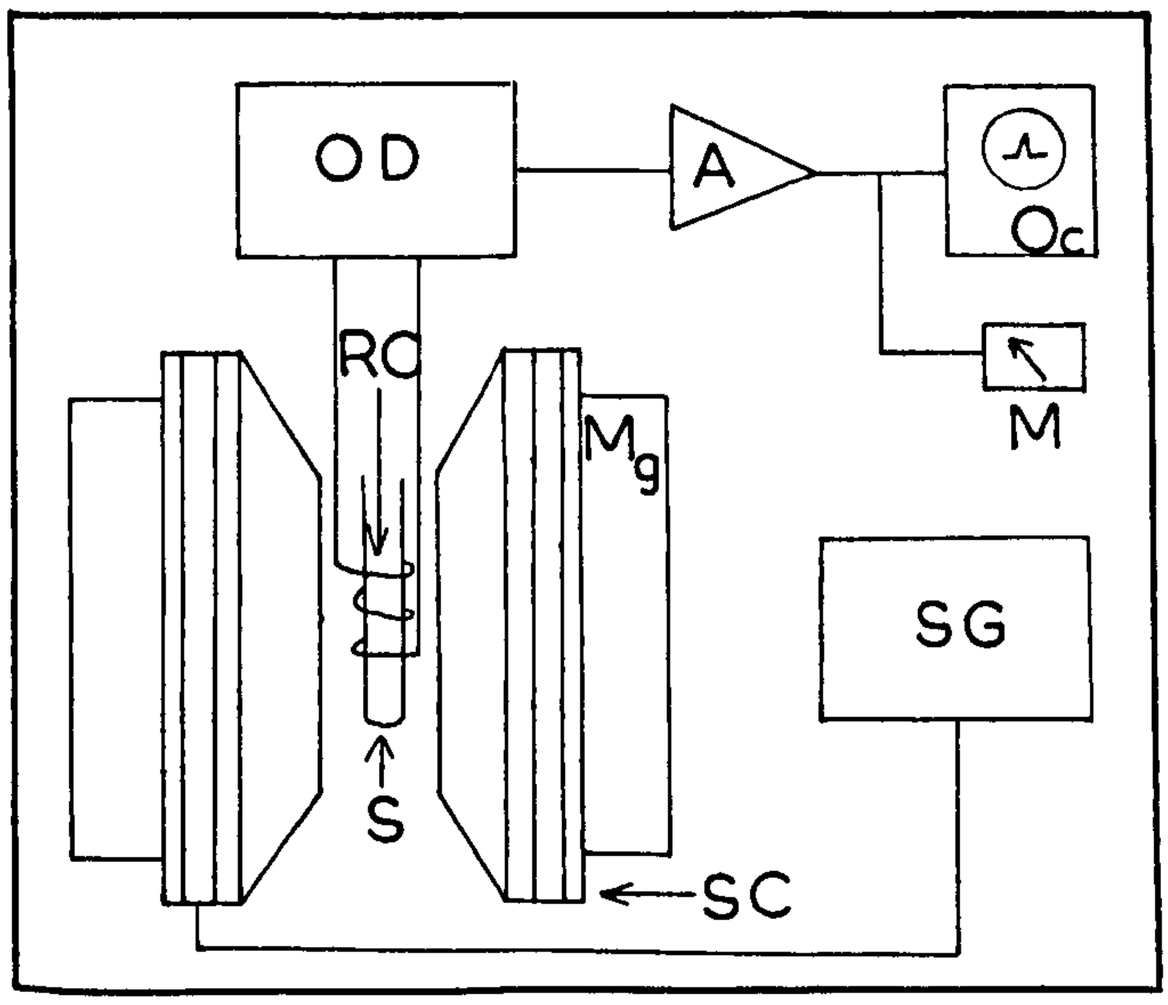

Fig. 17. Basic arrangement of the Newport nuclear magnetic resonance spectrometer.

SG, sweep generator; SC, sweep coils;
Mg, magnet; S, sample tube;
RC, radio frequency coil;
OD, oscillator detector; A, amplifier;
O_c, oscilloscope; M, meter.

The homogenous magnetic field is swept over a 2 gauss range at approximately 640 gauss by means of modulation coils on the magnet.

A small sample assembly was used for all studies, allowing a sample quantity of equivalent volume to 2 ml of water to be used. Care was taken to ensure that for any particular material the sample only occupied the bottom half inch of the sample tube. This ensured that all the material came within the vertical limit of the RF field.

It was initially established that saturation was absent in the type of materials examined. Such saturation indicates the inability of the free hydrogen protons to return rapidly to their stable, low-energy state following an absorption of radio frequency energy. As the varied magnetic field passes through the resonance level on the return portion of its sweep, these protons, still being in the high energy state, do not contribute to the signal size which records energy absorption. In all the materials examined in this study, however, it was ascertained that at the RF level used no saturation occurred, and the signal size was assumed, therefore, to be directly proportional to the number of mobile protons present.

All readings were taken at an RF level of 100 μA , the automatic loss control circuit operating at the high loss level. The output signals were electronically integrated over an interval of 33 seconds, triplicate readings being taken on all samples. The mean value of these readings was finally corrected by subtraction of a background reading determined at the same time as each set of test readings.

When feasible, the instrument was calibrated against a water

reference sample containing a small amount of a para-magnetic salt (manganese chloride), before a series of test readings were taken.

A sample of the material under examination and known to be of a high moisture content was used at the beginning of each series of readings to position the resonance signal within the gate of the instrument. The reference oscillator circuit was then adjusted, this procedure ensuring that all readings could be taken at the same frequency.

Preparation of signal size-moisture content calibration curves was effected as follows.

Samples with a range of moisture contents between zero and 100g/100g dry matter and at approximately 10g/100g intervals were prepared by the direct addition of water from a microsyringe. Each sample was than accurately weighed into a stoppered glass sample tube of known weight. Even distribution of the water within the sample was ensured by holding the tubes in an air tight container for seven days at $4^{o}C$. The tubes were then warmed to ambient temperature, readings taken, and moisture contents determined immediately afterwards. The mean corrected readings were expressed in terms of NMR read out units per unit dry weight and plotted against moisture content to give a calibration curve.

Low temperature NMR examinations were conducted during a stepwise cooling and/or warming of the magnet and samples in a freezing cabinet. In order to reduce any possible interference in the properties of the magnet assembly such as might be caused by the close proximity of the freezing cabinet body, the magnet was suspended with nylon webbing in the centre of the cabinet's air space.

Readings were taken at $5^{o}C$ or $10^{o}C$ intervals, allowing

12 hours for equilibration at each temperature before reading. The minimum temperature attained was -80°C, although orange albedo and its constituents were only examined down to -60°C, the minimum temperature attainable in the freezing cabinet which was used in the earlier examinations.

In all cases duplicate samples at each moisture content were examined, the moisture contents covering a range from below the known unfreezeable water level of the material to a value of about 20g/100g dry matter above it. Moisture content determinations, were conducted on the samples at the end of the cooling and/or rewarming cycle. The mean of the two readings at each moisture content was expressed in terms of NMR readout units per unit dry weight and these were plotted against temperature.

5.4 Differential Thermal Analysis

DTA was used to determine the quantity of unfreezeable water in all the materials under study. The presence of freezeable water was indicated by an endothermic peak obtained on the differential temperature record. The moisture content at which this could just no longer be detected was taken to represent the unfreezeable water content.

Preparation of Samples:

Samples of different moisture contents were prepared by the direct addition of water, or in certain cases a 2% NaCl solution*, using a microsyringe. The water was distributed throughout the sample by a thorough mixing process, and the sample was then placed in a stoppered glass tube which was subsequently sealed with transparent adhesive tape. Further equilibration of moisture in the sample was allowed to take place over a 7 day storage period at $4^{o}C$, the sample tubes being placed in an airtight container.

Macro-scale DTA:

An iron-constantan differential thermocouple circuit was used in conjunction with one of two, 50 microvolt full scale

* For examination by macro-scale DTA a 2% NaCl solution was used to humidify potato samples from which soluble material had been removed. This enabled any freezing peak to be observed at the salt eutectic temperature of $-21.5^{o}C$, thus avoiding the possibility of interference from the thawing of any water present in the reference material at $0^{o}C$.

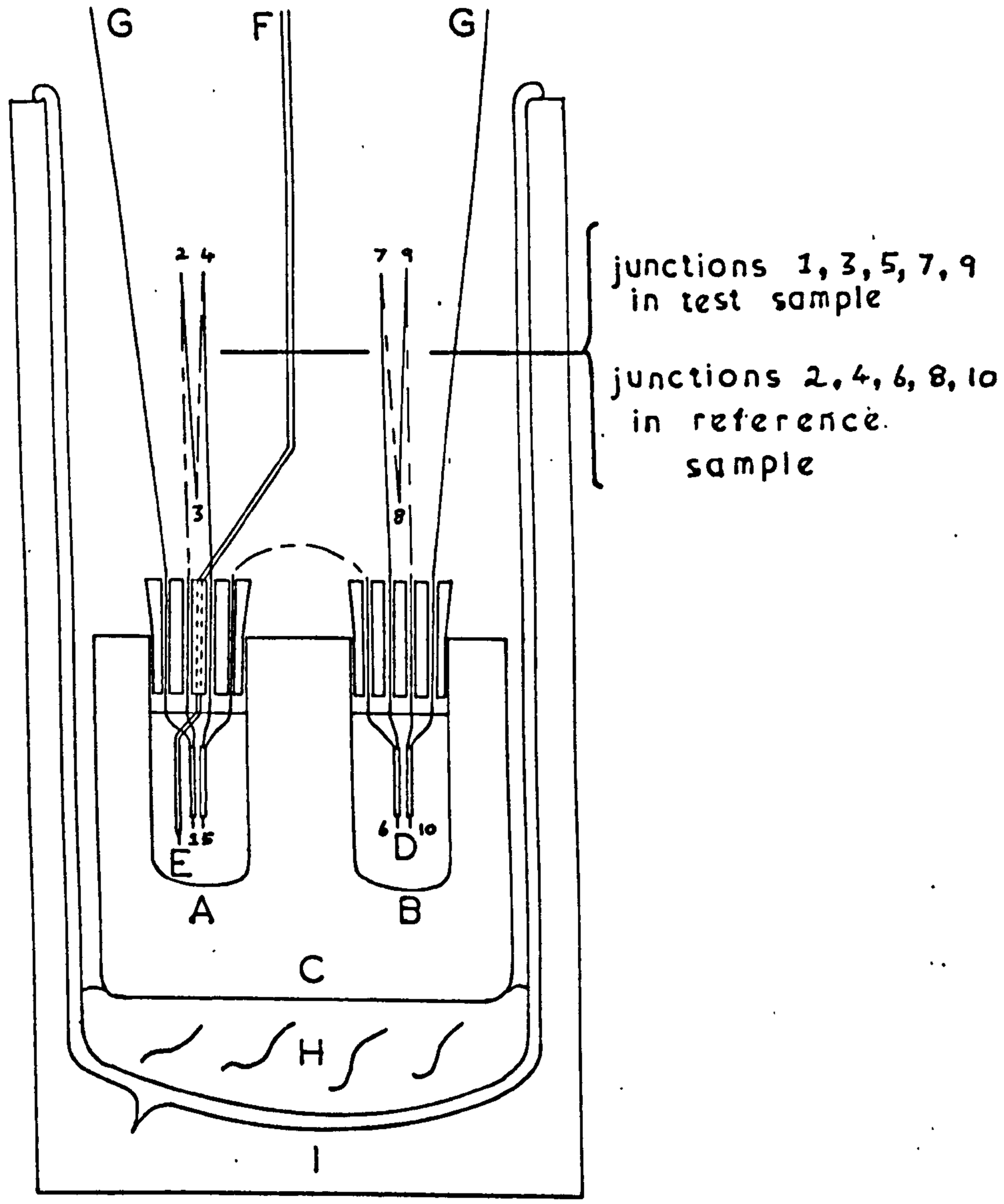

Fig. 18. Schematic diagram of assembly used for differential thermal analysis on the macro-scale.

A, test sample; B, water free sand-sawdust reference sample;
C, aluminium DTA block;
D, five-couple iron-constantan thermocouple assembly;
E, copper-constantan temperature measuring thermocouple;
F, leads from temperature measuring thermocouple;
G, leads from differential temperature couple assembly;
H, cotton wool; I, Dewar flask.

deflection, potentiometric recorders (Honeywell Electronik 8 point, and 12 point, Potentiometric Strip Chart Recorders, Honeywell Controls Ltd., Newhouse, Lanarkshire).

After considerable preliminary investigations, high sensitivity was found to be achieved by the use of a multi thermocouple system. This is illustrated in Fig. 18, and consists of five identical couple pairs connected in series. This system was developed in preference to a single, thin or thick gauge, iron-constantan couple pair; a three-couple pair system; and a manufactured thermopile containing 10 thermocouple pairs set in a solid head, all of which were found to be less sensitive under identical conditions. In addition a thermistor circuit was tried, but was found to be of such a high sensitivity as to be unmanageable with the level of signal amplification of the potentiometric recorders used.

Both component metals of the five-couple system were used in the form of insulated 24 gauge wire, meeting at silver soldered junction points. Alternate junction points were built up into two fine probes, each containing five junctions, insulation between each, and cohesion of the end of the probe being achieved by the use of fine coatings of epoxy resin glue. The individual wires of each probe passed through a rubber bung which allowed easier handling of the system and provided a means for rigidly positioning the probes in the sample, as well as closing individual sample chambers during a DTA run. Connection to the recorder was made by the first iron component of couple pair 1, and the second iron component of couple pair 5. In addition, a copper-constantan couple for direct temperature measurement was introduced alongside the probe which was inserted in the test

sample under examination. Cold junction reference compensation was built into the potentiometric recorder and calibration of the direct temperature reading circuit was occasionally checked against an ice-water mixture and an acetone-solid carbon dioxide mixture.

The presence or absence of freezing water in each sample under study was determined using this five-thermocouple circuit in the apparatus shown in Fig. 18.

The sample under examination (5.0g or, in the case of high bulk density materials, a constant weight equivalent to the volume of the reference sample) was tightly packed into one of the four chambers symmetrically drilled in the cylindrical aluminium DTA block. Tight packing eliminated any trapped air, ensuring even conductivity of heat throughout the sample and minimizing the response time to any changes occurring.

In the opposite chamber a well mixed sand (1.0g)-sawdust (4.5g) reference sample was introduced and similarly packed into place. This reference material had been found by preliminary tests to closely resemble, in thermal characteristics, the type of material to be examined and to give a satisfactorily linear, but sloping, differential temperature baseline over the range of temperatures used in these investigations (-78°C to 0°C).

Thus one cell accommodated two reference samples and two test samples.

The thermocouples, plus bungs, were placed in position immediately after filling each chamber in order to avoid moisture loss from the test material or moisture gain by the reference sample.

The complete cell was then cooled in crushed solid carbon dioxide and upom attainment of a constant temperature at approximately

-78°C was rapidly transferred to a previously cooled, jar-type Dewar flask from which all the solid carbon dioxide had been removed. This was then placed in an insulated box, or in a freezing cabinet which had also been previously cooled to approximately -78°C.

The cell was then allowed to warm up to ambient temperature, this occurring at an almost constant rate of approximately 4°C per hour, the precise symmetry of the cell ensuring, as far as was possible, that both test and reference samples experienced identical conditions, thus minimizing any slope in the differential temperature baseline.

During this warming cycle the temperature differential between test and reference samples was recorded, readings for any one thermocouple circuit being automatically taken every two minutes on the 8 point recorder, and every one minute twelve seconds on the 12 point recorder. The presence of a phase change due to thethawing of ice formed in the test sample was shown as a peak on the differential temperature-time curve recorded. In many cases, however, only the return leg of the peak was clearly shown.

At the end of a warming cycle, and as soon as the cell was at ambient temperature, duplicate moisture determinations were carried out using the complete sample. Following this procedure avoided any likelihood of condensation occurring on the samples when the bungs were removed.

Micro-scale DTA:

A Du Pont 900 Thermal Analyser (Du Pont Co. (U.K.) Ltd., Hitchin, Herts.) became available during the course of this work and this instrument was used for most of the later thermo-analytical

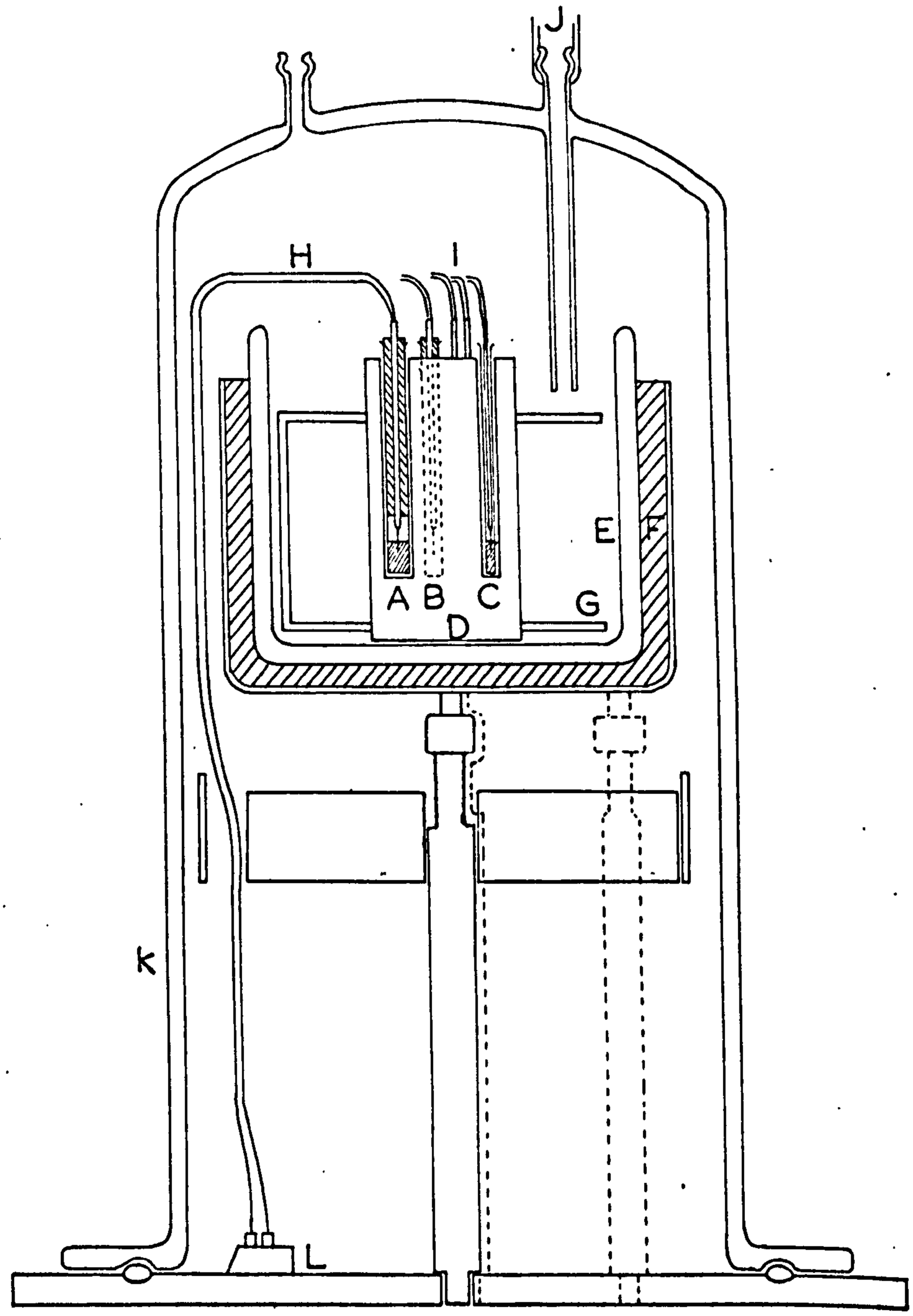

Fig. 19. DTA cell of Du Pont 900 Thermal Analyser with Quick-cool attachment

A, test sample; B, dry glass beads reference sample;
C, temperature thermocouple reference sample;
D, heating block; E, Dewar flask;
F, metal lined asbestos jacket;
G, metal spacer and block support;
H, differential thermocouple leads;
I, thermocouple and heater block leads;
J, liquid nitrogen inlet port; K, bell jar;
L, thermocouple lead junction box with Thermal Analyser.

investigations. The DTA head was used in conjunction with a 'Quick-cool' accessory, as illustrated in Fig. 19.

In this instrument a test and reference sample are both heated under identical conditions at a pre-selected constant rate of between 1°C and 30°C per minute. The heating is carried out in a heating block, in which the test and reference samples, contained in small glass tubes, are placed.

A single chromel-alumel thermocouple is placed in each sample and the temperature differential between them is plotted against temperature on an X-Y recorder. The temperature is recorded by a third thermocouple placed in a separate reference sample in the heating block and which experiences identical conditions to the other two samples. An ice-water mixture, external to the instrument is used as a cold reference junction.

If the heat capacity and thermal conductivity of both sample and reference are identical outside the region of any phase change, the differential temperature against temperature trace will be a straight, non-sloping, line. If these characteristics change linearly with temperature, but at a different rate, a straight, sloping line will result. If the change is quadratic, or of a higher order, a curved line will be obtained. Rapid heating rates tend to give large sharp peaks, slower heating rates provide greater detail but differential temperature values are lower and some peaks may be lost.

The instrument is able to plot changes in differential temperature against temperature over a wide range, from -180°C to 500°C, at different levels of sensitivity. On the temperature axis (X axis) one inch of the ten inch scale can be made to represent from 10°C to 200°C. On the differential temperature

axis (Y axis) one inch can be made to represent from 0.1°C to 10.0°C. Provision is made to allow any part of the total, two-dimensional, range at any given sensitivity, to be brought within the physical limits of the X-Y recorder.

All examinations reported in this study were conducted between -118°C and 0°C using a heating rate of 20°C per minute at a temperature scale sensitivity of 10°C per inch and differential temperature scale sensitivity of 0.2°C per inch. Commencement of examinations at -118°C allowed the system to be observed under constant conditions, having allowed any initial thermal disturbance at the beginning of heating to be resolved.

The sample under examination (2.0mg $\pm$ 0.2mg) was introduced into the bottom of a glass sample tube and a hollow ceramic sleeve placed on top of it. The tip of a thermocouple was embedded in the sample and the complete sample tube was placed in the heating block. A reference sample of dry glass beads was prepared in the same way and changed periodically.

The heating block and samples were then rapidly cooled by pouring liquid nitrogen into the surrounding Dewar flask (Fig.19). A temperature of -100°C was reached in three minutes and -196°C in about seven minutes. A short equilibration time at -196°C was allowed by using excess liquid nitrogen. When all the coolant had evaporated, heat was applied; the heat input being controlled so as to maintain a steady rate of temperature increase. At -118°C the scale sensitivity was set to 10°C per inch and a record obtained while heating to 0°C.

Duplicate moisture determinations were carried out on separate portions of the sample under examination.

PART 3

R E S U L T S

The results from the experimental work fall naturally into four sections.

1) A study of the water relations of the major macromolecular constituents of plant materials, that is cellulose, pectin and starch, as obtained from commercial sources.

2) A study of the water relations of a simple natural product, orange albedo, and of its two major components, cellulose and pectin, with reference to the role these play in the hydration properties of the intact material.

3) A study of the water relations of a more complex plant material, the potato, with special reference to the role of its constituents in its overall hydration properties.

4) A brief study into the effects of various solutes on the water relations of the pure constituents examined in Section 1).

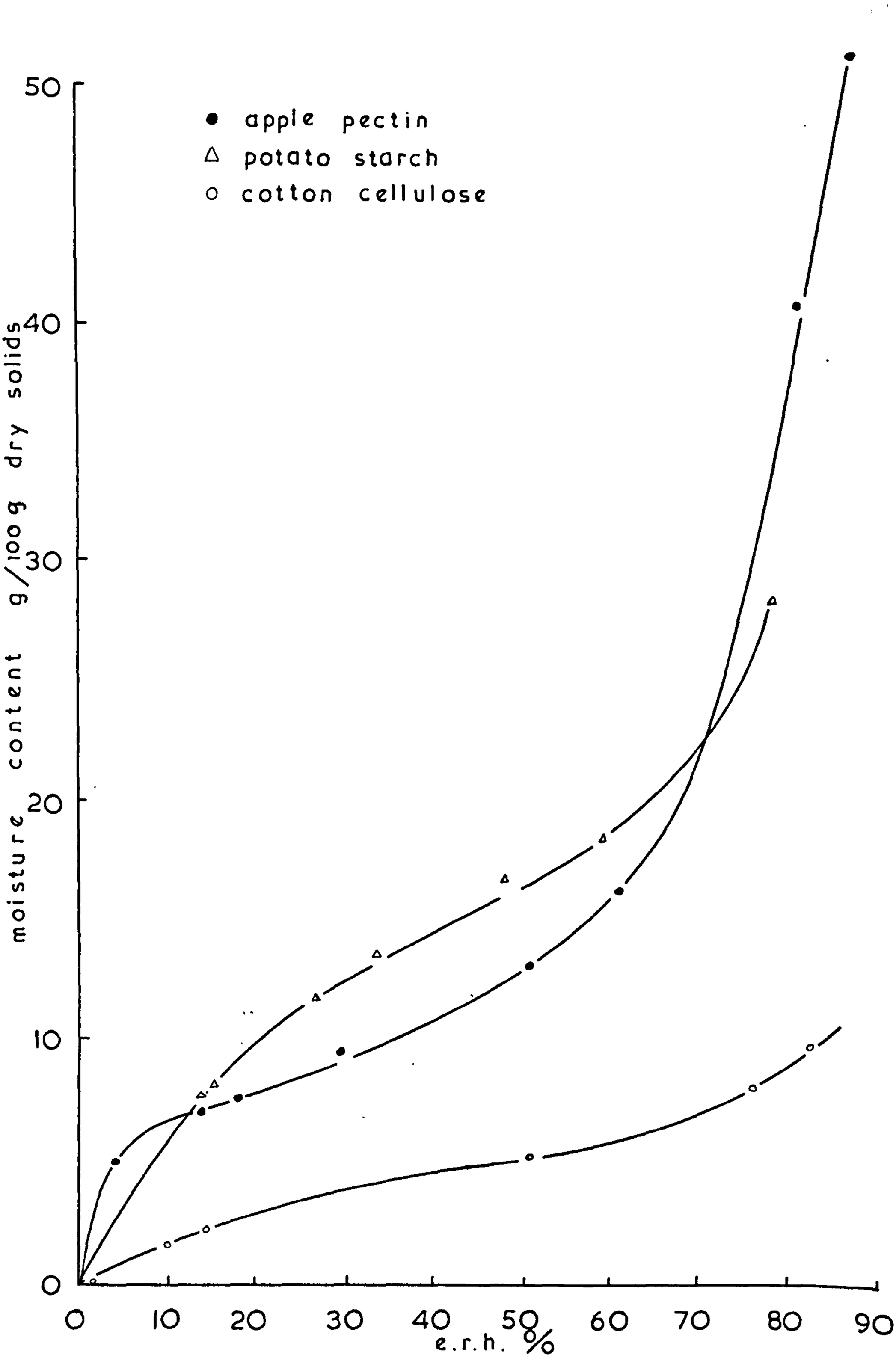

Fig. 20. Sorption isotherms of cotton cellulose, apple pectin, and potato starch prepared using the manometric method at 25°C.

6.0. THE WATER RELATIONS OF THREE MAJOR MACROMOLECULAR CONSTITUENTS OF PLANT MATERIALS

The water relations of cotton cellulose, apple pectin and potato starch were examined by preparation of sorption isotherms, using both the manometric and desiccator methods; by micro-scale differential thermal analysis; and by nuclear magnetic resonance at both ambient and lower temperatures.

6.1 SORPTION STUDIES

Desorption isotherms obtained at 25°C for cotton cellulose, apple pectin and potato starch using the manometric method are shown in Fig. 20.

All three isotherms are sigmoid and are of the kind which Brunauer et al[288] designated as type II. This isotherm type has been interpreted[284] as indicating the formation of multi-layers of absorbed water molecules upon an initially absorbed monomolecular layer.

At any one equilibrium relative humidity (ERH) within the range illustrated, potato starch and apple pectin both absorb considerably more water than does cotton cellulose. Potato starch sorbs more water than apple pectin between an ERH of about 12% and of 70%.

Figs. 21-23 illustrate isotherms prepared using the desiccator method for cotton cellulose, apple pectin and potato starch, respectively.

Isotherms for cotton cellulose were prepared at 4°C and 25°C, and those for apple pectin and potato starch at 4°C, 25°C and 44°C.

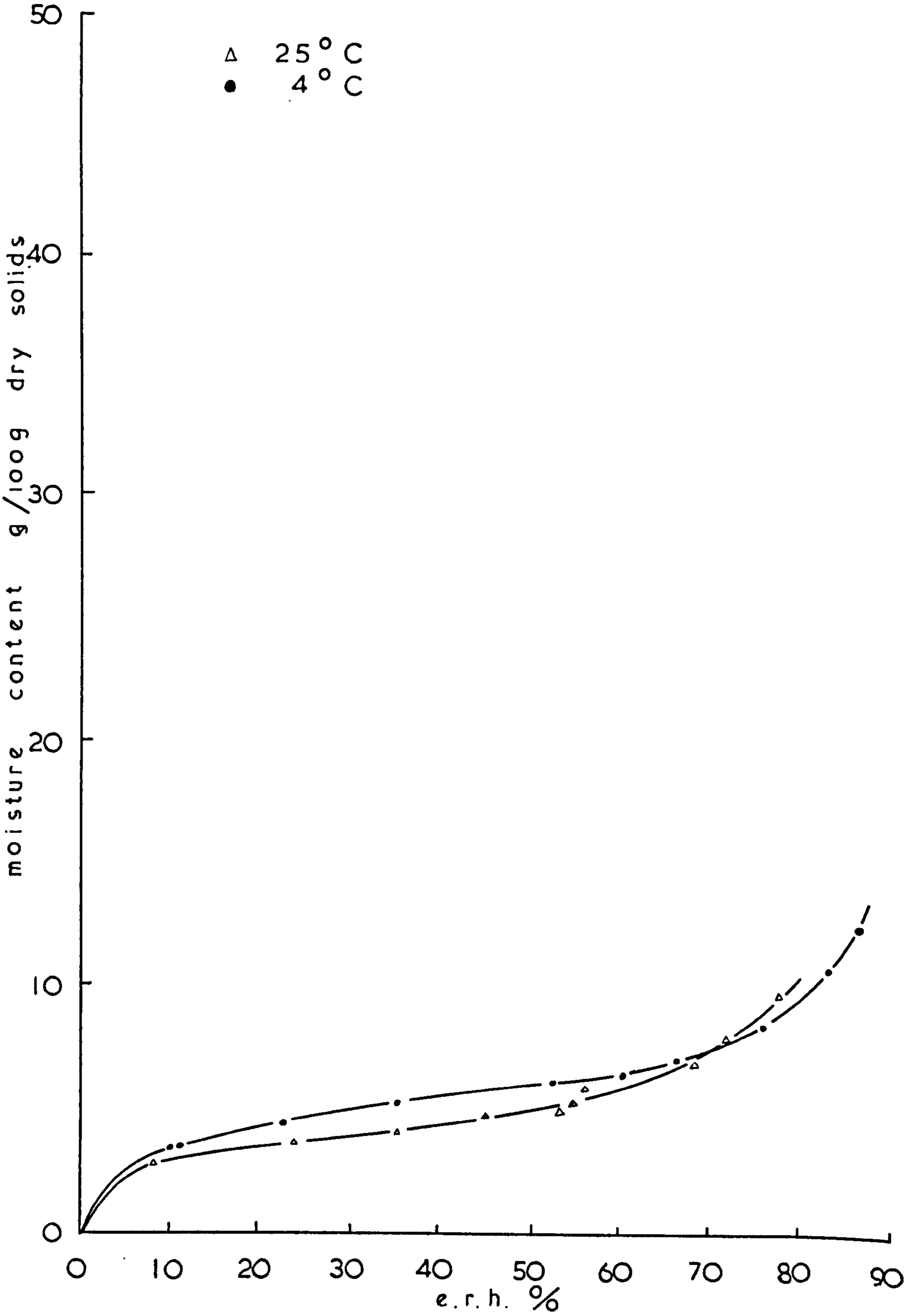

Fig. 21. Sorption isotherms for cotton cellulose prepared at 4°C and 25°C using the desiccator method.

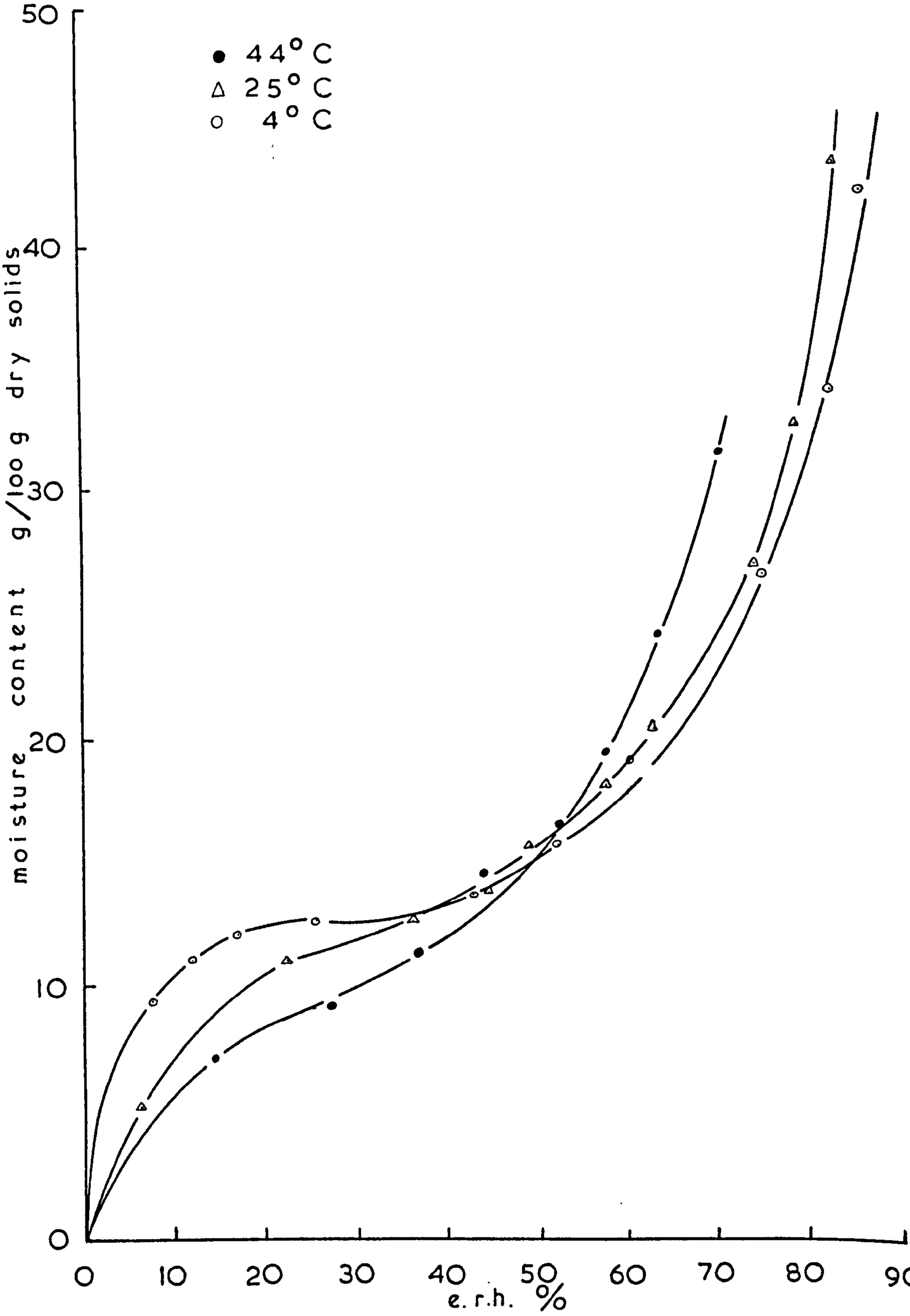

Fig. 22. Sorption isotherms of apple pectin prepared at 4°C, 25°C and 44°C, using the desiccator method.

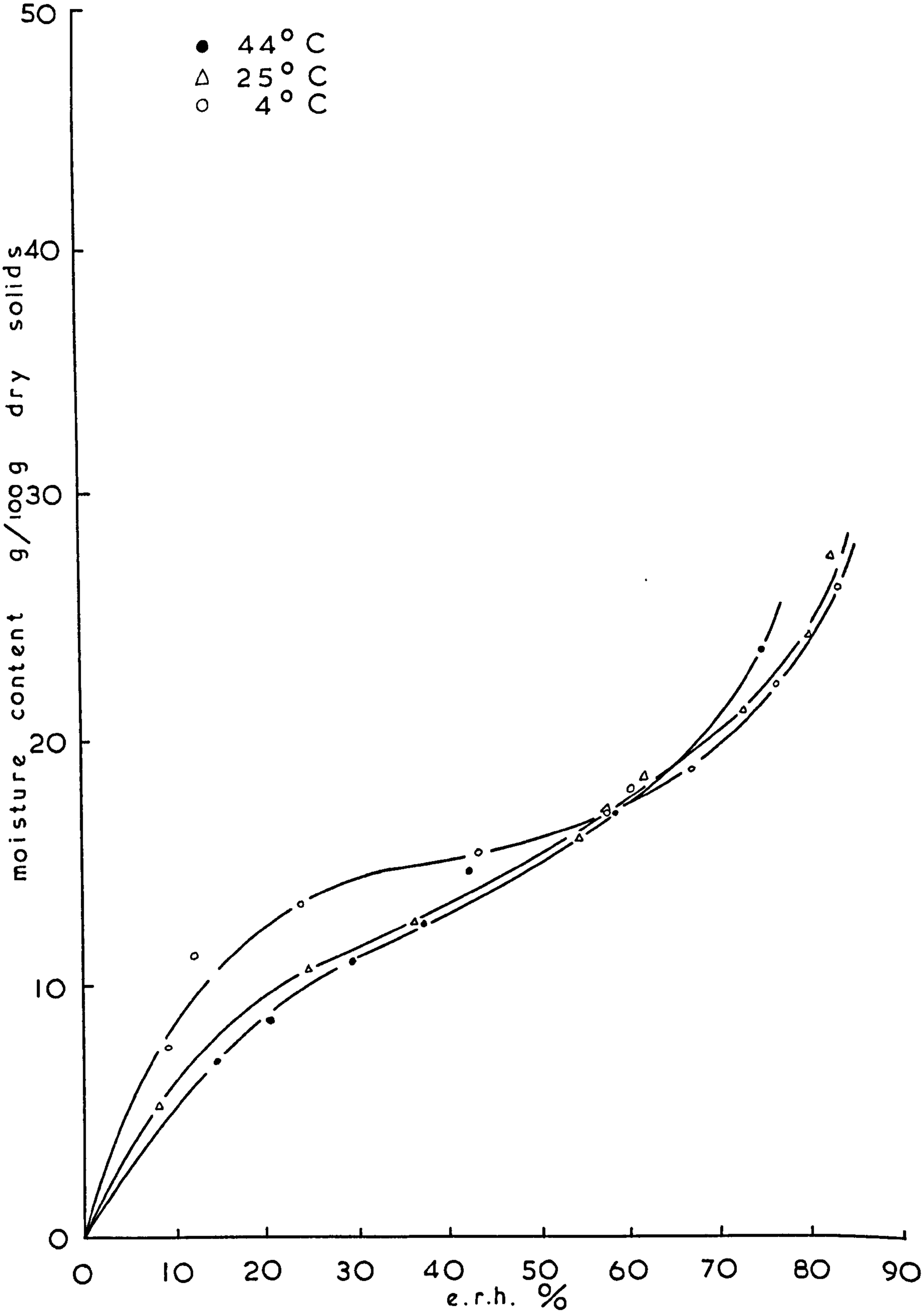

Fig. 23. Sorption isotherms of potato starch prepared at 4°C, 25°C and 44°C using the desiccator method.

All the isotherms are sigmoid, and the same major differences between the three materials, as were illustrated in Fig. 20, are again evident. Comparison of the isotherm for apple pectin and potato starch in the middle of the ERH range shows a reversal of the previously noted difference, however. This reflects the change in behaviour of the two materials under the desorption process involved in the manometric method, and the predominantly absorption process of the desiccator method, potato starch exhibiting a greater degree of hysteresis than apple pectin.

For each of the three materials it can be observed that at low moisture contents a change in temperature is directly related to a change in ERH. At higher moisture contents this effect is apparently reversed. In terms of actual vapour pressure, however, an increase is still obtained, as expected, with an increase in temperature. This reflects the temperature dependence of the vapour pressure of pure water.

Using the sorption data presented in Figs. 21-23 an attempt was made to elucidate the mechanism of water uptake, and its quantitative aspects, by the application of several different isotherm equations. The thermodynamic function of heat of absorption, Qs, was also determined as a function of moisture content.

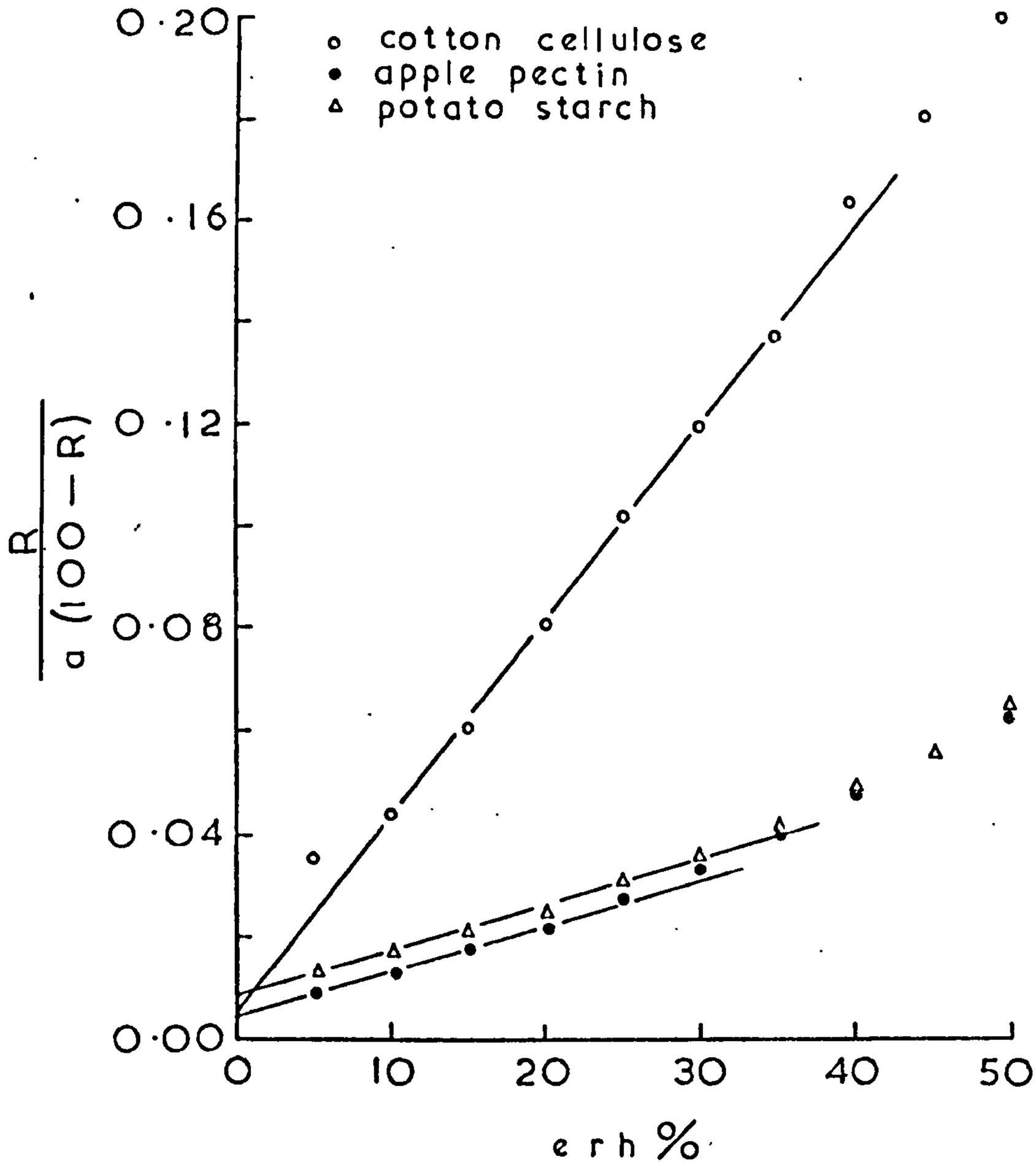

Fig. 24. BET plots on sorption data of cotton cellulose, apple pectin and potato starch.

The application of Salwin's modification of the BET isotherm equation (Eq. 4) to the sorption data obtained at 25°C for cotton cellulose, apple pectin and potato starch is illustrated in Fig. 24. It can be seen that the points fall on a straight line in the ERH range between 10% and 35%, 5% and 25% and 5% and 30% respectively. This indicates the limited range of applicability of the isotherm equation.

Calculated values for the moisture content corresponding to the completion of a monomolecular layer, are 2.66g/100g, 11.65g/100g and 11.13g/100g respectively.

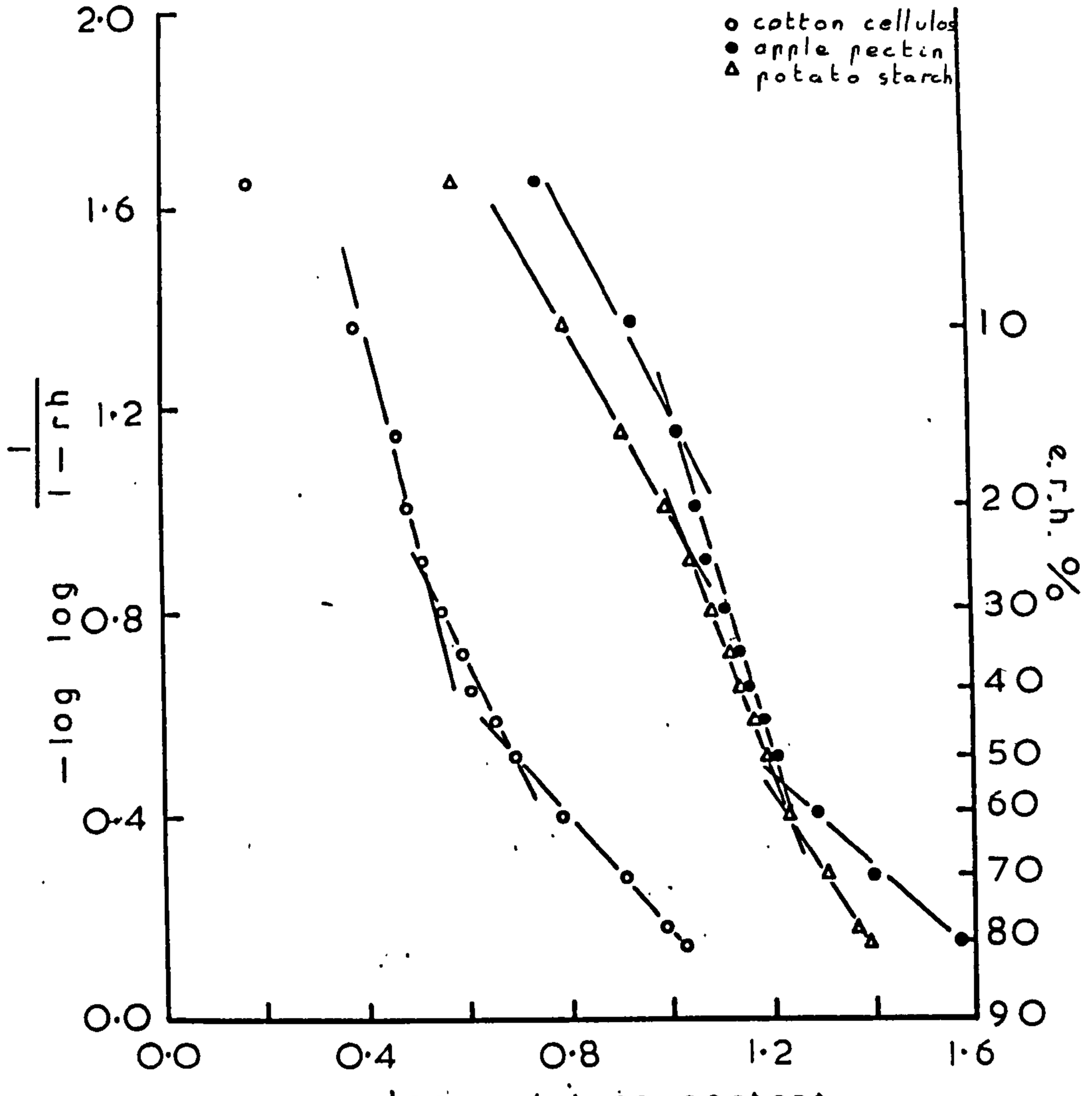

Fig. 25. Henderson plots on sorption data of cotton cellulose, apple pectin and potato starch.

Fig. 25 illustrates the application of Rockland's modification of Henderson's isotherm equation (Eq. 12A) to the sorption data obtained at 25°C for cotton cellulose, apple pectin and potato starch. In each case the points describe a flattened sigmoid curve, this being most apparent in the case of cotton cellulose. It seems reasonable, however, to follow the procedure used by Rockland[267], and to draw a number of straight lines, three in each case, through the points, The two points of intersection, expressed in terms of ERH, fall within the range of a) 14% to 28% and b) 43% to 60%.

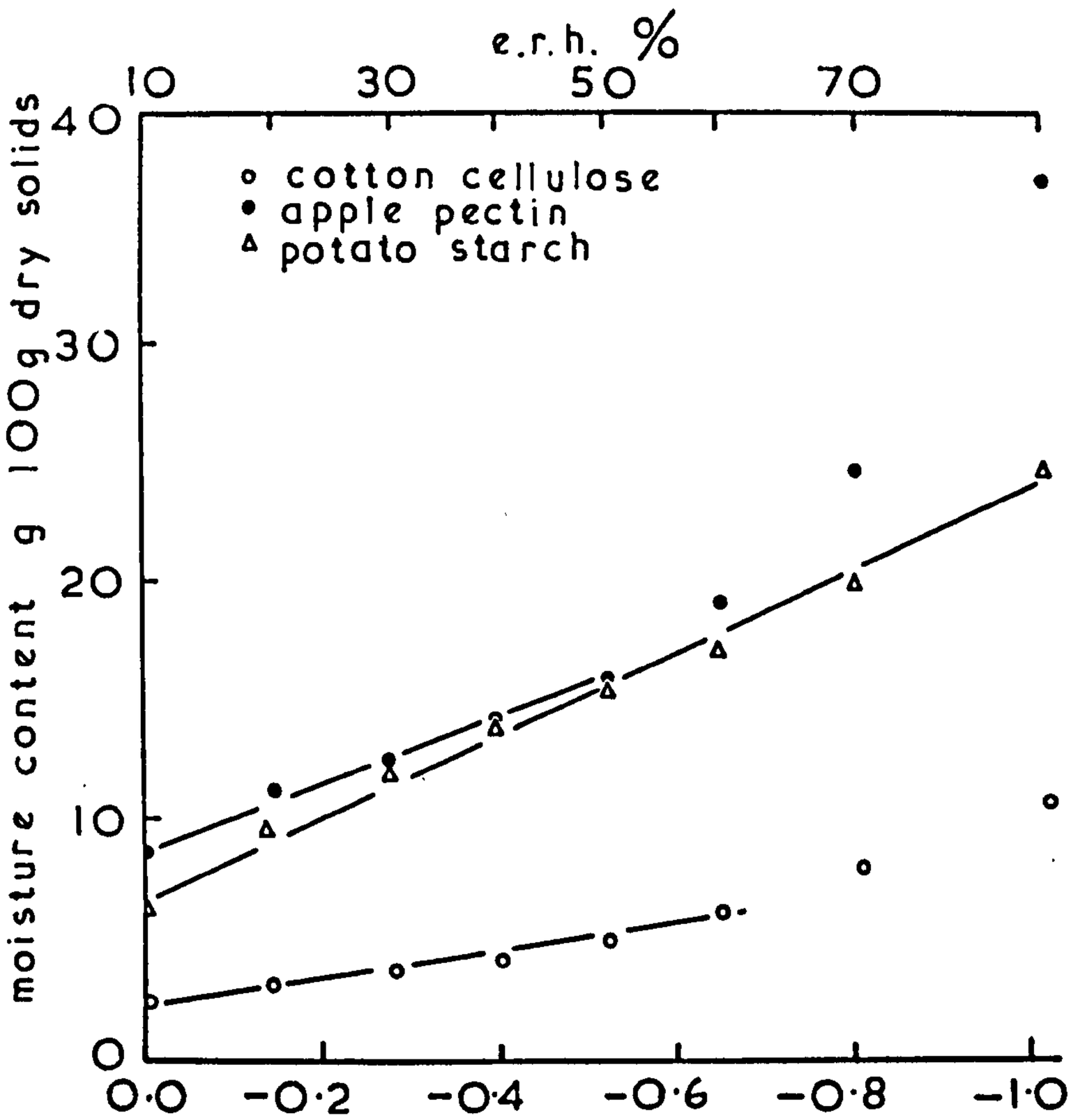

Fig. 26. Bradley plots on sorption data of cotton cellulose, apple pectin and potato starch.

The sorption data obtained at 25°C for cotton cellulose, apple pectin and potato starch, and treated by the application of Bradley's isotherm equation (Eq. 6) ~~is~~ are shown in Fig. 26.

In the case of cotton cellulose the points lie on a straight line between an ERH of 10% and 60%; for apple pectin between 10% and 50%; and for potato starch between 10% and 80%. Above the straight line portion and therefore the limit of applicability of the equation, a lower ERH or actual vapour pressure is observed at any given moisture content than would be predicted if the concept of multilayer formation, upon which the equation is based, still held true.

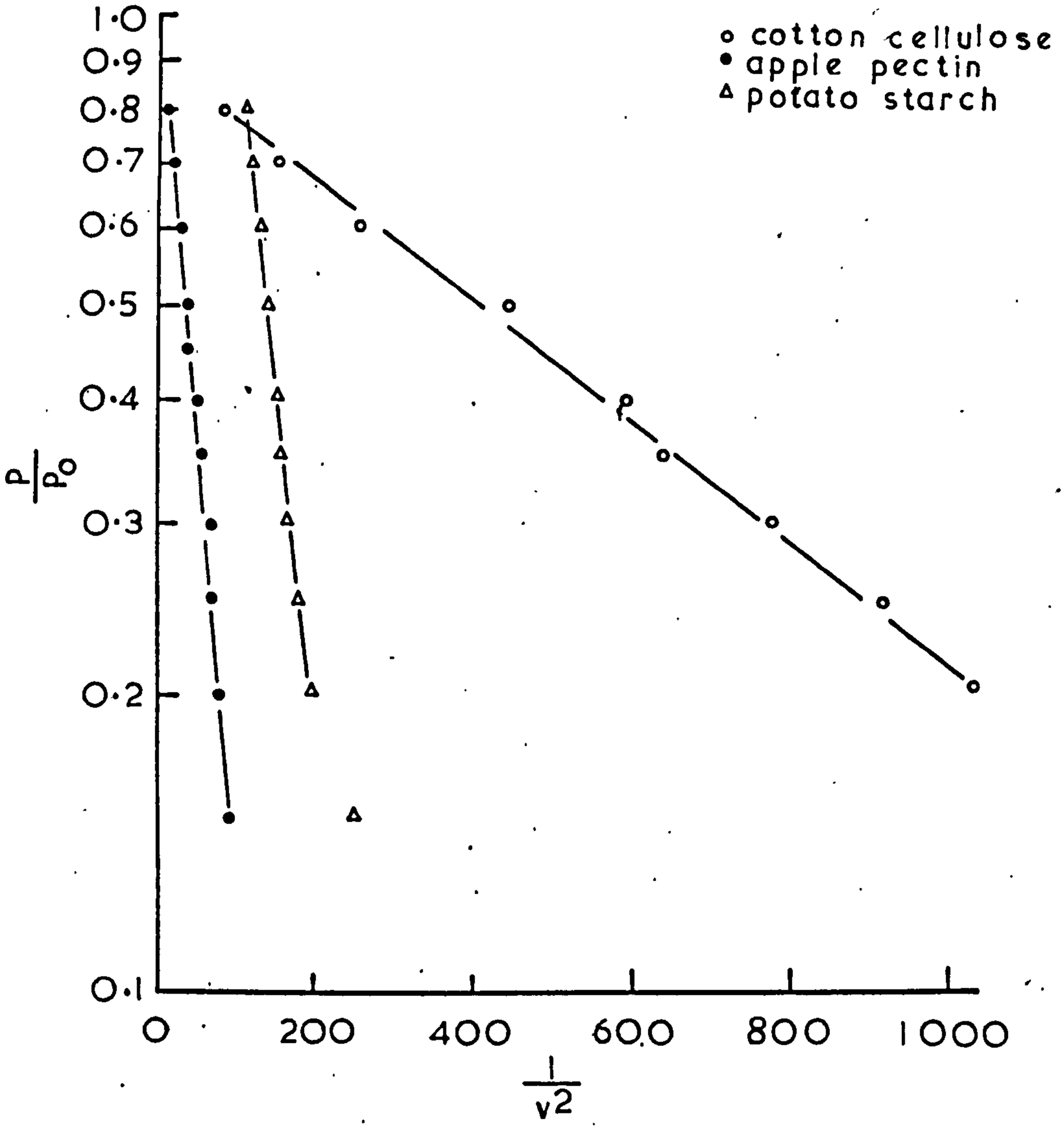

Fig. 27. Harkins-Jura plots on sorption data of cotton cellulose, apple pectin and potato starch. ($\frac{1}{v^2}$ scale displaced by 100 units for starch plot).

The ~~capillary~~ condensation approach to sorption, as used in the development of the Harkins-Jura equation (Eq. 9) is illustrated in Fig. 27 applied to the three pure plant constituents.

In all cases the equation appears to be applicable at high equilibrium relative humidities, down to 15% in the case of apple pectin, below 20% in the case of cotton cellulose and down to 25% in the case of potato starch. These corresponds to moisture contents of 10.4g/100g, 11.05g/100g and 3.10g/100g, respectively.

It has been shown by Liang[303] that the square root of the slope of the linear section is equal to a, where a, is the monolayer value. From Fig. 27 moisture contents corresponding to this monomolecular layer of 2.45g/100g for cotton cellulose, 8.75g/100g for apple pectin, and 9.06g/100g for potato starch can be derived.

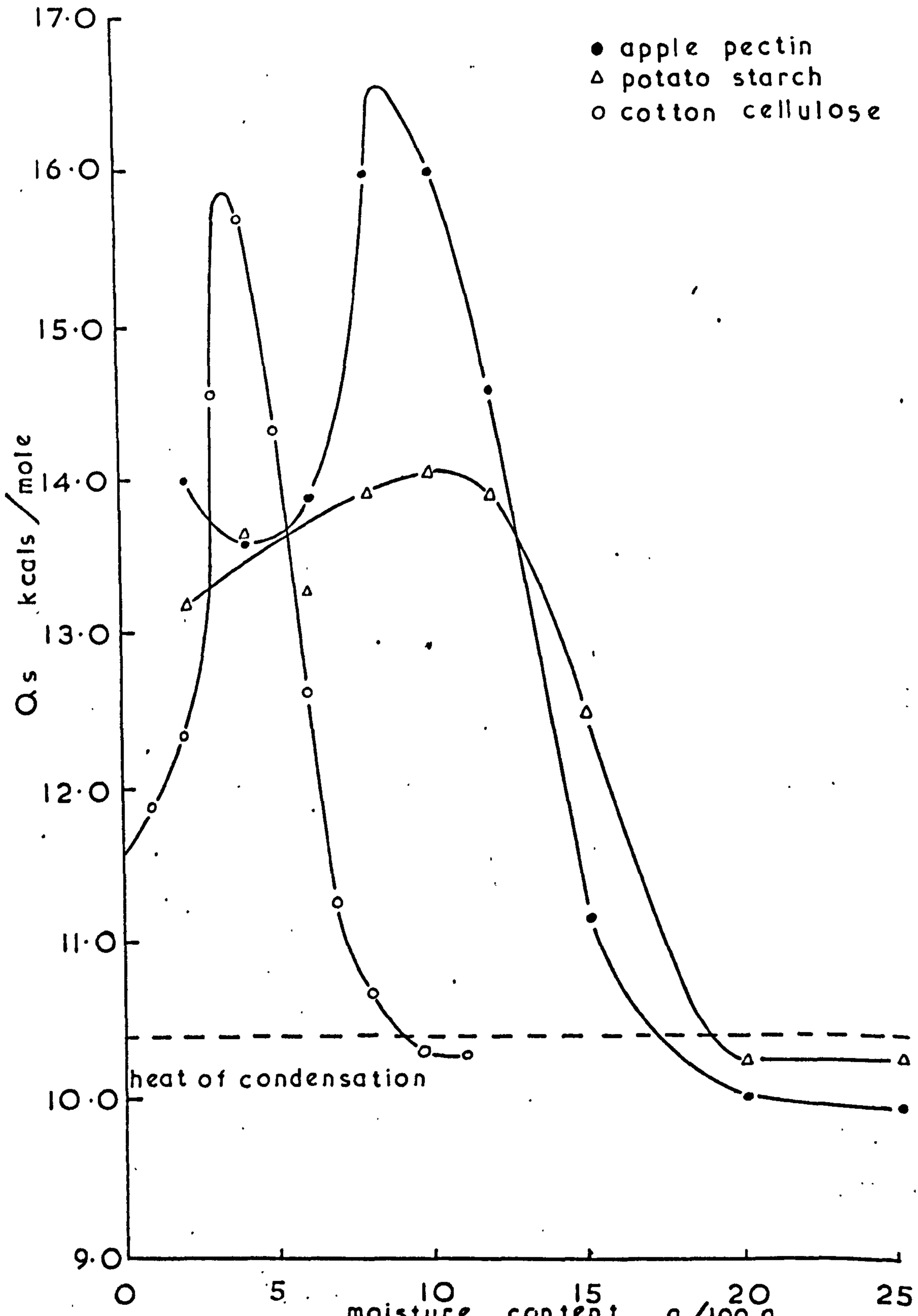

Fig. 28. Change in heat of association, Qs, with moisture content for cotton cellulose, apple pectin and potato starch.

The change in the heat of association, Qs, as a function of moisture content for cotton cellulose, apple pectin and potato starch are illustrated in Fig. 28.

The Clausius-Clapeyron equation (Eq.15) was used to derive values for Qs, the sorption data obtained at $4^{o}C$ and $44^{o}C$ being used in the case of apple pectin and potato starch and those obtained at $4^{o}C$ and $25^{o}C$ in the case of cotton cellulose.

A maximă in terms of Qs is reached in each of the curves before they fall to a constant value close to that of the heat of condensation of water (10.4 kcals/mole at $29.0^{o}C$[182]).

These maxima occur at moisture contents of 3.5g/100g in the case of cotton cellulose, 8.5g/100g in the case of apple pectin, and 10.5g/100g in the case of potato starch. The moisture contents at which the heat of absorption first reach a constant value close to the heat of condensation of water are 9.5g/100g, 20g/100g and 20g/100g, respectively.

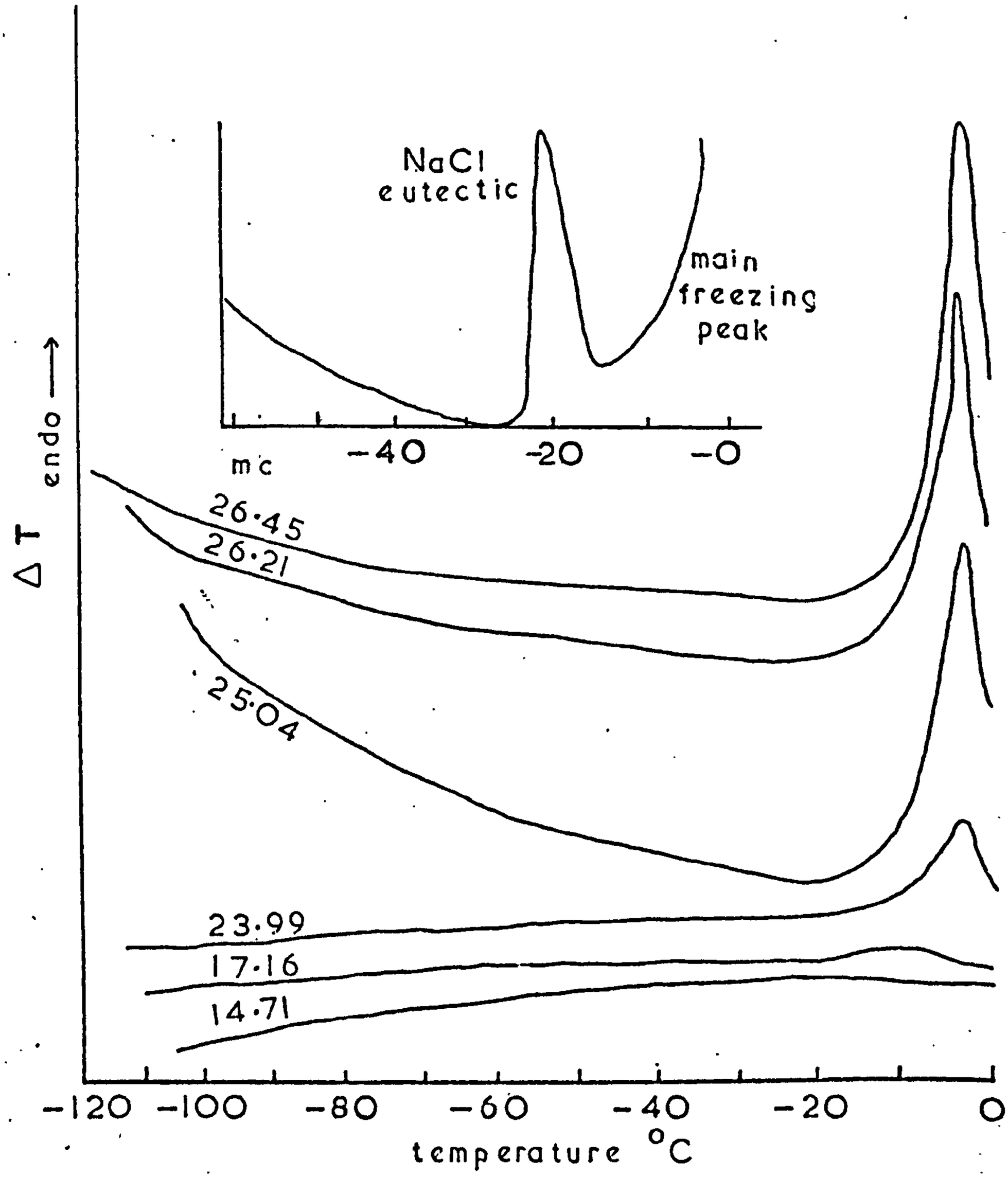

Fig. 29. Differential thermal analysis on the microscale of cotton cellulose hydrated to different moisture content (mc). Inset shows thawing peak of 20 µl of a 1% NaCl solution.

6.2 DIFFERENTIAL THERMAL ANALYSIS

Fig. 29 illustrates the series of curves obtained from the differential thermal analysis on the micro-scale of cotton cellulose hydrated to different moisture contents.

In the samplesof higher moisture content a distinct endothermic peak is visible in the region between $0^{o}C$ and $-10^{o}C$. Comparison of these curves with that obtained upon the warming of 20 µl of a 1% NaCl solution (see inset), indicates that the observed phenomenon is due to the thawing of ice formed from free solvent water.

With a reduction in moisture content the endothermic peak is reduced in size, and while still observable at 17.16g/100g, can only be detected as an inflection point at 14.71g/100g. The moisture content at which the presence of freezing water can no longer be detected has been taken as just below 14.71g/100g. Below this level all the water is considered to be unfreezeable, at least down to $-198^{o}C$.

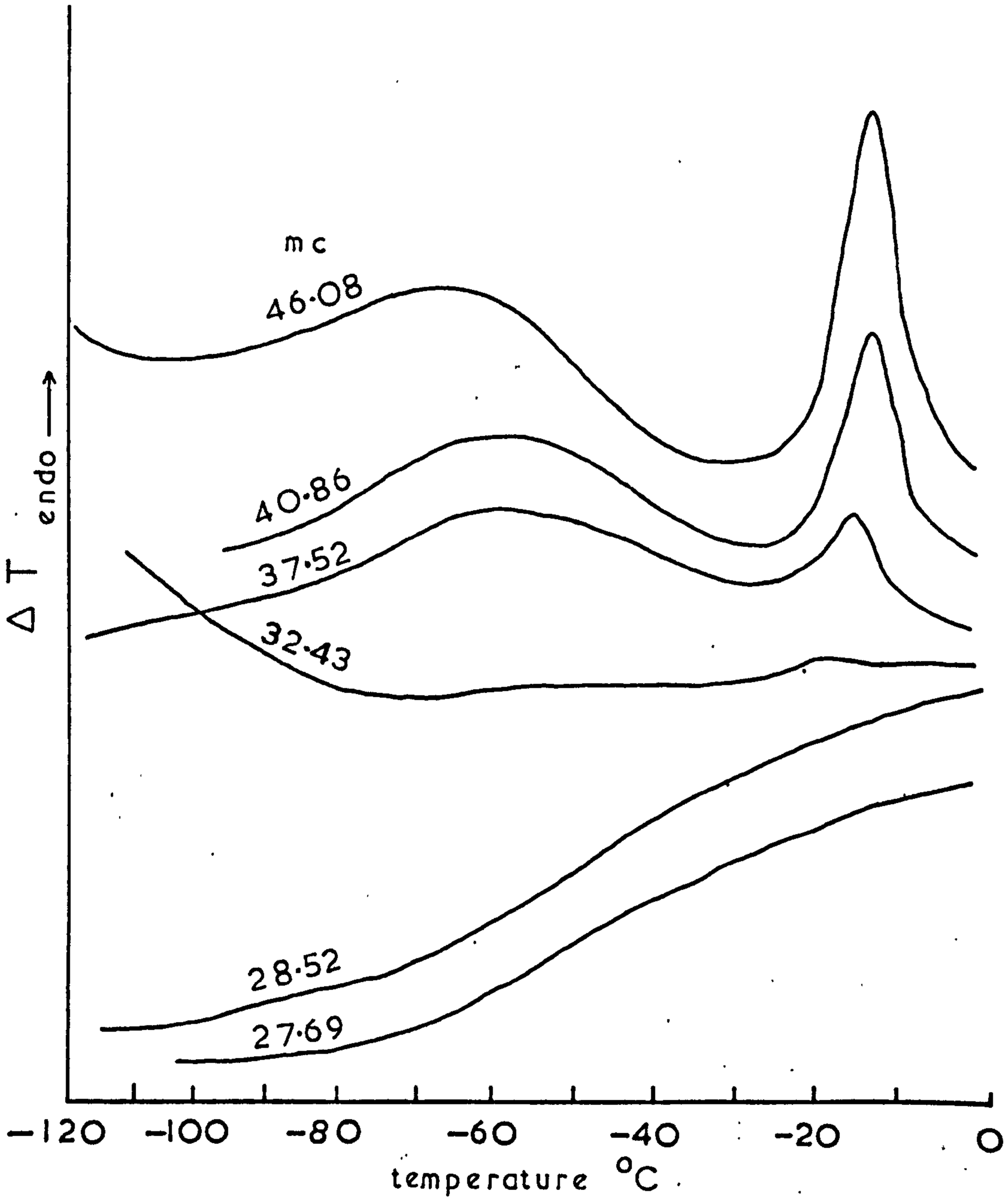

Fig. 30. Differential thermal analysis on the micro-scale of apple pectin hydrated to different moisture contents (mc).

A similar reduction in the size of the peak attributable to the thawing of ice formed from free, solvent water upon a reduction in moisture content is also observed in the case of apple pectin, as shown in Fig. 30. The moisture content at which the presence of freezing water can no longer be detected has been taken as occurring within the range between 32.40g/100g and 28.5g/100g.

An additional thermal effect, occurring at a lower temperature than the peak described above, is observable in the higher moisture content samples. This takes the form of a broad endothermic disturbance which increases in size with an increase in moisture content. For want of a better precise term this effect will be described as the 'hump' phenomenon.

The 'hump' is visible in the temperature range between -80°C and -50°C in those samples hydrated above the unfreezeable water content. With an increase in moisture content the temperature of onset of this endothermic effect and that coinciding with the mid-point of the 'hump' are both reduced.

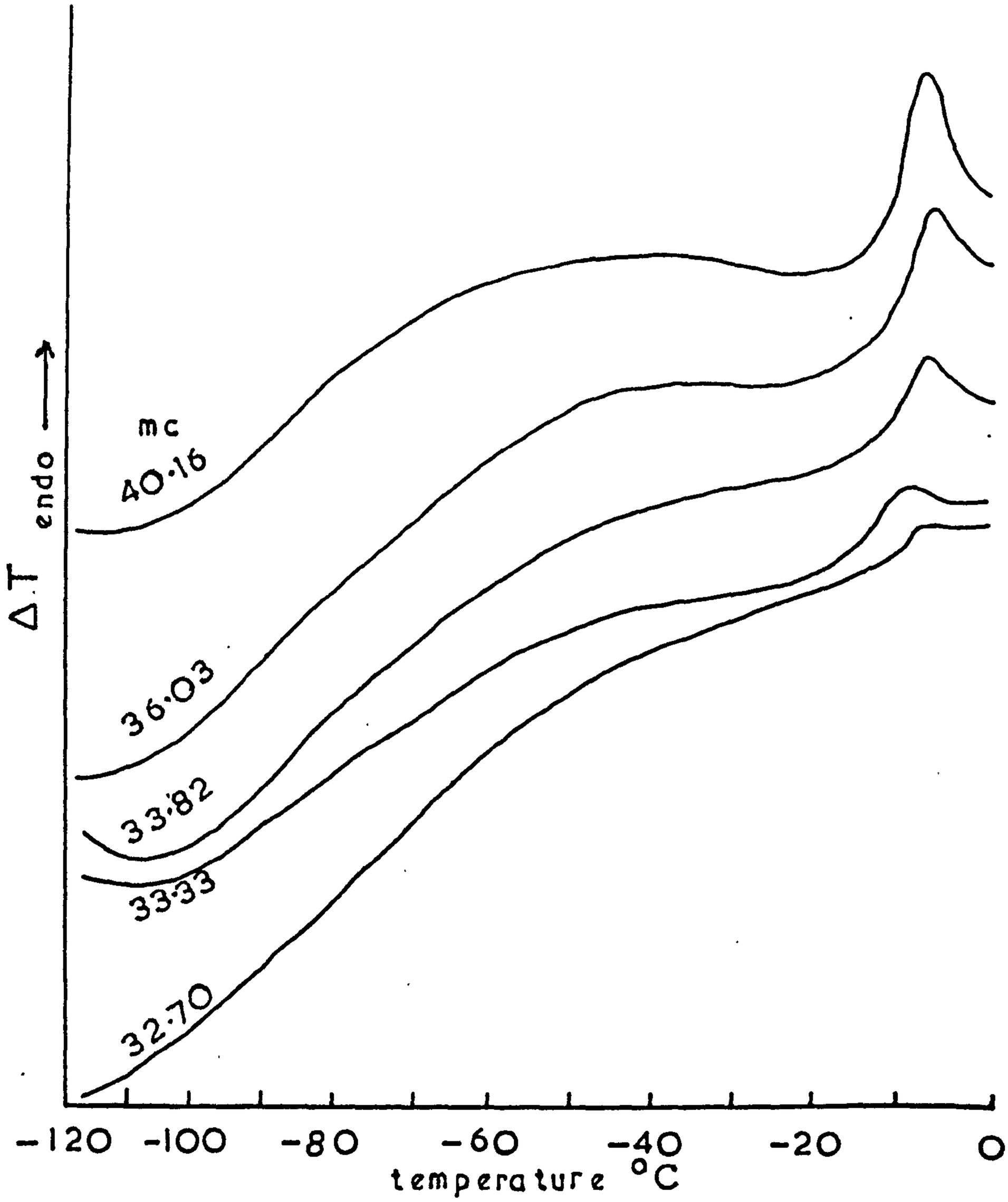

Fig. 31. Differential thermal analysis on the micro-scale of potato starch hydrated to different moisture contents (mc).

As with cotton cellulose and apple pectin, Fig. 31 illustrates that the presence of free solvent water is detectable in relatively moist samples of potato starch, a sharp endothermic disturbance just below zero being observed as ice formed from this water thaws. In this case, the presence of the freezing water can no longer be detected below 32.70g/100g dry matter.

Although the presence of an additional endothermic 'hump' at lower temperatures is not so clear in this material as was shown in Fig. 30 for apple pectin, a distinct change in the shape of the curve below the main thawing zone is observable with an increase in moisture content.

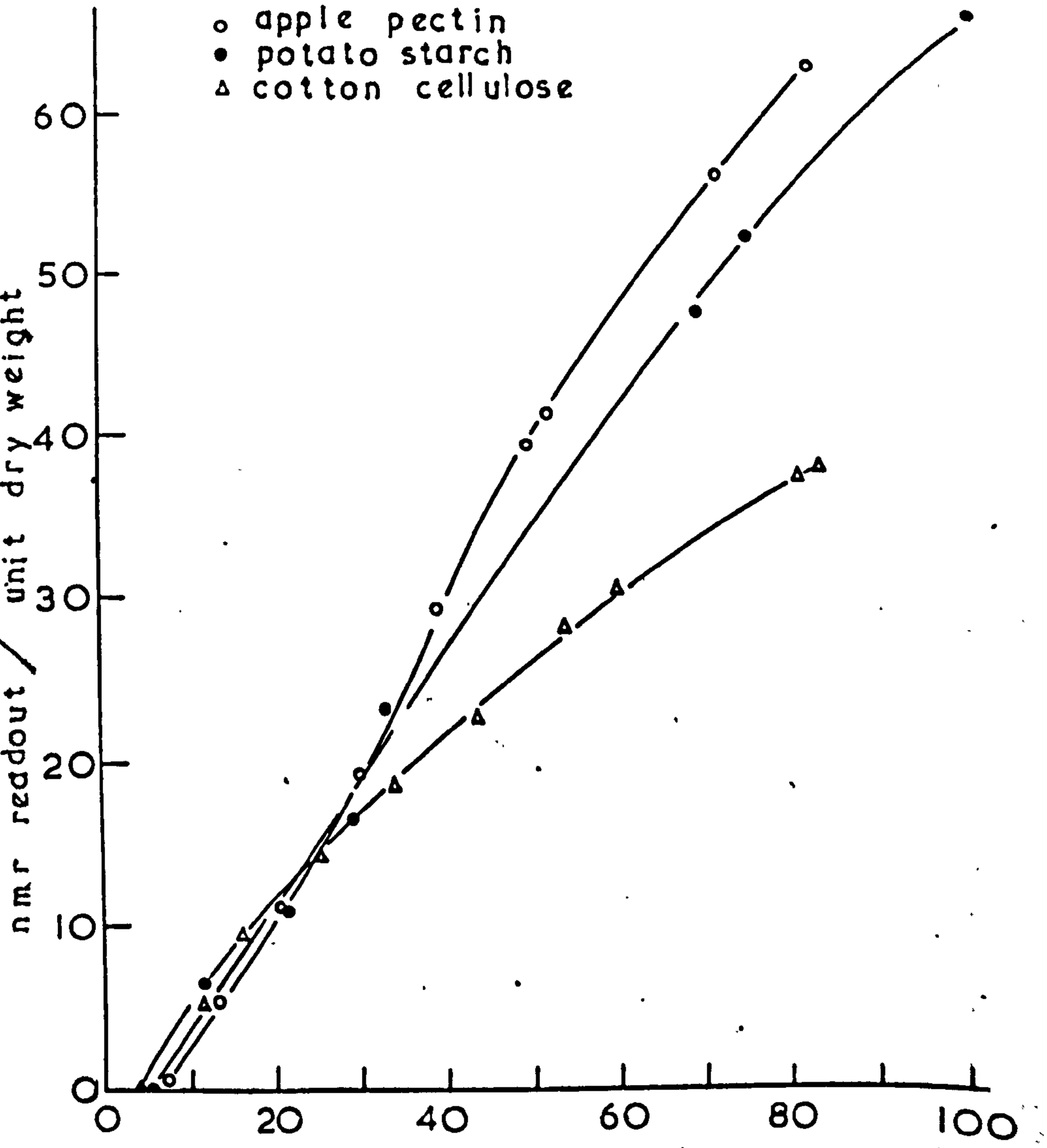

Fig. 32. Nuclear magnetic resonance - moisture content calibration curve for pure plant constituents, determined at ambient temperature.

6.3 NUCLEAR MAGNETIC RESONANCE

Low resolution nuclear magnetic resonance was used to examine cotton cellulose, apple pectin and potato starch as the material was cooled from ambient temperature to -80°C and, at less frequent intervals, during subsequent rewarming.

Initially, however, an NMR signal size - moisture content calibration curve was prepared for each of the materials at ambient temperature. These are presented in Fig. 32, signal size being expressed on a unit dry weight basis.

None of the calibration curves are linear over the complete moisture content range illustrated.

The curve for potato starch is straight over much of its length, deviating slightly at higher moisture contents.

In the case of apple pectin the points describe a flattened sigmoid shape. This coincides with the curve for potato starch at low levels of hydration but differs from it by a constant amount at higher levels.

The curve for cotton cellulose is concave to the moisture content axis over its entire length.

None of the three curves pass through the origin but all appear to bisect the moisture content axis. In the case of cotton cellulose this occurs at approximately 4.5g/100g, in that of apple pectin at approximately 6.5g/100g and for potato starch at approximately 5.5g/100g. This indicates that below these moisture contents the water present is unable to resonate and to produce a visible signal.

The results of the low temperature examination of cotton cellulose, apple pectin and potato starch, hydrated to a range of moisture contents encompassing the expected unfreezeable water

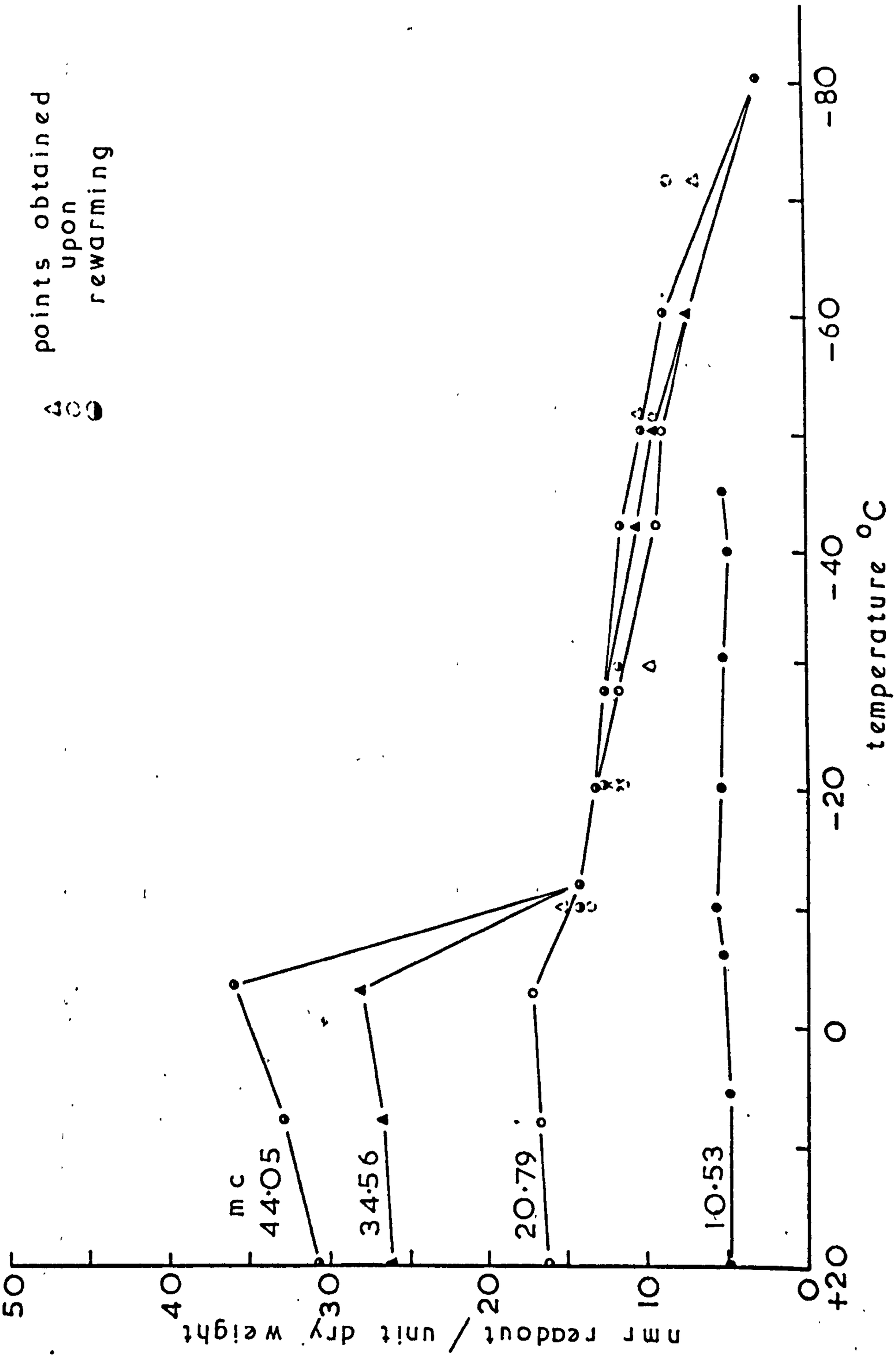

Fig. 33. Low temperature nuclear magnetic resonance examination of cotton cellulose hydrated to different moisture contents (mc).

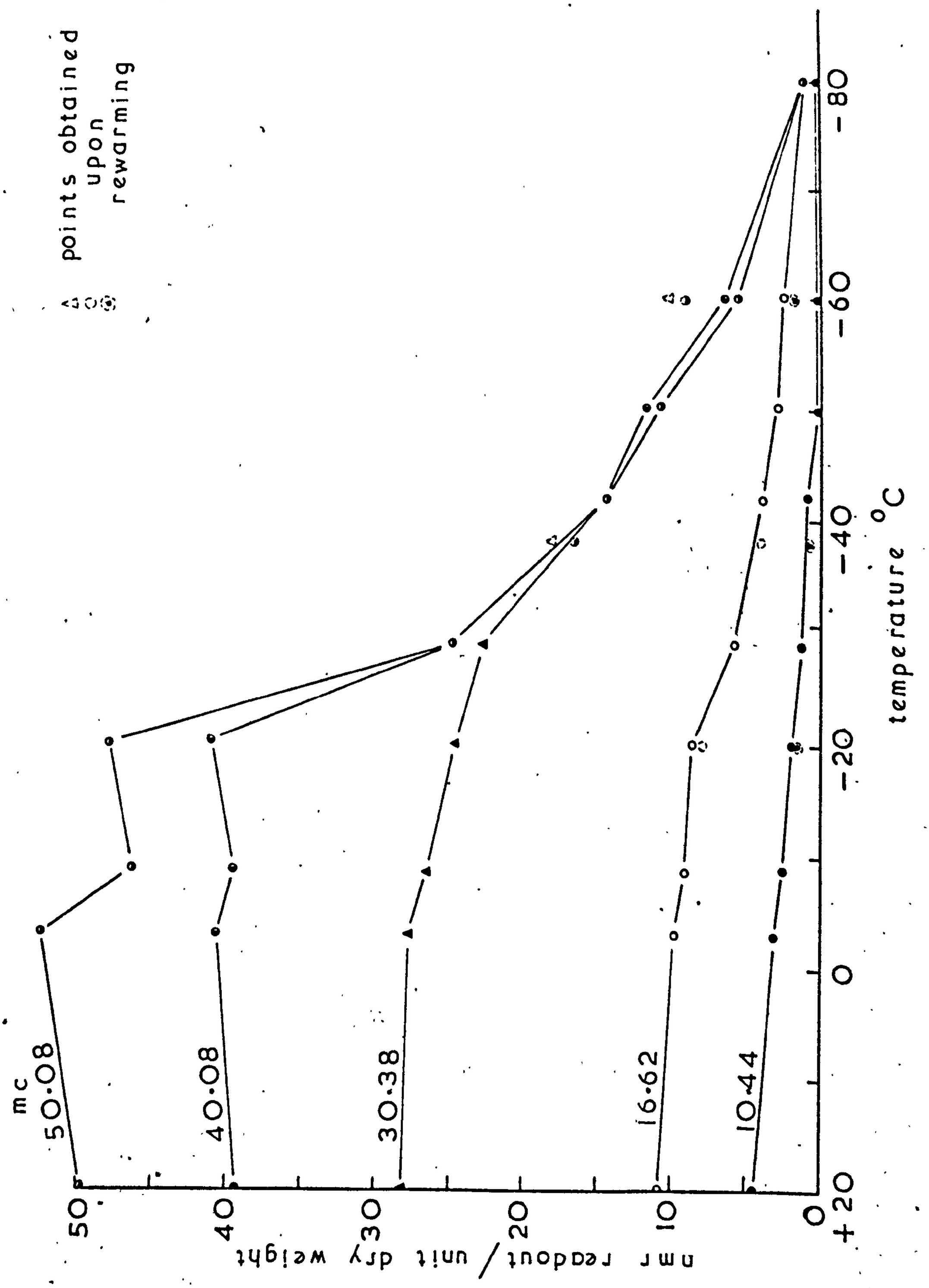

Fig. 34. Low temperature nuclear magnetic resonance examination of apple pectin hydrated to different moisture contents (mc)

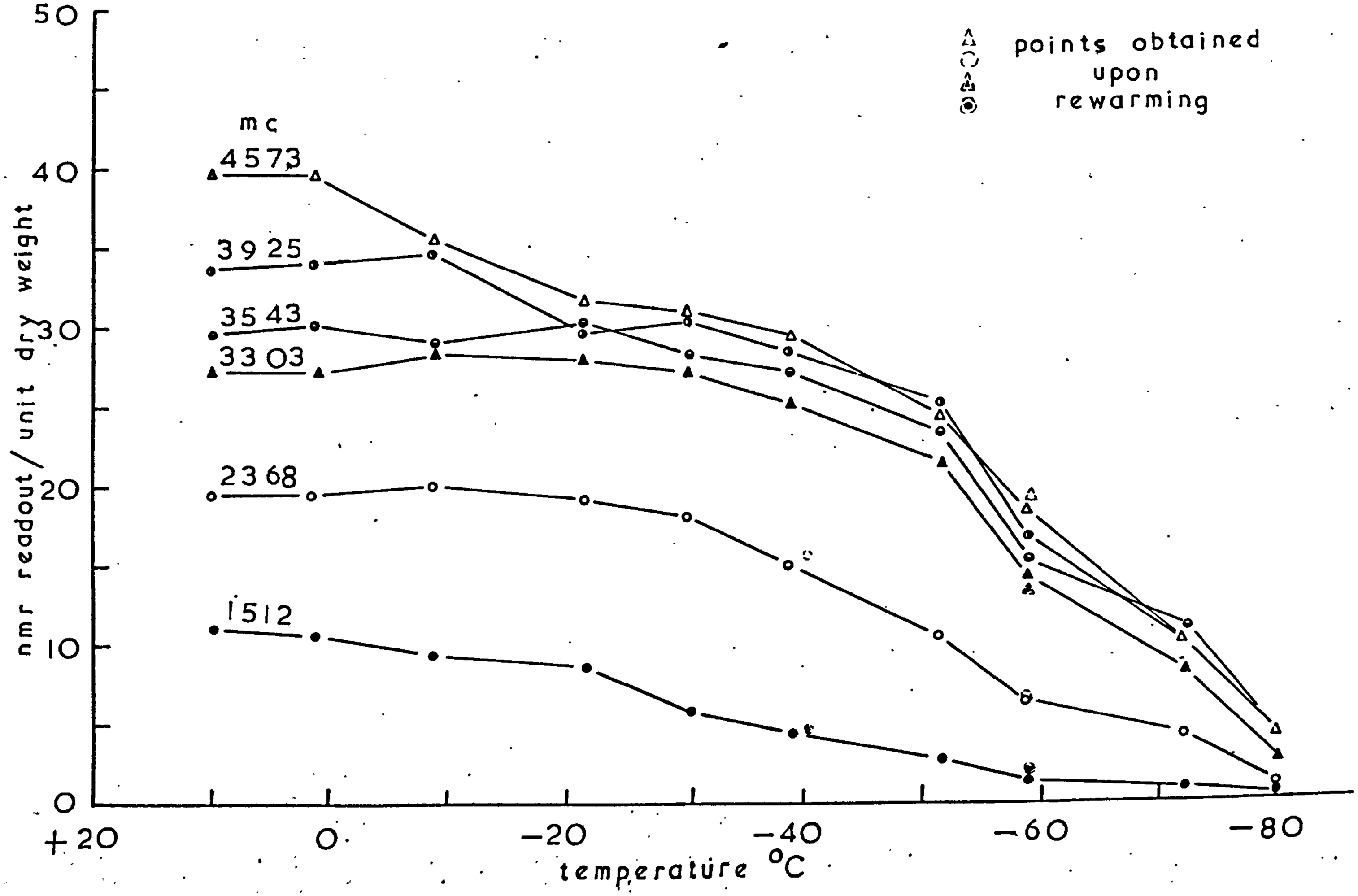

Fig. 35. Low temperature nuclear magnetic resonance examination of potato starch hydrated to different moisture contents (mc).

level, are shown in Figs. 33-35.

In all cases it is observed that the signals from samples above a certain moisture content become almost coincident at some point below 0°C. Above this temperature, signal size is dependent upon moisture content; below this point, however, the size of the common signal falls gradually with a further reduction in temperature to reach very low levels at -80°C.

The sharp reduction in signal size below zero is taken in each case to arise from the removal of readily-freezeable water in the form of ice.[130,131] Below the temperature of the virtual coincidence of the signals it is regarded that the non-freezing water is responsible for the energy absorption still recorded.

The accuracy with which an unfreezeable water content can be quoted, however, is dependent upon the increment of moisture contents used in the study, and upon the sensitivity of the instrument.

For cotton cellulose the signals from samples of a moisture content of approximately 20g/100g and above are almost coincident below -12°C.

For apple pectin the signals from samples of a moisture content of approximately 30g/100g and above are coincident below -28°C.

For potato starch the signals from samples of a moisture content of approximately 33g/100g and above are very similar below -21°C.

Samples with moisture contents below this critical level show no sharp reduction in signal size as the temperature is reduced through the main freezing zone.

The signal size is reduced gradually with a further reduction in temperature, the signal from all samples, irrespective of initial moisture content, coming to approximately the same low level at -80°C.

Upon subsequent rewarming from -80°C it can be observed that generally the signal size is almost identical to that obtained upon cooling, although in some cases the signal at a given temperature during rewarming is actually somewhat higher than that obtained at the same temperature during the cooling process. This indicates the reversible nature of the change which results in a reduction in signal size at low temperature.

The results from the various studies just reported, and values derived from them, are summarized in Table 9.

TABLE 9

WATER RELATIONSHIPS IN COTTON CELLULOSE, APPLE PECTIN AND POTATO STARCH

	Cotton Cellulose	Apple Pectin	Potato Starch
Calculated monomolecular layer by BET equation	2.66	11.65	11.13
Calculated monomolecular layer by Harkins-Jura equation	2.45	8.75	9.06
Moisture content at which Qs, reaches maximum	3.5	18.5	10.5
Moisture content at which Qs is approximately equal to heat of condensation of water	9.5	20.0	20.0
Unfreezeable water content by microscale DTA	<14.7	32.4-28.5	<32.70
Unfreezeable water content by low temperature NMR	<20	30	33

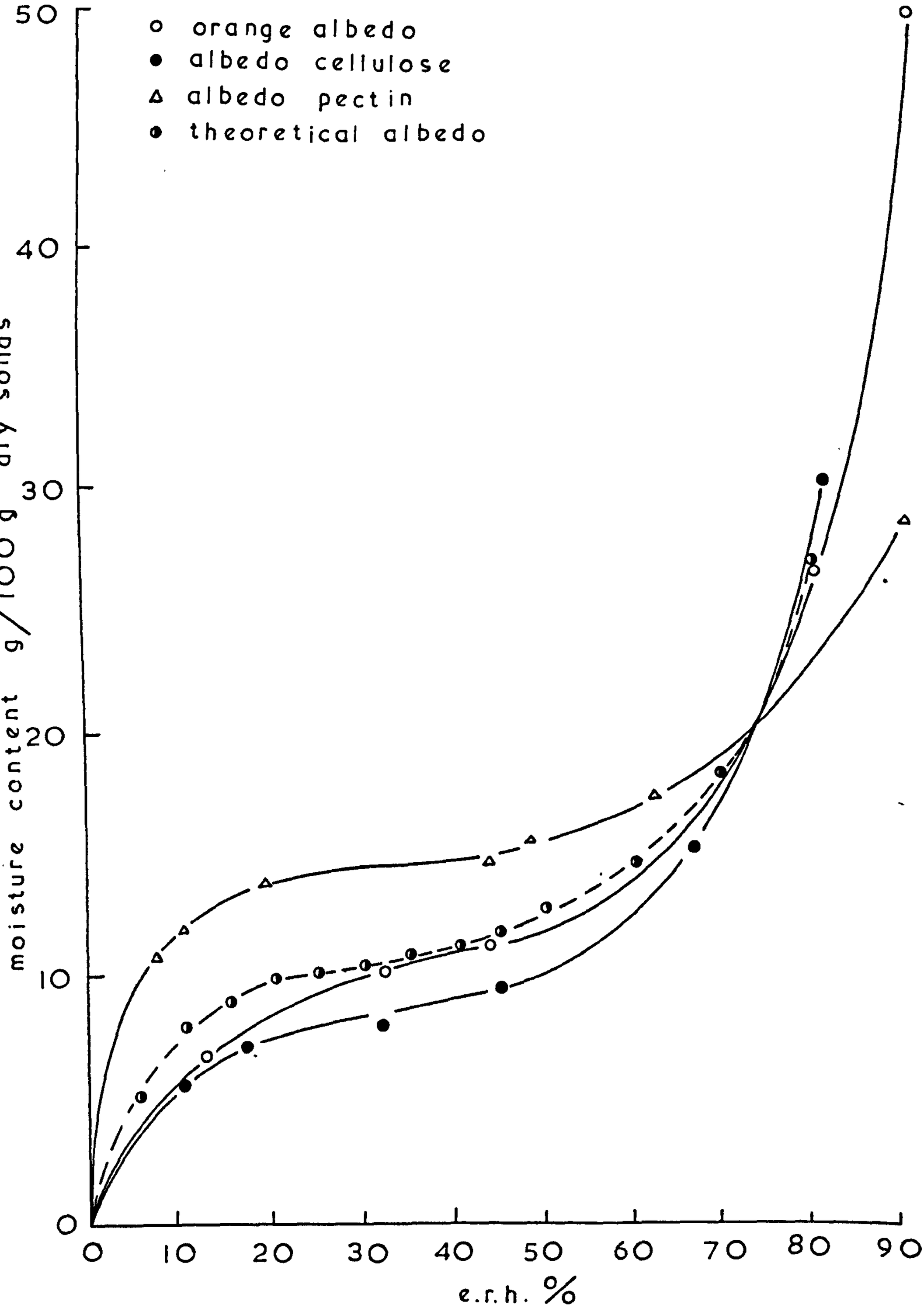

Fig. 36. Sorption isotherms for orange albedo and albedo pectin and cellulose prepared using the manometric method at 25°C. Also shown is a theoretical albedo isotherm constructed on a proportional basis from the cellulose and pectin isotherms.

7.0 THE WATER RELATIONS OF ORANGE ALBEDO AND ITS TWO MAJOR MACROMOLECULAR CONSTITUENTS

The hydration characteristics of orange albedo and of the pectin and cellulose fractions prepared from it were examined by the preparation of sorption isotherms using the manometric method; by micro-scale differential thermal analysis; and by nuclear magnetic resonance at low temperatures.

7.1 SORPTION STUDIES

The sigmoid desorption isotherms obtained at 25°C for orange albedo, and albedo pectin and "cellulose" are shown in Fig. 36.

Also illustrated is a theoretical isotherm constructed on a proportional basis from the isotherms of the "cellulose" and pectin components of the albedo. These were found to constitute 37.0% and 18.7% of the total albedo on a dry weight basis, the "cellulose" fraction probably containing a range of carbohydrate material as well as pure cellulose. Kefford[349] reports a detailed analysis of albedo from Navel oranges as yielding 19.84% total pectin and 29.18% remaining non-soluble carbohydrate material which includes cellulose and hemicellulose.

As in the case of the commercially obtained pure constituents there is a considerable difference between the isotherms for cellulose ahd pectin. The theoretical albedo isotherm, in which the soluble solids present are not taken into account, runs just above the actual albedo isotherm over nearly the complete ERH range illustrated.

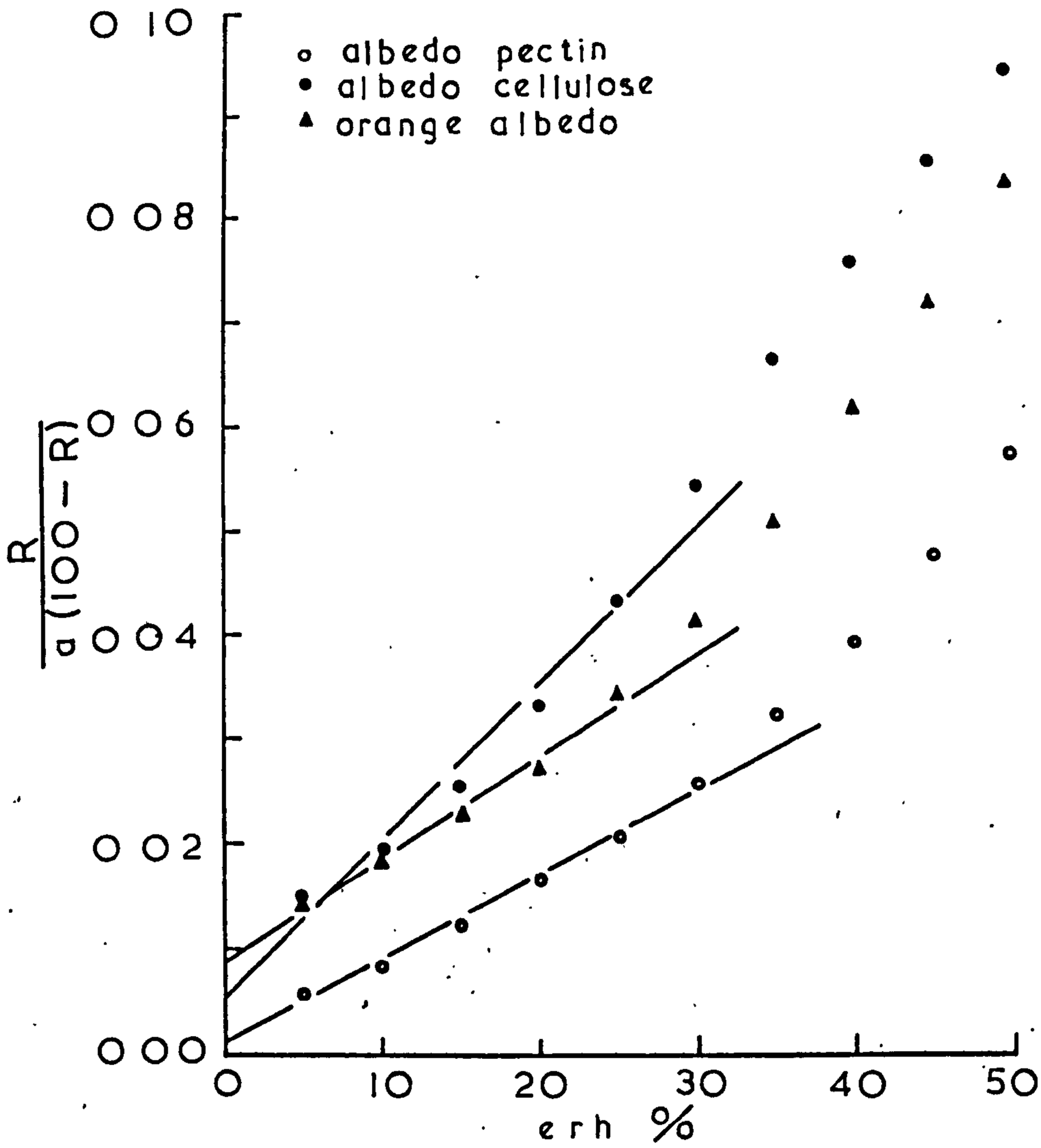

Fig. 37. BET plots on sorption data of orange albedo, orange cellulose, and orange pectin.

Sorption data determined for orange albedo, orange cellulose and orange pectin and to which Salwin's modification of the BET isotherm equation (Eq. 4) has been applied are illustrated in Fig. 37.

It can be seen that the points fall on straight lines over the ERH ranges of 5% to 25%, 10% to 25% and 5% to 30%, respectively.

Calculated values for a, the moisture content corresponding to the completion of a monomolecular layer, are 9.43g/100g, 6.06g/100g and 12.35g/100g, respectively.

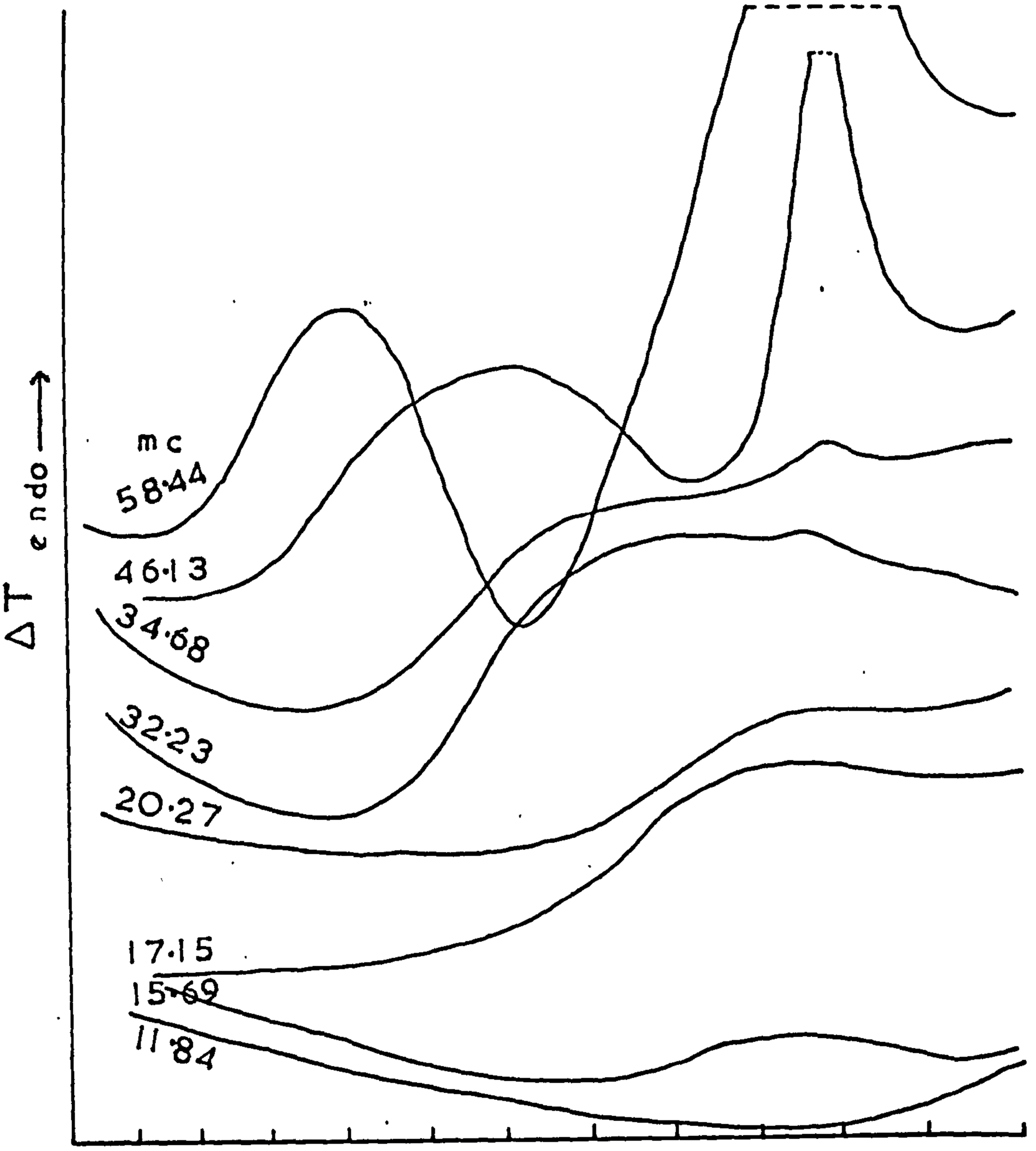

Fig. 38. Differential thermal analysis on the micro-scale of orange albedo hydrated to different moisture contents (mc).

7.2. DIFFERENTIAL THERMAL ANALYSIS

Fig. 38 shows the series of curves obtained from the examination of orange albedo, by differential thermal analysis on the micro-scale.

In the samples of ~~a~~ higher moisture content a distinct endothermic peak is visible in the temperature range between -40°C and -20°C, the apex of the peaks occurring at about -25°C. As previously explained when examining the results from the study of pure plant constituents this peak is attributable to the thawing of ice formed from free solvent water.

The moisture content at which the presence of freezing water can no longer be detected is taken to lie below 32.23g/100g dry matter.

An additional thermal effect occurring at a lower temperature than the thawing peak of the free solvent water is again clearly distinguishable at levels of hydration above the unfreezeable water content. This 'hump' occurs at a progressively lower temperature with an increase in moisture content.

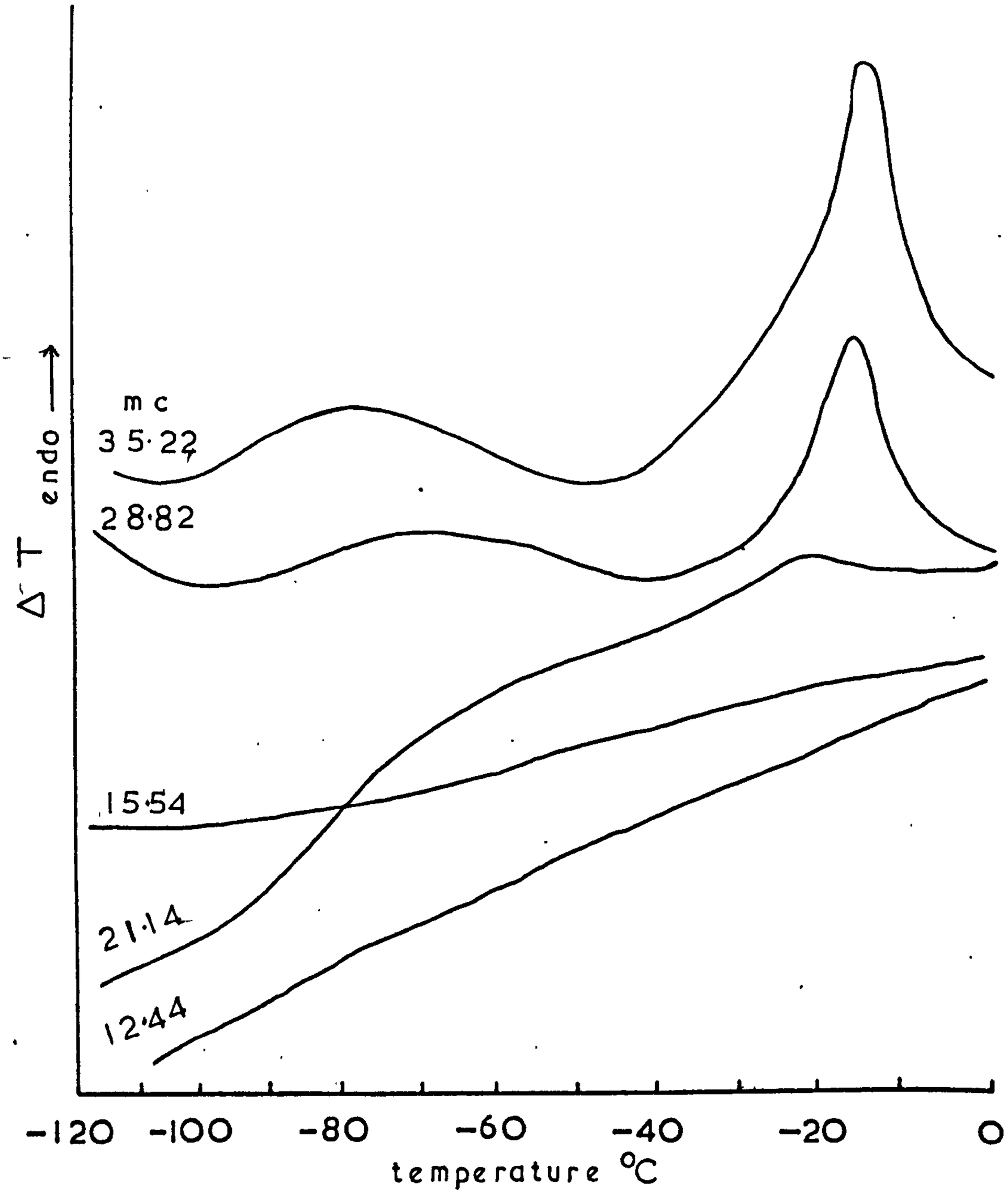

Fig. 39. Differential thermal analysis on the micro-scale of orange albedo cellulose hydrated to different moisture contents (mc).

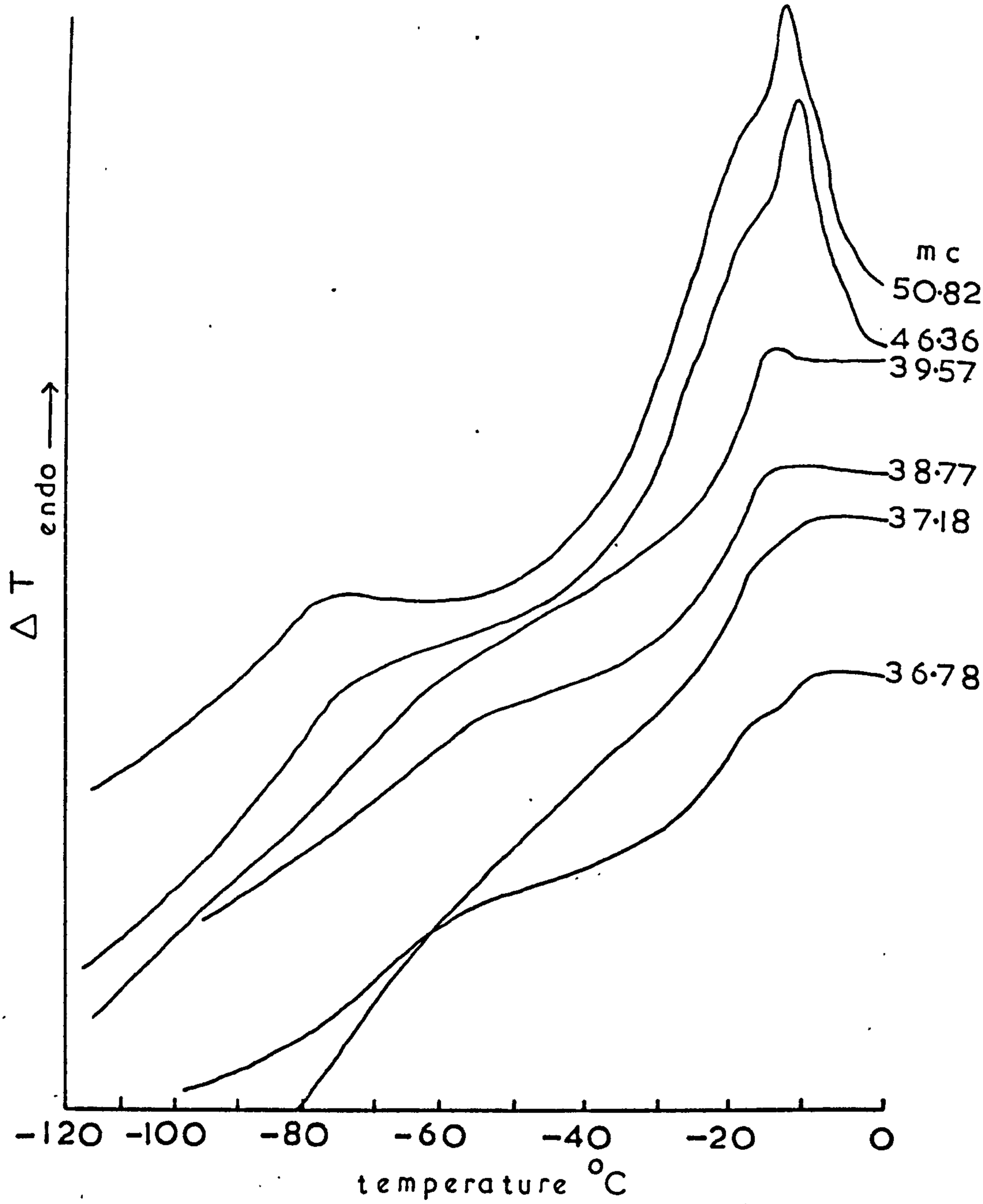

Fig. 40. Differential thermal analysis on the micro-scale of orange albedo pectin hydrated to different moisture contents (mc).

In Figs. 39 and 40 the curves obtained during a similar differential thermal analysis of orange cellulose and orange pectin are presented.

The moisture contents at which the presence of freezing water can no longer be detected is between 21.13g/100g and 15.54g/100g in the case of the cellulose, and just below 36.78g/100g in the case of the pectin.

In addition, the appearance of a 'hump' at moisture contents above the unfreezeable water level is again demonstrated.

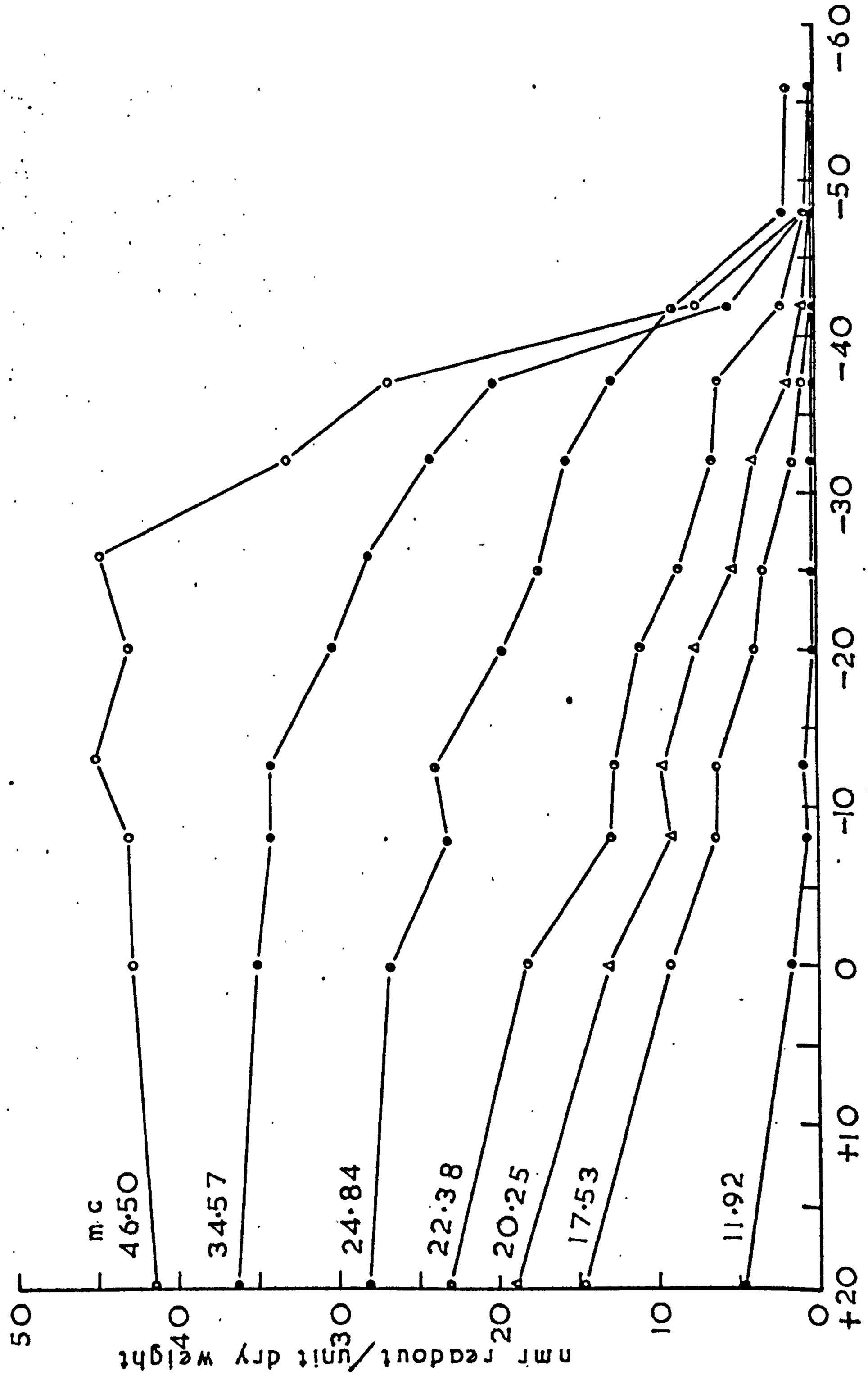

Fig. 41. Low temperature nuclear magnetic resonance examination of whole orange albedo hydrated to different moisture contents (mc)

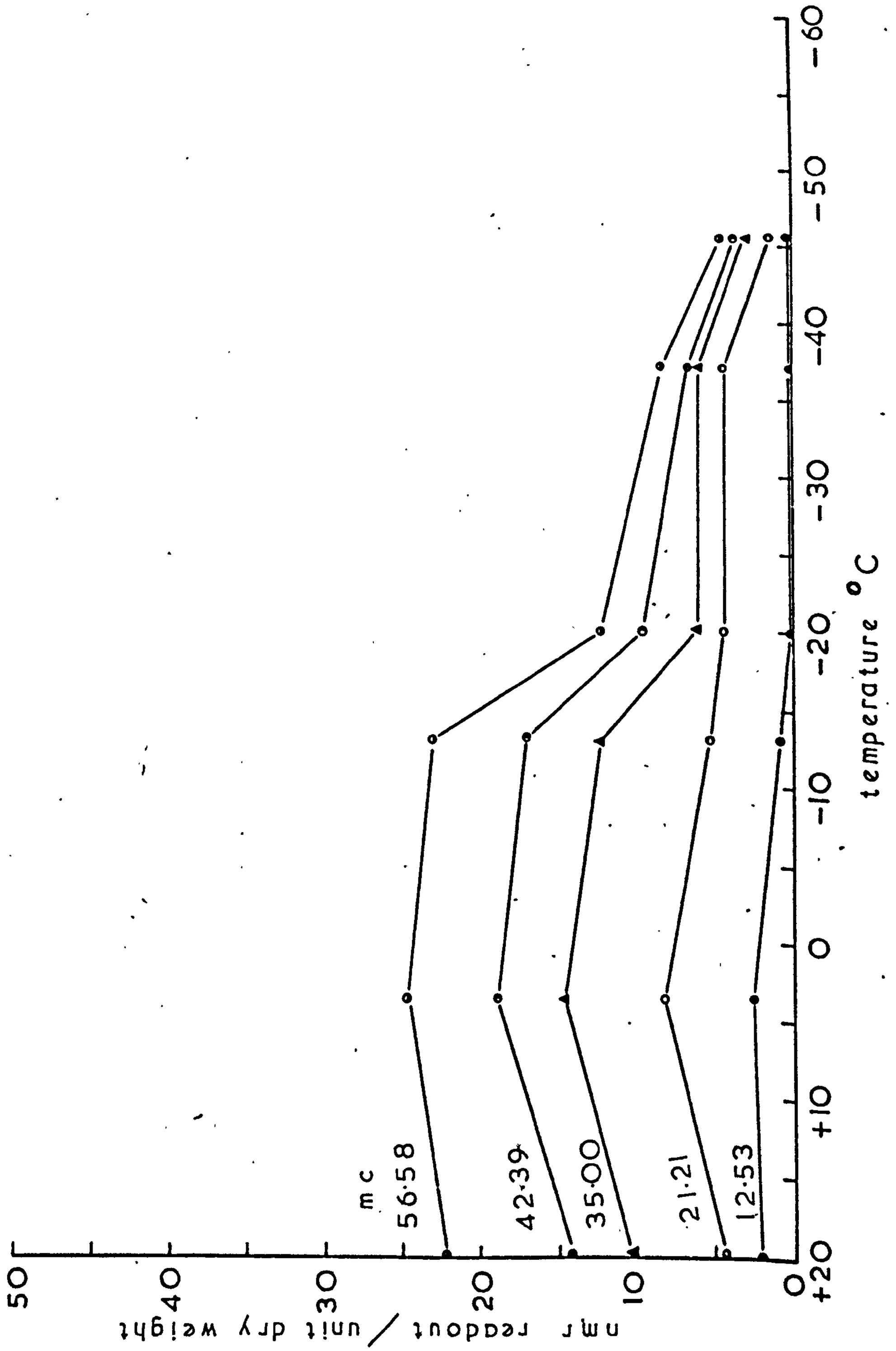

Fig. 42. Low temperature nuclear magnetic resonance examination or orange albedo cellulose hydrated to different moisture contents (mc).

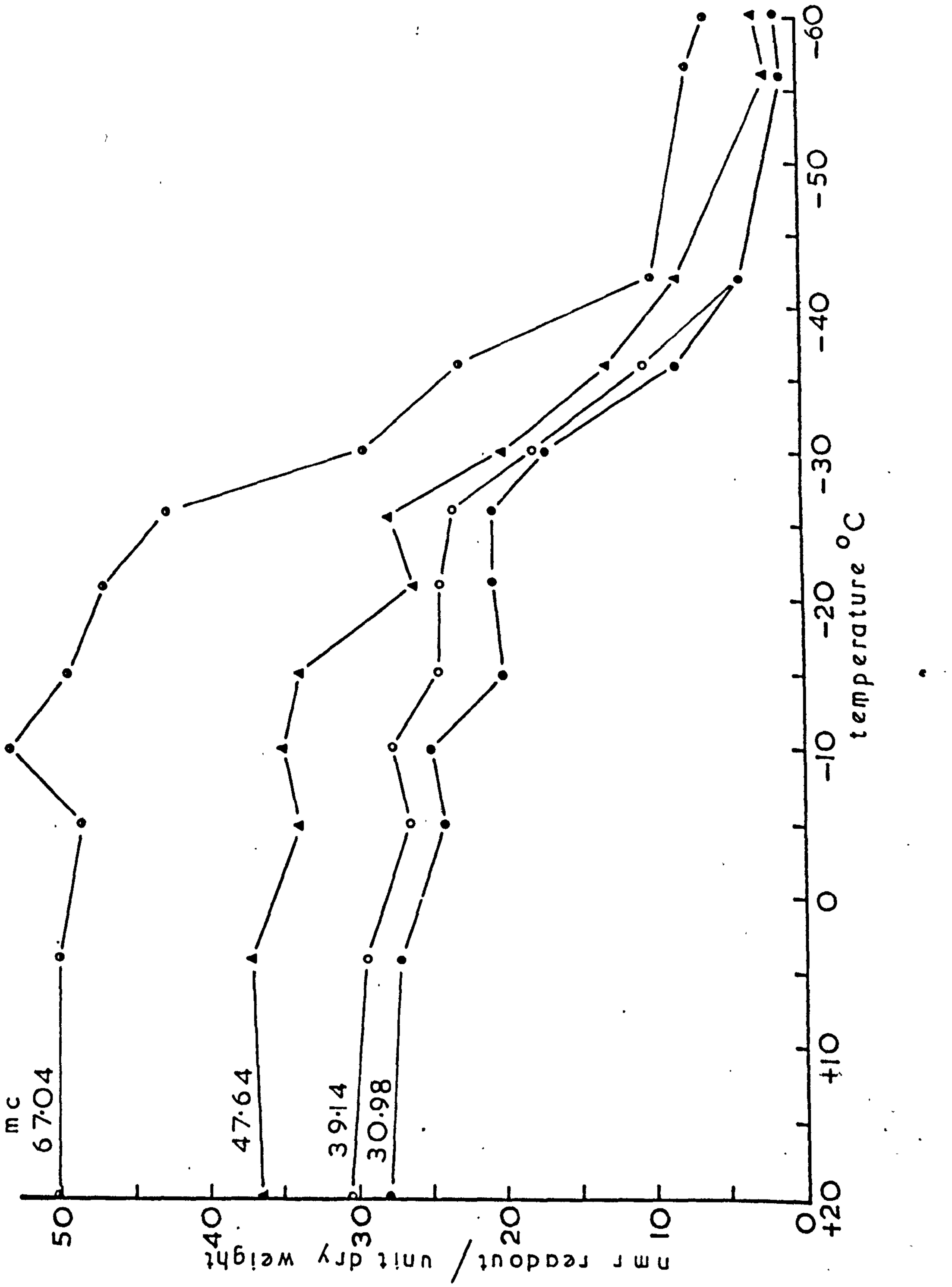

Fig. 43. Low temperature nuclear magnetic resonance examination of orange albedo pectin hydrated to different moisture contents (mc).

7.3 NUCLEAR MAGNETIC RESONANCE

Low resolution NMR was used to examine orange albedo, orange cellulose and orange pectin as the material was cooled from ambient temperature to -45°C or to -60°C. These materials were hydrated to a range of moisture contents encompassing the expected unfreezeable water content and the curves obtained during the examination are illustrated in Figs. 41-43.

In each case it can be observed that the signals from samples above a certain moisture level ~~become virtually coincident at~~ show a similar downward trend below a characteristic sub-zero temperature, but that above this point initial moisture content determines signal size. This behaviour is similar to that observed during the low temperature NMR examination of the pure plant constituents.

For orange albedo the signals from samples of a moisture content of approximately 25g/100g dry matter and above run close together below -40°C.

For orange cellulose the signals from samples of a moisture content of approximately 21g/100g dry matter and above ~~are similar below -20°C. It is not possible to say that these are coincident in this case.~~ show a similar downward trend below -20°C, while, in this case, being far from coincident.

For orange pectin the signals from samples of a moisture content of approximately 30g/100g dry matter and above are similar below -26°C.

Below the particular temperature quoted in each case the signal falls gradually with further reduction in temperature to reach a value similar to that achieved by samples with a moisture content below the unfreezeable water level when cooled to the same temperature. There is no sharp reduction in signal size as these

latter samples are cooled through the main freezing zone, such as would be consistent with the rapid freezing of free water.

The results from the application of the three methods of examining water relations used in the study of orange albedo, orange cellulose and orange pectin are summarized in Table 10.

TABLE 10

WATER RELATIONSHIPS IN ORANGE ALBEDO, ORANGE CELLULOSE AND ORANGE PECTIN

	Orange Albedo	Orange Pectin	Orange Cellulose
Calculated monomolecular layer by BET equation	9.43	12.25	6.06
Unfreezeable water content by micro-scale DTA	32.23	just below 36.78	21.13-15.54
Unfreezeable water content by low temperature NMR	25	30	21

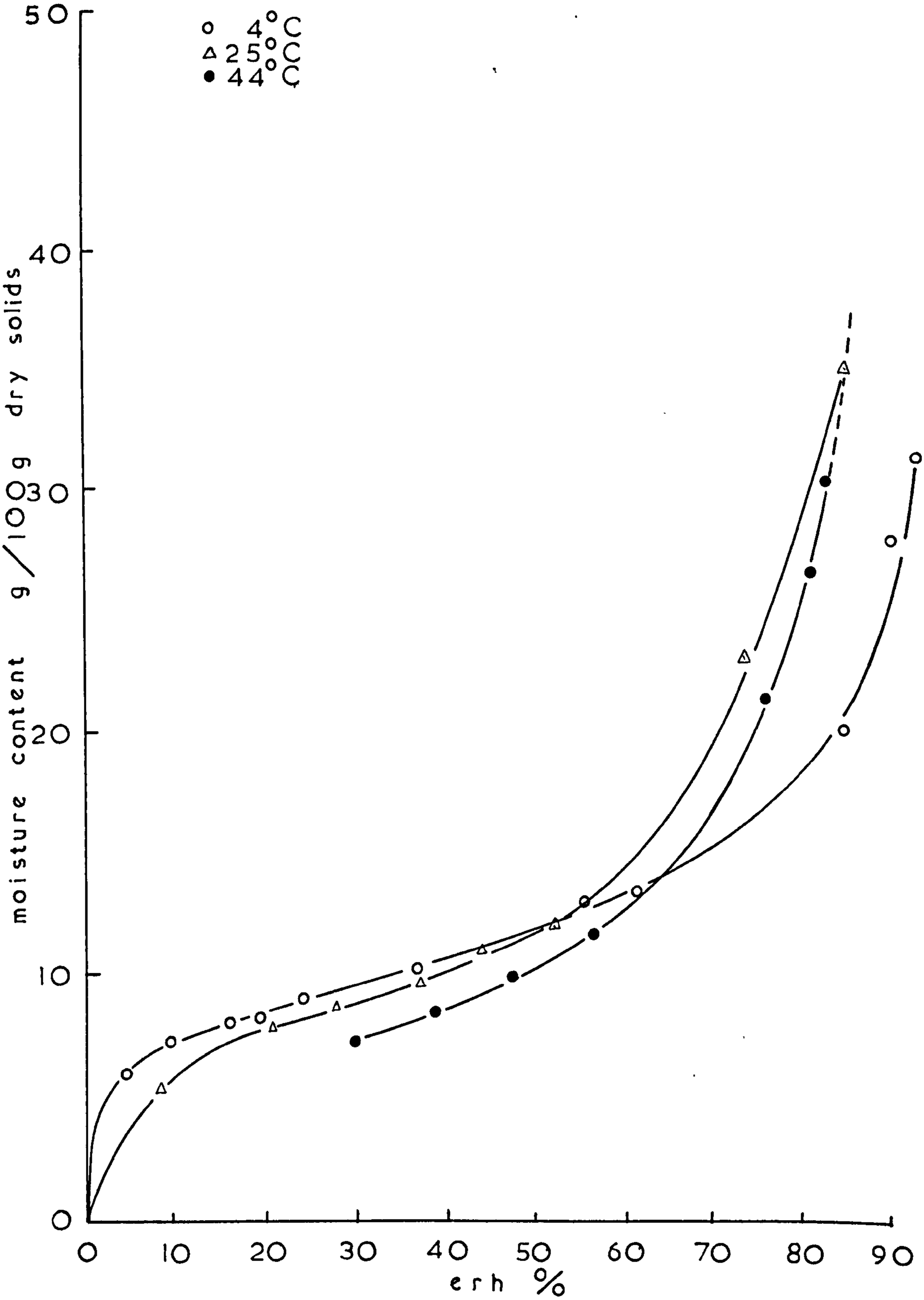

Fig. 44. Sorption isotherms for Whole Pentland Dell potato prepared using the desiccator method at 4°C, 25°C and 44°C.

8.0 THE WATER RELATIONS OF WHOLE POTATO TISSUE AND THE ROLE PLAYED BY ITS CONSTITUENTS

The hydration characteristics of the potato were examined by the preparation of sorption isotherms and by both micro and macro-scale differential thermal analysis. A qualitative analysis of the role of the major constituents of the tuber in determining its overall water binding properties was also attempted.

Sorption studies were conducted on two different potato varieties, Pentland Dell and Red Craigs Royal, and on their three major macromolecular constituents, starch, pectin and cellulose. Pentland Dell is known to have a high insoluble pectin content[347] and it was suggested[348] that Red Craigs Royal might be expected to be different in terms of cell wall composition from a variety high in insoluble pectin.

In addition, the fractions obtained from the stepwise degradation of whole Pentland Dell, as outlined in Fig. 15 (p.125), were examined by the preparation of sorption isotherms and by both micro and macro-scale differential thermal analysis.

8.1 SORPTION STUDIES.

Fig. 44 illustrates the sorption isotherms prepared for Whole Pentland Dell potato at 4°C, 25°C and 44°C using the desiccator method.

Below an ERH of 57% it can be seen that at 44°C the material sorbs less water at any given ERH than it does at lower temperatures, or, at a given moisture content, the material possesses a higher equilibrium relative humidity than at a lower temperature. Above a moisture content of approximately 11g/100g an apparent reversal of this effect shows itself, although as in the case of the pure plant constituents examined in Section 1.0 the water at this higher temperature still exerts a greater vapour pressure.

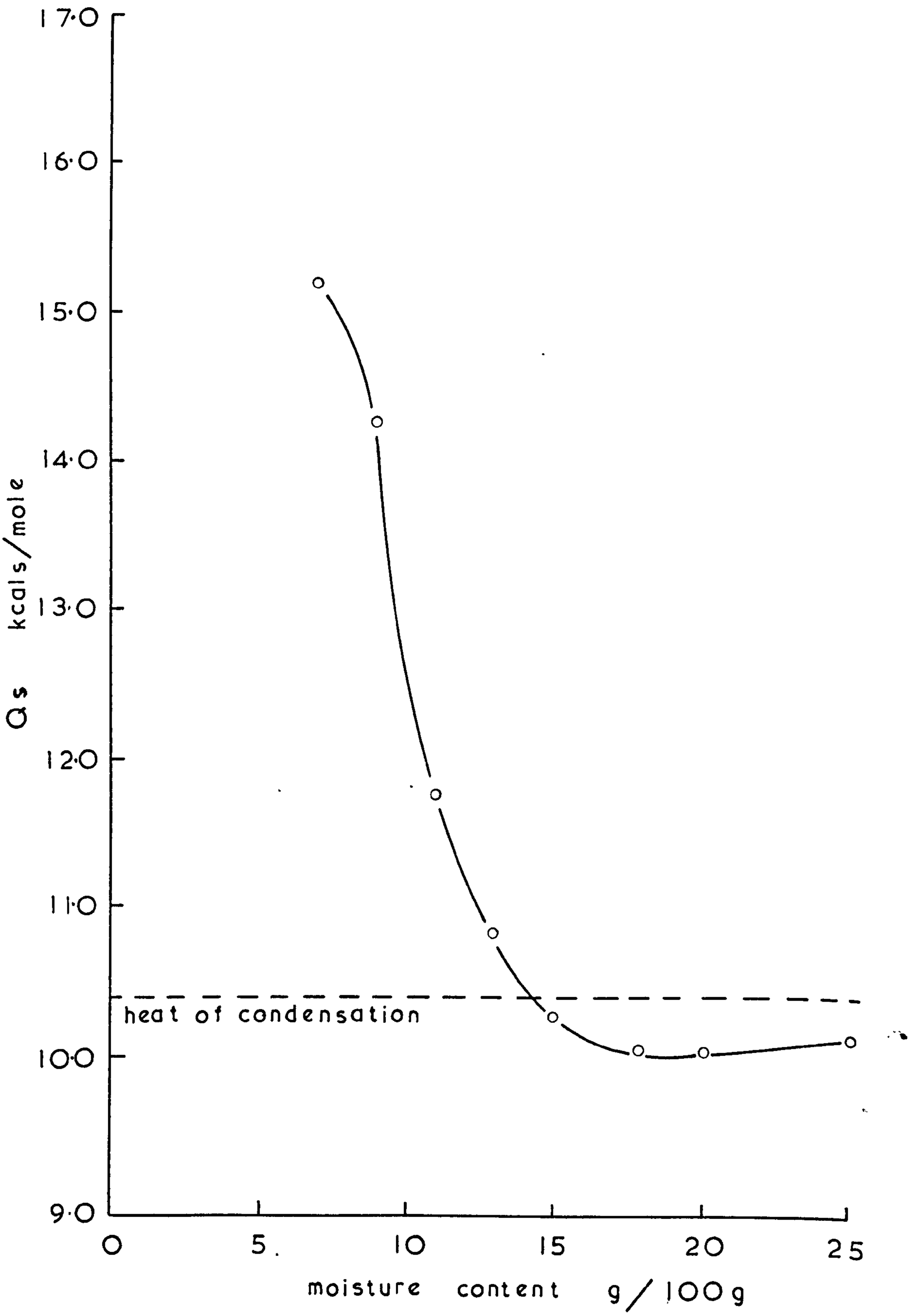

Fig. 45. Change in the heat of association, Qs, with moisture content for Whole Pentland Dell potato.

The change in the heat of association, Qs, as a function of moisture content is illustrated for Whole Pentland Dell in Fig. 45. The sorption data obtained at 4°C and 44°C were used to derive the plotted values for Qs by application of the Clausius-Clapeyron equation (Eq.45).

Lack of data obtained at 44°C does not allow the curve to be continued to complete dryness, but it can be seen that with an increase in hydration from an initial low level there is a decrease in the heat of association until it reaches a value close to the heat of condensation of water at 18g/100g dry matter.

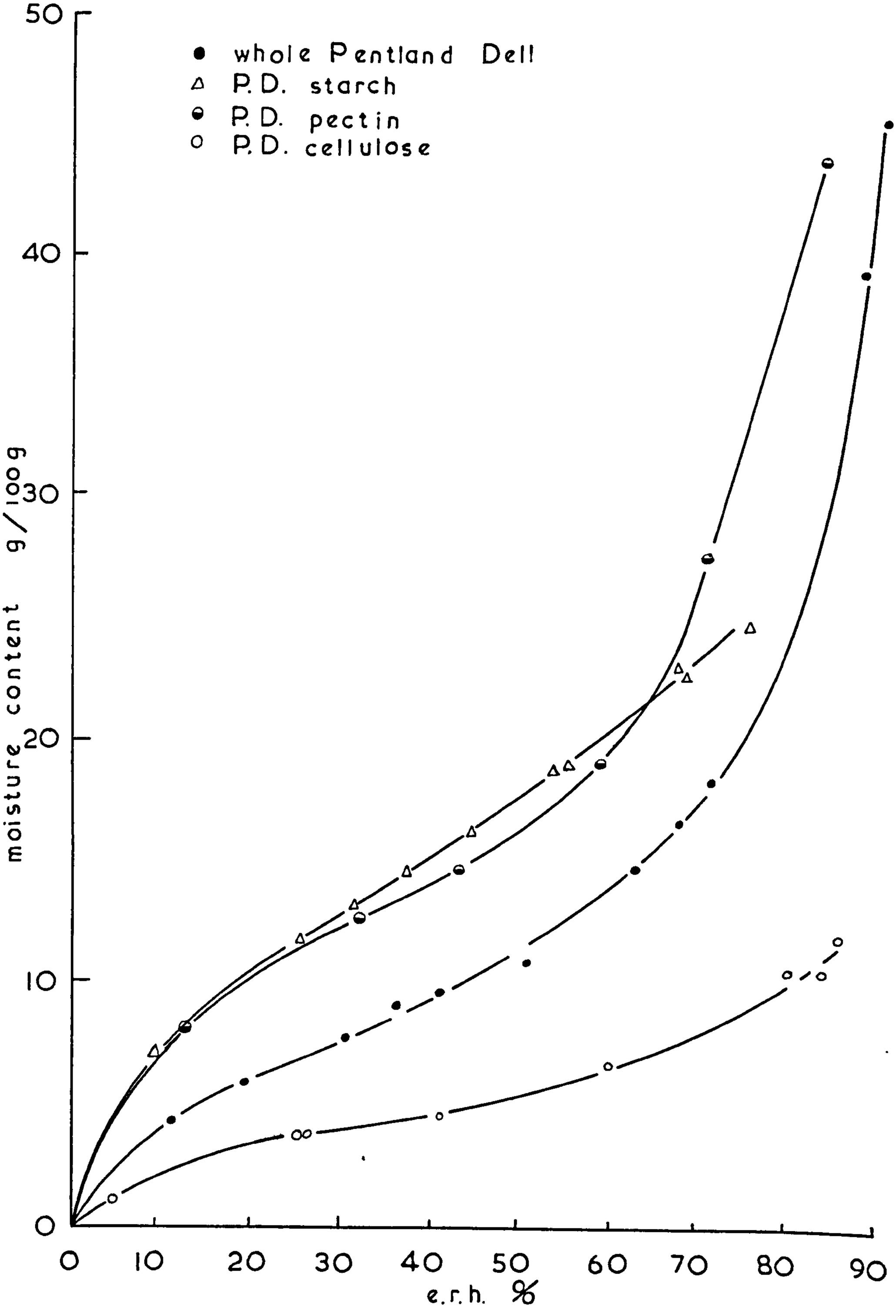

Fig. 46. Sorption isotherms for Whole Pentland Dell potato, and Pentland Dell starch, pectin and cellulose, prepared using the manometric method at 25°C.

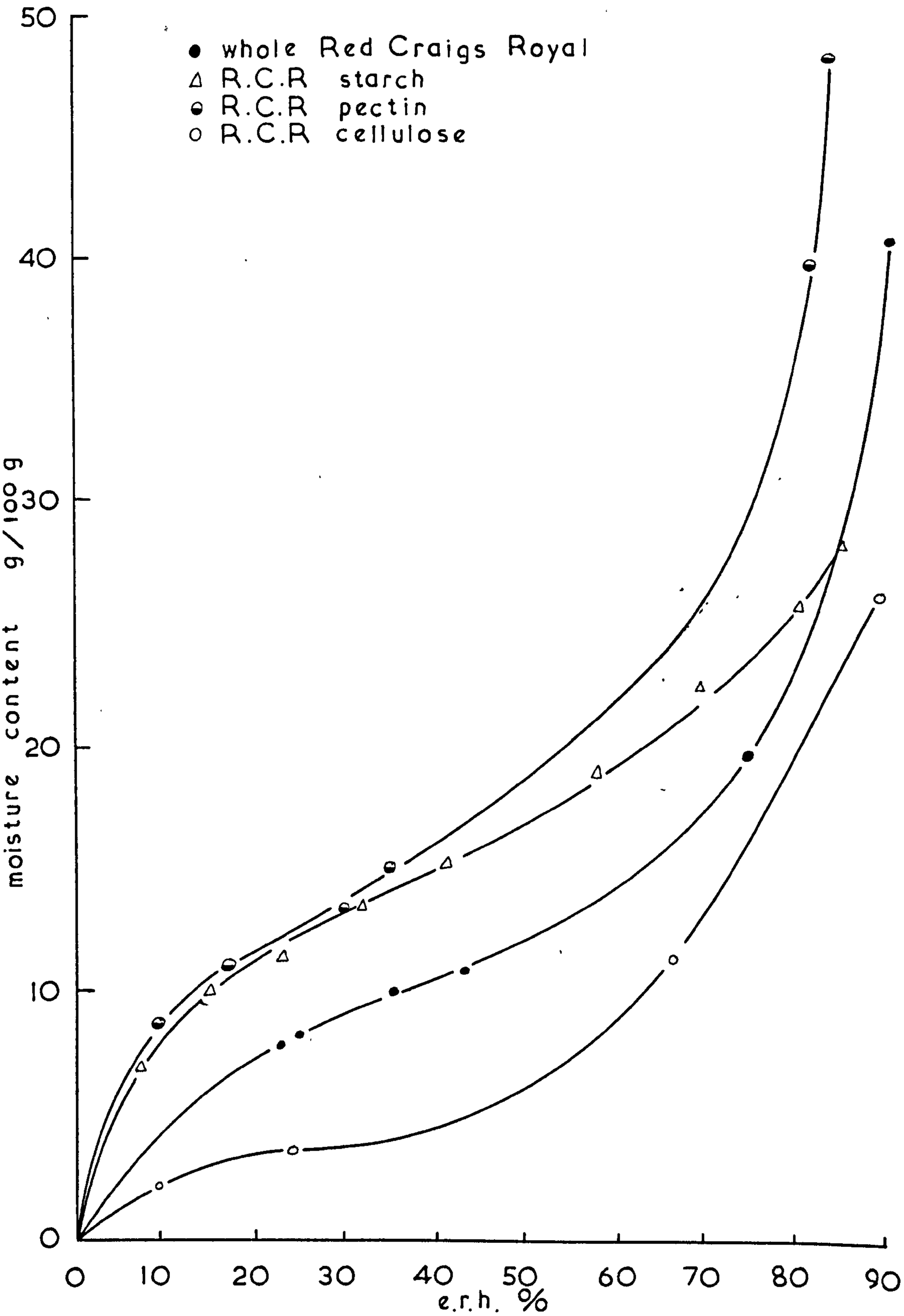

Fig. 47. Sorption isotherms for Whole Red Craigs Royal, and Red Craigs Royal starch, pectin and cellulose, prepared using the manometric method at 25°C.

The sorption isotherms determined at 25°C for each of the two varieties of potato and for the starch, pectin and cellulose fractions prepared from each of them are illustrated in Figs. 46 and 47.

In both cases the cellulose material sorbed considerably less water at any given ERH than either of the other two macromolecular constituents or than that associated with the intact potato.

The pectin fraction absorbed more water than the whole potato over the entire equilibrium relative humidity range illustrated.

It can be observed that the starch has very similar sorptive properties to the pectin at low ERH's but that above 40% ERH in the case of Red Craigs Royal, and 60% ERH in the case of Pentland Dell, the isotherms for the two materials diverge, starch sorbing less water than pectin at any given ERH.

Comparing the isotherms for the corresponding material from each of the two varieties of potato, it can be seen that these are similar over the complete ERH range shown. Whole Red Craigs Royal and its major constituents have a slightly higher level of hydration at any given ERH.

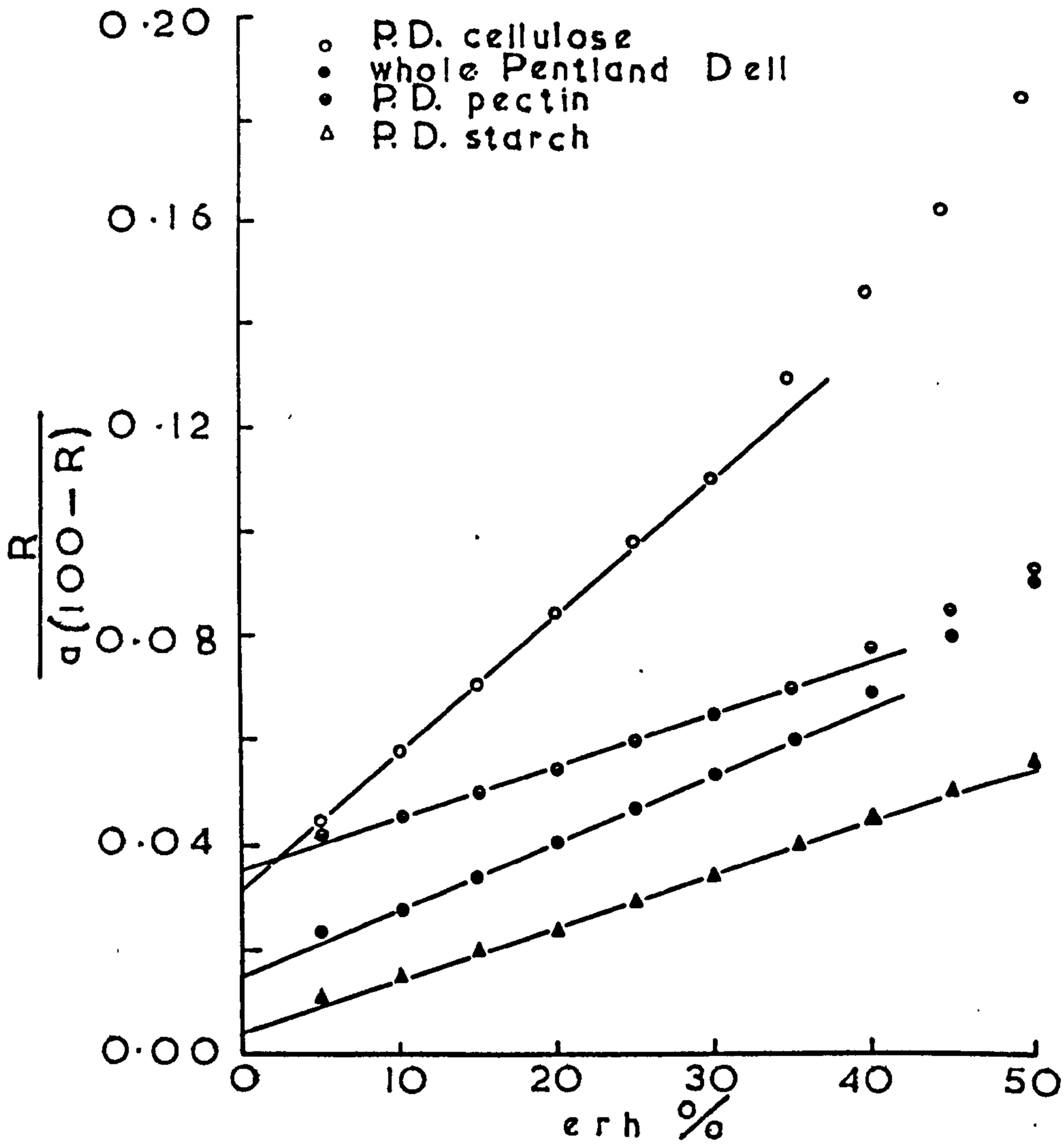

Fig. 48. BET plots on sorption data of Whole Pentland Dell and Pentland Dell starch, cellulose and pectin. Pectin plots are displaced by 0.03 ordinate units to allow greater clarity.

The application of Salwin's modification of the BET isotherm equation (Eq. 4) to the sorption data presented in Fig. 46 is shown in Fig. 48.

Calculated monomolecular layers and the ERH range over which the plots describe a straight line are given below.

	Range of Applicability % ERH	Value for Calculated Monomolecular layer g/100g dry matter
Whole Potato	10 - 35	7.14
Potato Starch	10 - 45	9.52
Potato Pectin	10 - 35	9.83
Potato Cellulose	5 - 30	3.42

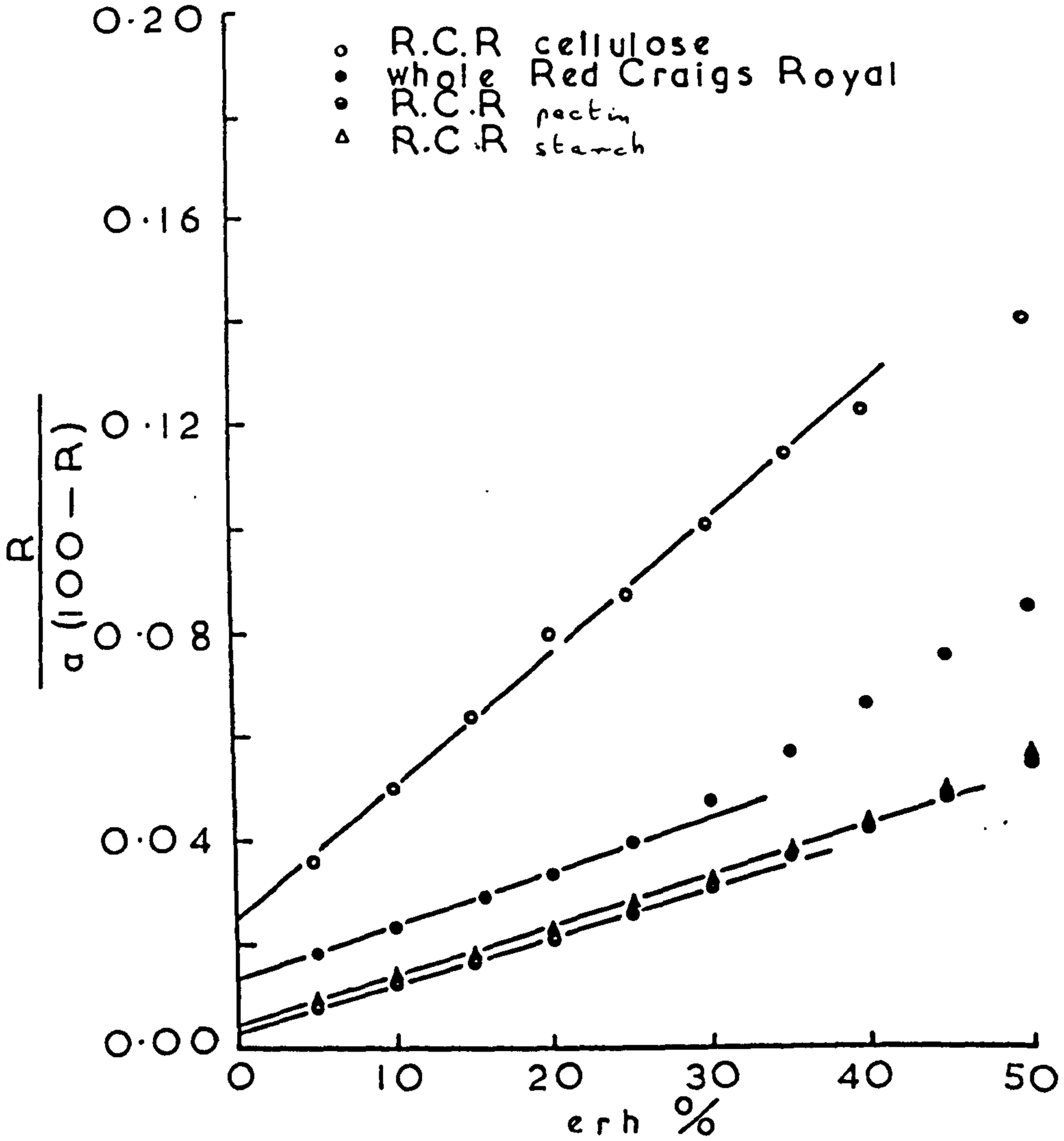

Fig. 49. BET plots on Whole Red Craigs Royal and Red Craigs Royal starch, cellulose and pectin.

The application of Salwin's modification of the BET isotherm equation (Eq. 4) to the sorption data for Red Craigs Royal and its macromolecular constituents is shown in Fig. 49.

Calculated monomolecular layers and the ERH range over which the plots describe a straight line are given below.

	Range of Applicability % ERH	Value for Calculated Monomolecular Layer g/100g dry matter
Whole Potato	5 - 25	8.62
Potato Starch	5 - 40	10.07
Potato Pectin	5 - 35	9.94
Potato Cellulose	5 - 35	3.62

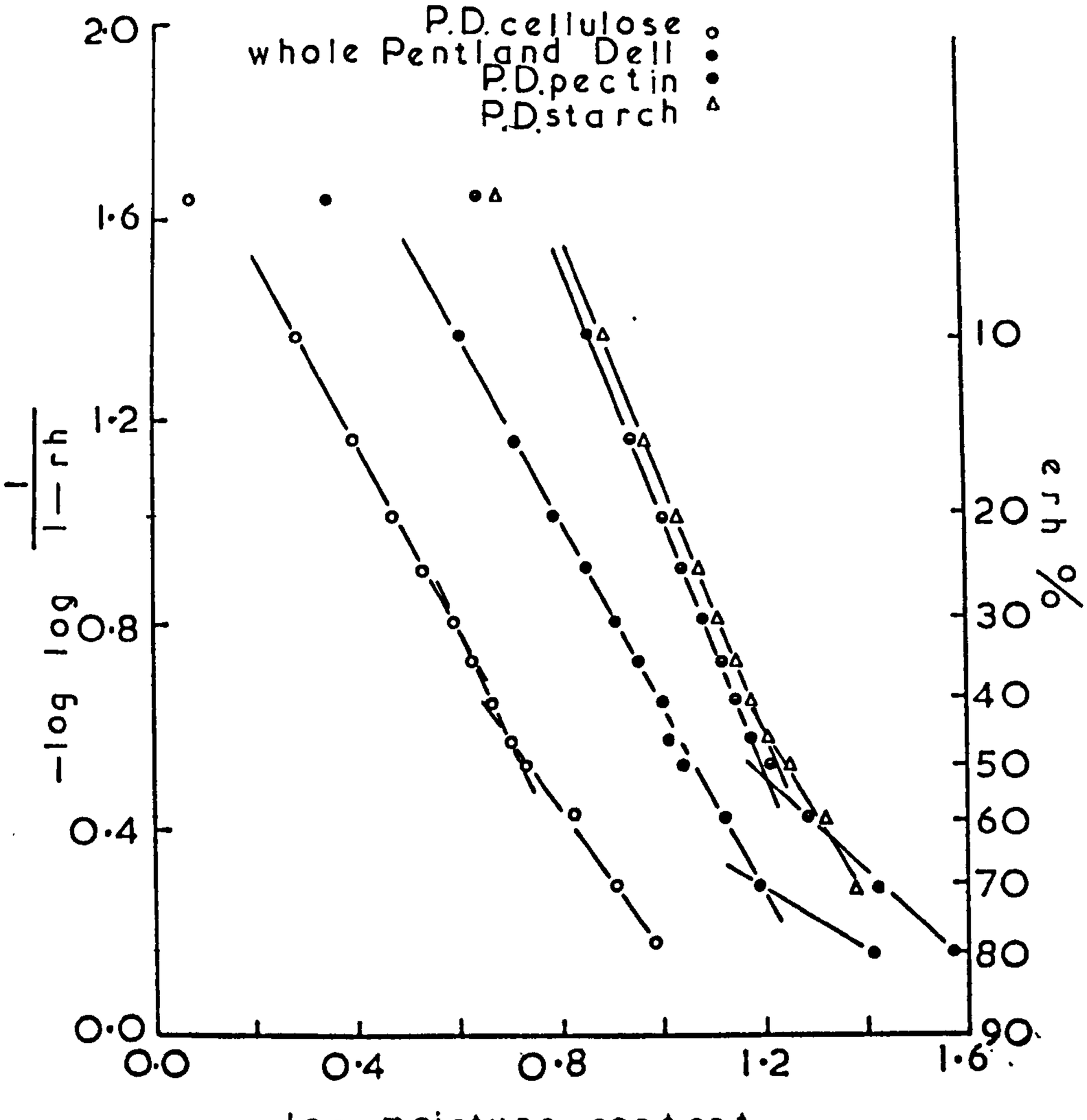

Fig. 50 Henderson plots on sorption data of Pentland Dell starch, pectin, cellulose and whole potato.

The application of Rockland's modification of Henderson's isotherm equation (Eq. 12A) to the sorption data determined for whole Pentland Dell and for its starch, pectin and cellulose constituents is illustrated in Fig. 50.

When the points plotted for either whole potato or for the pectin or starch fractions are joined, two intersecting straight lines result. The intercepts of these lines occur at points on the ordinate equivalent to ERHs of 70%, 53%, and 45%, respectively.

In the case of cellulose it seems reasonable to draw three straight lines through the plots, although it is perhaps debatable whether the upper two lines could be better represented as a single line. The resulting points of intersection occur at an ERH of 30% and of 45%.

The absence of a third straight line at a low ERH in the case of the starch and pectin plots, when compared to those for the commercially obtained materials as shown in Fig. 25, reflects the smoother shape of the isotherms for the former materials and the absence of an obvious inflection point at these ERH levels, such as would result in an intercept between two straight lines on a Henderson plot. These smooth curves can be expected on a desorption isotherm in materials which exhibit hysteresis rather than the better defined sigmoid curve obtained upon absorption.

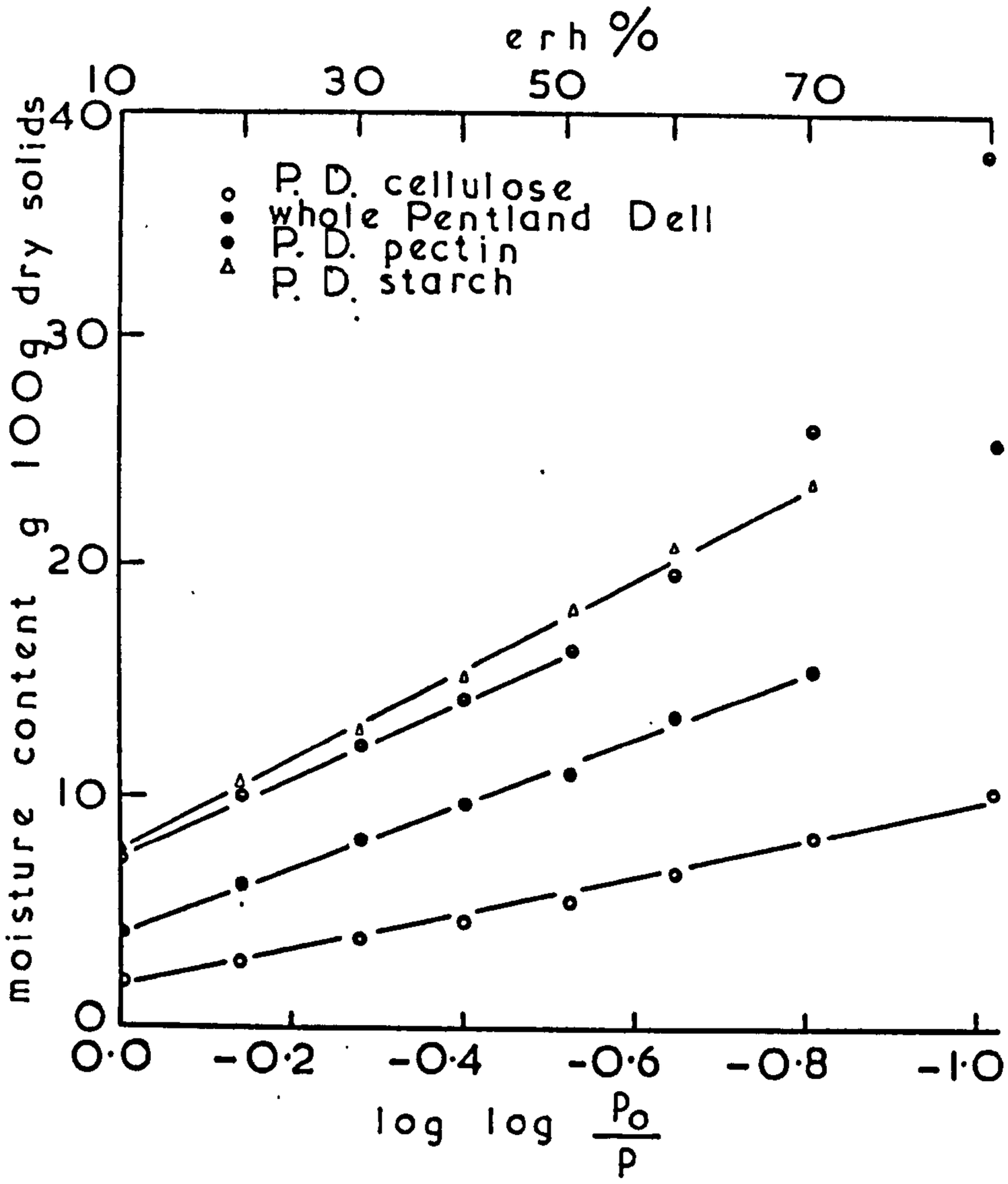

Fig. 51. Bradley plots on sorption data of Pentland Dell starch, pectin, cellulose and whole potato.

Fig. 51 illustrates the application of Bradley's isotherm equation (Eq. 6) to the sorption data prepared for whole Pentland Dell and Pentland Dell pectin, starch and cellulose.

In all cases, a straight line can be drawn through the points for any one material over the lower humidity range. This extends to 50% ERH in the case of pectin, to 70% in the case of whole potato, to 80% in the case of cellulose, and over the complete range (0% to 70%) in the case of starch.

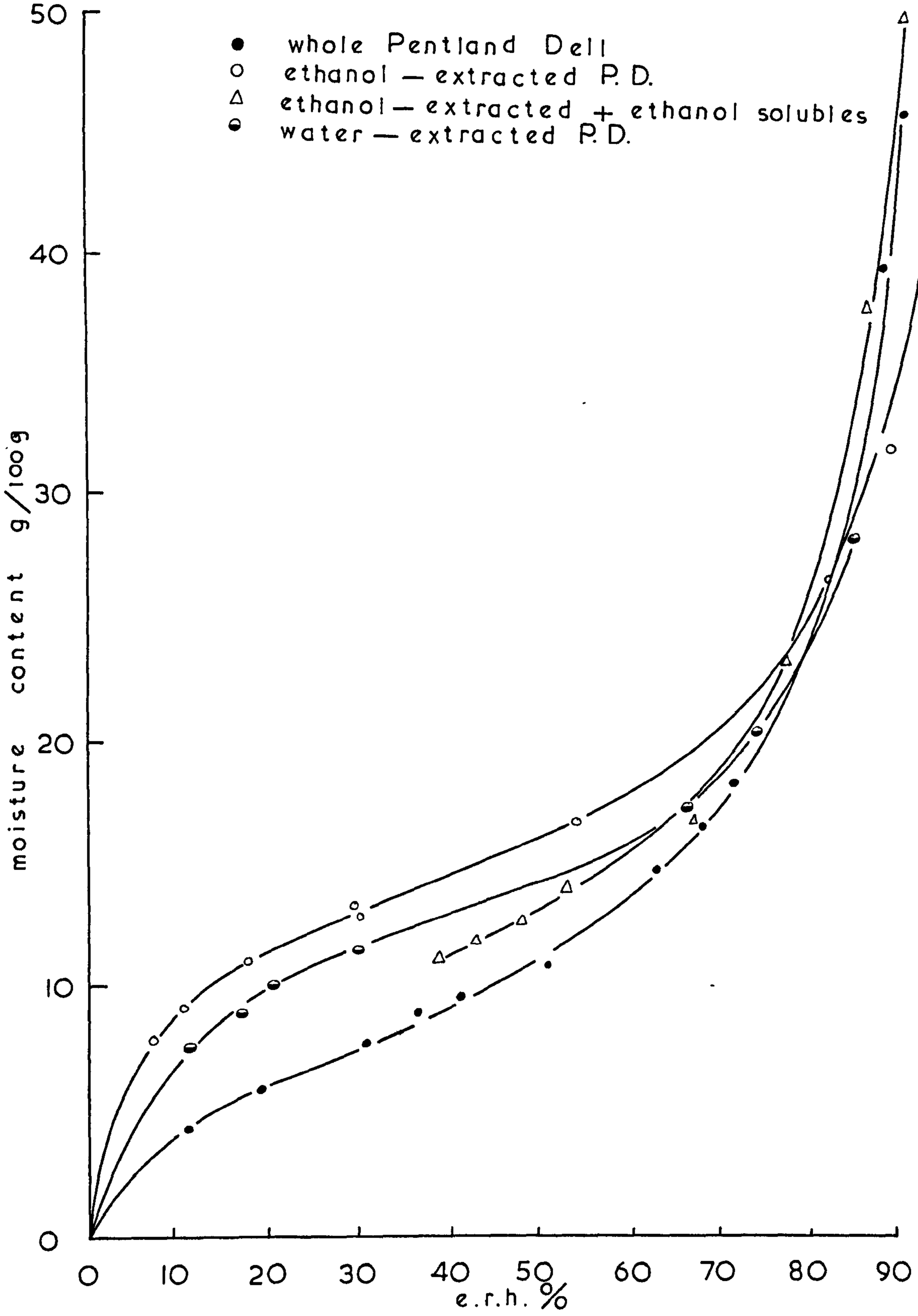

Fig. 52. Sorption isotherms of Whole Pentland Dell potato, Water-Extracted Pentland Dell, Ethanol-Extracted Pentland Dell and Ethanol-Extracted Pentland Dell with added back Ethanol Soluble Material, prepared using the manometric method at 25°C.

In Fig. 52 sorption isotherms prepared at 25°C using the manometric method are presented for whole Pentland Dell, Water-Extracted Pentland Dell, Ethanol-Extracted Pentland Dell, and for this latter fraction to which the recovered ethanol-soluble materials had been added back to give a concentration of 7.5% on a dry weight basis. This concentration was equivalent to the total soluble solids content of the whole tuber, as determined before the extraction procedure was commenced.

It can be observed that both extraction processes resulted in the material absorbing more water at any given ERH than the intact potato. At a high ERH the effect is reversed, possibly due to a solution effect of the sugars present in the whole tuber. The difference in sorption characteristics at these high levels of relative humidity are not great, however.

Addition of the removed soluble materials to the Ethanol-Extracted fraction causes a depression in the extent of sorption below 75% ERH. The resultant isotherm lies approximately half-way between the isotherm for the extracted material, and that of the whole potato.

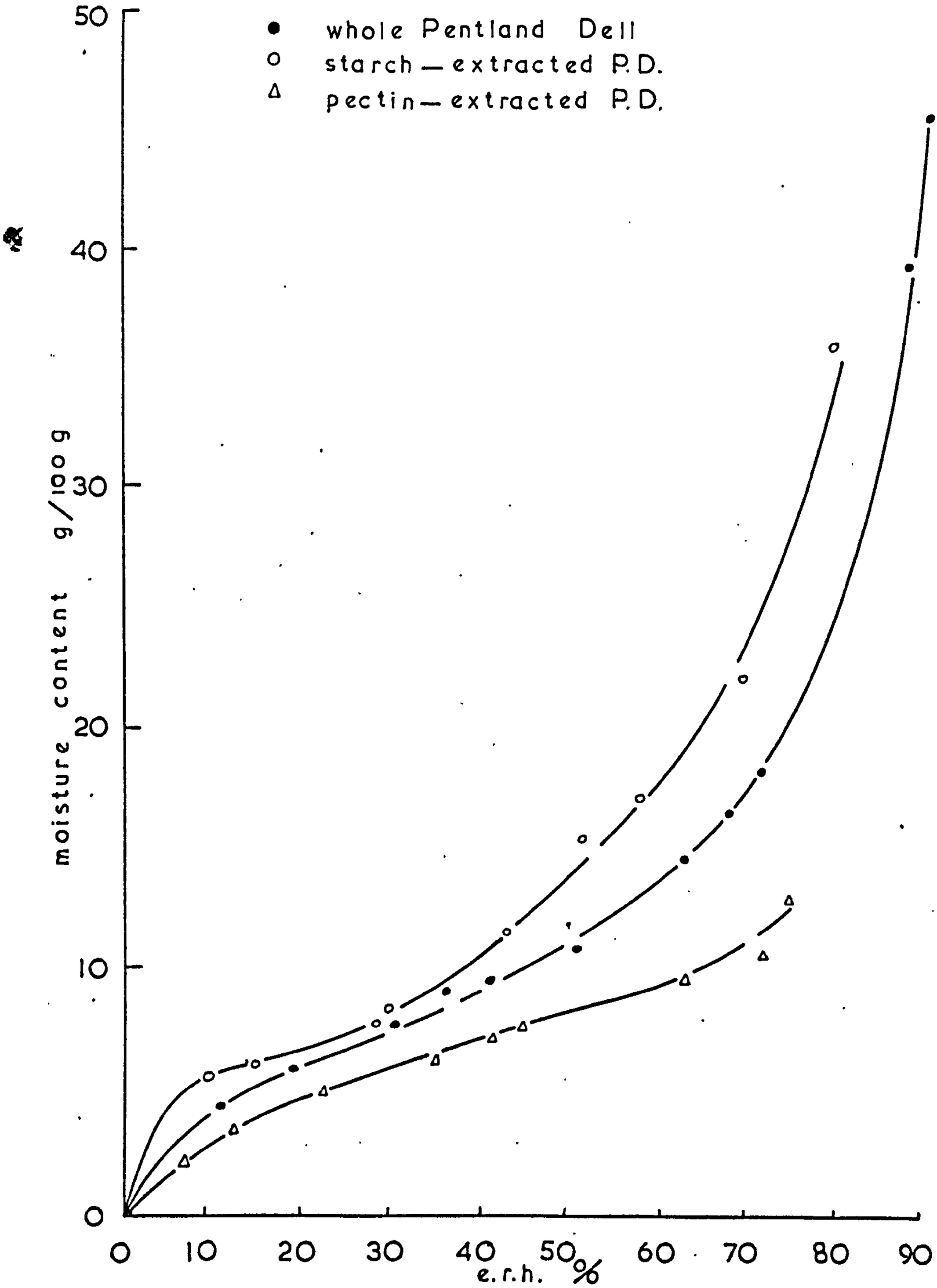

Fig. 53. Sorption isotherms for Whole Pentland Dell, Starch-Extracted Pentland Dell and Pectin Extracted Pentland Dell, prepared using the manometric method at 25°C.

The isotherms for whole Pentland Dell potato, Starch-Extracted Pentland Dell and Pectin-Extracted Pentland Dell, all prepared at 25°C using the manometric method, are presented in Fig. 53.

Compared to the isotherm for the Water-Extracted potato as illustrated in Fig. 52, it can be observed that starch removal causes a reduction in water binding capacity, although the insoluble pectin, hemicellulose, and cellulose, which remain in the Starch-Extracted fraction, still associate with more water at any given ERH than does an equivalent amount of whole potato.

Removal of the insoluble pectin causes a further depression in sorptive capacity and this Pectin-Extracted fraction is close in character to purified cellulose.

The calculated moisture content corresponding to a mono-molecular layer, as defined by the BET equation, for each of the extracted fractions obtained from whole Pentland Dell are given below.

Whole Pentland Dell	7.14g/100g
Ethanol-Extracted Pentland Dell	9.81g/100g
Ethanol-Extracted Pentland Dell plus Ethanol Soluble Material	8.75g/100g
Water Extracted Pentland Dell	9.21g/100g
Starch Extracted Pentland Dell	6.40g/100g
Pectin Extracted Pentland Dell	5.42g/100g

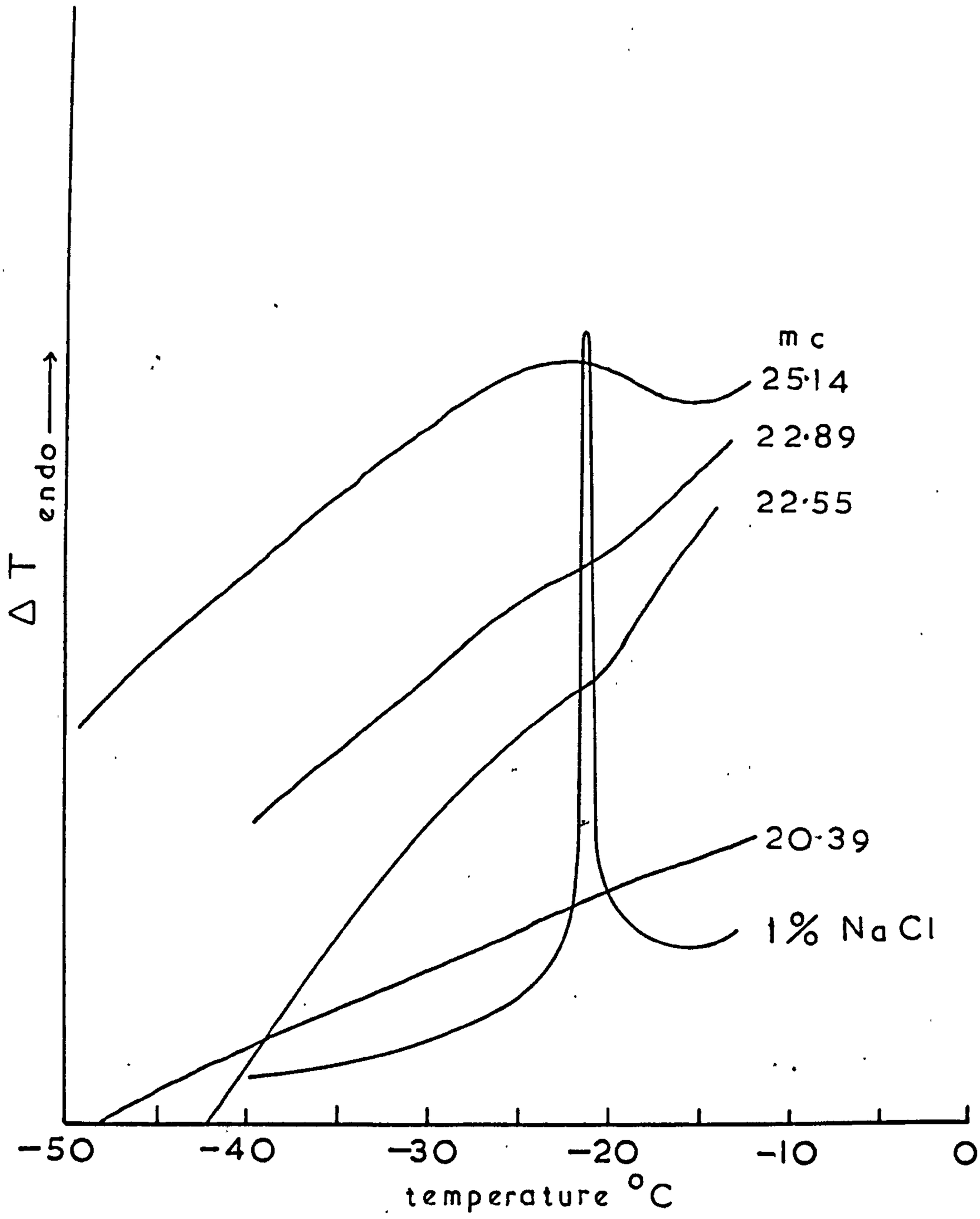

Fig. 54. Differential thermal analysis on the macro-scale of whole Pentland Dell potato hydrated to different moisture contents (mc). Also shown is the thawing peak of 5 ml. of a 1% NaCl solution.

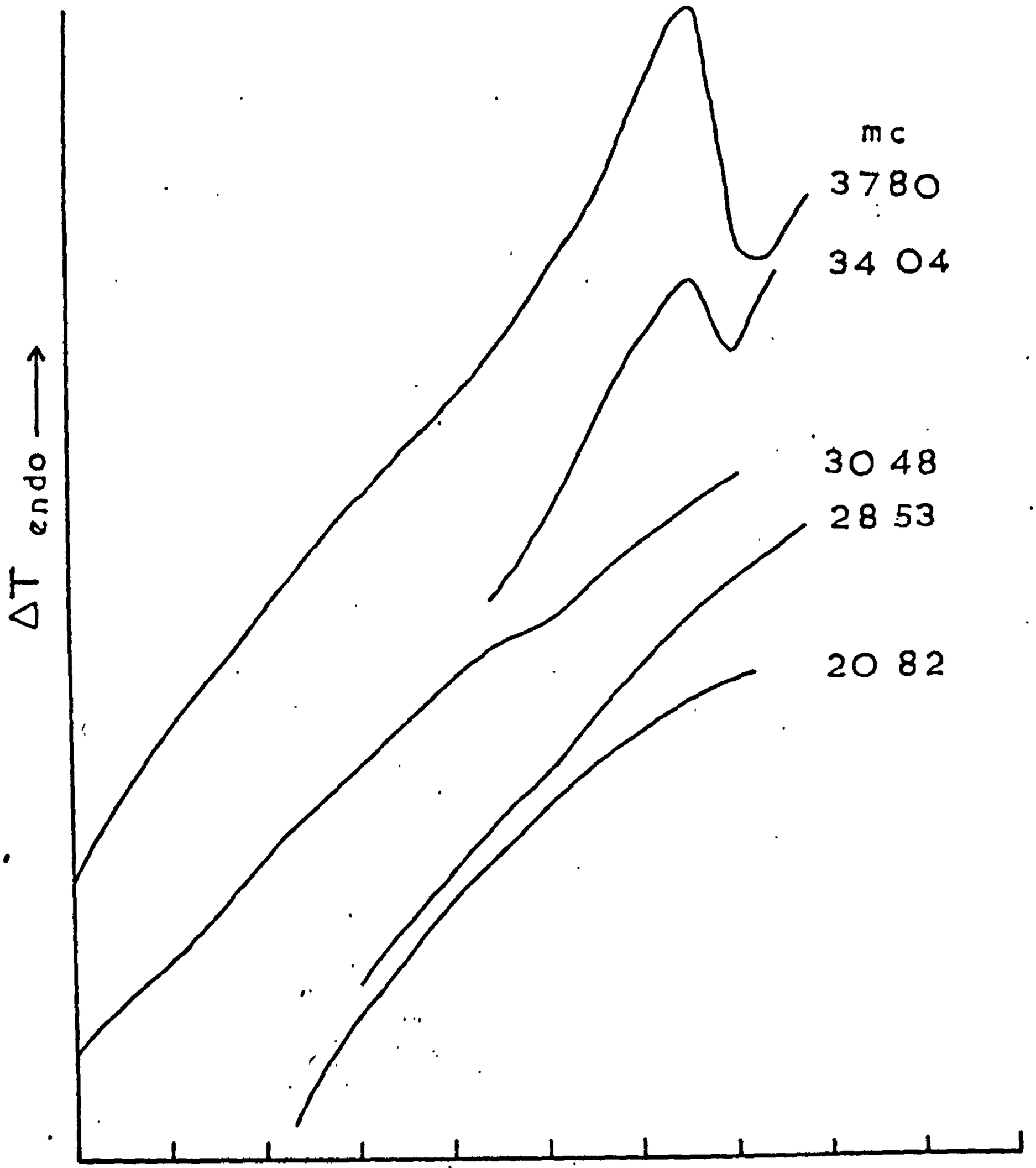

Fig. 55. Differential thermal analysis on the macro-scale of Ethanol-Extracted Pentland Dell hydrated to different moisture contents (mc).

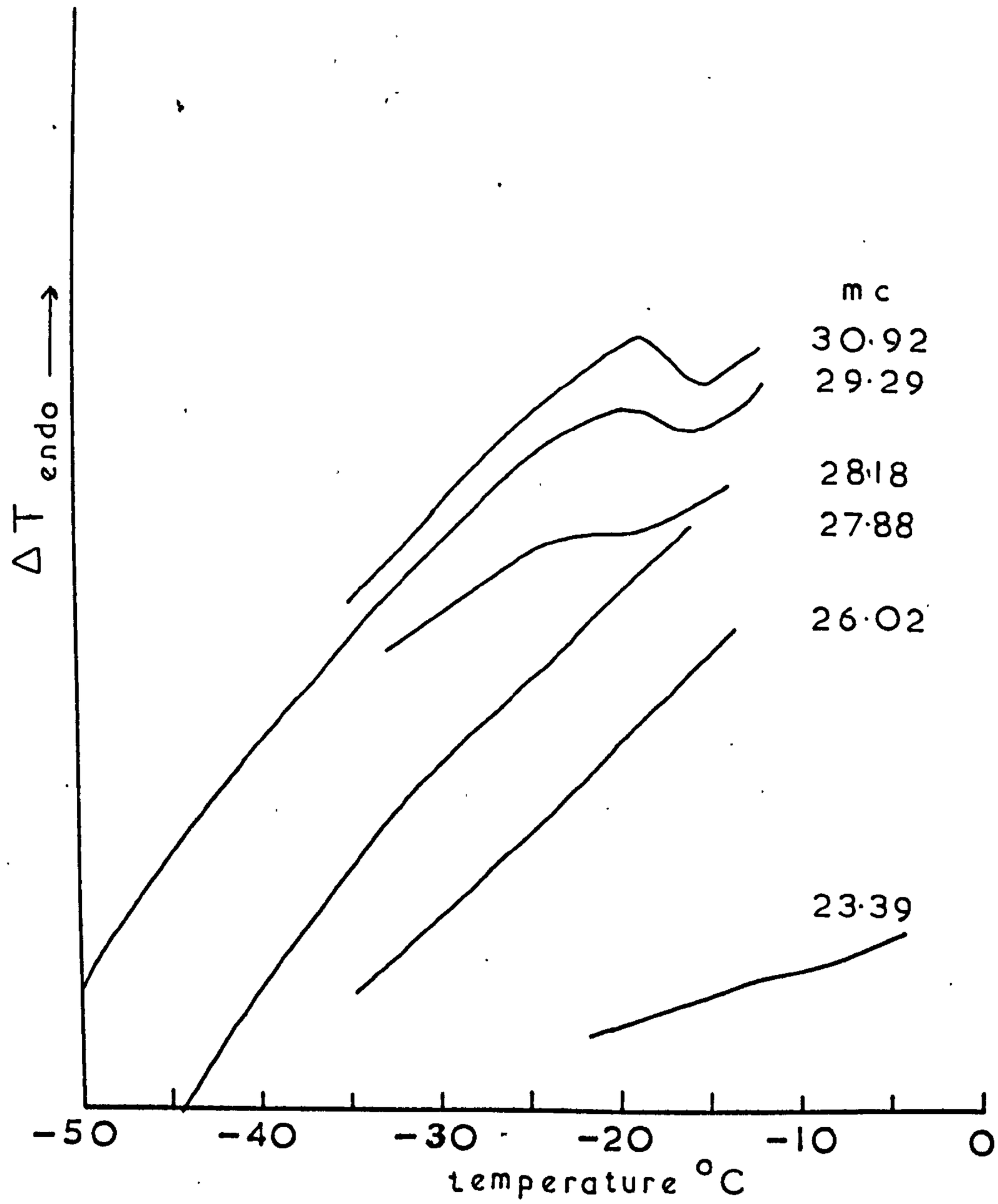

Fig. 56. Differential thermal analysis on the macro-scale of Water-Extracted Pentland Dell potato hydrated to different moisture contents (mc).

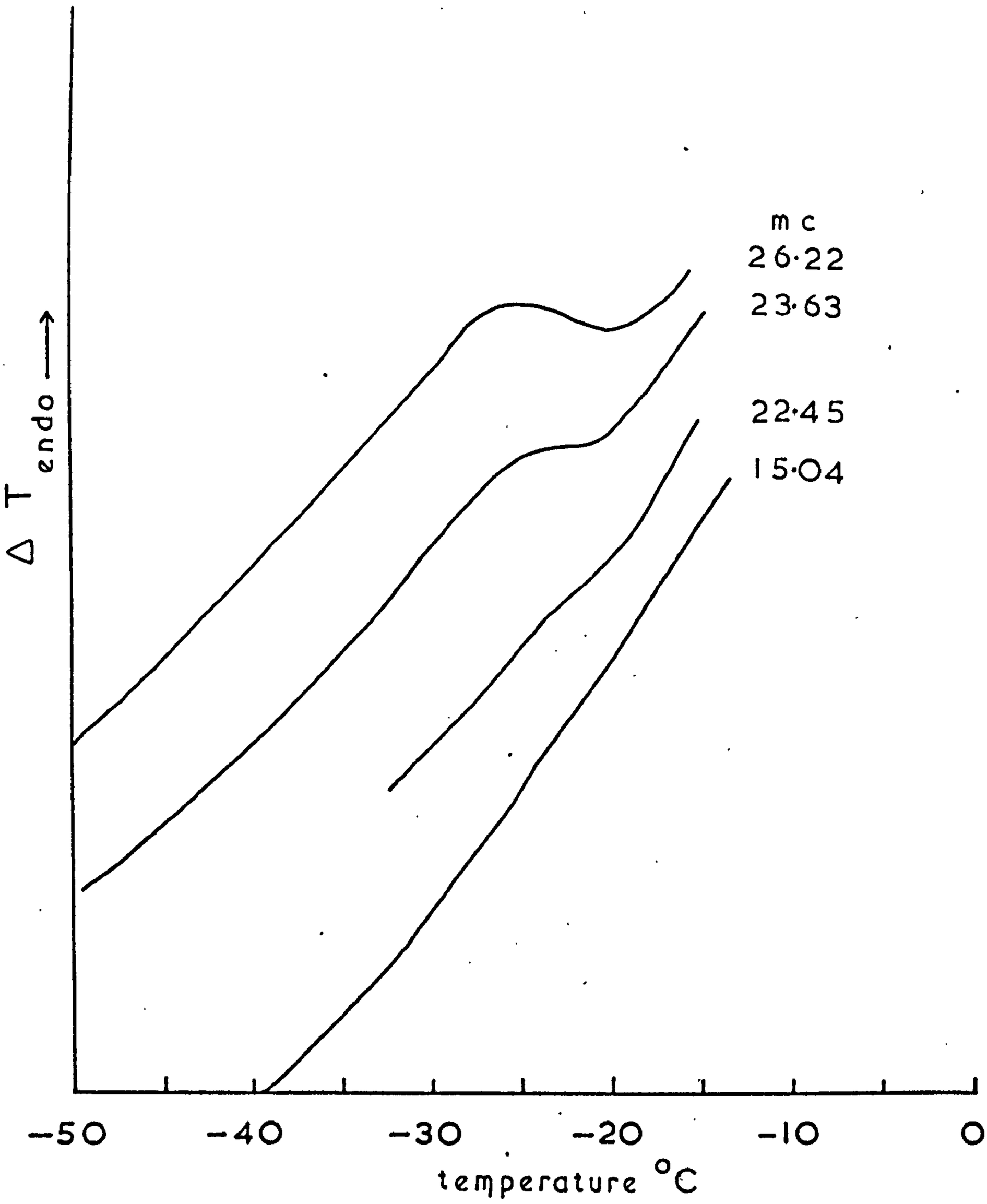

Fig..57. Differential thermal analysis on the macro-scale of Starch-Extracted Pentland Dell potato hydrated to different moisture contents (mc).

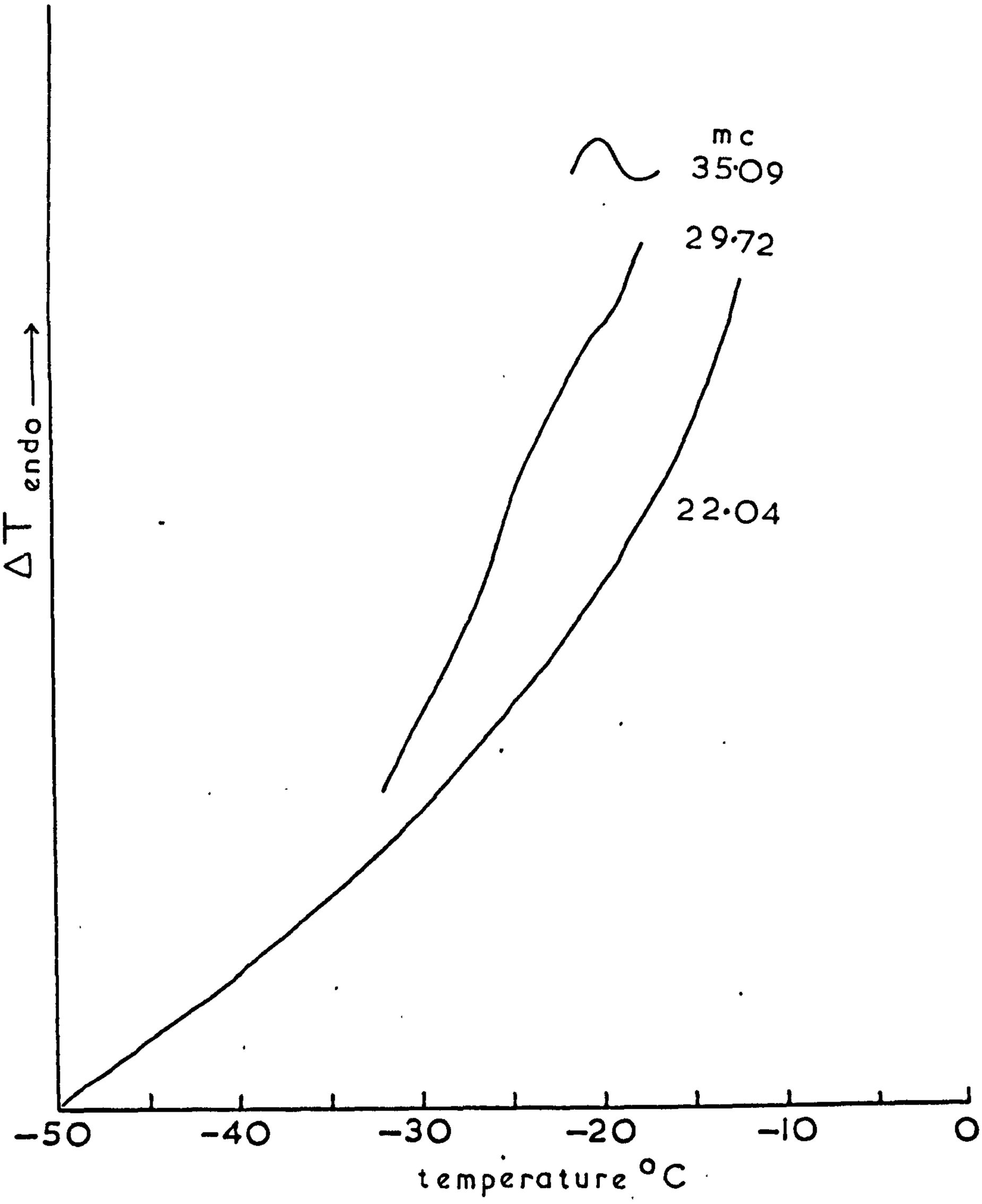

Fig. 58. Differential thermal analysis on the macro-scale of Pectin-Extracted Pentland Dell hydrated to different moisture contents (mc).

8.2 DIFFERENTIAL THERMAL ANALYSIS

Figs. 54-58 illustrate the series of curves obtained from the macro-scale differential thermal analysis of whole Pentland Dell, and the extracted fractions obtained from this material. In each case a series of samples was prepared, each sample with a different moisture content, the range used encompassing the expected unfreezable water level.

The curves show the change in temperature differential between the test sample and an inert reference sample, as represented as a function of change in actual temperature, as the material was warmed from -50°C to 0°C.

In any particular DTA run four samples were simultaneously examined, but due to differences in the behaviour of each differential system it was often not possible to obtain all four records on scale at any one time until a temperature considerably above the initial starting temperature had been reached. For this reason records are not complete over the entire range in some cases.

A 0.2% NaCl solution was used to hydrate all the samples of extracted potato so that the peak due to the thawing of free, solvent water in these materials could be observed in the region of the NaCl eutectic temperature of -21.5°C. In samples of high moisture content this peak is distinctly visible in the temperature region between approximately -30°C and -5°C. With a reduction in moisture content the temperature at which the thermal disturbance is observed is also reduced due to the greater concentration of the salt present.

The moisture content below which the presence of free solvent water can no longer be detected in each of these materials, lies within the particular range quoted below.

Whole Pentland Dell	22.55g/100g-20.39g/100g
Ethanol Extracted Pentland Dell	Just below 28.53g/100g.
Water Extracted Pentland Dell	26.02g/100g-23.39g/100g
Starch Extracted Pentland Dell	< 22.45g/100g
Pectin Extracted Pentland Dell	(29.72g/100g-22.04g/100g)

Difficulty in obtaining a sufficient quantity of Pectin-Extracted material to allow a more comprehensive range of samples to be studied is responsible for the limited range of results shown for this material.

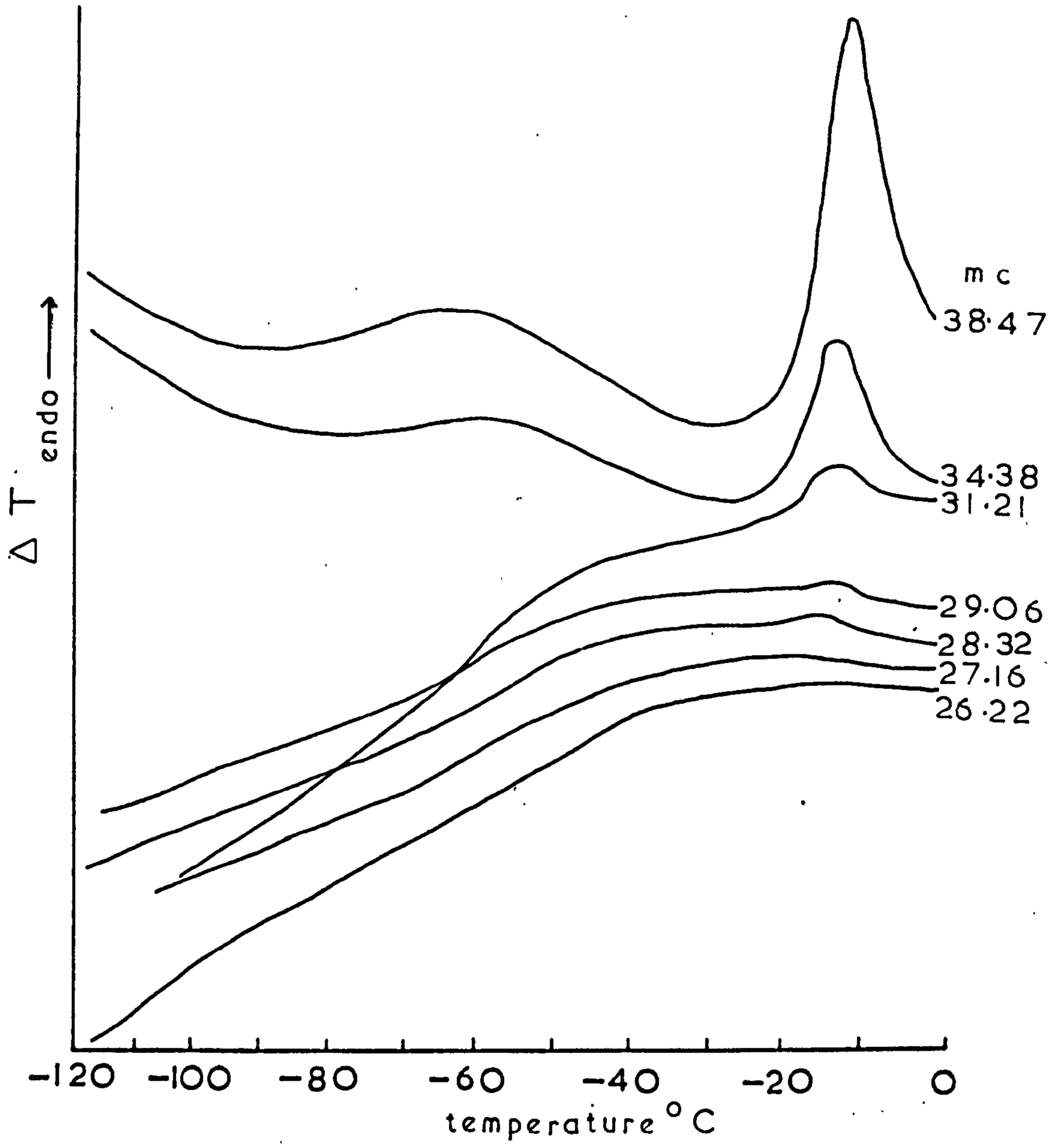

Fig. 59. Differential thermal analysis on the micro-scale of whole Pentland Dell potato hydrated to different moisture contents (mc).

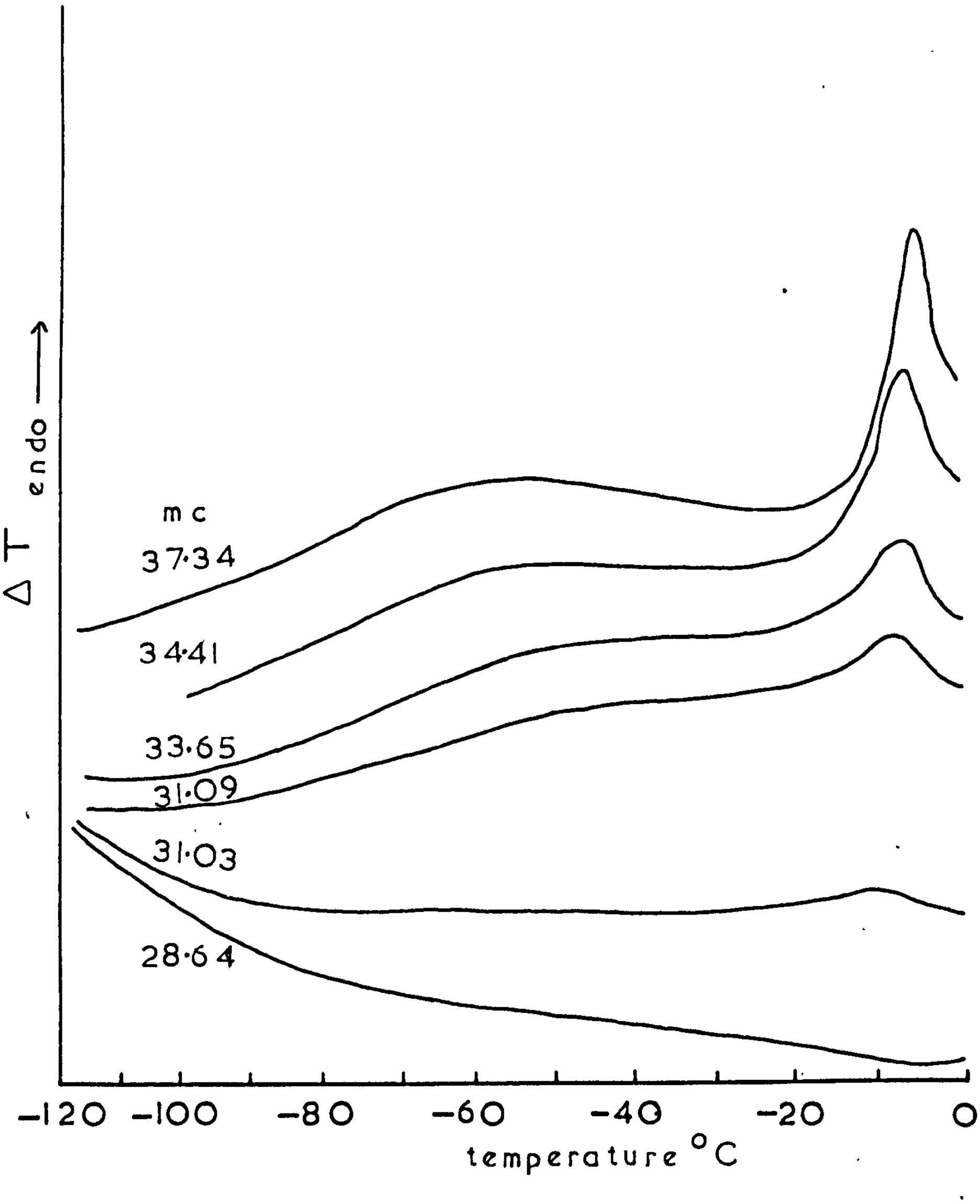

Fig. 60. Differential thermal analysis on the micro-scale of Ethanol-Extracted Pentland Dell hydrated to different moisture contents (mc).

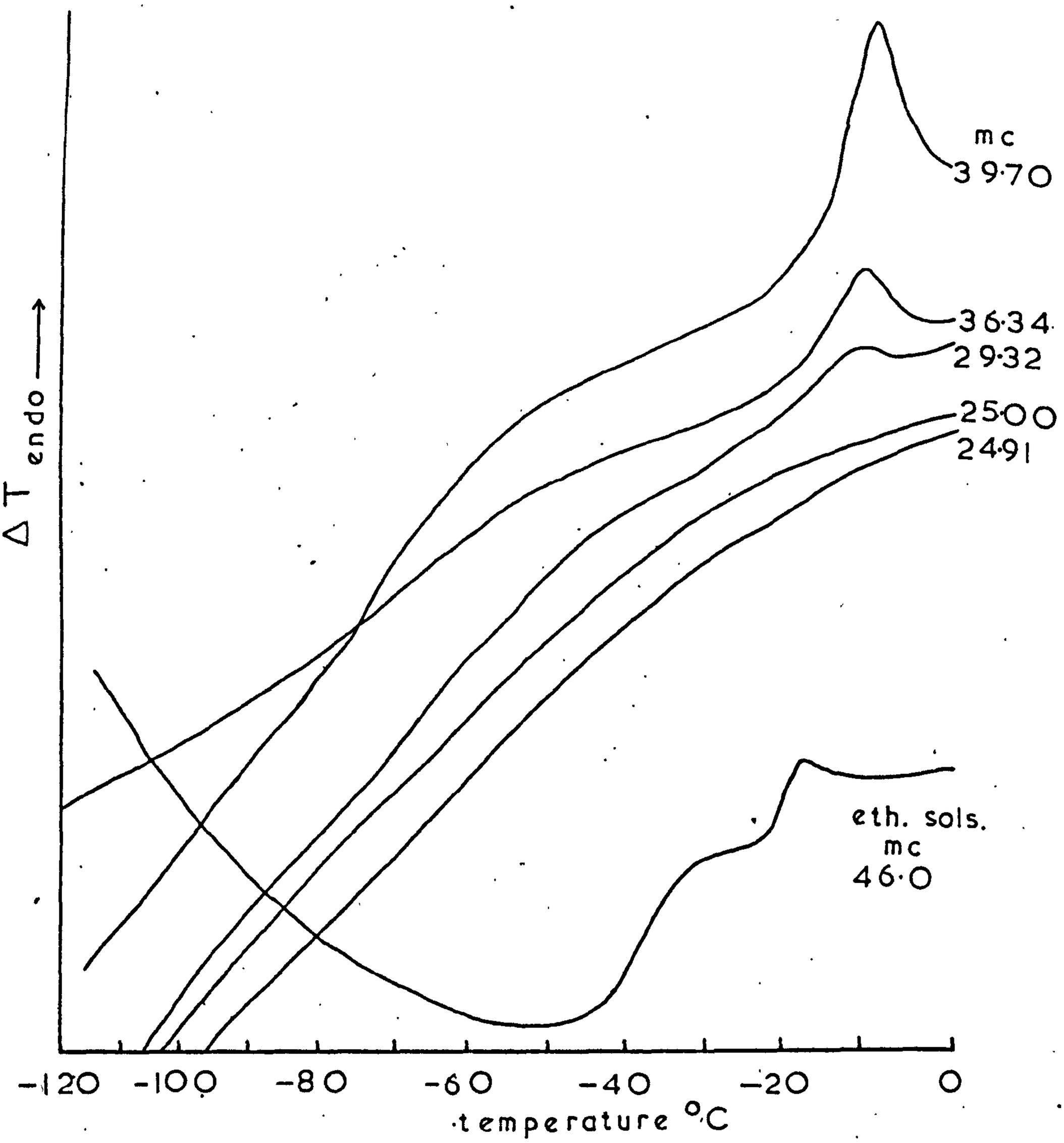

Fig. 61. Differential thermal analysis on the micro-scale of Ethanol-Extracted Pentland Dell plus added Ethanol Soluble Material from Pentland Dell, hydrated to different moisture contents (mc).

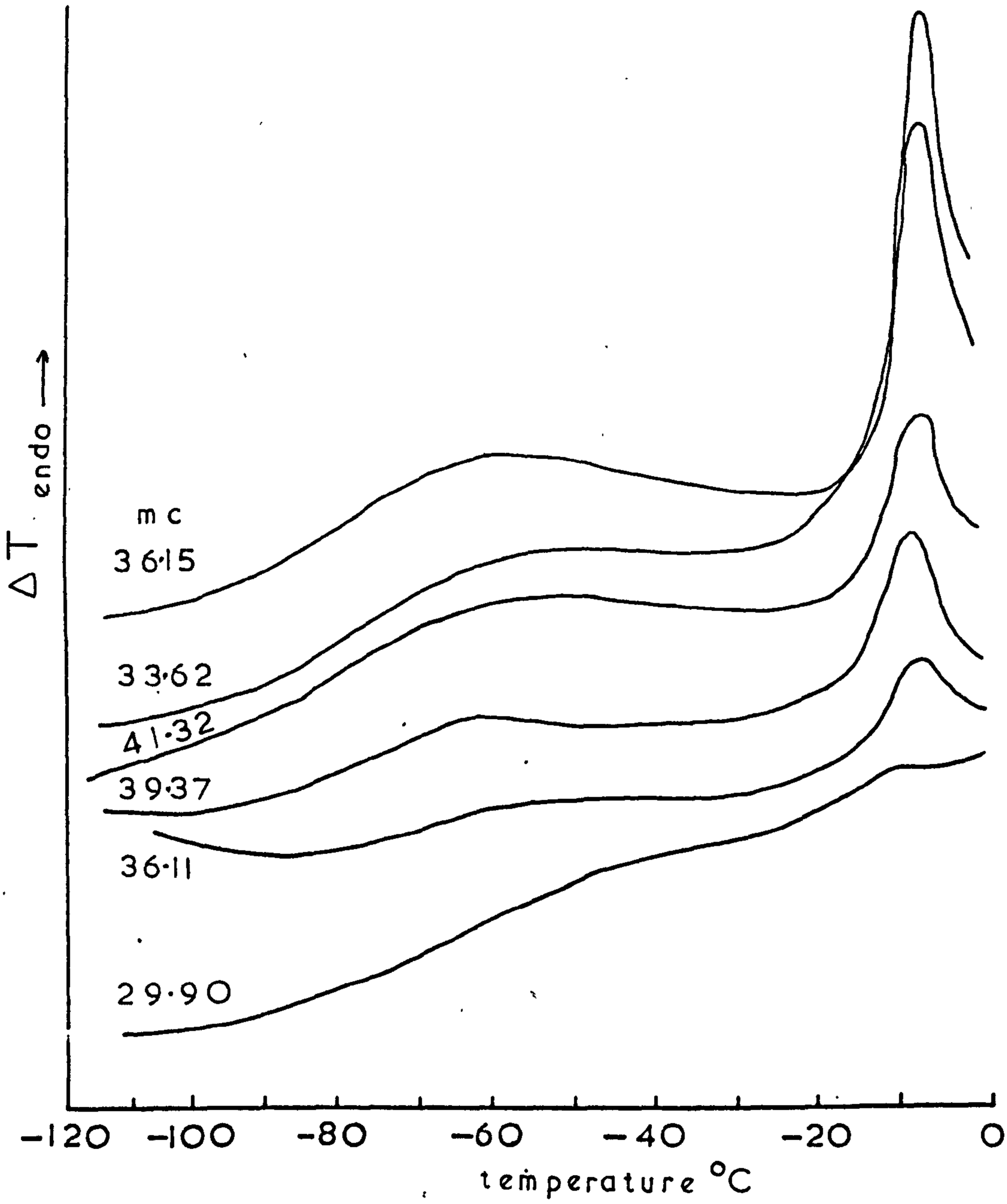

Fig. 62. Differential thermal analysis on the micro-scale of Water-Extracted Pentland Dell hydrated to different moisture contents (mc).

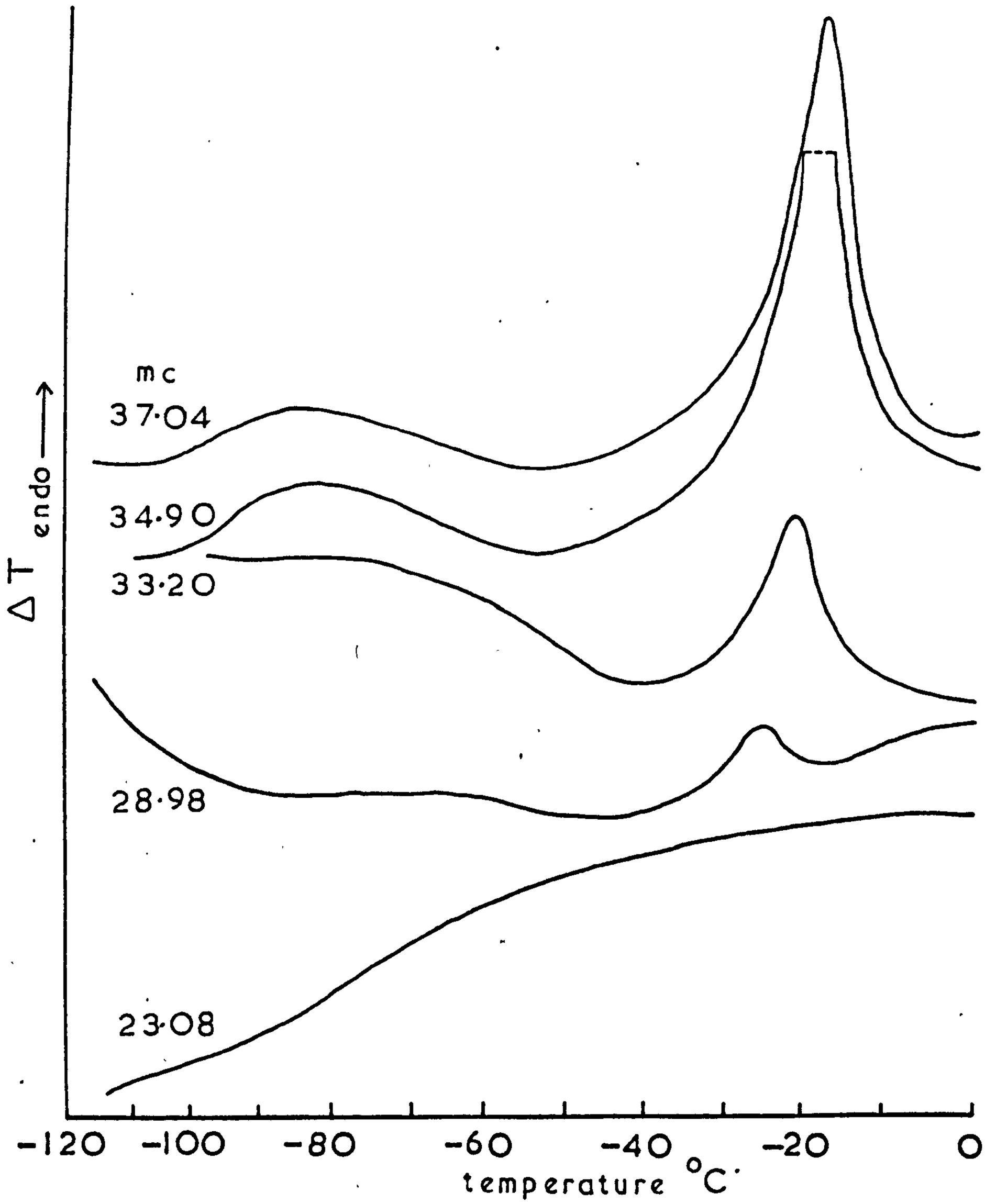

Fig. 63. Differential thermal analysis on the micro-scale of Starch-Extracted Pentland Dell hydrated to different moisture contents (mc).

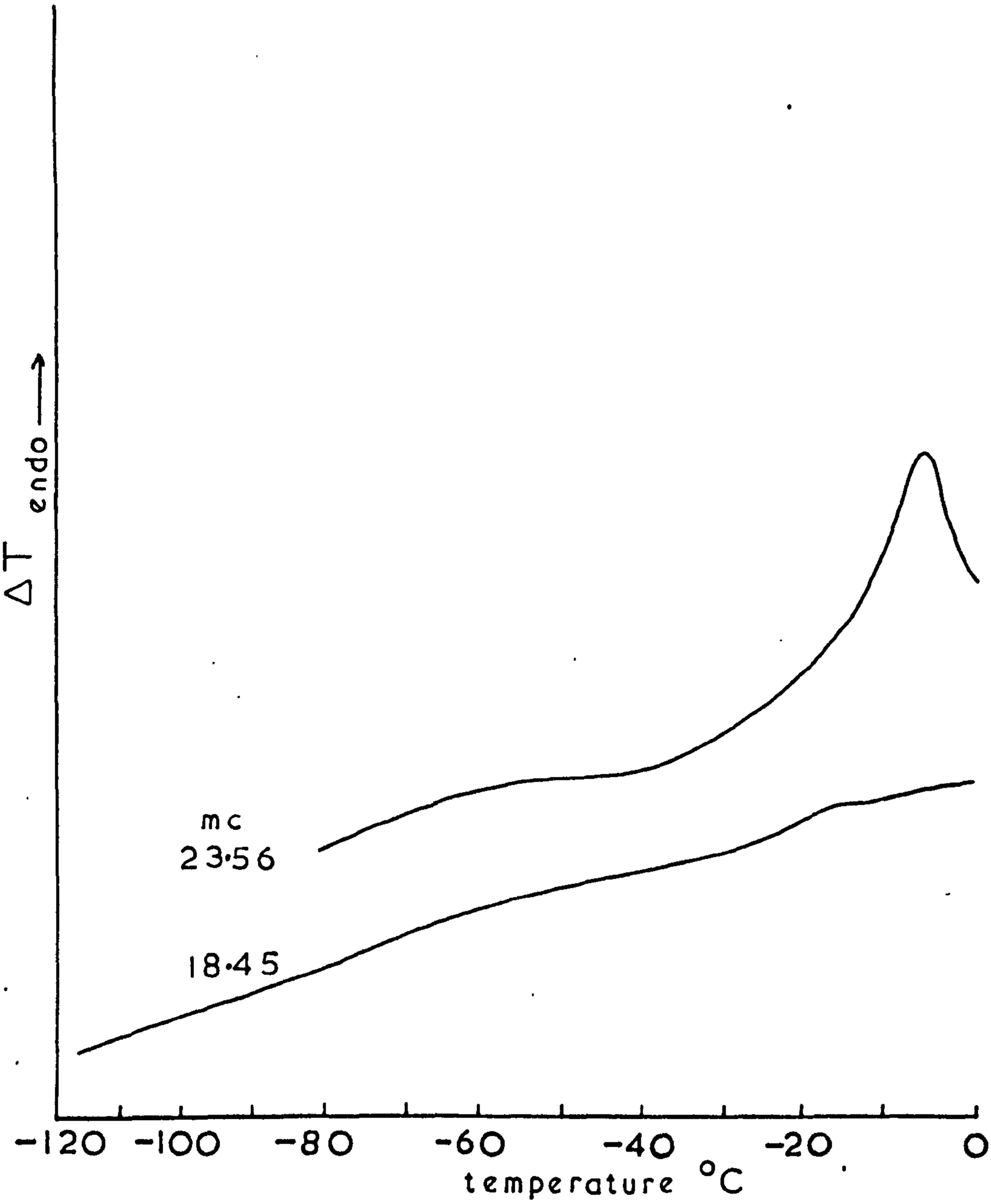

Fig. 64. Differential thermal analysis on the micro-scale of Pectin-Extracted Pentland Dell hydrated to different moisture contents (mc).

The results of the micro-scale differential thermal analysis of samples of whole Pentland Dell and of the fractions obtained from the stepwise extraction of this material are shown in Figs. 59-64. The curves obtained from a similar examination of Ethanol-Extracted material with the removed solubles added back to give a concentration of 7.5% on a dry weight basis are also shown.

In common with the results previously presented for the examination of other materials by the same method, an endothermic peak can be observed in some cases in the region between -30°C and 0°C. This occurs in the samples with a high moisture content and is attributable to the thawing of ice formed from free, solvent water.

The moisture content, below which the presence of this water can no longer be detected in each of these materials, lies within the particular ranges quoted below.

Whole Pentland Dell	27.16g/100g - 26.22g/100g
Ethanol-Extracted Pentland Dell	31.03g/100g - 28.64g/100g
Ethanol Extracted Pentland Dell plus Ethanol Soluble Material	29.32g/100g - 25.00g/100g
Water Extracted Pentland Dell	< 29.90g/100g
Starch Extracted Pentland Dell	28.98g/100g - 23.08g/100g
Pectin Extracted Pentland Dell	<18.45g/100g

In samples containing freezable water a second endothermic change is visible during warming. As previously observed in these cases the temperature of the mid-point of this 'hump' occurs at a lower value with an increase in moisture content,

an associated increase in size also being noted.

A summary of the results presented in this section are tabulated below.

TABLE 11

WATER RELATIONS IN THE POTATO

	Calculated Monomolecular Layer by BET Equation g water/100g dry matter	Unfreezable Water Content by DTA g water/100g dry matter	
		Macro-scale	Micro-scale
Whole Potato: PD	7.14	22.55-20.39	27.16-26.22
: RCR	8.62		
Potato Starch: PD	9.52		
: RCR	10.07		
Potato Pectin: PD	9.83		
; RCR	9.94		
Potato Cellulose: PD	3.42		
: RCR	3.62		
Ethanol Extracted PD	9.81	<30.13	31.03-28.64
Ethanol Extracted PD plus Ethanol Soluble Material	8.75	-	29.32-25.00
Water Extracted PD	9.21	26.02-23.39	<29.90
Starch Extracted PD	6.40	<22.45	28.98-23.08
Pectin Extracted PD	5.42	(29.72-22.04)	<18.45

PD = Pentland Dell

RCR = Red Craigs Royal

The difference in unfreezable water values determined by micro-scale and macro-scale differential thermal analysis reflects the greater sensitivity of this latter system, except in the case of Pectin Extracted Pentland Dell.

9.0 THE EFFECT OF SOLUBLE MATERIALS ON THE WATER RELATIONS OF POTATO STARCH AND COTTON CELLULOSE

A brief examination was made of the effect of two ionic solutes, NaCl and CsI, and of a known clathrate former, tetrahydrofuran, $(CH_2)_4O$, on the water binding properties of the potato starch and cotton cellulose examined in Section 6.0.

The effect of the addition to these two pure plant constituents of the ethanol soluble material extracted from whole Pentland Dell potato, which was added to give a final concentration of 15% on a dry weight basis, was also investigated.

NaCl (100 moles per 10^5g macromolecular material) was examined in order to compare its effects, when added to these plant materials, to those reported by other authors for casein[197] and for water-flour doughs.[95] Reference to Table 5 indicates that NaCl should have neither an overall structure-making effect, nor an overall structure breaking effect on the presence of water in the system.

CsI, added in the same proportions as the NaCl, was examined for the purpose of comparison, and would be expected to exert a predominantly disruptive effect on the water structure (Table 5).

Tetrahydrofuran was also added in the same molar concentration as were NaCl and CsI.

The treated potato starch and cotton cellulose were subsequently examined by differential thermal analysis on the micro-scale in a manner identical to that conducted on other materials examined in this study. In addition, sorption studies were carried out using the manometric method. These, however, were confined to the NaCl and CsI treated starch and cellulose, and to the starch following the addition of the ethanol-soluble potato

material. It was not possible to prepare isotherms for the tetrahydrofuran treated material due to the high vapour pressure and volatility of this additive.

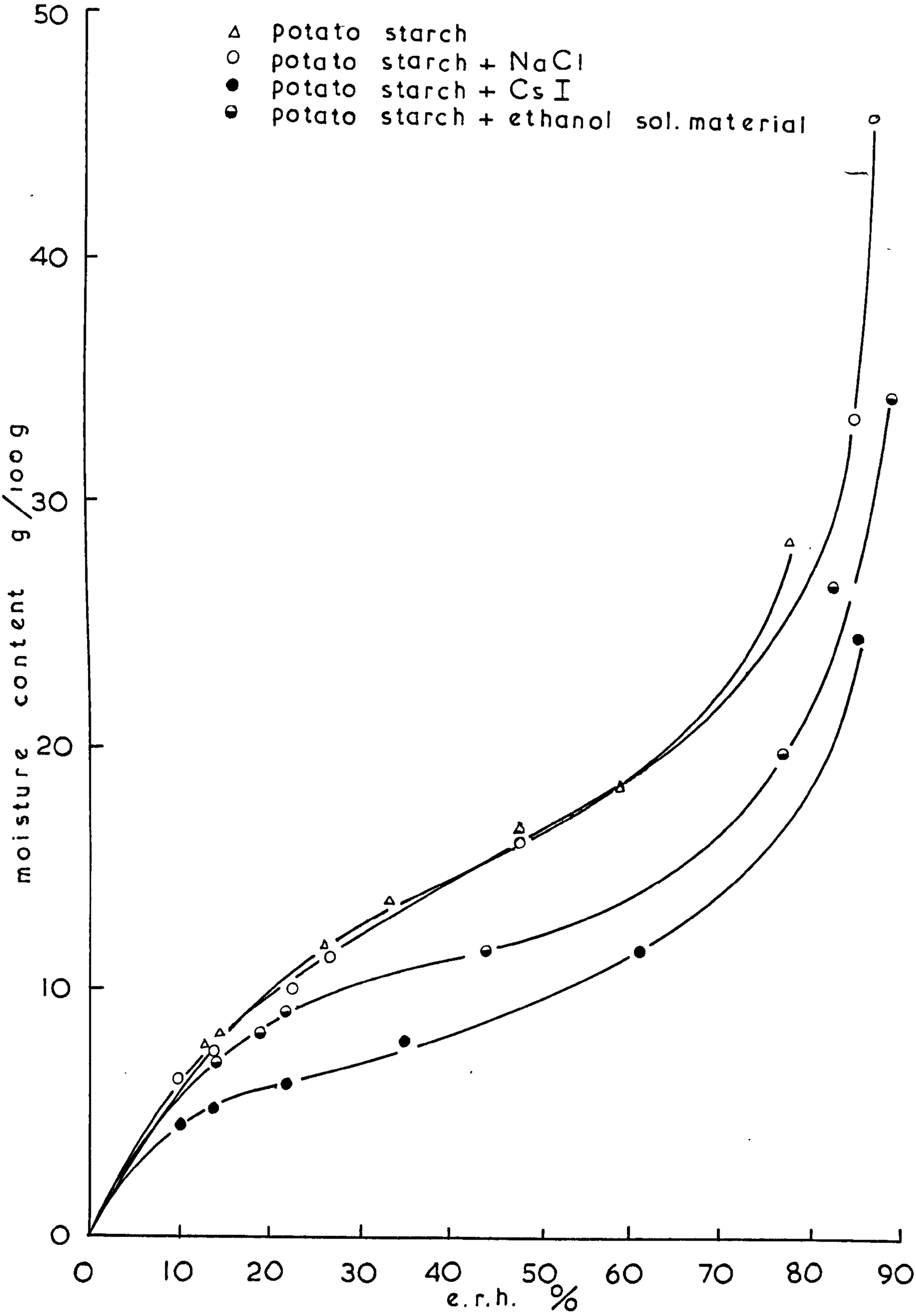

Fig. 65. Sorption isotherms of pure potato starch, plus added NaCl, CsI, and ethanol soluble material from Pentland Dell potato, prepared using the manometric method at 25°C.

9.1 SORPTION STUDIES

Fig. 65 illustrates the effect upon the shape of the sorption isotherm of the addition to potato starch of NaCl, CsI, tetrahydrofuran and Ethanol-Soluble Material from Pentland Dell potato.

It can be observed that the addition of NaCl has very little effect on sorption characteristics. In contrast, CsI appears to cause a marked reduction in the amount of water absorbed at any given equilibrium relative humidity.

The ethanol soluble material from Pentland Dell potato, mainly low molecular weight carbohydrates, similarly causes a depression in the amount of water associated with the macromolecule at any given ERH.

The moisture content corresponding to the completion of a monomolecular layer, as calculated using Salwin's modification of the BET equation (Eq. 4) is presented below for each of the starch plus solute mixtures.

Pure Potato Starch	11.13g/100g
Potato Starch + NaCl	10.44g/100g
Potato Starch + CsI	4.95g/100g
Potato Starch + Ethanol Soluble Potato Material	7.69g/100g

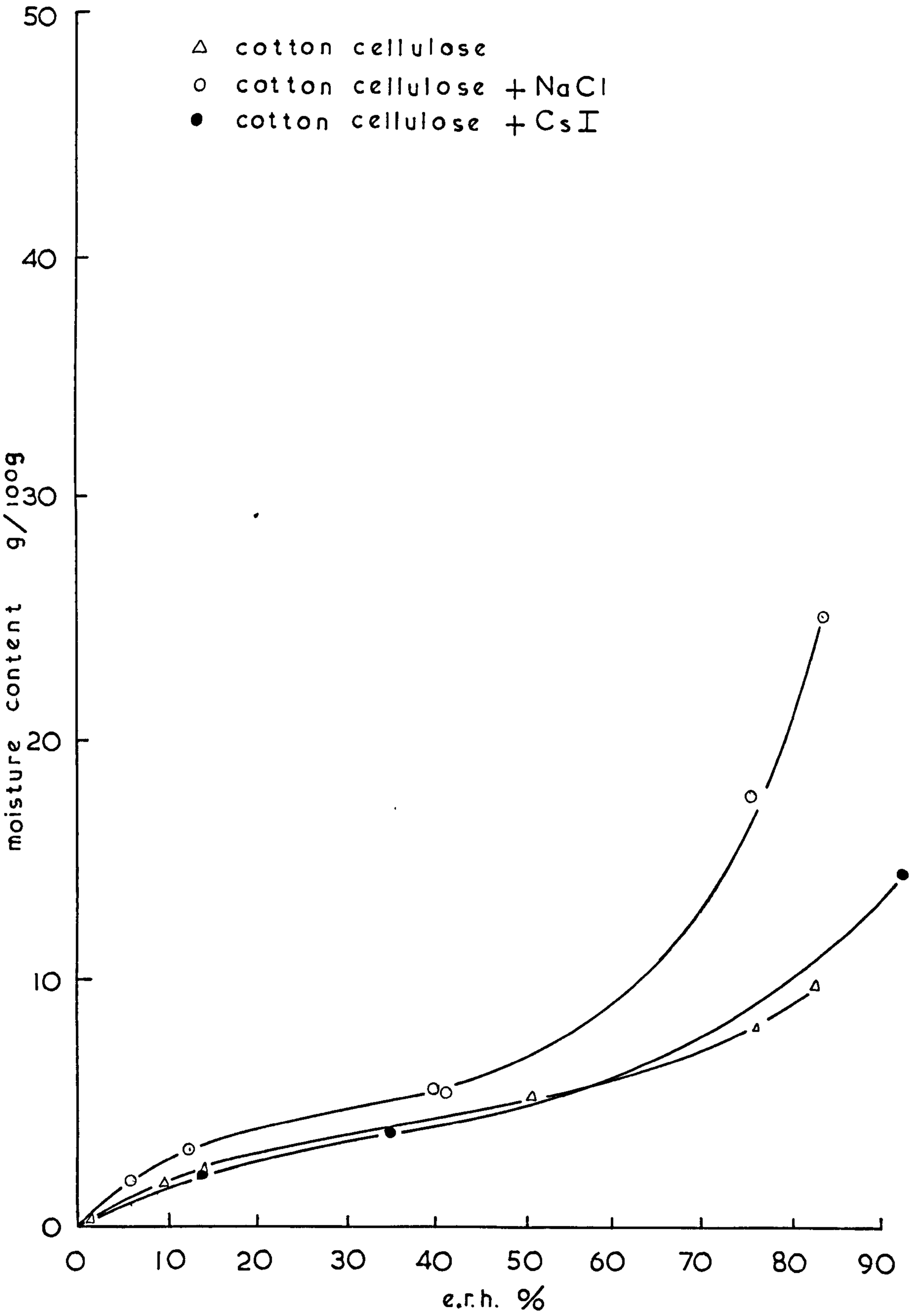

Fig. 66. Sorption isotherms of pure cotton cellulose and with added NaCl and CsI, prepared using the manometric method at 25°C.

The effect of the addition of NaCl and CsI on the form of the sorption isotherm for cotton cellulose can be see in Fig. 66.

In this case the addition of NaCl has increased the amount of water sorbed at any given ERH over that sorbed by the pure cellulose, while the presence of CsI has a slightly depressive action on sorption characteristics at low ERH values. The differences in the two isotherms in this latter case are small, however.

The moisture content corresponding to the completion of a monomolecular layer, as defined by the modified BET equation (Eq. 4), is presented below for each of the cellulose-soluble systems.

Pure Cotton Cellulose	2.66g/100g
Cotton Cellulose + NaCl	3.99g/100g
Cotton Cellulose + CsI	3.45g/100g

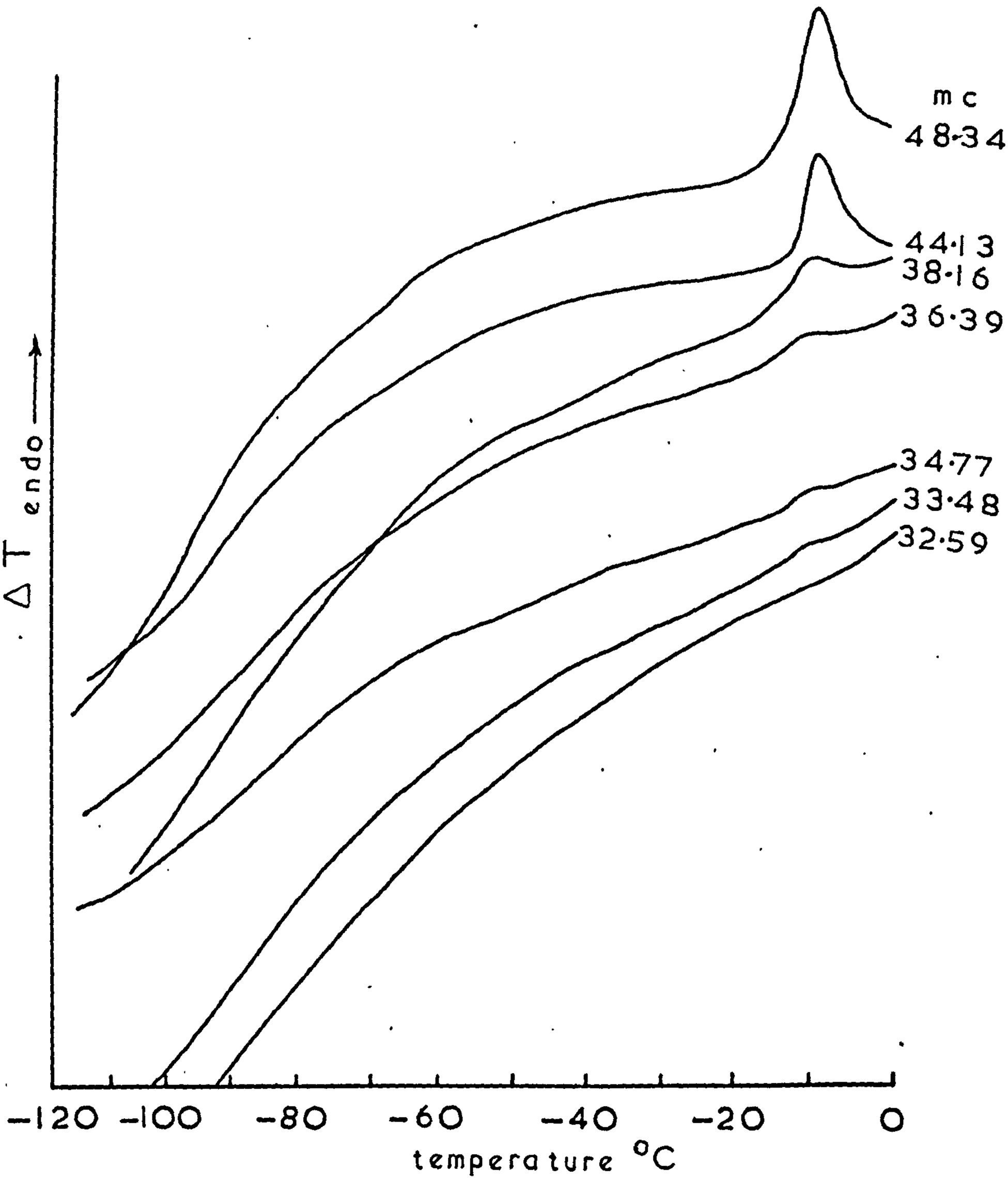

Fig. 67. Differential thermal analysis on the micro-scale of potato starch with added NaCl, hydrated to different moisture contents (mc).

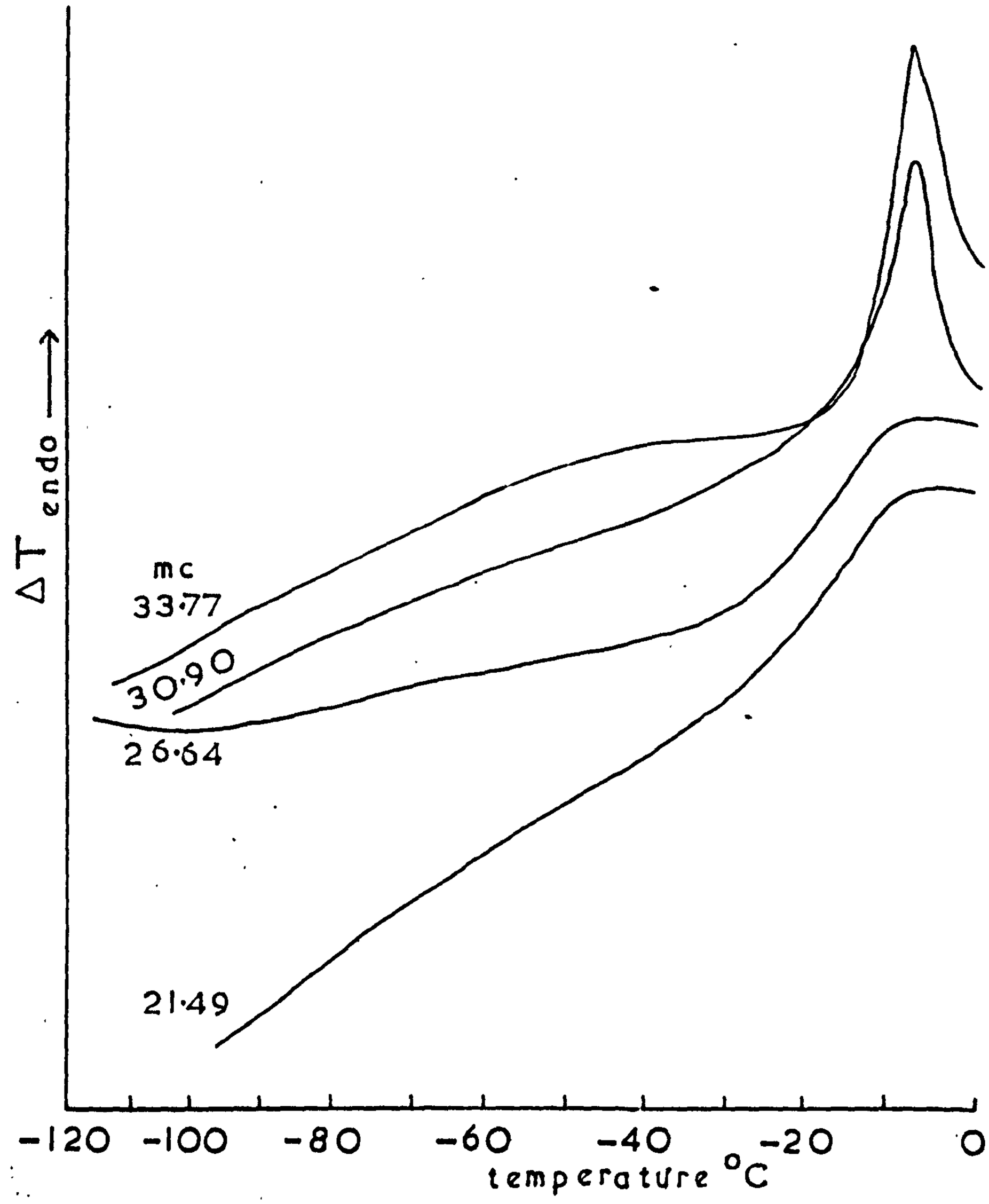

Fig..68. Differential thermal analysis on the micro-scale of potato starch with added CsI, hydrated tp different moisture contents (mc).

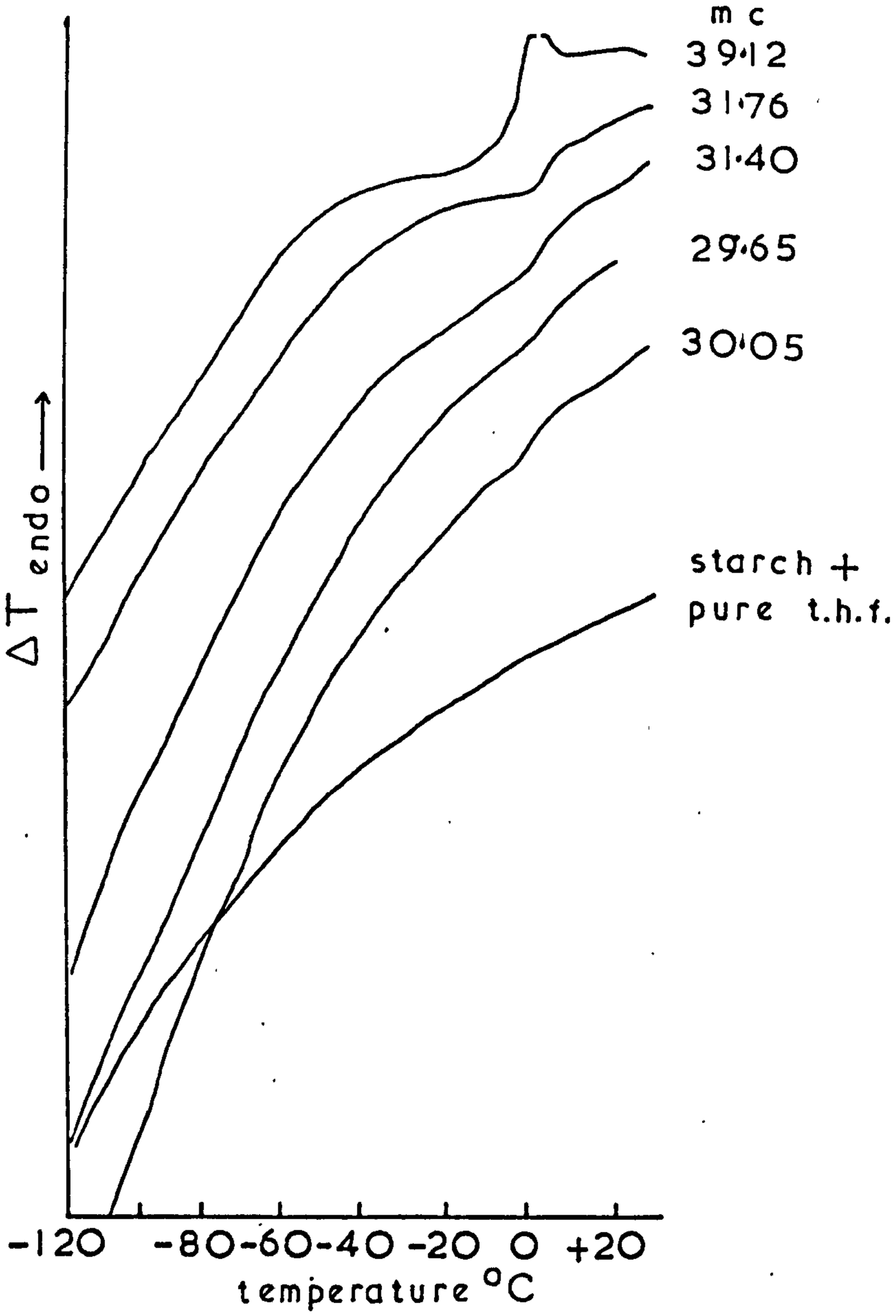

Fig. 69. Differential thermal analysis on the micro-scale of potato starch with added tetrahydrofuran (thf) hydrated to different moisture contents (mc).

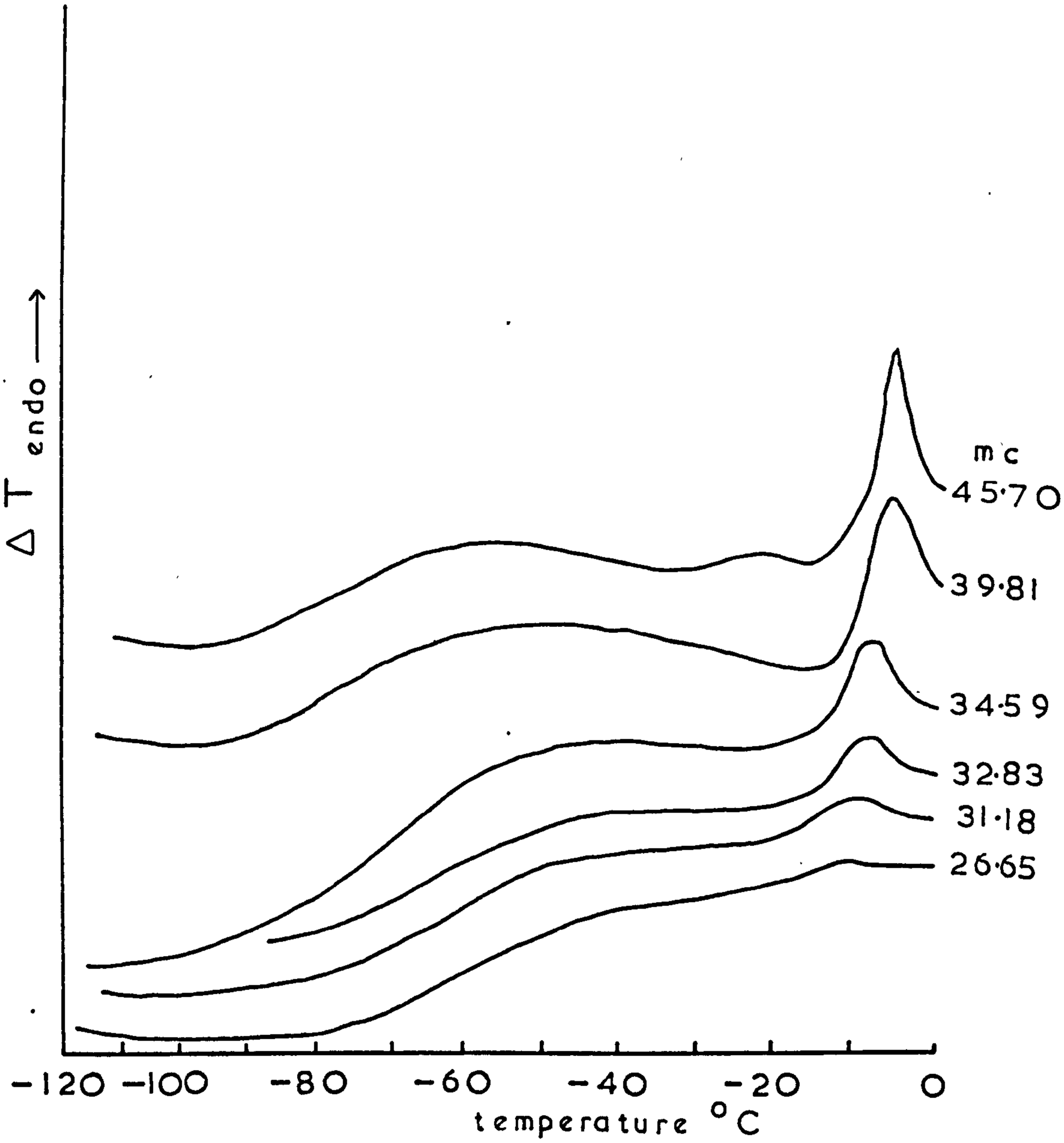

Fig. 70. Differential thermal analysis on the micro-scale of potato starch with added Ethanol Soluble Material from Pentland Dell potato, hydrated to different moisture contents (mc).

9.2 DIFFERENTIAL THERMAL ANALYSIS

Figs. 67-70 illustrate the results from a differential thermal analysis, on the micro-scale, of potato starch treated with NaCl, CsI, tetrahydrofuran, and Pentland Dell soluble material, respectively.

These curves exhibit the same general characteristics as those previously presented for other materials, and which have been fully described before.

The interval of moisture content, between which the presence of freezing water can no longer be detected, is given below for each of the starch-solute materials.

Potato Starch + NaCl	33.48g/100g- 32.59g/100g
Potato Starch + CsI	30.90g/100g - 26.64g/100g
Potato Starch + Tetrahydrofuran	Just below 29.65g/100g
Potato Starch + Ethanol soluble Potato Material	Just below 28.65g/100g

The equivalent value for pure potato starch as presented in section 1.0 is 32.70g/100g dry matter.

The addition of NaCl appears to have little effect upon the unfreezable water content, although the addition of either CsI, tetrahydrofuran or ethanol soluble potato material causes a depression in this value compared to pure potato starch.

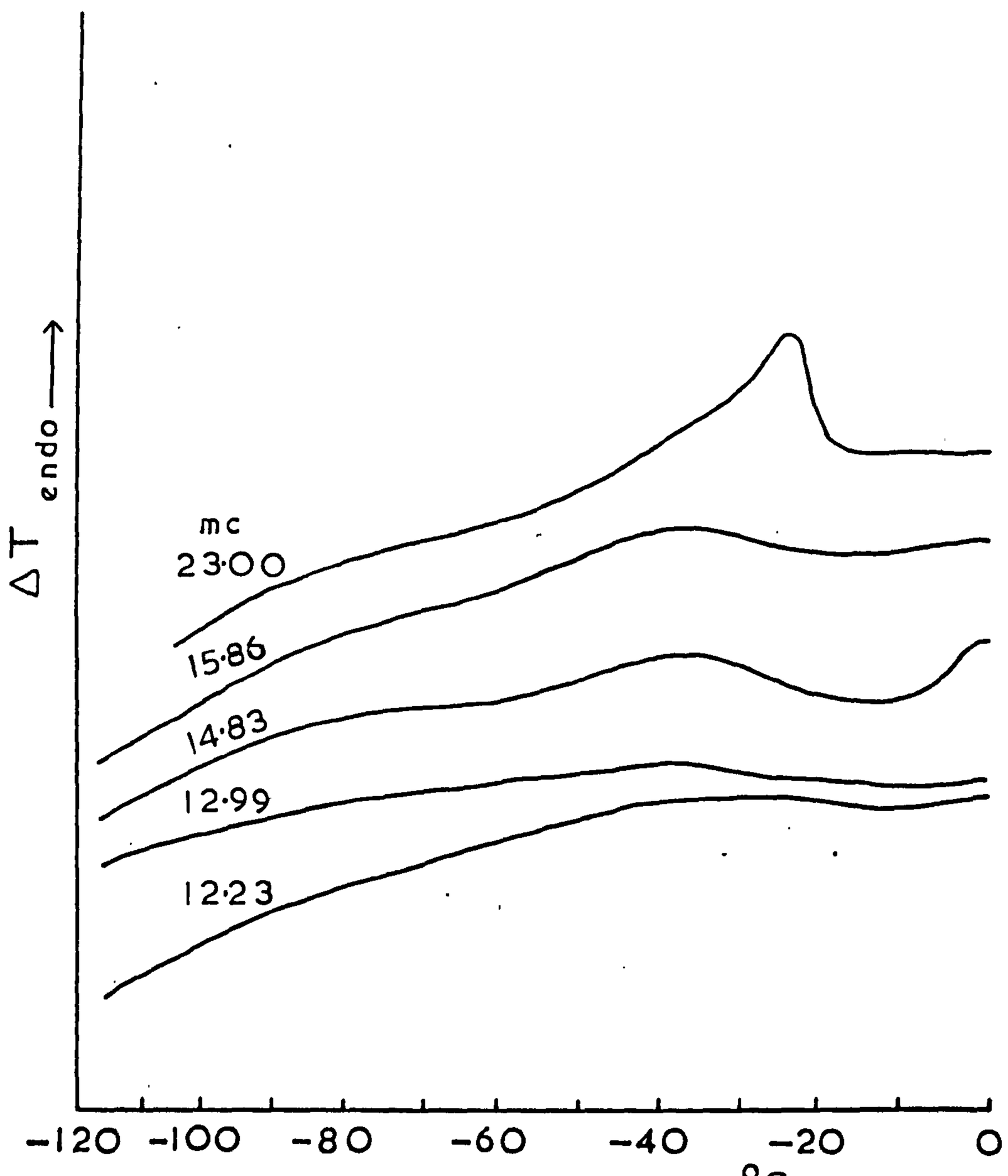

Fig. 71. Differential thermal analysis on the micro-scale of cotton cellulose with added NaCl, hydrated to different moisture contents (mc).

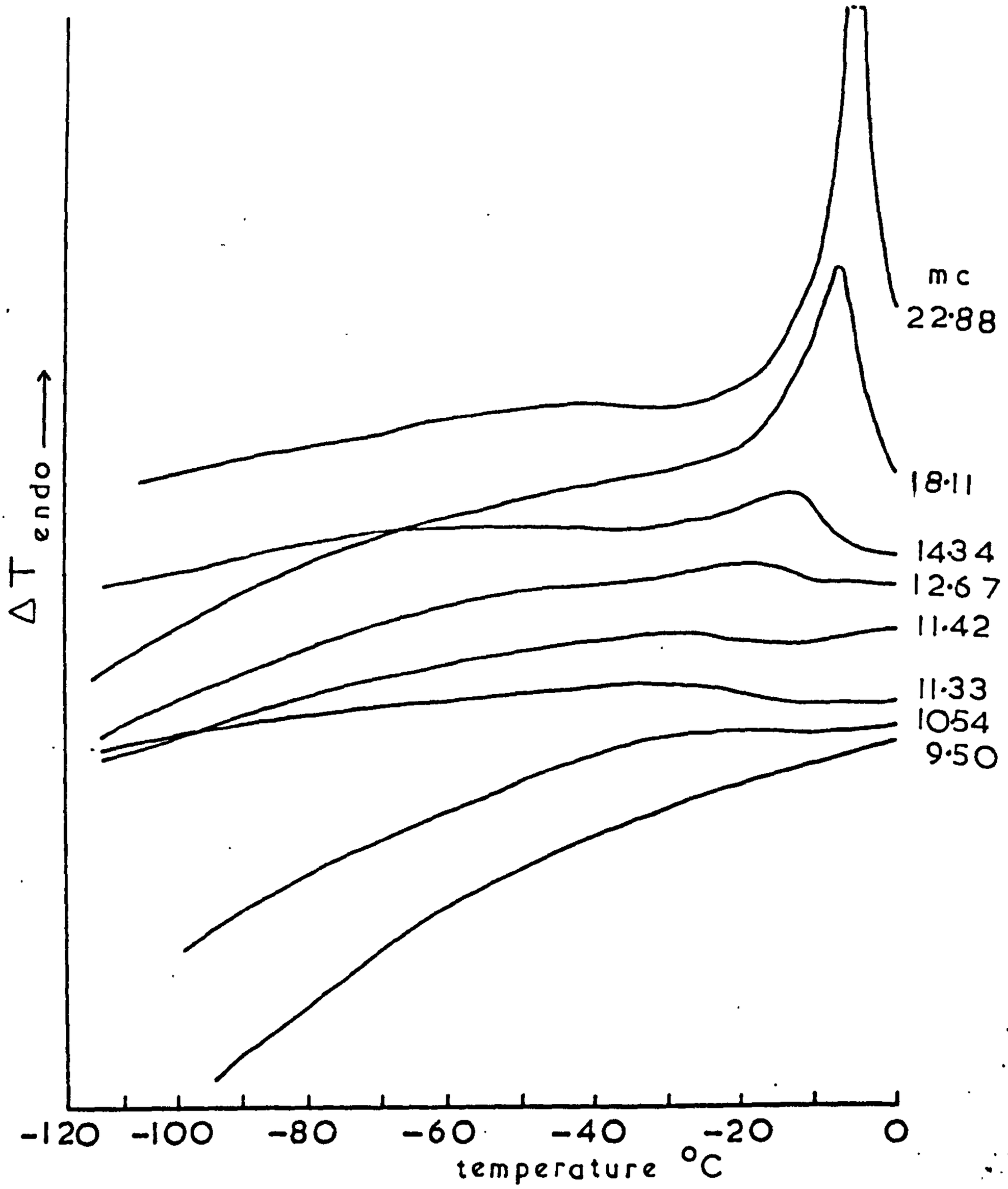

Fig. 72. Differential thermal analysis on the micro-scale of cotton cellulose with added CsI, hydrated to different moisture contents (mc).

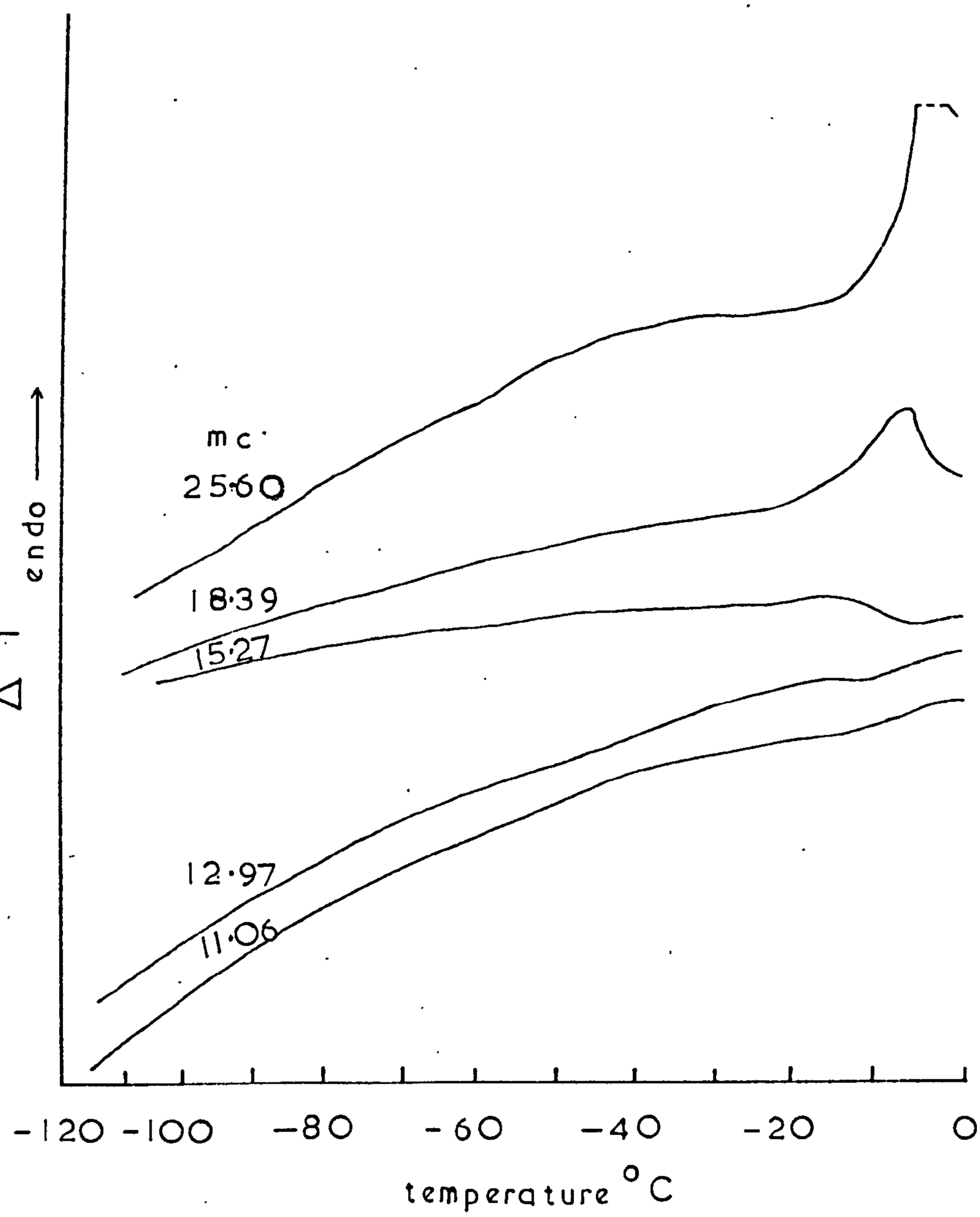

Fig. 73. Differential thermal analysis on the micro-scale of cotton cellulose with added tetrahydrofuran hydrated to different moisture contents (mc).

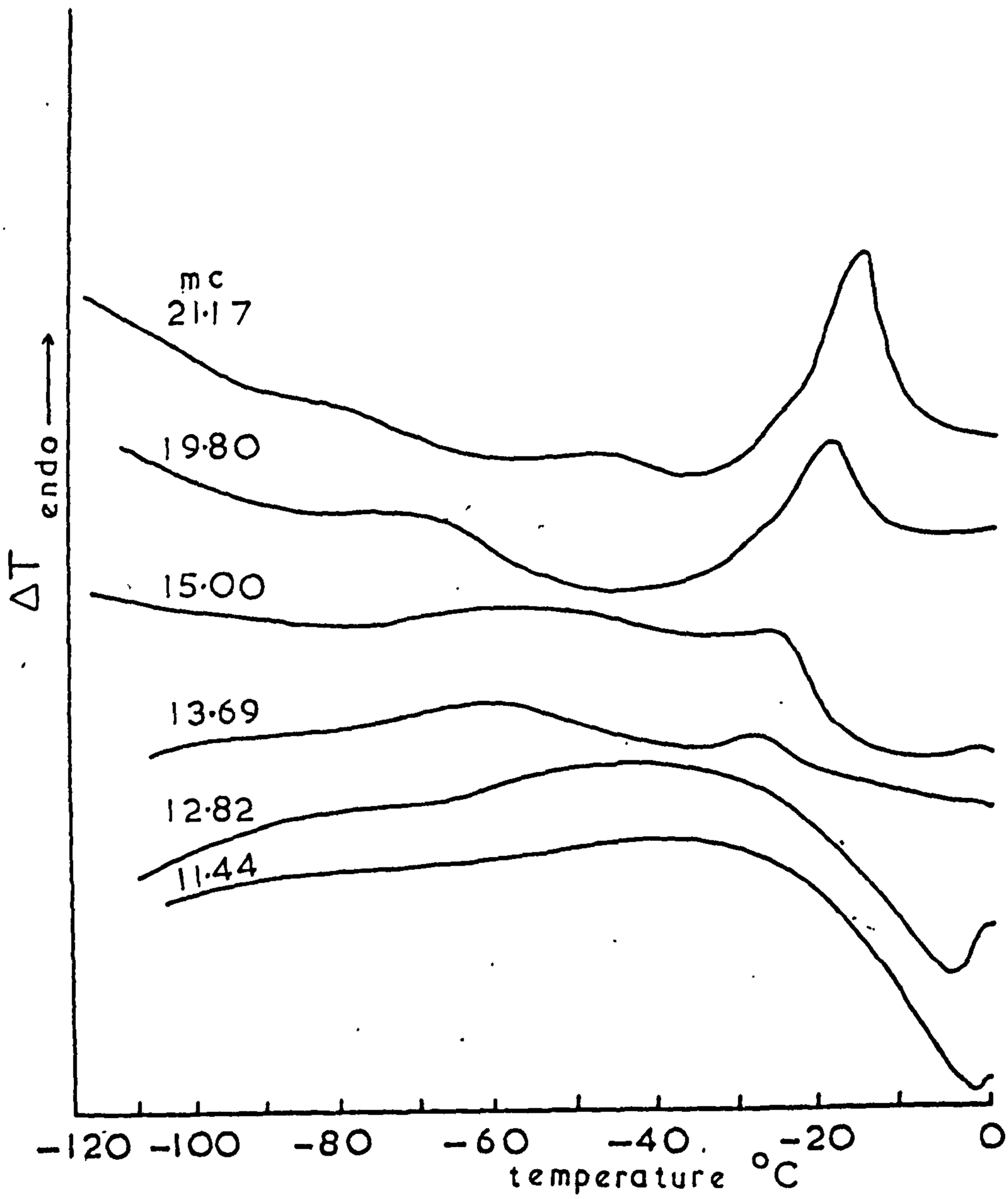

Fig. 74. Differential thermal analysis on micro-scale of cotton cellulose with added Ethanol Soluble Material from Pentland Dell potato, hydrated to different moisture contents (mc).

The effect of the addition to cotton cellulose of NaCl, CsI, tetrahydrofuran or ethanol soluble material from Pentland Dell potato, as investigated by micro-scale DTA, is shown in Figs. 71-74, respectively.

Once again these curves are similar to those previously presented for other material examined by this method, and the moisture content range within which the unfreezable water level lies is given below for each system investigated.

Cotton Cellulose + NaCl	15.84g/100g - 12.99g/100g
Cotton Cellulose + CsI	11.32g/100g - 10.54g/100g
Cotton Cellulose + Tetrahydrofuran	12.97g/100g - 11.60g/100g
Cotton Cellulose + Ethanol soluble Potato Material	13.58g/100g - 12.83g/100g

The equivalent value for pure cotton cellulose as presented in Section 1.0 is $\leq$ 14.7g/100g dry matter.

The addition of either CsI, Tetrahydrofuran or ethanol soluble potato material to cotton cellulose causes a depression in the unfreezable water content of the material. The addition of NaCl results in little change in the unfreezable water content within the limits of the moisture range quoted.

A summary of the results of the investigation into the effect of various solutes on the water relations of potato starch and cotton cellulose is presented in Table 12.

TABLE 12

THE EFFECT OF SOLUTES ON THE WATER RELATIONS OF POTATO STARCH AND COTTON CELLULOSE

	Calculated Monomolecular Layer by BET Equation g water/100g dry matter	Unfreezable Water Content g water/100g dry matter by Micro-scale DTA
Potato starch	11.13	< 32.70
Potato starch + NaCl	10.44	33.48-32.59
Potato starch + CsI	4.95	30.90-26.64
Potato starch + tetrahydrofuran		< 29.65
Potato starch + ethanol soluble potato material.	7.69	< 28.65
Cotton cellulose	2.66	< 14.70
+ NaCl	3.99	15.84-12.99
+ CsI	3.45	11.33-10.54
+ tetrahydrofuran	-	12.97-11.60
+ ethanol soluble potato material	-	13.58-12.83

PART 4

D I S C U S S I O N

TABLE 13

INTER-RELATIONS OF IMMOBILIZED WATER FRACTIONS IN PLANT MATERIAL

	I BET Monomolecular Layer g/100g solids	II Heat of Association approx. equal to Heat of Condensation g/100g solids	II as factor of I	III Unfreezable Water Level by micro-scale DTA g/100g solids	Mean of III as factor of I
Starch:					
Potato	11.13	20.0	1.80	<32.70	2.94
Pectin:					
Apple	11.65	20.0	1.72	32.4-28.5	2.61
O. Albedo	12.25			<36.78	3.00
Cellulose:					
Cotton	2.66	9.5	3.57	<14.7	5.53
O. Albedo	6.06			21.13-15.54	3.03
O. Albedo	9.43			<32.23	3.41
P.D. Potato	7.14	18.0	2.52	27.16-26.22	3.74
Eth. Ex. P.D.	9.81			31.03-28.64	3.04
Water Ex. P.D.	9.21			<29.90	3.25
Starch Ex. P.D.	6.40			28.98-23.08	4.06
Pectin Ex. P.D.	5.42			< 18.45	3.40

10.0 DISCUSSION

10.1 THE CONDITION OF WATER IN PLANT SYSTEMS

Both the sorption studies and the low temperature examinations of intact plant systems and their macromolecular constituents allow examination to be made of the condition of water in these materials. In particular, analysis is possible of that fraction which has classically been differentiated from the remainder by virtue of its specific association with the polymeric components, or due to the influence of the fine structure.

This association results in a restricted degree of mobility of the water, as reflected in its longer residence time at the macromolecular surface than in the bulk liquid. Depending on the criterion used to define mobility, however, different fractions can be distinguished. Correlating the various results in the way presented in Table 13, it is obvious, nevertheless, that irrespective of the nature of the material, these different quantities bear a similar relationship to one another. The rather higher values obtained in the case of cotton cellulose result from the small monomolecular layer value, which itself reflects the sharp shoulder found on the isotherm at low moisture contents (Fig. 21).

A relatively large amount of water, for example 6.25% of the total water in fully hydrated Pentland Dell potato, cannot be frozen out between 0°C and -198°C, as determined by differential thermal analysis and confirmed in certain cases by nuclear magnetic resonance at low temperatures. With the expected exception of cotton cellulose and the high cellulose

Pectin-Extracted Pentland Dell, unfreezable water values for all the materials examined fall within the range between 23g/100g and 37g/100g dry matter. This is in general agreement with the unfreezable water values for a variety of whole plant foodstuffs reported by other authors and presented in Table 6 (p.71).

Cellulosic materials, as a result of their relatively high degree of crystallinity have fewer freely available potential sites for water-immobilization. If unfreezable water can be related, either directly or indirectly, to the number of such sites it would be expected that the quantity of this water, expressed on a unit dry weight basis, would be less in these cases.

The process of freezing results in a loss of mobility of individual water molecules and the formation of an ordered crystal lattice of well defined characteristics. The inability of a certain portion of water to freeze, but which can, nevertheless, be determined as water by any recognised analytical method, indicates that at the freezing point it is already in a restficted condition. This may not necessarily be comparable to ice but its energy state is such that upon a substantial reduction in temperature no enthalpy decrease, reflecting an increase in hydrogen-bonding, is observed.

Within this fraction of unfreezable water a smaller quantity is differentiated by application of the BET theory. This describes the formation of a monomolecular layer as the first stage of absorption of vapour by solids, although this layer is not a continuous film, nor does it necessarily represent a situation where every potential water-immobilization site is occupied or fully satisfied. In all the

materials examined in this study, application of the BET isotherm equation to the respective sorption data was found to give a linear plot over the lower ERH range, thus allowing calculation of a value for the monomolecular layer in each case. With reference to those materials for which thermodynamic functions have been calculated (Fig. 28 in the case of cotton cellulose, apple pectin and potato starch, and Fig. 45 in the case of Pentland Dell potato) it can be seen that at moisture contents corresponding to the monomolecular layer the heat of association between the water and the solid is greater than the heat of condensation of water. This indicates the presence of a specific energy-involving interaction between the two components.

The heat of association curves derived for each of the plant constituents examined are similar in character although it must be borne in mind that the accuracy of thermodynamic functions derived from sorption data have been criticized[71,77] as being of a low order. These curves reach a maxima in terms of Qs as the moisture content is increased from dryness, and then fall rapidly to reach a value similar to the heat of condensation of water as hydration proceeds, and before the moisture content corresponding to the unfreezable water level is reached. A similar peak has also been observed by other workers studying amylose and amylopectin[147]; various pectic substances[182], and gelatin and agar systems.[170] Hill, Emmett and Joyner[349a] have explained a peak occurring in the entropy function at a similar moisture content to one occurring in the enthalpy function, which is closely related to the heat of association, as indicating a strong binding of the sorbate molecules to the solid surface.

The proximity of the apices of the peaks observed in this study to the moisture content corresponding to the calculated BET monomolecular layer in each case (Table 9) is therefore significant.

It would, however, be too categorical to over emphasise the importance of this correlation as such peaks in the heat of association curve have not been shown in all cases where mono- [illegible] formation can also be reasonably postulated to occur (for examp[illegible]ee Fish, 1957[146]).

The shortage of sorption data at 44°C in the case of the whole potato (Fig. 44) does not allow the heat of association curve to be continued to very low moisture levels. Although no peak is shown in the curve, the magnitude of the energy function at low moisture contents is of the same order as are those for the pure constituents.

It is significant to note that the heat of association coinciding with the maximum in each curve lies between 3.6 and 6.2 kcals/mole above the heat of condensation of water. This is similar to the energy involved in the formation of a hydrogen-bond in pure water, that is approximately 5 kcals/mole, and the total heat of association at this point is similar to that of the latent heat of sublimation of ice.[146]

By expressing the respective BET monomolecular layer values for potato starch and apple pectin on a molar basis (assuming 75% esterification in the latter[185]), as shown in Table 14, it is found that at the monolayer, approximately one mole of water is associated with one glucose or uronide residue.

TABLE 14

WATER RELATIONSHIPS IN POTATO STARCH AND APPLE PECTIN EXPRESSED IN TERMS OF MOLES OF WATER PER MOLE MONOMER

	Potato Starch	Apple Pectin
BET Monomolecular Layer	0.997	1.217
Point of Disappearance of Differential Heat of Binding	1.797	2.088
Unfreezable Water Content	2.992	3.180

The thermodynamic results suggest that, as it is unlikely on steric grounds that each water molecule in the monolayer becomes associated with the polymer through a similar number of hydrogen-bonds and of equivalent strength as are found in ice, every water molecule will form two hydrogen-bonds, each slightly stronger than those found in the bulk liquid where a single molecule participates, on average, in 1.5 hydrogen-bonds. If this is the case the postulated 1:1, water to monomer ratio further suggests the possibility that two of the three potential binding sites on each residue in the case of starch and cellulose, and two of the three, or possibly four, sites in the case of pectin, are available for hydrogen-bonding with water.

In view of the interference of steric factors in the case of cellulose, and not knowing the precise degree of crystallinity, it is not meaningful to express the results for this material in terms of molar proportions of water and glucose residues.

The 1:1, water to monomer ratio suggested to occur in the case of starch is in agreement with a similar ratio proposed by Fish[146], and is not necessarily incompatible with the 2:3, water to monomer ratio postulated for granular starch[40,147,170] where steric considerations related to the degree of crystallinity could account for a reduction in the number of freely available binding sites.

In the case of natural citrus pectins with a high degree of esterification a similar 1:1 ratio has been suggested to exist at the monolayer by Bettelheim and Volman.[182] Approximately the same ratio has been found in the present work for apple pectin. Comparison of the monolayer value for this latter material with those for orange albedo pectin and for the two varieties of pectin from the potato studied, show that an approximate 1:1, water to monomer ratio is also found in these materials.

The presence of supposedly sharply delineated fractions of water, such as the BET monomolecular layer, are difficult to reconcile with, for example, the smoothly changing values of the heat of association. These values might be expected, rather, to change in a stepwise manner were such sharp distinctions between water fractions truly possible. Nevertheless, these derived values are useful as indicators of the approximate moisture contents at which recognisable changes in the general state of the constituent water take place. Bearing this in mind, Table 13 shows that, with the obvious exception of cotton cellulose, the BET monomolecular layer value constitutes between one third and one quarter of the unfreezable water present. Similarly at levels of 1.75 of the monolayer in the case of the commercial potato starch and apple pectin, and 2.5 of the monolayer in the

TABLE 15

CORRELATION BETWEEN EQUILIBRIUM RELATIVE HUMIDITY AND VALUES REFLECTING THE CONDITION OF WATER IN PLANT MATERIALS

Material	ERH, %, Corresponding to				
	BET Monolayer	Disappearance of Differential Heat of Association	Unfreezable Water Level	Intercept of Henderson plots	
Cellulose:					
Cotton	7.75	74	>80	26	50
Pentland Dell	22.5			30	45
Red Craigs Royal	19.5				
Orange Albedo	13.0				
Starch:					
Potato (commercial)	29.0	70	>80	24	62
Pentland Dell	17.5				45
Red Craigs Royal	16.0				
Pectin:					
Apple	28.0	62.5	78	15	55
Pentland Dell	19.0				53
Red Craigs Royal	13.0				
Orange Albedo	12.0				
Whole Pentland Dell	29.5	76	82		70

case of Pentland Dell potato, the heat of association becomes equivalent to the heat of condensation of water.

The absence of additional peaks in the heat of association curve, such as were observed by Bettelheim and Volman[182] in the case of pure pectic acid and de-esterified sodium pectate, and by Masuzawa and Sterling[170] in the case of agar and carboxymethyl cellulose, suggests that no more specific binding sites are made available as absorption continues. The continuing presence of a positive differential heat of association in that water constituting a further 0.8 moles of water per mole of glucose monomer in the case of potato starch, and 1.1 moles/mole uronide residue in the case of apple pectin indicates, nevertheless, that energy changes greater than those involved in condensation are still involved in the sorption of water molecules above the level of monomolecular layer formation. Thus, it would appear that a quantity of water approximately equal in amount to the monomolecular layer will be involved in the second discernible stage of sorption. The diffusion experiments of Duckworth and Smith[41] show, however, that this water is nevertheless capable of acting as a solvent at ambient temperatures, while the more highly associated monomolecular water is not.

An attempt to correlate this differentiation of water fractions with the existence of different straight line sections of the Henderson isotherm, as proposed by Rockland[267,286] (Local Isotherm Theory), were not successful. It can be seen from Table 15 that, although on the one hand the first intercept points between the straight line plots fall within the same ERH range corresponding to the completion of the monomolecular layer, the second intercepts are in general below the point where the

differential heat of association falls to zero. In addition, for any one material, specific agreement between the first intercept point, in those cases where it could in fact be detected at all, and the BET monomolecular layer is not good.

It is interesting to note at this point that application of the Bradley isotherm equation to the sorption data for starch, as illustrated in Figs. 26 and 51, results in a straight line relationship over the complete ERH range studied in this case, that is up to a moisture content of at least 23.5g/100g dry matter. This is in accord with Bradley's[37] hypothesis of the formation in such a system of polarized multilayers of water molecules, and suggests that multilayer formation could occur up to a moisture content equivalent to the unfreezable water level. A similar straight line relationship over a wide ERH range has been shown for Pentland Dell cellulose (Fig. 50) and claimed for other two component systems by Ling[35,350] in the case of gelatin, and by Duckworth[108] for starch and cellulose among other materials. An upward deviation from linearity above an ERH of 50% can be observed in the Bradley plots on the pectin data (Figs. 26 and 51) and also in the case of Pentland Dell potato (Fig. 51) above 70% ERH. Duckworth[108] indicates that in other multicomponent food systems a straight line relationship is only observable, if at all, up to moisture contents of between approximately 10g and 15g/100g dry matter, corresponding to an ERH of between 50% and 70%. This deviation is due to a lower observed vapour pressure than that needed for continued linearity.

In the multicomponent systems, and quite possibly in the

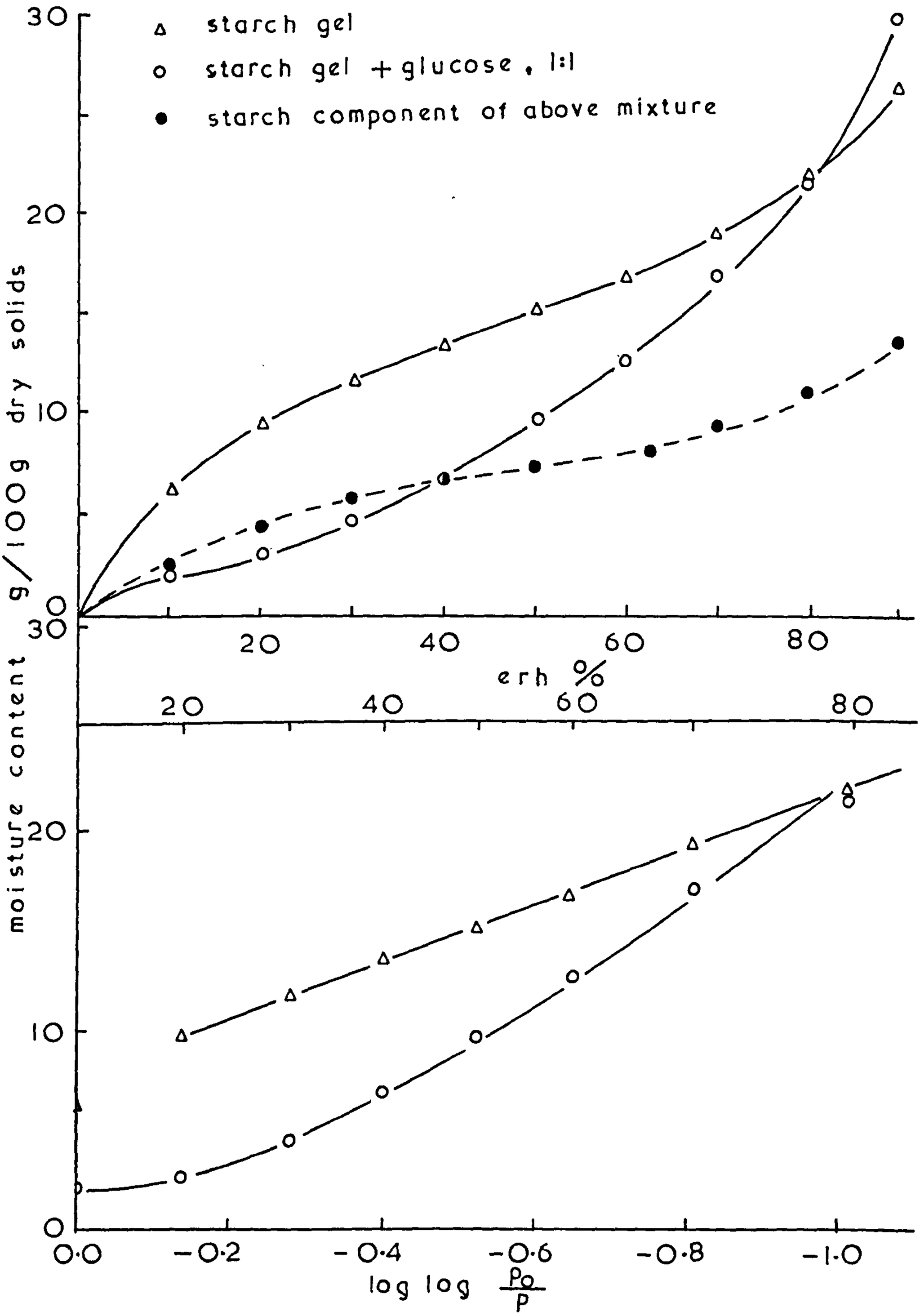

Fig. 75. Sorption data and Bradley plots of starch gel and starch gel with added glucose. From Saravacos and Stinchfield, 1965[113].

case of pectin, in addition to the macromolecular constituents there will be significant quantities of low-molecular-weight soluble materials present. Such substances, in solution, would cause a depression in vapour pressure in accordance with Raoult's Law and would be consistent with the deviation from linearity observed in the Bradley plots above moisture contents where free solvent water is present. This point is well illustrated in Fig. 75 where isotherms for starch gel and a 1:1 starch gel-glucose mixture are shown, (after Saravacos and Stinchfield[113]), together with the respective Bradley plots.

Application of the Harkins-Jura isotherm equation, which was developed from a potential approach based on condensation, to the pure plant constituents (Fig. 27) results in linear plots being obtained down to moisture contents below those where the Bradley equation suggests multilayer formation is still occurring. The apparent conflict which arises between these two approaches emphasises that no derived value should be taken as absolute when considering the state of water in a material. In complex biological materials, however, it is perfectly plausible to envisage an overlapping of multilayer and condensation effects.

The change in properties between different portions of water present in plant systems of low moisture content have previously been shown by other authors using nuclear magnetic resonance and by measurements of dielectric properties (Section 1.0). The NMR calibration curves conducted on the pure plant constituents examined in this study, and shown in Fig. 32, indicate that a small quantity of water, less than the monomolecular layer in the case of potato starch and apple pectin,

is restricted to the extent of not giving an observable signal on the low-resolution instrument used. A similar pattern of results is illustrated by Shaw, Elskin and Kunsman[175] for starch and pectin, although these authors extrapolated the calibration curve to pass through the origin, a marked deviation from the linearity shown between 7g and 20g/100g dry matter being necessary to achieve this. The associated changes in signal line width illustrated by these authors in the case of starch, and which show a sharp increase in width as the moisture content falls below 15g/100g dry matter, explain the disappearance of the signal reported in this present examination. This line broadening is attributable to an increased restriction of the water molecules which again is in accord with the relatively high heat of association observed at these low moisture levels.

Referring to Table 13 it can be seen that in all the materials examined the amount of water immobilized against freezing is greater than that which has a measurable differential heat of association at ambient temperatures. This apparent reduction in the amount of restricted water with an increase in temperature is explainable in the light of the hypothesis put forward by Riedel.[59] In this he postulates that at temperatures above -100°C both quasi-chemically combined water and unfrozen capillary solvent water will be present, the relative proportions of the two being temperature dependent and the amount of the latter increasing with an increase in temperature. Above the main freezing-thawing zone one would expect this latter fraction to be indistinguishable from the bulk of the non-restricted water in terms of the presence of any specific energy-involving interactions beyond those found in the bulk liquid. The

influence of the physical forces which reduce its mobility to the extent of it not freezing are reflected, however, in its lower vapour pressure as seen from the sorption isotherm.

The reduction in the amount of combined water with increase in temperature, as postulated by Riedel[59], is well illustrated by the temperature dependence of the sorption process, as represented in Figs. 21-23 and Fig. 44. At any given low ERH the amount of water decreases with increase in temperature due to the thermal disruption of the water-macromolecule hydrogen-bonds.

Analysis of the results from the nuclear magnetic resonance studies at low temperatures lends further support to the idea of a temperature-dependent equilibrium between combined and capillary water.

With reference to Figs..33-35 and 41-43 it has been shown that a certain amount of water present in the systems examined does not freeze in the main freezing-thawing zone, this quantity being in agreement with that determined within narrower limits by differential thermal analysis. The gradual reduction observed in the signal attributable to this water upon a further reduction in temperature could plausibly be accounted for by the slow formation of additional ice. The absence of any hysteresis effect, however, as observed in those experiments when the signal was monitored during subsequent rewarming (Figs. 33-35), indicates that this explanation is not valid. If ice formation did occur at these lower temperatures, thawing would not be expected until the main freezing-thawing zone was reached. Such behaviour is not indicated by the similar pattern of results obtained upon rewarming as were found upon initial cooling.

An alternative explanation, and one which fits Riedel's hypothesis, is suggested from studies reported in the literature of the observed change in signal line width with reduction in temperature in cod muscle[130], and in ribonuclease and bovine serum albumin.[107] In both cases the signal attributable to water persisting below 0°C was found to broaden as further cooling took place. Such a broadening would result, in the case of the low-resolution instrument used in the present study, in part of the signal not being detected within the gate. An increasing degree of restriction of the unfreezable water, manifest in the conversion of capillary water to combined water, would result in the observed reduction in signal size with a reduction in temperature and would be consistent with the change proposed by Riedel.[59]

Having rejected the idea that any significant irreversible freezing out of water occurs below the main freezing-thawing zone, an explanation must be offered of the low temperature thermal phenomenon observed during the differential thermal analyses on the micro-scale. (The broad rounded 'hump' occurring over the temperature range between -90°C and -40°C). In this case too, Riedel's proposed temperature dependent change in the proportions of combined water and unfrozen capillary water provides an interpretation of this phenomenon. It is also possible, however, that in relatively high moisture content samples part of the disturbance is due to a devitrification effect occurring upon warming, as suggested by Ladbrooke and Chapman.[117]

Inherent in Riedel's hypothesis is the definition of a minimum quantity of unfreezable water which will remain in this state

however low the temperature is taken. Above -100°C this will be composed of both combined water and unfrozen capillary water. At very low temperatures he further suggests that in samples with a moisture content in excess of this minimum quantity, there will be a reversible formation of additional combined water from ice present in the sample. Upon rewarming, this additional combined water will refreeze, to thaw at a temperature closer to zero. This latter refreezing process, together with an increased heat demand occurring as a result of the progressive dissociation of the original quasi-chemically combined water, could account for the observed endothermic effect under discussion.

The absence of the 'hump' phenomenon noted in the present investigation at moisture contents below the unfreezable water level, conforms with this interpretation - all the water present at these moisture contents being permanently unfrozen.

With reference to Fig. 6 it can be observed that the higher the initial moisture content above the unfreezable water level, the lower is the temperature at which the ice curve, E, is bisected upon rewarming. The crossing of this curve represents the point at which the conversion of reversibly-formed combined water to ice begins. Thus, at this temperature it would be expected that the onset of any thermal disturbance, caused by the change in the energy state of the water, would commence, The observed decrease in the temperature coinciding with the apex of the 'hump' following increases in moisture content above the unfreezable water level (for example as illustrated in Fig. 39) is consistent with this interpretation.

Ladbrooke and Chapman[117] observed by differential scanning calorimetry an exothermic change during the warming from -100°C

of a 40% and a 60% gelatin gel. They attributed this phenomenon to crystalline rearrangements occurring in the ice present in the samples. This suggestion is obviously very different from the nature of the changes proposed by Riedel and work is currently being conducted in this Department in an attempt to resolve the apparent anomaly between the suggested exothermic and endothermic changes occurring below the main freezing-thawing zone, and to determine the role in the 'hump' phenomenon observed in the present study.of the proposed crystalline rearrangements occurring in any ice which may be present.

In addition, a very recent report[353] has come to my notice which suggests that the low temperature endothermic phenomena under discussion is due to a glass transition in the relatively small amount of vitreous ice which it is postulated forms at moisture contents above the unfreezable water content. This change is then followed by a devitrification and melting process at higher temperatures. Sterling[354] has also very recently postulated from X-ray diffraction evidence the existence of vitreous ice in highly concentrated biological gels. It is important, however, to bear in mind the levels of hydration which are being considered compared to those very low levels with which Riedel is concerned in postulating his theory of the changing amounts of combined water. Much active discussion and current work is found in this general field, however, and no absolute answers can yet be given to certain aspects of the observed effects reported in this study.

10.2 THE CONTRIBUTION OF INDIVIDUAL CONSTITUENTS TO THE HYDRATION PROPERTIES OF INTACT MULTI-COMPONENT PLANT SYSTEMS

The examination of the three major macromolecular constituents of plant material has shown a consistent pattern to be present in their relative water binding properties (Figs. 20,36,46 and 47; Tables 9,10 and 11). Both starch and pectin have similar hydration characteristics although the isotherm of the latter material enters the third, steeply rising, portion of the curve at a lower ERH than does the corresponding isotherm for starch. In contrast, cellulose associates with considerably less water than do either starch or pectin, at least below an ERH of 70%.

These differences are illustrated in the isotherms presented in Figures 52 and 53 for the extracted fractions of Pentland Dell potato. In those fractions from which the low-molecular-weight soluble solids have been removed, the influence of the high percentage of starch in the system is noticeable. When this starch is removed the greater percentage of cellulose present is reflected in a depression of the isotherm toward the ERH axis. Removal of the remaining pectic material results in an isotherm similar to those of the purer cellulose materials studied.

A similar comparison between the behaviour of these three polymers is also illustrated by Stitt[205], although the starch and pectin isotherms shown exhibit a more pronounced difference than has been shown in the present study. For each material, however, a variability in characteristics can be expected between samples from different sources, due to factors of crystallinity, purity, and to minor differences in composition.

In all plant foodstuffs in a low moisture condition, therefore, there are macromolecular components present with significantly different hydration properties in terms of their moisture content-ERH relationships. When evaluating the significance of this it is more useful to consider the situation from the point of view of equilibrium relative humidity as a) the degree of restriction of the water present in each component is not necessarily reflected in differences in moisture content;and b) the overall moisture content of a multi-component system will not be the same as each of its constituents, these contributing to the total according to their relative proportions in the whole. In a dried material in an equilibrium condition, however, while the amount of water associated with each constituent will be different, the activity in each case will be the same at any given point over the complete humidity range. Although this does not necessarily mean that at a low moisture content the same stage in the absorption process will have been reached in each component, in the case of starch, cellulose and pectin it might be expected that the water present would be in a similar condition.

In starch and cellulose the only sites for the immobilization of water are hydroxyl groups. In the case of natural pectins, which usually have a high degree of esterification, it has been suggested that the methyl group will completely block the carboxyl oxygen in terms of its availability to bind water,[182] and thus, in these materials also, the hydroxyl groups of the molecules will certainly provide the major points of association with water. If the hydroxyl groups present in each of these

three materials are similar, therefore, it is likely that the water associated with them will be in a similar condition at any given humidity, the differences in the amount of associated water purely reflecting the number and availability of potential immobilization sites in the different polymers.

In view of the importance which has been placed upon the existence of the monomolecular layer as providing protection against certain types of deteriorative changes during the storage of dehydrated foods, it is pertinent to establish whether, in the plant materials studied, there is much variation in the equilibrium condition of relative humidity at which the layer is complete. Reference to Table 15 shows that with the previously explained exception of cotton cellulose, the monolayer values for the starches, pectins and celluloses from various sources fall within a 17% range below 29.5% ERH. Although this is a relatively large variation it is present in the case of all three constituents, the overall range being similar in each case. Taking into account the degree of imprecision involved in the determination of such a value this suggests that the process of water uptake in the three constituents is comparable and that at any one defined level during the hydration process the water is in a similar condition and possesses a similar activity.

The definition of the lower ERH limit within this range, or, rather, the moisture content of a complete system coinciding with this level, may be a useful parameter when considering dehydration processes, describing as it does the point below which no solvent water should be present in association with any of the components. Although the value of defining any such precise moisture content

is limited when considering large scale commercial processes, it would, nevertheless, allow a rapid method for the determination of a derivable moisture content to be achieved by the dehydration process. This would simply involve equilibration of the material over a saturated salt solution maintaining the required humidity and be followed by a moisture content determination, thus obviating the necessity for complete sorption data to be obtained.

In plant systems rich in other macromolecular constituents, especially proteins, the use of such an ERH value to derive dehydration parameters may not be applicable due to differences in sorption characteristics. It must also be borne in mind that the likely non-equilibrium situation encountered immediately after drying may create locally high concentrations of water even though the overall moisture content will be at the required level.

The possibility that the monolayer value as such could be calculated from the known monomolecular layer levels of its major macromolecular constituents, or indeed that a complete isotherm could be derived in this way, does not appear to be feasible. This is due mainly to the difficulty in predicting the influence of the low-molecular-weight soluble constituents present.

The fact that materials high in soluble solids have a lower water holding capacity than those high in macromolecular constituents has been shown by Salwin (Fig. 12). In addition, Saravacos and Stinchfield have illustrated the changed sorption characteristics of starch gel upon addition of glucose, as shown in Figure 75. A comparison of the present results in this connection with those reported in the literature, indicate that the effect of the soluble solids, which consist mainly of low-molecular-weight materials and various ionic solutes, is a very

complex one, some of the results obtained showing a certain lack of consistency.

With reference to Figures 46 and 47, it would be expected that due to the high proportion of starch present in potato (60-80% of the dry matter[252]) the isotherm of the intact material would reflect the behaviour of this major constituent. This is not the case, however, and the significant difference between the two isotherms suggests that the low molecular weight soluble materials have a depressive action on the sorptive capacity of the intact tissue, greater than that expected on grounds of proportionality.

As can be seen from Figure 52 and Table 11, removal of both ethanol-soluble and water-soluble solids from Pentland Dell potato increases the amount of water associated with the remaining material at any given ERH below 70%. A certain increase would be expected due to the higher proportion of macromolecular constituents in 100g of the extracted material, but the observed effect is greater than could be explained in this way.

Referring to Figure 52, at 50% ERH there are 16g of water present in 100g of dry Ethanol-Extracted Pentland Dell. When the soluble material removed during the preparation of this fraction was added back to give a final concentration of 7.5% on a dry matter basis, (that concentration present in the intact material), as expected, there was a reduction in the water holding capacity of the mixture compared to that of the extracted fraction. At the same ERH the 92.5g of extracted material present in 100g of the mixture would be expected to be associated with 14.8g of water. If the 7.5g of soluble material made a positive

contribution to the water holding capacity, even if this was of a low order, the water held by 100g of the mixture would therefore be greater than 14.8g. In fact it is considerably below this value suggesting that the soluble material in some way depresses the water holding capacity of the macromolecular constituents of the potato.

A similar addition of Ethanol-Soluble Material from Pentland Dell to both potato starch gel and cotton cellulose, but in the higher ratio of 15:85, solubles to insolubles, had a comparable effect on sorption characteristics (Fig. 65 and Table 12).

The influence of the presence of low-molecular-weight carbohydrate materials and their solution effects at higher humidities upon the shape of an isotherm is shown in Fig. 75. This illustrates the effect upon starch gel of the addition of an equal weight of glucose, as reported by Saravacos and Stinchfield.[113] At low equilibrium relative humdities, however, in contrast to the situation in whole potato, the addition of glucose has only a very slight depressive effect on the amount of water sorbed by 50g of the gel (illustrated by the dotted line).

In the case of orange albedo the isotherm of the intact material is similar to that of a theoretical albedo isotherm constructed from the data of the pectin and "cellulosic" constituents. In this intact material, however, these constituents compose only just over 50% of the total solids and thus their contribution to the water held by 100g of the whole albedo will be approximately half that illustrated for the theoretical system. It would appear, therefore, that the soluble solids present in the intact material make a positive contribution to the amount of water sorbed over the complete ERH range (Fig. 36). Several other

factors must be borne in mind, however.

In making comparisons between intact biological systems and those which have undergone an extraction procedure which will alter the fine structure, for example the removal of pectin, account must also be taken of such purely structural considerations. The diffusion studies of Duckworth and Smith[41] have suggested that the more highly structured nature of cell wall material, which is composed in the main of cellulose and pectin, is more conducive to holding water at moisture contents above the monomolecular layer than, for example, the gel structure of cooked potato starch. In the intact albedo, therefore, the insoluble constituents of which are principally cellulose and pectin, structure could be a significant contributory factor in relation to the hydration properties. Indeed, the difference between that water held by the macromolecular constituents and that held by the whole material is most marked above the monolayer region, although this may be partly explained by the solution effects of the soluble solids.

A further variable factor which must be considered is the ill-defined effect of ionic solutes.

Tracey[95], studying a system of plant origin (water-flour dough), suggests that the presence of salts reduces the amount of immobilized water due to a disruptive effect on the ordered structure of that liquid adjacent to the macromolecular surface, the ions themselves possessing an opposing structuring action. In a previous study conducted in this Department[343], the addition of sodium and magnesium chlorides was found to cause a slight increase in the quantity of unfreezable water present in potato. The degree of significance to be attached to these results was

doubted at the time, however, and in view of the known strong hydration capacity of magnesium ions[189], it might be expected that a significant effect would accompany its addition, or at least a difference in the magnitude of any effect when compared to that of sodium would be observed. Gál and Signer[197] studying an animal protein which possesses considerable numbers of ionic side chain groups found that the addition of salts increased the level of hydration at any given water activity. The mechanism proposed in this case was an interaction between the ions and the charged sites on the protein chain which, while preventing a direct association between water and the polymer, nevertheless allowed water to be ordered by the ions, as they would be in simple solution. Nemitz[118] also found the presence of salts in a high protein system increased the amount of immobilized water.

The overall picture obtained from the brief examination into the effects of added salts conducted in the present work (Figs. 65 and 66; Table 12) is consistent with the known behaviour of these materials in solution.

NaCl is considered to have an overall structure making effect, that is to create a net entropy decrease in water, while CsI has the opposite effect (Table 5). If these ions are introduced into a water poor system and a change in hydration properties is observed, it would be expected that whatever the mechanism of their action NaCl would tend to have a greater effect than the CsI due to the known differences in hydration potential.

In the present study little change was found in hydration

characteristics upon the addition of NaCl, although, if anything, a slight increase in water binding capacity was observed. The addition of CsI reduced the amount of associated water in the case of starch and had a similar, but less noticeable effect in the case of cellulose.

In these materials where there are no ionic groups present a direct interaction between the macromolecule and the added salts is unlikely. If a mechanism, as proposed by Tracey[95], is operative, one might expect the magnitude of the disruption effect would reflect the polarizing power of the added ion, this author suggesting that the removed water, in contrast to that influenced by clathrate formers, would not be associated with the salt to the extent of it being unavailable to affect the rheological properties of a dough.

The results observed in the present examination suggest, however, that different polarization properties of the various added solutes will determine the extent of the influence on the overall condition of the water in the system. While the ions will have a disruptive effect on the water directly associated with the polymer, it would appear that those capable of strongly polarizing the removed water molecules will in effect counteract their initial action by influencing the liquid in their own right. This would produce an effect similar to that proposed by Gál and Signer[197], but without any direct interaction with the macro-molecule. The surface charge density of the ion will dictate whether or not an overall increase or decrease in the amount of water present at any given ERH will be observed. CsI, for example,

which has a net structure-breaking effect on water when in simple solution, increases the mobility of the water present at low moisture contents, while NaCl, a net structure-maker, has little overall effect on this parameter.

In order to confirm this suggested mode of action of ions present in low moisture content macromolecular systems, a considerably more comprehensive study is needed. Nevertheless it is obvious that the soluble constituents of plant materials, both ionic and non-ionic, will affect the hydration characteristics of the macromolecular components and of the complete material in a way which may be both considerable in magnitude and complex in nature.

The introduction of known clathrate formers into a food system containing water has been shown to increase the amount of immobilized water.[95,212-215] This phenomenon will only be observed, however, under conditions which favour the formation of stable clathrate lattices; especially low temperature. The lack of a positive effect found in the present study upon the addition of tetrahydrofuran to either potato starch or cotton cellulose suggests that, under the experimental conditions employed during the preparation of the sample and the determination of unfreezable water, clathrate formation was not initiated.

10.3 CONCLUSIONS /...

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1. A similarity has been established between the water binding properties of starch and pectin.

2. Cellulose associates with considerably less water than either starch or pectin until high humidities have been reached. This reflects the lower number of specific sites for water immobilization due to the relatively high degree of crystallinity.

3. It is suggested that the process of sorption is similar in both starch and pectin.

4. The first stage in absorption involves the formation of a monomolecular layer of water. It is suggested that a 1:1, water to monomer ratio is present in this layer, each water molecule forming two hydrogen-bonds and each monomer unit possessing two available hydroxyl groups for hydrogen-bond formation. This monomolecular water has a heat of association with the macromolecule considerably greater than the heat of condensation of water.

5. The second stage in absorption involves the formation of an additional layer of water approximately equivalent in size to the monomolecular layer. This water has a lower, but still positive, differential heat of association although it is suggested that no new sites for hydrogen-bonding become available on the macromolecule as sorption proceeds.

6. A further quantity of water is distinguishable along with that in the first and second layer by virtue of its inability to freeze. This water has no differential heat of association with the macromolecule and it is suggested that it may be equated to the unfreezable, capillary water proposed by Riedel.

7. The total quantity of unfreezable water is between three to four times greater than that in the monomolecular layer.

8. Evidence in support of a temperature dependent equilibrium between quasi-chemically combined water and unfreezable capillary water has been obtained from differential thermal analysis, nuclear magnetic resonance and sorption studies.

9. It is suggested that in dehydrated plant systems with a low protein content a single specific value of equilibrium relative humidity equivalent to a moisture content in the region of the monomolecular layer value may describe a condition of maximum stability.

10. Due to the complex influence of both ionic and non-ionic low molecular weight soluble constituents it would not appear feasible to accurately predict the sorption characteristics of whole plant material from gross compositional data.

11. It is suggested that the influence of ionic solutes on the water immobilization properties of macromolecular systems depends upon their ability to orient water molecules in their own right.

S U M M A R Y

SUMMARY

This study was conducted in order to add to the knowledge concerning the condition of water in plant materials and to investigate the role of their major constituents in relation to hydration characteristics.

The water binding properties of three major macromolecular components of plant materials; cellulose, pectin and starch; obtained both from commercial sources and extracted from potato and orange albedo were investigated. Sorption studies at a range of temperature; differential thermal analysis on the micro-scale; and both ambient and low temperature examinations by nuclear magnetic resonance allowed a comprehensive qualitative and quantitative picture to be constructed of the state of water in these materials, and in particular at low moisture contents and at sub-zero temperatures.

A similarity in behaviour between starch and pectin was established and the considerably reduced ability of cellulose to immobilize water shown. It is suggested, however, that the process of absorption is similar in all three materials and proposals are made concerning the quantitative relationships between starch and water, and pectin and water during the initial stages of hydration.

It is suggested that if the monolayer region describes a condition of optimum stability in dehydrated plant materials with a low protein content, this layer will be complete in all the constituents within the same narrow ERH range and that

determination of the moisture content equivalent to the lower limit of this range offers a more easily obtainable parameter for dehydration processes.

Evidence in support of a temperature dependent equilibrium between quasi-chemically combined water and unfreezable capillary, solvent water was obtained.

An examination of a simple, natural system, orange albedo was conducted. The discrepancy between the amount of water held in the intact material and that proportion held by its major macromolecular constituents, pectin and cellulose, was attributed to the positive contribution of the soluble solids and of structure to the water binding capacity.

A more complex plant system, the potato, was also studied. The influence of its constituents on the hydration properties of the complete material was shown during a stepwise extraction of the tuber. In contrast to albedo the soluble solids were found to have an apparent depressive effect on the water binding properties of the macromolecular constituents.

A brief study was conducted into the effect of ionic solutes on the hydration characteristics of starch and cellulose. A mechanism based upon the difference in surface charge density is proposed for their observed action.

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