Physicochemical studies of wheat proteins and dough

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Physicochemical Studies of Wheat Proteins and Dough.

A Thesis

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by

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Preface

The work presented in this thesis is an account of the results obtained in a cooperative research project between the Bread Research Institute of Australia and the University of New South Wales. The author was seconded to the University for a period of ten terms to enable him to work under the direct supervision of Professor A.E.Alexander. He then continued his investigations at the Institute during another five terms.

The research project on which the author was engaged during this time, forms part of the programme of basic research at the Institute. This programme aims at an elucidation of the reasons responsible for differences in the bread making performance of wheat flours and the handling properties of the doughs made from them. The Director of the Institute assigned to the author the task of developing, under the guidance of Professor A.E.Alexander, the application of monolayer techniques to the study of wheat gluten, the protein complex of wheat flour in which differences in baking quality and dough behaviour are thought largely to reside.

Monolayer techniques were chosen as they offer several distinct advantages in the physicochemical investigation of an insoluble protein such as gluten. No surface chemical study of wheat gluten has previously been reported.

In the course of this investigation a study was made of our present knowledge of the chemistry and physical

chemistry of wheat gluten. A synopsis of this is presented in the introductory chapter to this thesis.

Chapter II contains a description of the materials used in this work. The preparation and analysis of gluten dispersions is detailed in Chapter III while Chapter IV discusses the surface chemical (monolayer) techniques and the apparatus employed.

In the following chapter the development of acid ethylene chlorhydrin as a new dispersant for gluten and other cereal proteins is reported. Chapter VI describes preliminary studies in the spreading of gluten dispersions as monolayers, and the suitability of dispersions in various media for this purpose.

Chapter VII contains the results obtained in a detailed study of gluten films spread from acid chlorhydrin. A comparative study of films of the proteins extracted with acid chlorhydrin from five different types of wheat flour and from the flours of rye, barley, oats and carob bean germ is described in Chapter VIII.

Chapter IX is introduced by a brief summary of the main features of protein monolayers. Differences between these and gluten films are pointed out and are then discussed in detail. Chapter X contains conclusions and suggestions for future work.

An Appendix presents in a concise form the mathematics of the damped oscillating surface torsion pendulum which have not previously been treated in a similar manner.

The thesis concludes with the author's expressions of

gratitude to all those who have given him guidance and assistance and have made this work possible.

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Summary.

Monolayer studies were carried out on wheat gluten and other cereal proteins to gain an understanding of the factors responsible for variations in the bread baking value of the different cereals and of different types of wheat flour.

Films were spread at the air/water and the oil/water interface and were examined by the techniques of the film balance (surface pressure – area isotherms) and the oscillating surface torsion pendulum (surface viscoelastic properties).

Ethylene chlorhydrin containing hydrochloric acid (acid chlorhydrin) was introduced as a new dispersant for gluten and other cereal proteins. The gluten molecules appeared to be more completely unfolded in this dispersant than in sodium salicylate or in aqueous acid or alkaline dispersions.

Gluten films were found to be considerably more stable than the films formed by most other proteins while at the same time showing greater compressibility. It is believed that portions of the strongly bonded coherent gluten film can be displaced from the interface with relative ease in the form of loops or folds.

As with other proteins, a change from the A/W to the O/W interface resulted in the expansion of the pressure-area isotherms and in enhanced viscoelasticity commonly ascribed to elimination of Van der Waals attraction between nonpolar side chains.

The areas of close-packing and of minimum compressibi-

lity were larger than those found with most other proteins and it is suggested that the reason for this might be found in the high proline content (ca.11%) of gluten.

Interesting hysteresis phenomena were observed on gluten films at the A/W interface.

Ionic strength (0.02 – 0.1 μ) and pH (1–12) had no effect on the stability of the films at either interface. When spread under 10% sodium salicylate, however, a very expanded film showing low stability and hardly any viscoelasticity was obtained. The remarkable stability of gluten films is therefore likely to be due to unusually strong hydrogen bonding probably involving the amide groups of the numerous glutamine residues (ca. 33%) in the chain.

When spread under 24% urea, however, gluten films were stable and viscoelastic and it is thought that an interplay exists between hydrogen bonds and ionic linkages.

That these latter play a considerable role in the intermolecular bonding of gluten films is shown by the marked dependence of the viscoelastic properties on the pH and ionic strength of the substrate. The highest viscoelasticity was observed around pH 7.5 which may be regarded as the film isoelectric point of gluten. The decrease in viscoelasticity away from the isoelectric point was more pronounced on the acid side. It is thought that this can be explained in terms of the dissociation of the ionogenic groups of the side chains. The viscoelasticity, at the same area and pH decreased with an increase in the ionic strength.

The viscoelastic properties of gluten films showed a

spontaneous increase with the age of the film while the interfacial pressure remained constant. This increase is believed to reflect intermolecular bond formation. The effect of changing the pH of the substrate again indicated that an interplay between hydrogen bonds and ionic linkages might be involved.

Great film stability and compressibility were found to be common features of gluten and of the protein extracted by acid chlorhydrin from wheat flour and from rye, barley and oat flour. While, however, the pressure—area curves of these proteins were very similar, the viscoelastic film properties of the various plant species showed considerable differences. The bread cereals, wheat and rye, were clearly differentiated from the non—bread cereals, barley and oat, in their dependence on the pH of the substrate. The effect was very marked in the first group and much less so in the second. This suggests that the bread baking value of the different cereals may in some way be linked with the number of charged groups in the chain.

No clear differentiation was obtained in the film properties of the proteins from different wheat flours and this must await further detailed study.

The protein extracted from the germ of the carob bean, reported to possess gluten forming properties, was also examined but found to be different from the cereal proteins.

Note.

The terms "surface" or "interface" in the wider sense refer to both the air/water (A/W) and the oil/water (O/W) interface. In this sense they are often omitted altogether if no confusion with "bulk" is likely to arise. In the narrower sense "surface" refers to the A/W and "interfacial" to the O/W interface.

For easier reference, tables are referred to by the number of the page on which they are found. Thus "Table 78" properly reads: "the table found on page 78". For the same reason graphs bear the number of the page they follow, from which they are distinguished by small letters. Thus "Fig.35a" reads: "the graph following page 35". If there are more than one graph on the same page or more than one graph follow a page, these are distinguished by the letters b,c, etc.

References are indicated by raised figures. A list of the full references is given at the end of the thesis.

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Professor A.E.Alexander has introduced the author to surface chemistry and has guided and counselled him for many years. Not the least of important lessons he has taught the author is that results can often be obtained by simple means. To him the author is profoundly grateful.

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The Staff of the Institute is to be thanked for selecting samples and carrying out routine cereal analyses on them.

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Chapter I.

Introduction.

From the colloid chemical point of view bread is a solid foam, i.e. a coarse dispersion of a gaseous phase (air) in a solid continuum. Bread is baked from yeasted dough which is itself a type of foam, the gaseous phase being carbon dioxide from the metabolism of the fermenting yeast cells, whilst the continuous phase consists essentially of a visco-elastic aqueous gel of protein and starch. Under the influence of heat in the baking even this gel is "set", retaining, however, a considerable amount of elasticity. In the course of the baking and the subsequent cooling process the carbon dioxide gas is exchanged against air by diffusion and the result is the light, porous structure which we associate with a good loaf of bread.

In order to yield satisfactory bread, the protein-starch hydrogel of the dough must possess considerable gas retention capacity coupled with a high degree of elastic extensitibility. Wheat owes its preeminence as a bread making cereal to its unique combination of these desirable features. Rye has only a limited bread baking value and no risen loaf of bread can be produced from either barley, oat, maize or rice. Although all species of the genus Triticum (wheat) are suitable to some extent, from a practical point of view, bread making is confined to the numerous varieties of the species Tr. vulgare or common wheat.

The ability of a wheat flour to produce a well-risen loaf of bread, usually termed its "baking strength"[137b], as well

as the rheological or handling properties of the dough made from it, vary greatly according to the variety, and within varieties, depend considerably on climatic and soil conditions.

Only a limited amount of control is available to the manufacturer over the baking strength and dough properties of a flour although these have a decisive influence on the manufacturing process as well as on the quality of the finished product. The correct design and operation of bakery machinery must depend on an appreciation of dough rheology.

An understanding of the factors responsible for the differences in flour quality is therefore a basic problem of the baking industry. This has become more pressing in our own times partly because of the competitiveness of the market and the high quality standards set by a modern industrial community, partly because of the technological necessity to standardize within relatively narrow limits the properties of mechanically processed raw materials.

The problem is of particular importance in this country where the industry, while in the full swing of mechanisation, has to cope with a rather wider variation in flour quality than most other similarly developed communities.

1. The Role of Gluten in Determining Flour Quality.

Wheat flour consists of the endosperm of the wheat kernel, the bran and germ being almost completely removed in the milling process. The chief constituents of the endosperm are protein and starch. Hess[117] has shown that the starch granules in the endosperm are completely surrounded

by protein. In milling, a portion of the protein is released as discreet particles and can be separated from the heavier starch by differential sedimentation in nonaqueous solvents[117,118] or by separation in a stream of air[104].

The endosperm structure is carried over into the dough where the hydrated protein forms a matrix in which the partly swollen starch granules are embedded. This structure has been demonstrated in a series of black-and-white and coloured microphotographs (obtained by differential staining)[101-104,118] and it is not surprising that the protein complex of wheat flour dough is mainly held responsible for its unique properties.

This view is based on two prima facie facts. Firstly, the proteins can be isolated from wheat flour dough as a coherent rubberlike elastic mass, the gluten, simply by kneeding it under water to remove the starch. This was demonstrated as early as 1745 by Beccari[21]. No gluten can be washed out from any of the other cereals including rye, although "glutens" of much inferior cohesiveness and elasticity can be obtained from these cereals by dispersion and precipitation of the proteins[52,53,99,100,140]. Secondly, glutens washed from the doughs of different types of flours vary in their mechanical properties, the differences by and large paralleling those of the doughs, while the amount of gluten isolated is often found to correlate with the strength of the flour.

From an early date, therefore, the interest of cereal

chemists and technologists has been focussed on gluten. For a time the amount of gluten that could be obtained from a flour was utilized as a measure of baking strength. However, the yield of washed gluten depends considerably on a number of factors[13,68,78] such as the temperature, pH and salt content of the wash solution, time of washing, etc. Consequently, gluten must be washed out under strictly standardized conditions, and the practice is now almost universally supplanted by the more reliable Kjeldahl nitrogen determination.

The addition of gluten of good quality to an inferior flour will raise its baking performance[4]. Experience has also shown[27a] that better wheats tend to have a higher protein content. The effect of protein quantity on loaf volume was demonstrated convincingly[70,231] on a series of flours with increasing protein content, obtained from the same starting material by air separation. However, protein content is definitely correlated with loaf volume only within the same variety[77], and it has long been realised that gluten quality is more important than quantity[77,201,209] in determining the bread making performance of a flour.

A large number of mechanical gluten testing instruments were developed from as early as 1848 (see review by Bailey[16] and some later work[120,224]). Although some proof was presented[204] that gluten and dough behaved in a similar way in certain rheological experiments and that differences could be explained by and large by the absence of the starch component in gluten, experience showed that the

correlation between gluten and dough properties was not satisfactory for practical purposes and gluten testing has been given up in practically all cereal laboratories in favour of physical dough testing. (For this see Kent-Jones and Amos[137a], the reviews of Bailey[16] and of Greup and Hintzer[94], and numerous more recent papers mainly in Cereal Chemistry, particularly by Hlynka and co-workers who emphasize the importance of relaxation studies).

It is often overlooked that the true quality of gluten is not evident until the dough has been subjected to fermentation. Gluten seems to exhibit its maximum strength when developed by yeast in the dough[18]. This realisation has led to the design of apparatus for the testing of yeasted rather than the customary unyeasted dough[137e].

The role of the starch component in determining baking strength cannot be neglected. Fuchs[83] has recently reviewed the subject and also showed that aerated loaves could be baked from starch (wheat, maize, etc.) and even from millet, pea and bean flour by the addition of swelling agents such as pregelatinised starches or certain cellulose derivatives. These "doughs", however, completely lacked the plastic properties of a wheaten dough.

While it is thus generally recognised that the baking strength of a flour and the physical properties of the dough are the result of an interplay of many factors, flour protein continues to be regarded as the constituent meriting primary attention.

However, despite the considerable amount of work expended on the investigation of the physical and chemical properties of wheat gluten, (probably the best investigated of all plant proteins), it was not found possible to advance a satisfactory explanation either for the distinctive position of the wheat proteins among plant seed proteins in general, or for the variations in bread making characteristics of flours from different wheat varieties and sources.

The body of work on the physics and chemistry of flour proteins accumulated over the years has been discussed repeatedly by different workers from slightly differing points of view[17,24,26,27,51,86,137b,153,175,186,216]. The following is an attempt to summarise the salient points only.

2. The Chemical Composition of Gluten.

Chemically, gluten is a cross-linked high-polymer of considerable complexity. Dry gluten contains[27d] about 75-85% protein, 8-10% starch, 1-2% reducing sugars, 2% cellulose and a small percentage of minerals. The water content of wet gluten is about 66%.

According to Osborne's classical scheme of characterizing cereal seed proteins[181], wheat flour contains 4-6% albumins (soluble in water), 4-6% globulins (soluble in dilute salt solutions), 40-45% prolamin (soluble in aqueous alcohol), and 40-45% glutelin (soluble in dilute alkali). The prolamin of wheat is called gliadin while the glutelin is termed glutenin.

Hand-washed gluten contains not only the gliadin and

glutenin fractions but also about half of the albumin and almost all of the globulins[186]. The proportion of starch remaining in the gluten depends on the efficiency of the washing process. At least some of the carbohydrates, however, appear to be bound chemically to the protein. The same is true of the lipids. Less lipids can be extracted from dough than from flour. The lipids seem to be associated with the more insoluble gluten fractions[179].

The amino acid composition of gluten as well as gliadin and glutenin have been determined by several workers using different techniques. The latest tabulation of these analyses is that by Bourdet[27b]. The most comprehensive study of the amino acid composition of gluten was carried out by Pence et al.[185], who investigated 17 different wheat varieties. They found no significant differences in the amino acid contents of flours from different wheat varieties. The averages of their results (slightly rearranged) are shown in Table 8. They represent probably the best available "building block" analysis of the gluten proteins.

Nothing is known yet of the sequence of these building blocks along the chain. Nakashima[173] reported the occurrence in gliadin of a tetrapeptide consisting of one glutamic acid, one tyrosine and two glutamine residues. Results of N-terminal amino acid analyses carried out on gliadin[64,65,139,192] failed to agree.

From the data in Table 8 the following conclusions may be drawn. Firstly, polar and non-polar side chains are present in about equal amounts. Secondly, the amount of

Table 8.

Amino Acid Composition of Gluten.

(Referred to a theoretical protein of 17.5% nitrogen).

Group	Amino acid	g/100g	moles/10^5g
(1) Large Nonpolar Side chains	Valine	4.7	40
	Leucine	7.6	57
	Isoleucine	4.6	35
	Proline	12.7	110
	Phenylalanine	5.4	33
	Tyrosine	3.1	17
	Tyrptophane	1.1	5
		39.2	297
(2) Small Nonpolar Side chains	Glycine	3.5	47
	Alanine	2.2	25
	Methionine	1.9	13
		7.6	85
(3) Nonionic Polar Side Chains	Serine	4.7	45
	Threonine	2.6	21
	Cystine	1.9	8
		9.2	74
(4) Cationic Side Chains	Lysine	1.8	12
	Arginine	4.7	27
	Histidine	2.3	15
		8.8	54
(5) Anionic Side Chains	Aspartic acid	3.7	28
	Glutamic acid	35.5	241
		39.2	269
Total		104.0	779
Ammonia		4.5	264

basic side chains is small (ca.7%). Thirdly, there is a very large proportion (ca.35%) of dicarboxylic acids, about 90% of which is glutamic acid. These, however, appear to be present overwhelmingly as amides (cf. total ammonia). Considerations of the isoelectric point (See Section I-3) and studies of dye-binding capacity[225] seem to support

the view that the actual number of anionic and cationic groups is about equal. Finally, gluten contains a substantial proportion of proline and a significant, although relatively low, amount of cystine.

From the considerable proportion of non-polar side chains one would expect Van der Waals dispersion forces to play a considerable role in inter- and intrachain binding. This is in some measure supported by the partial solubility of wheat flour proteins in organic solvents (cf. Sections V-1 and V-2).

Free acidic and basic groups are essential for the formation of ionic or salt linkages, the existence of which in gluten is suggested by the dispersibility in salt solutions and acids and alkali.

The high amide content of gluten is significant in view of the extraordinary molecular cohesion of the $-CONH_2$ group[40a] suggesting the possibility of strong hydrogen bonds involving the amide groups of the glutamine and asparagine residues along the chain[230].

On the other hand, proline, being an imino acid, is distinguished from the others by not having a bond forming hydrogen in the peptide backbone itself[184]. It also disrupts the regular sequence of the amino acids for sterical reasons[218]. A high proline content must therefore be expected to have a significant influence on the properties of the protein.

The cystine/cysteine content of gluten has, of course, aroused a good deal of attention since -S-S bridges are the

only likely covalent bonds to partàcipate in interchain linkage. There is no evidence for either ester or peptide interchain links in gluten.

No amino acid analysis of precipitated "glutens" of the other cereals are available. Cunningham et al. recently produced evidence[52] that the amide nitrogen content is highest in wheat gluten, intermediate in barley and rye, and lowest in oat "gluten". According to the data of Osborne and Clapp[182], the composition of the rye and barley prolamins closely resembles that of gliadin. The data collected by Bourdet[27] and the newer analyses of Folkes and Yemm[79] on barley prolamin (hordein) and glutelin(hordenin) also support the view that the cereal proteins are constituted on broadly similar lines.

Mention should be made here of the tree legume Ceratonia siliqua or carob bean. It was apparently first reported[199] by Sagi in 1933, that a coherent gluten could be washed from flour milled from the seed germ of this plant. So far, this material (and perhaps some related species, cf. Bienenstock at al.[23]) is the only non-wheat protein known to possess this property. Rice and Ramstad[193] reported that carob gluten differed from wheat gluten in containing much more arginine, aspartic acid and lysine; somewhat more glycine and histidine; somewhat less cystine, glutamic acid and phenylalanine; and much less proline. They concluded that similar physical properties may be shared by proteins of dissimilar amino acid composition.

3. The Physical Chemistry of Gluten.

The available chemical evidence therefore is not adequate to explain either baking strength or its variations. The physicochemical investigation of gluten is greatly handicapped by exactly that property which makes it so unique: its insolubility. This question will be reviewed in more detail in Section V-1. Progress in the physical chemistry of gluten (and the cereal proteins in general) closely paralleled on the one hand, the development of new methods of effecting solution or dispersion, and, on the other hand, the development of the modern colloid chemical techniques for the study of proteins and synthetic high polymers.

Practically coinciding with the classical chemical studies of Osborne[181] were the physicochemical investigations of Wood[232] and of Wood and Hardy[233] who first drew attention to the colloidal nature of gluten. Initially, interest centred mainly in the swelling and disintegration of gluten in aqueous solutions of acids, alcohols and various salts and in the viscosity of acidulated flour suspensions[137c]. While this work clearly demonstrated considerable differences in the glutens or flours from various wheat varieties, it remained of doubtful practical value for establishing correlation with baking strength. Only the Zeleny test[235] based on the sedimentation of lactic acid suspensions of wheat flour has recently come into some prominence as a quick grading test for wheats.

As the modern techniques of protein chemistry became available, these were naturally first applied to the alco-

hol soluble gliadin, at that time believed to be a well-defined protein of known amino acid composition which could be obtained in a relatively high state of purity. Glutenin was much less investigated, no doubt partly because its poorer solubility characteristics, and partly because of the early realisation[25] that glutenin is not a well-defined protein and that its properties depend to a considerable extent on the method of preparation.

The introduction of the so-called "neutral solvents" urea[42] and sodium salicylate[43] gave a new impetus to the investigation of gluten dispersions.

For detailed reviews, the reader is referred to the references on p. 6. The main outcome of these researches was that the earlier concept of the two distinct "insoluble" proteins gliadin and glutenin had to be abandoned. Current opinion favours the view that gluten is made up of a number of proteins although there is no unanimity on whether it consists of a relatively small number of distinct proteins or whether it consists of a "reversible complex made up of molecules that vary progressively and continuously in both chemical and physical properties."[41]

As Blish has pointed out[24], appearance of non-homogeneity may be due, in considerable measure, to aggregation, and to component interaction with "complex formation" rather than the actual existence of numerous individual components.

Gliadin is considered to be made up of smaller and more

symmetrical molecules than glutenin[20,142]. The molecular weight of gliadin has been determined by practically all the available methods. These measurements[14,95,141,145,203] place it between 26000 and 27000 although considerably higher values were reported by some workers[74,115,141], particularly those using osmometry[36,70]. The lowest molecular weight determined by physical measurements is 24000 obtained by Holme[123] from light scattering. In view of the often demonstrated inhomogeneity of gliadin[20,76,115,138,141–143,167,208] these values should, of course, be regarded as averages. No molecular weight determinations were reported on the glutenin fraction. In an ultracentrifugal study of gluten dispersed in sodium salicylate McCalla and Gralen[156] concluded the minimum molecular weight of gluten to be 35000. Electrophoresis in acid buffers[148,150,165] showed gluten to be inhomogeneous.

Another important result concerns the shape of the molecule. Axial ratios of from 10 to 26 were calculated for gliadin[20,30,164,176]. The values reported for gluten[20,41,51,84] ranged from 15 to 41. The frictional ratios of gluten fractions in sodium salicylate decreased with increasing weight[154] and this was regarded as an indication that aggregates were formed by side-to-side rather than end-to-end aggregation of the molecules.

Clearly, the gluten molecule appears to be much more elongated than the so-called globular proteins. This has led some workers[51,221] to regard gluten as intermediate between the globular and the fibrillar proteins.

Data on the isoelectric point of gluten are scanty and

conflicting. Maximum precipitation of gluten dispersed in formic acid[52] occurs at pH 5.5 – 6.0. Bungenberg de Jong and Klaar[35] considered the isoelectric point of gluten to be 6.1 (cf. Section I–4). Dill and Alsberg found[68] that gluten was most insoluble at pH 6.8, maximum yield of gluten being obtained when washed from dough with a dilute buffer of this pH. Values ranging from 5.7 to 6.3 were obtained from microelectrophoresis measurements of quartz particles coated with gluten[136].

Few attempts were made to correlate physicochemical measurements on gluten dispersions with baking quality. Electrophoretic studies of glutens from various wheats dispersed in acid buffer failed to reveal any significant differences[148]. In sodium salicylate[41], gluten was found to be electrostatically homogeneous with a high mean negative valence. This was attributed[154] to complex formation between the protein and the salicylate ion, precluding the use of salicylate dispersions for the purpose of electrophoresis.

Although earlier experiments indicated[84,112,196] that the "better" gluten would yield higher viscosities in salicylate dispersions, Udy found[223] that the relative viscosity of such dispersions was independent of the varietal source of gluten. He concluded that the electrostatic forces of interaction between the protein molecules were repressed by the high ionic strength of the medium. On the other hand dilute acetic acid dispersions of the same glutens differed in the relative viscosities. Udy did not attempt to

correlate these differences with flour quality.

4. Cross-linking in Gluten.

The physical properties of gluten must be determined essentially by the extent and nature of the cross-links in the network. Hydrogen bonds, electrostatic dipole interactions Van der Waals dispersion forces and ionic links as well as cystine bridges are involved. The inevitable breaking of many of these bonds on dissolving or dispersing wheat gluten severely limits the usefulness of the study of dispersions for the understanding of the network properties.

From the solubility characteristics of gluten (cf. Sections V-1 and V-2) it may be inferred that hydrogen bonds, Van der Waals attraction and salt bridges play a part in its intermolecular cohesion.

The importance of electrostatic attraction has been emphasized by Bungenberg de Jong and Klaar and by Hess from somewhat different points of view. The Dutch authors[35] believe that gliadin and glutenin are mixtures of closely resembling fractions with slightly differing physical properties and that they interact by reason of differences in electrical charges on the particles. Maximum interaction was shown to occur in the region between the isoelectric points of the two proteins, in which the gliadin carried a positive and the glutenin a negative charge.

According to Hess[117,118], the protein surrounding the starch granules in the endosperm (the "Haft", or "adhesion" protein) differs from the protein filling the intergranular

spaces (the "Zwickel" or "interstitial" protein), from which it is separated by a lipid layer. "Haft" protein is richer in lipids and has an isoelectric point near pH 7, while "Zwickel" protein has an isoelectric point near pH 6 and has no free amino groups reacting with formalin. Gluten is formed by electrostatic interaction between these two proteins which are not thought to be identical with glutenin and gliadin[119].

Since gluten contains about 2% of cystine, the possible role of disulphide bridges[195] has aroused considerable interest. These bridges are sensitive to oxidation and reduction. There is no lack of evidence that oxidising and reducing agents have a profound influence on dough and gluten properties. In fact, the action of the so-called bread improvers, chemicals capable of influencing the physical properties of dough in a marked manner when added in only minute amounts, is almost certainly an oxidation reaction. Despite considerable effort, however, a completely satisfactory explanation of how these effects are brought about has yet to be found[26,27,186,215,216]. This is due in large measure to the experimental difficulties with which the estimation of sulphydryl and disulphide groups in complex systems such as flour, dough or gluten, is fraught[186,216].

The view most favoured currently is that of a direct action of oxidising and reducing agents on the sulphydryl and the disulphide groups[58,144,160–162,187,216,217,223,234] respectively, although the earlier view[19,133] that redox reagents act by the inhibition and activation respectively

of proteolytic enzymes has not yet been completely disproved[26].

Pence at al. think it conceivable[186] that oxidising agents influence dough behaviour by way of the soluble proteins. The relation of these with baking strength is being investigated[188].

The possibility that reducing carbohydrates may act as cross-linking agents between gluten chains through their aldehyde groups and that this action may be influenced by redox reagents has lately aroused interest[121,122,174,197,198,226].

Flour lipids, which have considerable influence on baking quality, may also be affected by oxydizing reagents[46]. The role of the lipids is currently receiving much attention[34,46,163]. X-ray studies[221] revealed, in wheat grain, a spacing at 47 A not observed in any other cereals but common in all varieties of wheat examined. This spacing was found to be due to the "bound fat" (phospholipid) closely associated with the protein. The acetone extract from rye, but not from barley, oats or maize, also contained the 47 A spacing fat.

While in the past gluten has been regarded simply as a protein complex difficult to free of its lipid and carbohydrate contaminants, the recognition that it is in fact a lipo-glyco-protein complex, now seems to gain ground.

5. Surface Chemical Methods.

In his 1954 review on wheat gluten Blish stated[24a]: "Convincing solution of the problem of gluten structural composition and homogeneity apparently must await discovery

of appropriate solvents, or of new methods and criteria, or a combination of both". Reviewing the position in 1956, Bourdet[27c] still repeated the same words.

The work presented in this thesis is an attempt to introduce a new dispersant for cereal proteins and to widen the range of the available methods by the inclusion of surface chemical techniques, in particular those of spread monolayers. These techniques combine the advantages of speed and versatility with low cost of equipment and the need for only small quantities of test material.

The monolayer may be spread at a great variety of interfaces allowing experiments to be carried out under a range of conditions that is usually difficult to realise in bulk investigations. This is a particular advantage with proteins that are difficult to dissolve or disperse. Gluten stays in dispersion only within a relatively narrow range of conditions which cannot be altered materially without effecting precipitation. Furthermore, the study of such dispersions is always handicapped by the interference of the dispersant. When, however, a protein is spread as a monolayer, the dispersant is rapidly lost from the interface and the protein may be studied free of its influence.

In recent years, monolayer techniques have become a recognised tool of high polymer research. As an example of such studies on an insoluble protein, the work of Ellis and Pankhurst on collagen[72,73] should be mentioned.

No surface chemical studies have hitherto been reported on whole wheat gluten or any of the other cereal flour pro-

teins, although gliadin, and to some extent zein, the prolamin of maize, have been studied. The object of these investigations, however, was the development of monolayer techniques as a tool of general protein chemistry and not the study of gliadin as a constituent of wheat protein. Gliadin aroused much interest because of its solubility in alcohol.

The first spreading of gliadin films was reported by Gorter and Grendel[89,90]. This was followed by a detailed study of gliadin monolayers by Hughes and Rideal[124] using the techniques of surface pressure and surface potential measurements. In later years, a great many papers were published by various authors, dealing with surface pressure, surface potential and surface viscosity measurements on gliadin films[39,88,129–132,145–147,170,207]. Interfacial pressure–area curves at the water/benzene interface were also obtained[8]. Some work was carried out on mixed films of gliadin and cholesterol[146,207], and of gliadin and tannic acid[39,87].

In 1942 Blokker and Gorter[87] presented a comprehensive study of the influence of pH on the surface pressure, potential, compressibility and viscosity of gliadin films spread from aqueous ethanol solution.

The film molecular weight of gliadin was determined by Guastalla[95–98] as 26000–27000 in excellent agreement with bulk measurements. Guastalla's measurements were confirmed by Benhamou[22] who showed, however, that the molecular weight varied with the pH of the substrate. Quite recently Jaffe and de Coene[128] calculated axial ratios of gliadin

fractions from surface pressure measurements. Schulman and Dogan[206] studied the influence of chromium and copper ions on gliadin films.

Some of this work will be referred to in more detail in Chapter IX. Gliadin films were found to be more compressible than most other protein films[190] but in other respects no clear differences were revealed.

Gliadin, when doughed up with water, is inelastic, though extensible and does not show the remarkable properties of gluten. The work presented in this thesis, being primarily concerned with the problem of baking strength and its variations, was carried out on whole gluten rather than any gluten fraction.

The techniques used were those of spreading gluten films at an interface between water and air, or between water and oil, and recording changes in the surface pressure and the surface viscoelastic properties as a function of the available area per unit weight of protein. The apparatus and techniques employed are discussed in detail in Chapter IV. The following two chapters deal with the materials used and with the preparation and analysis of the dispersions.

Chapter II.

Materials Used

Reagents were of A.R. quality unless otherwise stated. Water was first deionised and then distilled from an all-glass still containing a trace of sulphuric acid.

1. Flours:

Wheat flours were milled on a Buhler experimental mill from sound whole grain of known variety. Samples were analyzed by the official methods of the AOAC[2] for protein content, moisture and diastatic activity. Doughs made from these flours were tested on the Chopin alveograph[137d].

These samples were selected so as to represent a gradation both with respect to "baking strength" and to "balance" (ratio of extensibility to stability, a measure of handling properties). The gradation is representative of the normal range of baking flours encountered in this country.

Rye, barley and oat flours were milled on an Allis Chalmers mill from whole rye, pearl barley and dehulled oats, and analyzed by the AOAC methods for protein content, moisture, ash, and fat content.

Defatted oat flour was prepared by extracting oat flour twice for five hours with redistilled petroleum ether (B.R. 40–60 °C).

Carob bean seeds were softened by boiling in water for 30 minutes and soaking in a fresh portion of water overnight. They were then opened up by hand, the germ picked out and dried over anhydrous calcium chloride. This procedure was thought preferable to carbonising the seed

coats in concentrated sulphuric acid[193]. The germ was ground in a laboratory hammer mill, bolted through 56 mesh nylon gauze, and analyzed for protein content, moisture and fat content.

The analytical and physical testing data are shown in Tables 22a and 22b.

Table 22a

Analytical Data of Wheat Flour Samples.

Classification	Strong	Weak	Medium	Harsh	Extensible
Alveogram 'strength'	84	13	48	46	35
" 'balance'	1.23	1.10	1.42	0.75	3.56
Diastatic activity	2.57	1.82	2.25	2.48	1.16
Extraction (%)	73	73	74	73	74
Protein (% dry basis)	11.71	9.92	12.50	10.60	10.12
Moisture (%)	15.0	12.8	14.4	14.6	14.0
Ash (% dry basis)	0.55	0.53	0.48	0.57	1.85
Fat (% dry basis)	1.91	2.06	1.80	1.97	1.85
Protein/fat ratio	6.12	4.82	6.94	5.38	5.46

Table 22b

Analytical Data of Non-Wheat Flour Samples

Cereal	Rye	Barley	Oat	Def.Oat	Carob
Extraction	19.8	26.2	52.9	—	—
Protein (% dry basis)	6.52	6.15	8.20	10.52	62.2
Moisture(%)	13.4	12.2	9.2	10.8	9.3
Ash (% dry basis)	0.38	0.59	0.74	—	—
Fat (% dry basis)	1.56	1.70	8.68	2.37	9.20
Protein/fat ratio	4.17	3.62	0.95	4.43	6.76

2. Dispersion Media.

Ethylene chlorhydrin (2-chloro-ethanol) was purified from laboratory grade material. When this was distilled under

atmospheric pressure, the distilling liquid became yellowish-greenish, the acidity rose and a dark brown oily fraction remained. Some of the hydrochloric acid invariably passed over into the distillate. Gross impurities were therefore removed by a preliminary distillation under reduced pressure. The sample was then dried and freed of acid over desiccated potassium carbonate and twice redistilled under a vacuum of about 18–22 mm Hg. The purified chlorhydrin had an acidity of less than 0.01% expressed as hydrochloric acid.

Dry hydrochloric acid gas prepared from sulphuric and aqueous hydrochloric acid as described by Vogel[229], was passed into the redistilled chlorhydrin until a concentration of 0.365% (0.1 M) was attained.

Solutions of lower hydrochloric acid content were prepared simply by dilution of the 0.1 M sample with purified chlorhydrin.

Acid alcohols were prepared by passing, in the same manner, dry hydrochloric acid gas into redistilled n-butanol, isopropanol and absolute ethanol freshly distilled from calcium oxide.

Solutions of other acids in purified chlorhydrin were prepared by adding the required amounts of concentrated sulphuric acid, anhydrous (90%) formic acid, glacial acetic acid and laboratory quality trichloracetic acid.

Sodium salicylate was of B.P. quality and was used in 10% aqueous solution, having been checked for melting point (acid) and purity (% sodium salicylate).

Aqueous solutions of 0.01 N hydrochloric, formic and

acetic acid and of sodium hydroxide were prepared and standardized in the usual way.

Concentrated lithium bromide solutions were prepared from B.P. reagent, filtered and standardized by the Mohr method.

3. Substrates.

Twice redistilled carbon tetrachloride was normally used as the oil phase. A few experiments were made with redistilled petroleum ether of B.R. 60°–80°C.

The aqueous substrates were mainly solutions of hydrochloric acid, sodium hydroxide and various buffers of different pH and ionic strength. A few experiments were also carried out using 10% sodium salicylate, 24% urea, 0.1 N sodium sulphite and 0.01 N sodium thioglycollate in glycine buffer of pH 10.6.

Dill and Alsberg's phosphate buffer[68] of pH 6.8 was used for most of the determinations at neutral pH. Gluten is claimed to be most insoluble at this pH which was therefore chosen as the most likely isoelectric point. The surface isoelectric point of gluten was later found to be somewhat higher but, for reasons of comparability, measurements were continued at this pH, which may be regarded as the lower limit of the surface isoelectric range. The buffer was adjusted to 0.1 and 0.02 μ with potassium chloride.

At pH 1, 2, 12 and 13, 0.1 and 0.01 N solutions of hydrochloric acid and sodium hydroxide were used respectively. The 0.01 N solutions were adjusted to ionic strength 0.02 and 0.1 with potassium or sodium chloride. The buffers used

at the other pH values were acetate (Walpole), glycine (Sørensen), phosphate and boric acid (Clark and Lubs) buffers, made up as directed by Vogel[228] and again adjusted to ionic strength 0.02 and 0.1 when required.

Veronal (Michaelis) buffers were found to give anomalous results.

Chapter III.

Preparation and Analysis of Dispersions.

Dispersions were prepared either from vacuum dried gluten or directly from flour. These dispersions had a protein content of about 5 mg/ml as determined from the Kjeldahl nitrogen figure using the customary factor[17a] of 5.7. After analysis the dispersions were diluted to about 0.4 mg/ml. Acid chlorhydrin and sodium salicylate dispersions were diluted with the same reagent. Aqueous acid and alkaline dispersions were diluted with acid-free chlorhydrin. All dispersions were stored in a refrigerator at about 5°C.

1. Preparation of Gluten.

Gluten was washed from the medium wheat flour following the standard procedure detailed in Cereal Laboratory Methods[1], except that Dill and Alsberg's[68] phosphate buffer was substituded for tap water. The gluten was dried in vacuo, powdered, bolted through mesh 56 nylon gauze and stored over phosphorus pentoxide in a vacuum desiccator. The protein content of the dried gluten was about 80%.

Acetone extracted gluten was prepared by Dr.H.Zentner of the Bread Research Institute by triturating wet gluten with several changes of acetone, filtering on a Buchner funnel and air drying.

2. Preparation of Dispersions.

Dispersions were prepared by extracting about 100 mg of dried gluten or an equivalent amount of flour for three days with 10 ml of the dispersing agent in glass stoppered

test tubes which were shaken at regular intervals. At the end of the extraction period, the tubes were centrifuged at 2000 rpm. for 20 minutes in an MSE Major centrifuge and the supernatant was decanted into another tared tube. The residue was shaken up with 3 ml of dispersant, recentrifuged and the supernatants united. This process was repeated once.

3. Analysis of Dispersions.

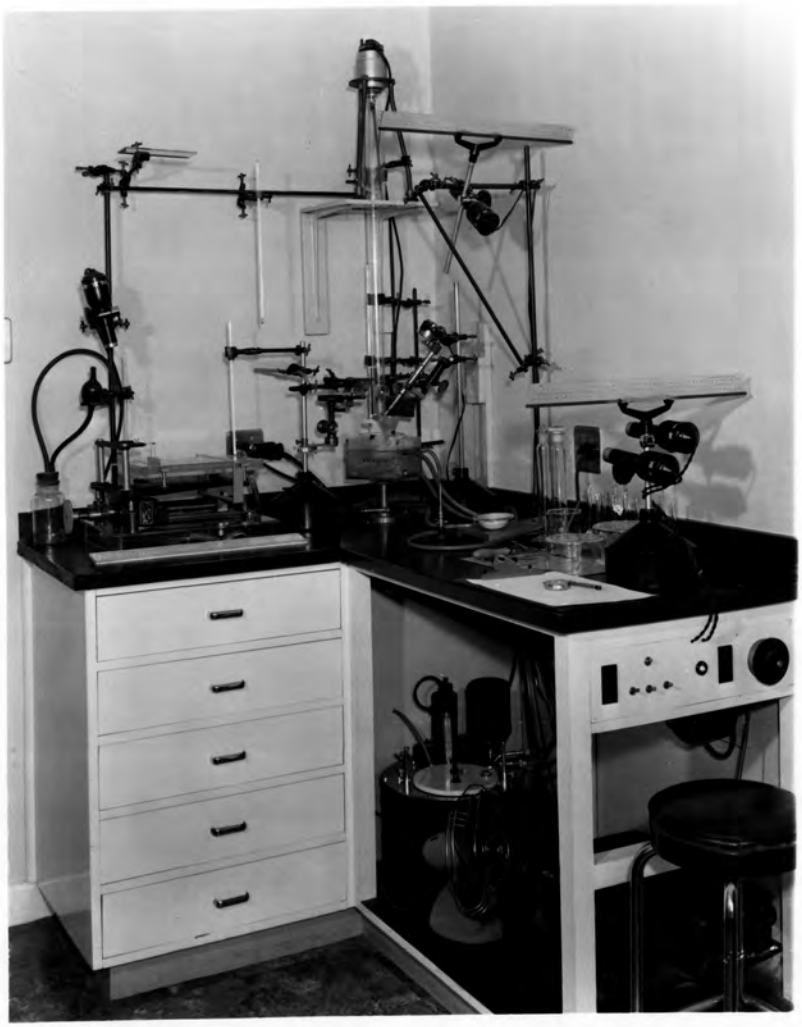
Protein concentration was determined by analysis of the combined supernatants in duplicate by a semi-micro Kjeldahl procedure. Two ml of the dispersion were digested in 50 ml Kjeldahl flasks with 5 ml of concentrated sulphuric acid and about 2 g of a 1:100 mixture of selenium and potassium sulphate as catalyst. Chlorhydrin was found to deplete the flasks of sulphuric acid, probably through the formation of volatile sulphates[135,202]. The addition of 5 ml of water overcame this difficulty as the chlorhydrin distilled off with the water before reacting with the sulphuric acid. The dispersions frothed badly but when the burner was turned off as soon as frothing began, the digestion could be continued without any trouble after a few minutes. The same procedure was followed with the alcoholic dispersions.

The digests were cooled, diluted with 10 ml of water, cooled again and transferred to a Mackenzie-Ohye[158] vacuum jacketed still. The ammonia liberated with 42% sodium hydroxide was steam distilled into sulphuric acid. This was titrated with N/8 standard alkali from a micro burette using methyl red-methylene blue indicator.

A reagent blank in duplicate was carried along through all stages.

The degree of dispersion was calculated from the concentration and the known amount and specific gravity of the dispersion, and the total amount of protein in the residue. Gluten residues were normally kjeldahled in toto, as described above. Flour residues were kjeldahled by duplicate aliquots in small weighing cups.

Fig.28a. Apparatus.



Chapter IV.

Surface Techniques and Apparatus.

Films were examined at the air/water (A/W) and the oil/water (O/W) interface. Surface and interfacial pressures were measured with two hanging plate type torsion strip film balances at the two interfaces. Interfacial viscosities and elasticities were measured with an oscillating surface torsion pendulum. These instruments are shown in Fig. 28a.

The dispersions were spread from an all-glass Burroughs-Wellcome "Agla" micrometer syringe mounted on a rack-and-pinion. The micrometer screw was advanced at the speed of 0.01 mm/sec by a small synchronous motor through a simple friction drive. This arrangement allowed the glass tip of the syringe to be brought into the desired position at the interface and to be held there with a minimum of disturbance throughout the deposition.

1. Surface Pressure Measurements at the A/W Interface.

Most measurements at the A/W interface were carried out in a 29 x 17 x 1 cm Perspex trough. A glass coil placed inside the trough and connected to a Hoeppler Ultrathermostat provided temperature control. Half inch wide glass strips were used for barriers. Both trough and barriers were waxed with paraffin in the usual way. The movable barrier was actuated either by hand or by a small reversible electric motor, propelling the barrier at the speed of about 0.16 cm/sec. A microswitch automatically stopped

the motor at either end of the course.

The distance of the movable from the fixed barrier, or the effective length of the trough, was read directly in cm from a Perspex scale mounted adjustably under the barriers alongside the trough so that its origin could be brought to coincide with the inside of the fixed barrier. The maximum distance between the barriers (spreading position) was 20 cm. The minimum distance was 2 cm and the trough therefore had a compression factor of 10.

The films were usually deposited at an initial area of 2.0 m^2/mg and compressed (or decompressed) in steps of 0.1 m^2/mg.

The surface pressure (i.e. the lowering of the surface tension of the clean surface, due to the film) was determined from the vertical movements of a thin 10 x 2.75 cm mica plate hanging from one arm of a simple torsion strip balance[6]. The other arm carried a small pan for counterweights. Both arms were of equal length (4 cm). A small mirror was mounted on the fulcrum. The movement of the plate was magnified by an optical lever system and read on an adjustable scale as the deflection (cm) of a hairline from zero (clean surface). The total length of the optical path was about 170 cm. The deflections were read to 0.5 mm and the sensitivity of the balance was of the order of 0.05 surface bars.

The deflections were converted into surface bars (dyn/cm) in the usual way by calibration with known weights. The calibration curve was a straight line over the required

range of 0 to 40 bars.

The mica plate was cleaned before each spreading by rubbing with very fine silicon carbide paper and rinsing. The surface was sucked free of gross contamination and swept with the aid of the barriers at least twice. Before spreading a blank compression test was carried out and the sweeping repeated if the deflection corresponded to more than 0.5 bars at 2 cm separation of the barriers.

A period of five minutes was found sufficient for the film to reach equilibrium after deposition. The interfacial pressure of protein films generally rises sharply[32,81] immediately after compression and then falls to a limiting value with time. The reverse happens on decompression. The effect is more pronounced the smaller the area and is believed to be due to molecular rearrangements. One minute was allowed after compression (or decompression) for this rearrangement to take place. Changes in the pressure after this period were found to be negligible.

Compressibility coefficients[7] were obtained from the pressure–area isotherms by graphical differentiation according to

$$c_s = \frac{1}{A}\frac{dA}{dF} \qquad (1)$$

Molecular weights and "gaseous areas" were calculated from the equation of Bull[31,32,33]

$$F(A - A_o) = KT \qquad (2)$$

where A_o is the "gaseous area", K is the appropriate form of Boltzmann's constant and T the absolute temperature. If F is in surface bars, A (and A_o) in m^2/mg, K becomes 8.32/MW where MW is the molecular weight. Provided the plot of FA against F is a straight line, A_o is simply the regression coefficient of the regression of FA on F, while MW is obtained from the intercept b of the regression line with the FA-axis as

$$MW = 8.32\ T/b \qquad (3)$$

The measurements were carried out by noting the areas at which deflections equally spaced by 0.1 cm (up to about 1 surface bar) were obtained. This procedure gives about 10 measurements in the required range and allows summation methods to be used in the regression analysis, resulting in considerable simplification of the computation.

2. Interfacial Pressure Measurements at the O/W Interface.

Measurements at the O/W interface were carried out in a glass crystallising dish of 12 cm diameter. As the area of the film was constant, the area per unit weight was varied by the successive addition of dispersion[8].

The films were usually spread at an initial area of 5.0 m^2/mg. This was decreased in steps of 0.5 to 2.0 m^2/mg, whence in steps of 0.25 to 1.0 m^2/mg, and finally in steps of 0.1 to 0.2 m^2/mg.

Normally the interface was that between carbon tetra chloride and water, although a few measurements were also made at the water/petroleum ether interface.

The balance used for the measurement of interfacial pressures was similar to the one described in Section IV-1, except for the following modifications. The optical path, about 100 cm long, was changed from vertical to horizontal movement by means of a small mirror inclined at 45° and placed just above the mirror mounted to the fulcrum. The balance could be pivoted around the vertical axis and could also be racked up and down to follow adjustments in the position of the interface which had to be made from time to time in connection with the operation of the torsion pendulum.

Instead of a mica plate, a 6 x 1 cm Teflon plate (about 1 mm thick) was used at the carbon tetrachloride/water interface. When measurements were carried out at the water/petroleum ether (or the air/water interface at constant area), a mica plate of the same dimensions was substituted. Because of the shorter plate and optical lever the sensitivity of this arrangement was slightly less, being of the order of 0,13 surface bars. The Teflon plate was cleaned before each spreading by boiling for about 5 minutes in a 1:2 mixture of concentrated nitric and sulphuric acid[82].

Calibration was carried out in the usual way and gave a straight line over the required range. The slope, however, depended on the position of the plate relative to the interface. This seems to be a consequence of the much greater buoyancy effect on the relatively thick plate at this interface.

One hundred mls were used of each phase and the height of the liquid layers was just under 1 cm. Care had to be

taken that the plate would not touch the upper water surface at maximum deflection.

3. Measurement of Viscoelasticity.

The interfacial pressure measurements at the O/W interface were usually carried out concurrently with the measurements made with the oscillating surface torsion pendulum. This consisted of a brass bob of 2.2 cm diameter and 2.8 cm height, suspended from a 40 s.w.g. eureka wire about 50 cm long. The pendulum had a moment of inertia of 65 g cm^2 and a period in air of 14.29 sec. A horizontal platinum needle 4 cm long was attached centrally to the bob about 2 cm below its lower end. The upper end carried a small pinvice gripping the torsion wire.

The other end of the wire was similarly gripped by another pinvice fixed to the shaft of a Magslip telemotor mounted on wall brackets. The stator windings of the motor were connected to the corresponding windings of a similar motor (transmitter), situated in the operating panel. An excitation potential of 50 V was supplied to both rotors from a Variac transformer. When the shaft of the transmitter was rotated through a small angle, its twin followed suit inducing the oscillation of the pendulum.

Both pinvices carried small mirrors. The oscillations of the bob could be followed (magnified) from the movements of a hairline reflected from the lower mirror onto a centimetre scale. The image of a hairline reflected from the upper mirror onto another scale allowed the torsion head to be returned to the initial position after imparting the

necessary angle of twist. The magnitude of this angle could be read on the upper scale. In this way the oscillations of the bob could be easily controlled and kept within both ends of the lower scale.

The crystallysing dish was placed in a temperature bath consisting of a Perspex box supported by a brass tube. This could slide up and down in another tube let into the bench and could be clamped in any vertical postion. By the use of a fine adjustment the height of the dish could be accurately regulated until the oscillating needle was brought exactly into the interface. A frosted lamp and shade placed behind the Perspex box illuminated the interface and facilitated its observation.

The arrangement had the advantage that no pendulum swings could result from a vertical translation of the oscillating system which would be necessary with a stationary dish. As a further precaution, the bob and wire were enclosed in a glass tube for protection against air drafts.

Before each spreading the oscillating needle was flamed with a micro burner and quenched in distilled water. Surface active impurities in the carbon tetrachloride and the aqueous substrates were allowed to collect on the surface of wide storage bottles, the solutions to be used being syphoned from below the surface.

The mathematics of the oscillating surface torsion pendulum are treated in the Appendix. The formulae derived there depend on the period T and the (decadic) logarithmic decrement λ of the damped oscillations as the observed variables.

They also depend on the apparatus constant H. This was obtained from

$$H = 2/l^2 \qquad (4)$$

where l is the length of the needle in cm. Although this can only be regarded as an approximation (cf. Appendix), any errors due to the inadequacy of Eq. (4) are not likely to affect the conclusions reached in this work as these are not based on absolute measurements.

The period of oscillation was determined by timing three full swings with a stop-watch to 0.1 sec. It should be noted that the period was found to decrease in successive swings when the films were highly elastic. The mean period determined from these swings is then only an experimental average.

At the begining of this work a pendulum having a period of about 7 seconds in air was used. The logarithmic decrement was calculated from the ratio of successive amplitudes on one side of the scale, according to

$$\lambda = \frac{1}{n-1} \log \frac{A - A_o}{A_n - A_o} \qquad (5)$$

where n is the number of swings, A the first, A_n the nth amplitude and A_o the rest point. It was found, however, that A_o did not remain constant when the oscillations were highly damped. This led to a decrease of the logarithmic decrement with a decrease in amplitude, suggesting "inverse structural viscosity" or "negative thixotropy". However, when λ was calculated from the mathematically equivalent expression

$$\lambda = \frac{1}{n-1} \log \frac{A - A'}{A_n - A'_n} \qquad (6)$$

where the primed A are the corresponding opposite amplitudes, the logarithmic decrements became constant. This formula was therefore adopted and the period in air was doubled for more convenient timing.

When determining the viscoelasticity of the films as a function of the area, measurements with the oscillating surface torsion pendulum were usually begun at an area of 1.50 m^2/mg. At higher areas changes in T or λ were too small to be measured except in a few cases. T and λ contrary to F, showed a marked increase with time. The measurements were therefore standardized by starting the oscillations five minutes after addition of dispersion.

Viscoelasticity measurements as a function of time were begun exactly five minutes after commencement of spreading and were repeated at five minute intervals for not less than 35 minutes.

A few measurements were also carried out in the trough with a simple hand-operated pendulum.

4. Temperature Control.

All measurements at the O/W interface were carried out at 25 $\pm$ 0.2 degrees centigrade by passing the circulation from a Hoeppler Ultrathermostat through the Perspex box surrounding the dish. This circulation also flowed through the heat-exchange coil in the trough, maintaining it at 24.6 $\pm$ 0.3 degrees. In hot wheather the thermostat was backed by a refrigeration unit through which part of the circulation was by-passed.

Chapter V.

The Dispersibility of Gluten.

Acid Chlorhydrin as a Cereal Protein Dispersant.

The difficulty of bringing cereal proteins, and in particular, wheat gluten into solution or dispersion, has been mentioned in the introductory chapter. In the first section of this chapter, the means available for bringing wheat proteins into solution or dispersion are briefly reviewed. The solubility characteristics of the other cereal proteins are largely similar. In the following sections, experiments are described in which acid chlorhydrin is introduced as a new dispersant for wheat gluten and cereal proteins in general. Brief mention is also made of the gluten dispersing action of anhydrous formic acid and of concentrated lithium bromide solutions containing isopropanol.

1. The Dispersibility of Wheat Proteins.

Aqueous ethanol and similar organic solvent systems[69] disperse variable amounts of the so called gliadin fraction, although virtually complete dispersion can apparently be effected in the presence of strong shearing forces as attained in a Waring Blendor[59,60]. The dispersing power of organic solvent systems in the absence of these forces is influenced by a change in practically every experimental variable[17,27] and strictly reproducible results are difficult to obtain even by rigorous standardization of procedure.

The same is true of inorganic salt solutions which, depending on the type of salt, concentration, etc. disperse a variable amount of the albumins and globulins along with

varying propertions of gliadin[92,107–110,183,188,189].

The dispersion of gluten in dilute alkali is complete but invariably accompanied by irreversible changes in the chemical and physical properties of the protein[25,44,45,47,196,200], although it has been claimed[28,59,60] that gluten can be recovered from dispersion at pH 11 with its properties unchanged if the dispersion is carried out at 0° C.

Dilute acids affect the flour proteins much less, although it is difficult to obtain complete dispersion. The work carried out on gluten[111,151,232,233], gliadin[75,220] and the flour proteins[52,53,99,100,159] has demonstrated that: the weaker (organic or phosphoric) acid is more effective than the stronger (mineral) acid; the more dilute acid is a better dispersant than the more concentrated acid; there is an optimum concentration for any given acid (the same is true for alkalis); this optimum extends over a wider concentration range with the weaker acid; the presence of salts greatly influences the action of the acid; and the protein may be precipitated from acid or alkaline dispersions by careful neutralisation or salting out.

The dispersing effect of acids seems to be largely but not simply one of pH. Acid dispersions, however, are very polydisperse[200]. They cannot be filtered because some of the particles are too large. Centrifugation always carries some protein down along with a certain amount of starch which is also dispersed[200].

Another disadvantage is the instability of these dispersions, as shown e.g. in a fall of viscosity with time[196]. The changes seem to be due to hydrolysis, probably[45] of an

enzymic nature since the dispersions can be stabilised[180, 223] by heating for a few minutes at about 95–100°C, although there is always a residual drop in viscosity even after heat treatment[178].

These various difficulties precluded a comprehensive study of whole gluten until the introduction of a new range of dispersants in the 1930's when Cook and Alsberg[42] reported that gluten dispersed readily in 24–30% urea, and Cook and Rose[43] reported its dispersibility in 8% aqueous sodium salicylate. Gluten also disperses in aqueous dimethyl formamide[227], guanidine hydrochloride[187], and solutions of a series of compounds related to sodium salicylate[93]. All of these reagents are known to break hydrogen bonds[48].

While globular proteins in general are denatured by urea or salicylate, this does not occur with gluten to anything like the same extent. Gluten could be salted out from salicylate dispersion with its properties unaltered[157]. The quality of the gluten recovered from urea and salicylate dispersions was superior to gluten recovered from acetic acid dispersion. Hydrolysis did not occur and higher viscosities were obtained in the neutral dispersants[44,45,196].

The work of Cook and Rose led to the extensive researches of McCalla and his coworkers[41,152–157,210,212] on salicylate dispersions of gluten. Although these produced many interesting results, it was found[156] that gluten is not wholly in molecular dispersion even in 12% salicylate. It also imparts electrophoretic homogeneity to gluten[41] and apparently obliterates varietal differences in viscosities[223].

While it does not contain nitrogen like urea or formamide, which makes an accurate estimation of the protein concentration by the Kjeldahl procedure difficult, it still has to be used in high concentrations. Urea always disperses some starch along with the protein but salicylate does not[194].

2. Dispersion of Gluten in Acid Chlorhydrin.

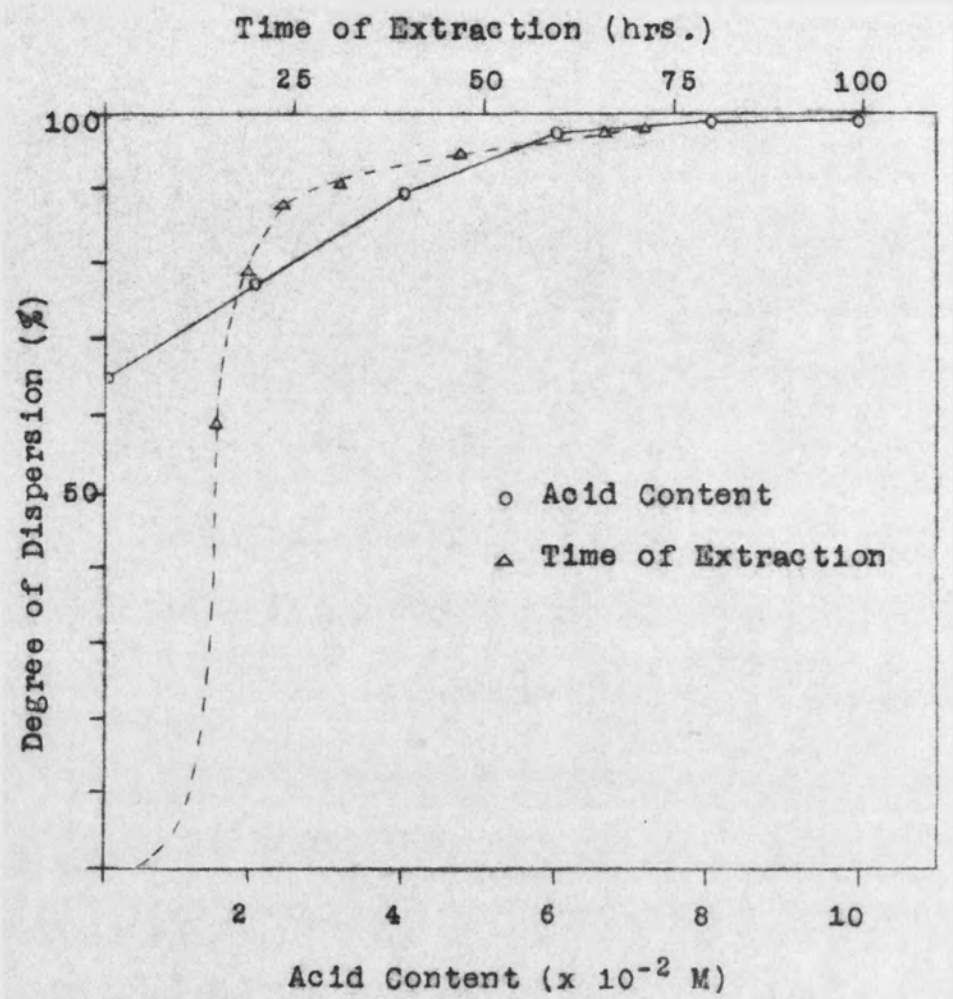
In 1955 Hess[118] reported the chance observation that native wheat flour protein could be dispersed in ethylene chlorhydrin and precipitated by the addition of diethyl ether.

As chlorhydrin appeared to be an attractive medium for the preparation of gluten dispersion for surface chemical studies in particular and physicochemical investigations in general, a series of experiments was carried out to obtain some information on the suitability of this solvent as a gluten dispersant.

First, dried gluten was shaken up for 3 days with a sample of unpurified laboratory grade ethylene chlorhydrin. The gluten particles first swelled, then disintegrated and were completely dispersed at the end of this period. Centrifugation removed some starch and also some protein along with it. The supernatant was much less turbid than a dispersion of the same concentration in 10% sodium salicylate and gave no iodine test.

When another gluten sample was heated with chlorhydrin at the temperature of boiling water, the gluten rapidly dissolved without agitation. The dispersion after centrifugation was clear although slightly brownish in colour.

Fig.41a. Effect of HCl Content and Time of Extraction on the Degree of Dispersion of Gluten in Chlorhydrin.


Time of Extraction (hrs.)
25
50
75
100
100
50
Degree of Dispersion (%)
o Acid Content
Δ Time of Extraction
2
4
6
8
10
Acid Content (x 10⁻² M)

No cloudiness appeared on cooling. In sodium salicylate the gluten did not dissolve but was easily dispersed by shaking after heating for 15 minutes.

However, when the experiment at room temperature was repeated with a redistilled sample of chlorhydrin, the swollen gluten particles did not disintegrate even after 3 days. Nevertheless, the clear supernatant after centrifugation contained about 63% of the protein. This suggested that the high degree of dispersion in the former case was due to the presence of hydrochloric acid in the sample. In fact, the acidity of several laboratory samples was found to range from 0.03 to 0.23% expressed as hydrochloric acid. The water content of these samples was 3–5%.

In the next experiment, the influence of the hydrochloric acid content of chlorhydrin on its ability to disperse gluten was investigated systematically. The gluten was dispersed at 25° C in carefully purified chlorhydrin containing increasing amounts of acid from 0 to 0.1 M concentration, and the degree of dispersion was determined as detailed in Section III–3. The results plotted in Fig. 41a clearly show that the dispersing power of ethylene chlorhydrin increases with its hydrochloric acid content and that dispersion is virtually complete in a 0.1 M solution of the acid. Even pure chlorhydrin, however, disperses a substantial amount of the gluten proteins, slightly in excess of the combined amount of the albumin, globulin, and gliadin fraction.

In order to see whether the combination of chlorhydrin with hydrochloric acid was unique, another experiment was

carried out using solutions of various acids in chlorhydrin on the one hand, and solutions of hydrochloric acid in chlorinated alcohols, on the other. At the same time, some information was also sought on the effect of heating. The dispersion was carried out at room temperature for 3 days and at the temperature of boiling water for 15 minutes. Sulphuric acid was used in 0.05 and 0.1 M, all other acids in 0.1 M concentration. The results are shown in Table 43. The butanol and the heated sulphuric acid dispersions were not analysed.

Table 43

Degree of Dispersion of Gluten in Various Acid/alcohol Dispersing Media.

Dispersant		% Dispersed	
Acid	Alcohol	Room Temp.	Heated
Hydrochloric	Ethanol (abs.)	0.6	—
"	Isopropanol	2.8	—
"	n-Butanol	(X)	—
None	Chlorhydrin	64.3	70.6
Hydrochloric	"	99.2	99.5*
Sulphuric, 0.05M	"	99.4	—
" 0.1M	"	99.0	—*
Trichloracetic	"	82.1	—
Formic	"	81.6	87.0

(X) no dispersion * dissolved

No swelling at all occureed and very little protein was dispersed in the unchlorinated alcohols. In chlorhydrin, sulphuric acid was about as effective as was hydrochloric acid although the gluten particles seemed to disintegrate

somewhat more rapidly. Formic acid is not as effective as the mineral acids, but definitely increased the degree of dispersion as is clear from comparison with the control. Trichloracetic acid, a powerful protein precipitant in aqueous solution, increased the dispersion in chlorhydrin slightly more than did formic acid. The last two and the acid free dispersion at the end of the extraction contained swollen particles that would not disintegrate on shaking.

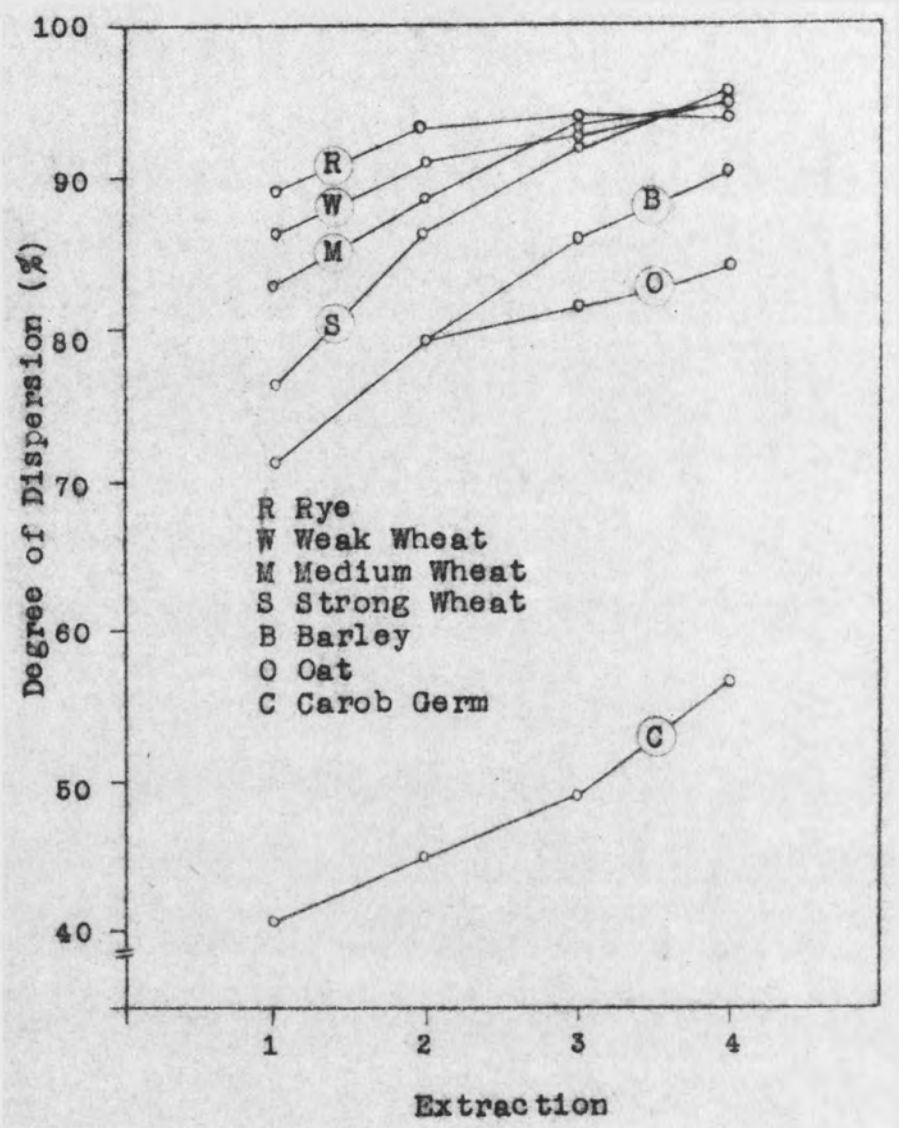
Heating always increased the degree of dispersion but the relative amounts dispersed were by and large the same. Solution only occurred in hydrochloric and sulphuric acid.

All other experiments were consequently carried out with a 0.1 M solution of hydrochloric acid in ethylene chlorhydrin (referred to simply as "acid chlorhydrin"). Although sulphuric acid would perhaps have given slightly more rapid and complete dispersion, its use was avoided because of the divalent anion.

The influence of the time of extraction on the degree of dispersion was studied next. Dispersion was carried out at 25°C and the tubes were agitated regularly. From time to time a tube was withdrawn, centrifuged and the degree of dispersion determined. The results plotted in Fig. 4a show that the greatest increase in the degree of dispersion occurred during the first 24 hours (overnight) and that dispersion was virtually complete in 3 days.

Finally a series of experiments was carried out in

Fig.44a. Extraction of Wheat and Other Cereal Flours with Acid Chlorhydrin.


100
90
80
70
60
50
40
Degree of Dispersion (%)
1
2
3
4
Extraction
R
W
M
S
B
O
C
R Rye
W Weak Wheat
M Medium Wheat
S Strong Wheat
B Barley
O Oat
C Carob Germ

order to obtain information on the effect of the manner and frequency of agitation on the degree of dispersion of gluten in acid chlorhydrin. These experiments indicated that shaking was more effective than simple mixing of the glass stoppered test tubes in which the dispersion was carried out. The degree of dispersion increased both with the intensity and the frequency of agitation.

3. Extraction of Flours with Acid Chlorhydrin.

Portions containing 100 mg of protein of the strong, medium, and weak wheat flour, and of rye, barley, oat, and carob germ flour were repeatedly extracted at 25° C with acid chlorhydrin. The cumulative degrees of dispersion achieved in four extractions are plotted in Fig. 44a.

Extraction of about 95% of the wheat and rye flour proteins was attained in four extractions. Of the wheat flours the weak flour was most and the strong flour least rapidly extracted. This was confirmed in a repeat experiment. Rye flour behaved like a rather weak wheat flour.

Extraction of barley (90%) and oat (88%) flours was slightly less satisfactory and only about 66% of the carob germ flour could be extracted under these conditions.

In another experiment, carried out with the harsh, medium, and extensible flours, the extensible flour was most and the harsh flour least rapidly extracted. Only two extractions were made in this experiment but the second one lasted for 17 days. The data from this and another experiment carried out with the medium wheat

flour, indicated that better yields might be achieved by longer extraction times.

4. Dispersion in Other New Gluten Dispersants.

Ellis and Pankhurst[73] successfully used anhydrous formic acid for the preparation of spreading dispersions of collagen. Dry gluten was found to disperse readily in the same reagent, dispersion being complete within a few hours. The usual ca. 1% dispersion was quite viscous and some creaming occurred on centrifugation. There was very little sediment, the anhydrous formic acid apparently dispersing starch as well as gluten. The dispersions could be diluted with chlorhydrin without precipitation of the protein. This was not possible with isopropanol.

Complete and ready dispersion of gluten was also obtained in a 56% solution of lithium bromide containing 8% isopropanol. Neither of these reagents is capable of dispersing gluten in the absence of the other.

As acid chlorhydrin appeared to be preferable for the spreading of monolayers, no further work was done on these dispersants.

5. Conclusions.

The degree of dispersion of gluten in acid chlorhydrin was invariably found to be 2–3% higher than in salicylate. Under identical circumstances, about 10% higher extractions were achieved from flours. Acid chlorhydrin can therefore be regarded as the more effective dispersant. As will be shown in Section VI–2, by surface chemical

methods acid chlorhydrin dispersions seemed to be superior to either salicylate or acid/alkali dispersions.

The yield from direct extraction of flour could probably be increased by longer extraction times, continuous agitation and perhaps by an increase in the relative amount of the extractant.

The dispersing action of acid chlorhydrin is certainly different from that of the other dispersants. The chlorhydrin itself is probably effective in breaking hydrogen bonds and in overcoming intermolecular attraction of the Van der Waals type. The acid might act as an additional hydrogen bond breaker.

That the acid is not ionised may be seen from the fact that trichloracetic acid enhanced the dispersion and that a substantial amount of water had to be added before the protein was precipitated by the now ionised trichloracetic acid. When the same amount of water was added to chlorhydrin containing hydrochloric acid, the dispersion became opaque but was perfectly stable. No precipitation of protein occurred over periods of many weeks.

The observation that gluten is completely dispersible in 56% lithium bromide containing 8% isopropanol, again points to the need of having a nonpolar solvent coupled with an agent capable of breaking hydrogen bonds.

Chapter VI

Preliminary Surface Chemical Studies.

Before commencing a more detailed study, certain factors affecting satisfactory spreading of gluten films were examined and the suitability of various dispersing agents for the proposed study were investigated. The effect of heating on the dispersions was studied and some exploratory experiments were carried out by spreading at low pH and under reducing substrates. Some experiments were made with gluten from which lipids had been removed by acetone extraction. An attempt was also made to determine the molecular weight of gluten with the aid of the film balance.

1. The Spreading of Gluten Dispersions.

Proteins generally do not spread satisfactorily at the A/W interface if unaided by a spreading agent. Spreading is generally better at the O/W interface[8,15,54]. The usual spreading agent is either a small amount (0.1%) of amyl alcohol[61,66,95-97], or about 60% isopropanol[8,213]. With gluten dispersed in acid chlorhydrin or sodium salicylate, no spreading agent was found necessary. Identical pressures were obtained whether a spreading agent was added or not. Obviously the chlorhydrin and the salicylate themselves act as spreading agents. Only a few minutes were required for the film to reach equilibrium at the usual initial areas of 2.0 (A/W) and 5.0 (O/W) m^2/mg, and no change in pressure occurred over periods of 30 to 45 minutes.

Dispersions in acids or alkali, however, only spread satisfactorily if a spreading agent was present. For reasons mainly of comparability chlorhydrin was used for this purpose as explained in Section III-2.

The high specific gravity of the dispersions (salicylate: ca. 1.06, chlorhydrin: ca. 1.19) presented certain difficulties at the O/W interface. When the interface was that between water and petroleum ether, the dispersion began to flow out of the syringe as soon as it touched the interface. Immediately after deposition, the syringe had to be withdrawn. The dispersion had a great tendency to sink to the bottom, particularly when spreading occurred against an already existing pressure as when making [illegible] of successive additions.

Although it was possible, with a little care, to deposit the film to areas as low as 0.2 m^2/mg if deposition was carried out in one operation, losses invariably occurred at the lower areas when deposition was carried out in successive stages. This situation was remedied by reversing the phases, i.e. by using carbon tetrachloride (specific gravity 1.58) as the hypophase and water as the epiphase. Since a mica plate was difficult to use at this interface, a Teflon plate was substituted and this worked very satisfactorily.

To prevent the dispersion from flowing out of the syringe when it was lowered through the aqueous layer, an air seal was applied by turning the micrometer screw back about 0.1 mm. The tip of the syringe was introduced

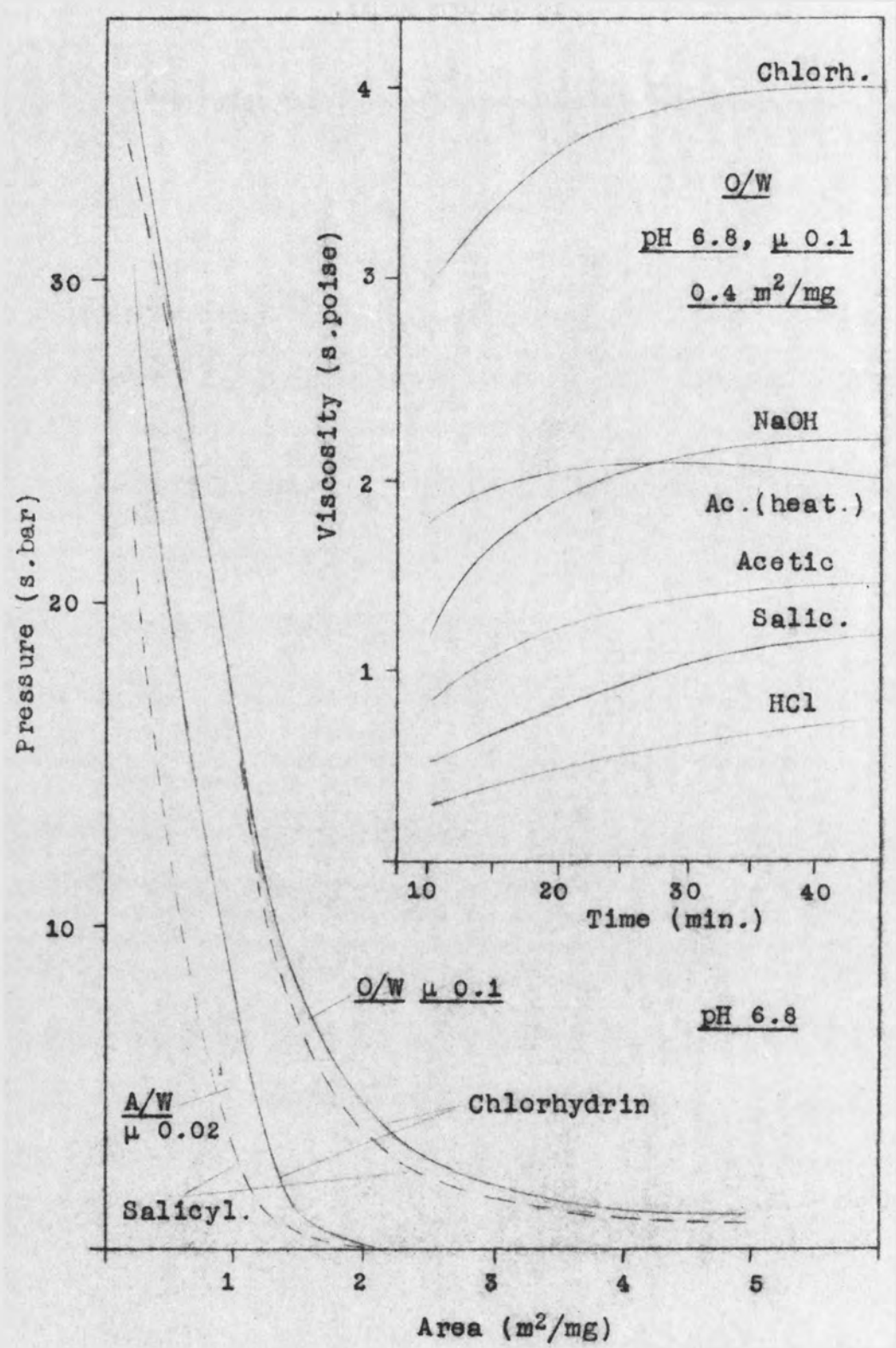
in this way just below the interface and kept in this position throughout the run. No dispersion flowed out spontaneously into the heavier carbon tetrachloride. Very reproducible results were obtained by this method.

To check whether the method of successive addition could safely be used with gluten dispersions, the pressure-area isotherm obtained in the usual way in the trough by compressing the film with the aid of the movable barrier was checked against the A/W isotherm in the dish at constant area by the method of successive addition. Down to about 1 m^2/mg the two isotherms were identical. At lower areas, i.e. when the film was close packed and successive spreadings occurred against increasing film pressure, the pressures obtained in the dish were somewhat lower than those measured in the trough.

Another isotherm was then obtained in the dish at the water/petroleum ether interface and compared with another at the carbontetrachloride/water interface. Again the two isotherms were identical down to about 1 m^2/mg whence lower pressures were found at the water/petroleum ether interface. The lower pressures in both cases were due to some loss of dispersion which could not be avoided even with the greatest care.

A final check was obtained by comparing the carbon tetrachloride/water isotherm with the pressures recorded in independent spreadings at selected areas. These were within the expected error and it was concluded that the method of successive addition could be used safely.

Fig.50a. Pressure-Area Isotherms and Viscosity-Time Curves of Gluten Films Spread from Dispersion in Various Media.


Chlorh.
O/W
pH 6.8, μ 0.1
0.4 m²/mg
NaOH
Ac.(heat.)
Acetic
Salic.
HCl
Viscosity (s.poise)
Time (min.)
O/W μ 0.1
pH 6.8
A/W
μ 0.02
Chlorhydrin
Salicyl.
Pressure (s.bar)
Area (m²/mg)

Concentrations of the spreading solution between 0.16 and 0.84 mg/ml were found to have no effect on the isotherms at the O/W interface. Surface pressure measurements at the A/W interface were independent of the initial spreading area within the range 2.0 to 4.0 m^2/mg. Interfacial viscoelastic measurements at the O/W interface were not influenced by the initial spreading area within the range 1.5 to 5.0 m^2/mg.

2. The Suitability of Various Dispersing Agents.

A series of experiments was carried out to see which of the dispersing agents capable of dispersing gluten virtually completely, would be most suitable for more detailed studies. Gluten dispersions were therefore prepared in acid chlorhydrin as well as in 10% sodium salicylate, 0.01 N hydrochloric, formic and acetic acid and sodium hydroxide as detailed in Section III -2. Monolayers were spread at the A/W and the O/W interface and the isotherms and rheological characteristics of these films were determined at various pH values and two ionic strengths.

Fig.50a shows the isotherms of the films obtained from the acid chlorhydrin and the salicylate dispersions at pH 6.8. The isotherms from dispersions in acids and alkalis were only slightly more expanded than those from salicylate and are therfore not shown. The films from the acid chlorhydrin dispersion are seen to be more expanded than those from the other dispersing agents at the A/W interface. At the O/W interface this difference

disppeared almost completely. Significant differences, however, were found at the same interface in the rheological features.

The interfacial viscosity and elasticity of the films spread from the various dispersions were first determined as a function of the area (not shown). As both properties were found to increase markedly with time, the time dependence of η_S and G_S were studied at the areas of 0.4 and 0.5 m^2/mg. The plots of η_S as a function of time are shown in the inset in Fig.50a as they most conveniently illustrate the differences. The behaviour at 0.5 m^2/mg was quite similar. The elasticities at both areas ran parallel with the viscosities. The viscosity curve of the formic acid dispersion was very near that of the salicylate dispersion and is not shown. The acid chlorhydrin dispersions produced films with far higher viscosities than did all others. A clear correlation may be seen between the viscosity of the film and the strength of the acid. It is interesting that the films spread from sodium hydroxide dispersion were more viscous than those from the acid dispersions. The salicylate dispersion yielded films of low viscosity.

All these observations seem to indicate that the gluten molecules are more completely unfolded in acid chlorhydrin than in the aqueous dispersants. With the strongly bonded gluten molecules the surface forces at the A/W interface might not be sufficient to cause complete unfolding and the film properties might reflect the state of

of uncoiling in the dispersion. At the O/W interface uncoiling would be added by elimination of Van der Waals attraction.

This view is partially supported by the effect of heating on the dispersions.

3. Effect of Heating.

Since observations on the bulk viscosity of acetic acid dispersions of gluten (cf. p.40) made it seem possible that the lower viscosity of the films spread from this medium might be due to enzymic hydrolysis which would conceivably be absent in acid chlorhydrin, films were spread from two more acetic acid dispersions, one heated for 15 minutes at the temperature of boiling water immediately after addition of the acid to the gluten, the other heated similarly on completion of the dispersion after centrifugation three days later. The η_S–t curves of these dispersions were identical but were different from those of the unheated dispersion as shown in the inset of Fig.50a. Although heating resulted in an increase in the viscosity of the film, the shape of the curve was altered.

Heating of salicylate dispersions caused a similar increase but the shape of the curve was not altered.

Heating of acid chlorhydrin dispersions in the same manner resulted in a decrease in the viscosity without altering the shape of the curve. The pressure–area curves were little different at the higher areas but below about 1 m^2/mg the heated chlorhydrin dispersions gave lower pressures than did the unheated ones at the same area.

If the view is accepted that the gluten molecules are not completely unfolded in the aqueous dispersions, the effect of heating might be understood in terms of an increase in the degree of uncoiling by thermal agitation. The fact that heating of the chlorhydrin dispersions did not result in higher viscosities might then be regarded as an indication that maximal uncoiling is attained in this reagent at room temperature.

The reason for the lower pressures and the decrease in viscosity experienced with heated chlorhydrin dispersions is not clear at present. Deamidation of the glutamine and asparagine residues might be involved.

4. Spreading at Low pH.

The isotherm of films spread from the salicylate, acid and alkali dispersions at the A/W interface at pH 2.1 were not different from those at pH 6.8 but the isotherms of films spread from acid chlorhydrin dispersions were slightly more expanded at pH 2.1 at areas above about 1 m^2/mg. Similar results were obtained at the O/W interface.

While thus spreading at low pH had little influence on the isotherms, a profound change occurred in the rheological curves when the films were spread at pH 2.1. Both the interfacial viscosity and elasticity were drastically reduced and became noticeable only at the lowest areas.

5. Spreading under Reducing Substrates.

In view of the effect redox reagents are reported to have on gluten quality, films were spread from dispersion

in chlorhydrin under 0.1 N sodium sulphite and under 0.01 N sodium thioglycollate (pH 10.6) at the O/W interface. These substrates had no effect on the pressure-area curves. The interfacial viscosities were somewhat reduced but there were no marked changes as one might be led to expect from a splitting of -S-S- linkages. This work, however, is not completed.

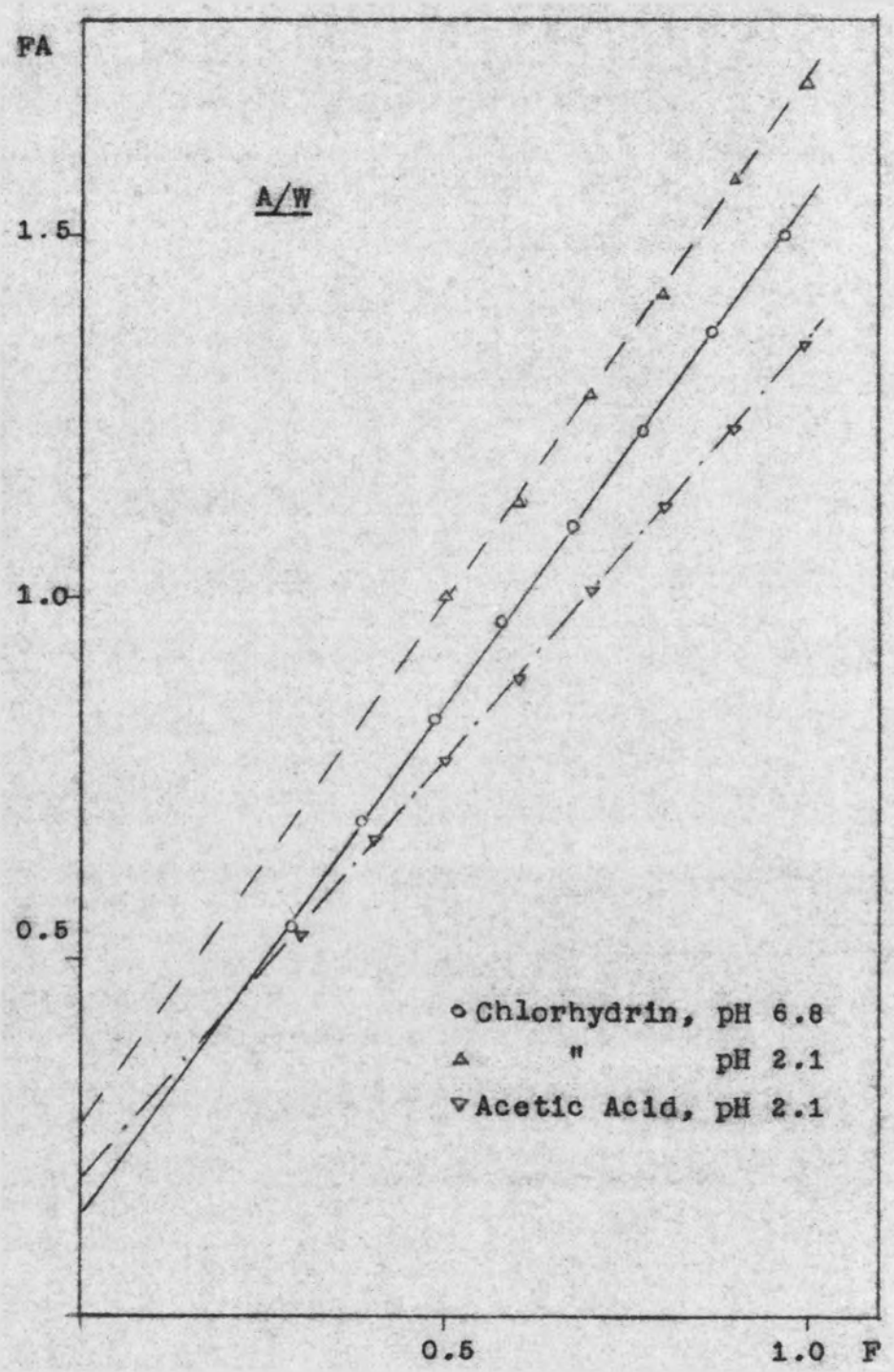
6. Acetone Extracted Gluten.

Films of acetone extracted gluten spread from acid chlorhydrin dispersions showed all the essential features of unextracted gluten, such as high stability, change of viscoelasticity with time, susceptibility to the pH of the substrate, etc. Further work on these lines was postponed until a systematic attack would be made on the role of lipids.

7. Molecular Weight Determinations.

One of the most useful applications of the surface film balance is in the determination of molecular weights of high polymeric substances. This method is speedy and requires only a small amount of material. In order to obtain accurate measurements, a sensitive balance must be used, capable of measuring surface pressures of the order of a few millibars. Special precautions must be taken to keep the surface free from adventitious impurities as even a small number of low molecular weight substances will cause an appreciable error. The same consideration applies to the dispersion or solution of the test material which must have a high degree of purity.

Fig.55a. FA-F Plots (Gluten).


FA
A/W
1.5
1.0
0.5
0.5
1.0
F
Chlorhydrin, pH 6.8
" pH 2.1
Acetic Acid, pH 2.1

The theory and techniques of molecular weight determination was originally developed by Guastalla[95–98] and by Bull[31–33] for proteins and has since been used by a number of workers for a variety of substances[11,37,66,67,105,168,169].

Although the equipment and the preparative techniques used in the present work could not be expected to yield true molecular weights, an attempt was made mainly as a guide for future work. The dispersions were those described in Section VI–2: Table 5 contains the molecular weights and gaseous areas (average of 2 to 3 determinations) calculated from the regression of FA on F (cf. Eqs. (2) and (3), p.31–32). Fig. 5a contains some of the plots of FA against F.

Table 5.

Molecular Weights and Gaseous Areas (m^2/mg) of Gluten in Various Dispersing Agents.

Dispersion	pH 6.8		pH 2.1	
	MW	A_o	MW	A_o
Chlorhydrin	15200	1.32	9700	1.43
Acetic Acid	15000	1.24	12600	1.17
Formic Acid	15000	1.11	10900	1.15
Hydrochloric Acid	15600	1.13	17200	1.19
Salicylate	15800	1.10	17000	1.14
Sodium Hydroxide	14500	1.08	17000	1.23

At pH 6.8 the film molecular weights obtained from dispersions in different agents were in good agreement although the actual values are almost centainly too low.

McCalla and Gralen estimated[156] the minimum molecular weight of gluten dispersed in sodium salicylate to be 35000 by ultracentrifugal studies and they concluded that gluten consisted of a spectrum of molecules varying progressively in molecular weight. The reason for the low molecular weights obtained here, apart from the possible effect of impurities, might well be the same. The film molecular weight would be largely determined by the lowest weight in a mixture.

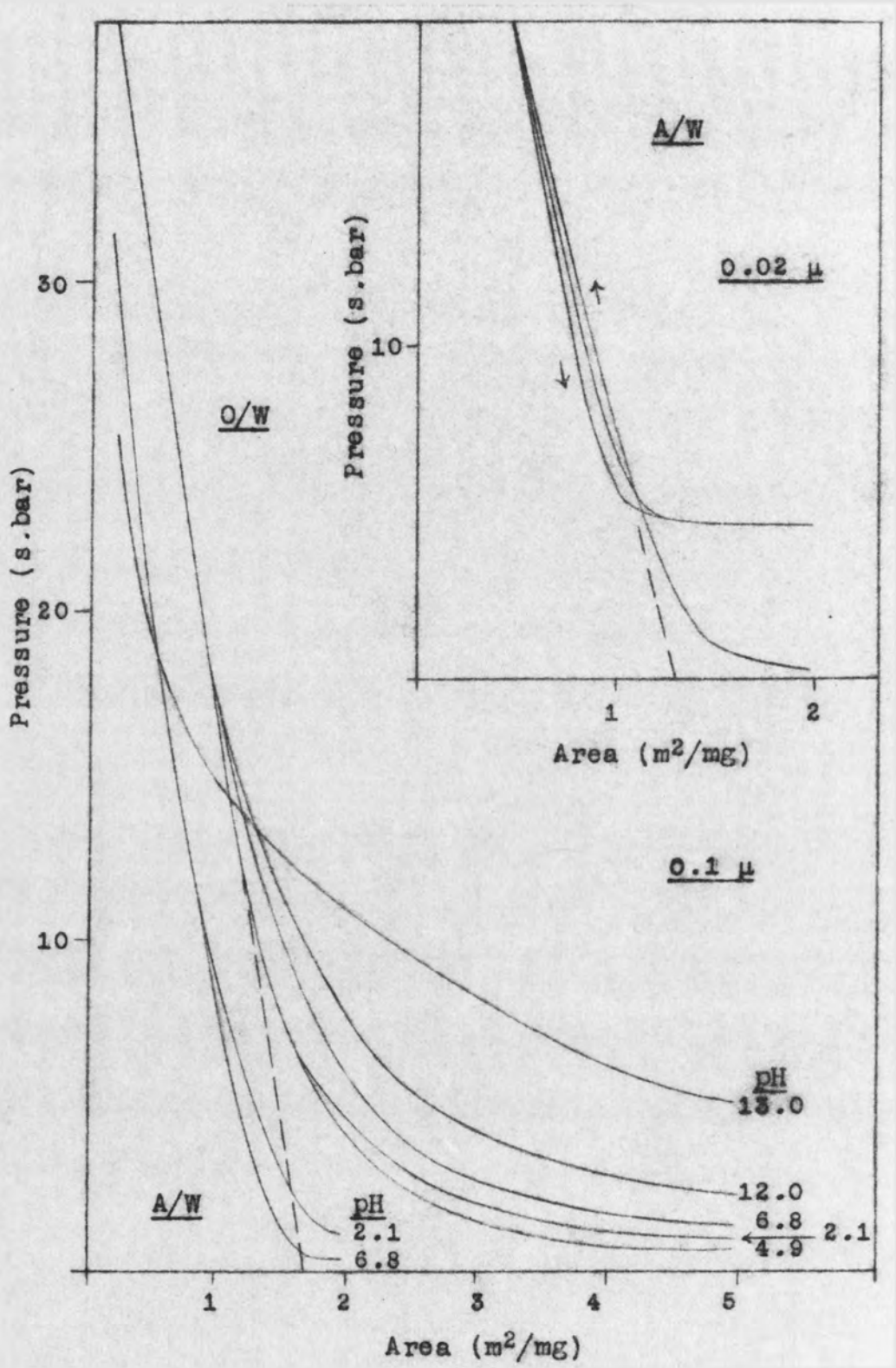
At pH 2.1 there is much more variation, the chlorhydrin dispersion giving the lowest molecular weight, followed by the acetic and formic acid dispersions. This trend follows the extent to which the pressure-area isotherms are expanded. Although the FA-F plots seem to lie on a straight line, there is sufficient evidence that had the pressure been measured at higher areas the plots would have curved away from the FA axis, thus giving a lower intercept and higher molecular weight. In accordance with the theory of Singer[211] as expounded in various papers by Davies and his associates[38, 55, 57], this would be a consequence of the enhanced flexibility of the molecule at this low pH as a result of intramolecular repulsion.

Further work on these lines had to be deferred until a more sensitive balance would be available. The data do suggest, however, that the gluten chains are most flexible,

indicating greater unfolding, in acid chlorhydrin dispersions, and more flexible in acetic and formic acid dispersions than in the others. This accords well with the interfacial viscosity data (cf. inset in Fig.50a).

The gaseous area was found to be substantially larger in the case of the acid chlorhydrin dispersion.

Fig.58a. Influence of pH on the Pressure-Area Isotherms of Gluten Films.


A/W
0.02 μ
Pressure (s.bar)
10
1
2
Area (m²/mg)
O/W
30
20
10
Pressure (s.bar)
0.1 μ
pH
13.0
12.0
6.8
4.9
2.1
A/W
pH
2.1
6.8
1
2
3
4
5
Area (m²/mg)

Chapter VII

Detailed Investigation of Gluten Films.

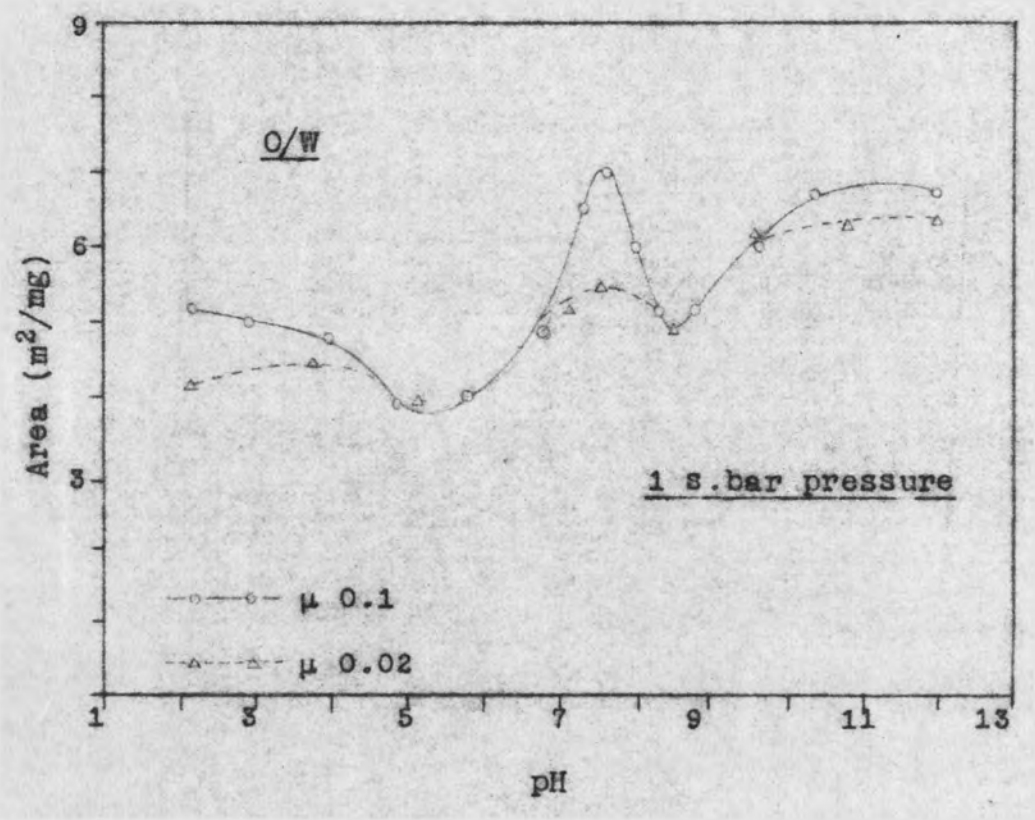
The studies on gluten films spread from dispersions in various media, reported in the previous section, indicated that films from acid chlorhydrin dispersions were more expanded and also more viscous and elastic than those from the other dispersions. Acid chlorhydrin dispersions also proved to be more stable. While in the other dispersions (undiluted, concentration ca. 5 mg/ml) some sediment always appeared after only a few days, no residue could be seen at the bottom of the acid chlorhydrin dispersions even after several months. No change seemed to take place in the surface viscoelastic properties of these dispersions over periods of at least several weeks. While the other dispersions were decidedly turbid, acid chlorhydrin dispersions were only slightly opaque and were water clear when diluted to the spreading strength of about 0.4 mg/ml.

In view of these facts a more detailed study of gluten films was carried out in acid chlorhydrin dispersions.

1. Influence of pH and Ionic Strength on the Pressure-Area Isotherms.

A number of isotherms were obtained by varying the pH at both interfaces at ionic strengths of 0.1 and 0.02 μ. Some of the 0.1 μ isotherms are plotted in Fig.58a. At 0.02 μ the picture was much the same. The films are of the vapour expanded type. The O/W isotherms are much more expanded than those at the A/W interface.

Fig.59a. Area at Constant Pressure-pH Curves (Gluten).


O/W
1 s.bar pressure
μ 0.1
μ 0.02
Area (m²/mg)
pH
9
6
3
1
3
5
7
9
11
13

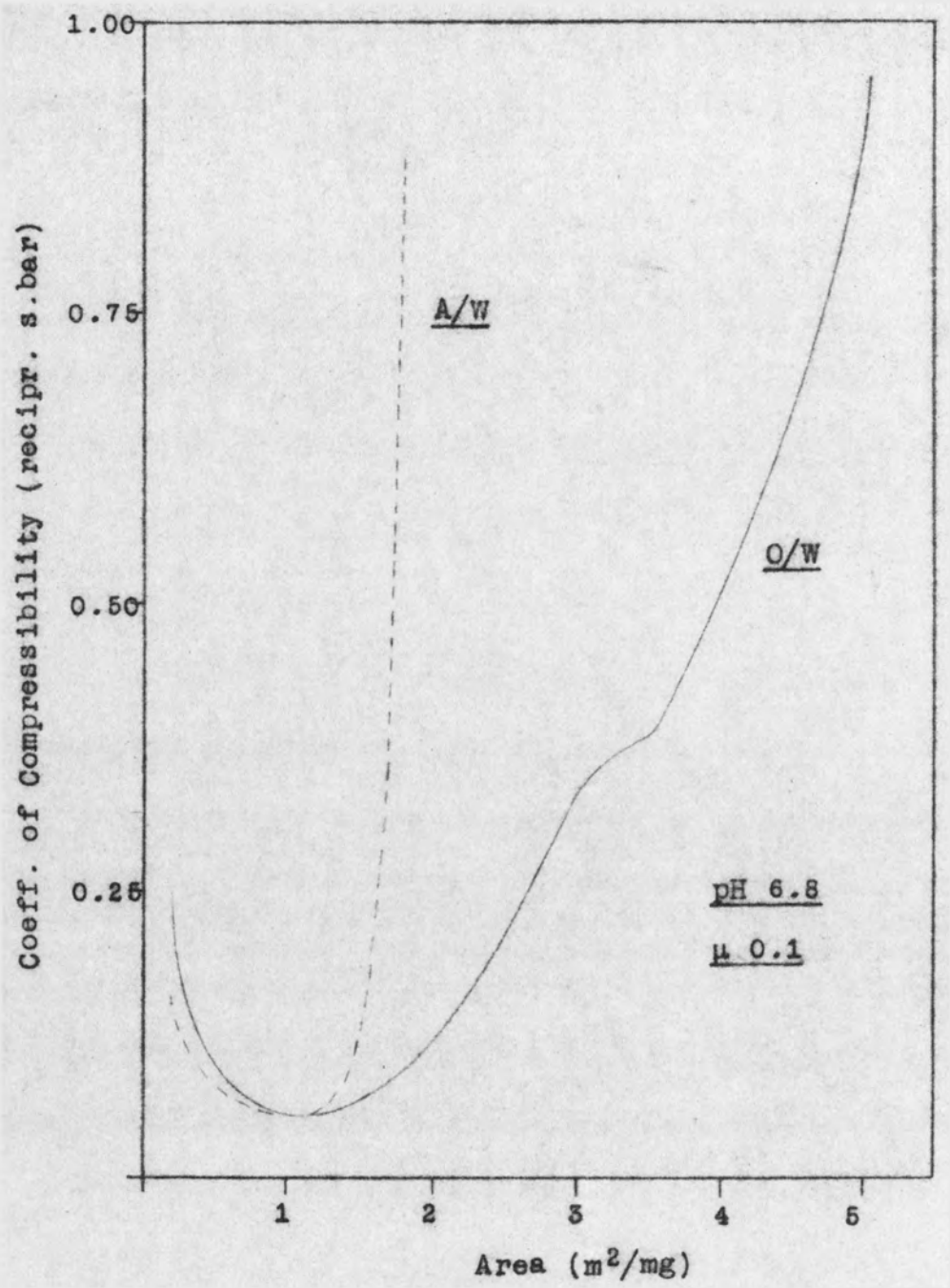
Rather high pressures were obtained at the O/W interface, the pressure at 0.2 m^2/mg being about 36 surface bars. The pressures at the A/W interface were slightly lower, about 32 bars at 0.2 m^2/mg. There was no indication of collapse at either interface.

At areas below about 1 m^2/mg, the isotherms were identical within the experimental error with the exception of the pH 13 isotherm. Extrapolation of the steep part of the curves below 1 m^2/mg to zero pressure gave areas of 1.33 m^2/mg at the A/W and 1.5¢ m^2/mg at the O/W interface. These are the areas at which the molecules may be regarded as being in the closest possible packing while still in the state of a monolayer. At higher areas, the film expanded to a greater or less degree as a function of pH. At pH 13 the isotherm is very expanded and quite different from that at pH 12.0, suggesting a breakdown of the film. The isotherm at pH 1.0 (not shown) was found to be normal.

The influence of pH on the expansion of the film at the O/W interface may be seen best from Fig. 59a where the area at 1 bar pressure is plotted against pH. Both the 0.1 μ and the 0.02 μ curves have minima at about pH 5.5 and 8.5 and maxima at about pH 7.5 and at both ends of the pH scale. While the minima are identical at the two ionic strengths, the maxima were less pronounced at 0.02 μ.

At the A/W interface, where the film could be decompressed by simply reversing the direction of travel of the movable barrier, gluten films showed interesting hysteresis phenomena. At pH 6.8 and an ionic strength of

Fig.60a. Compressibility-Area Curves (Gluten).


1.00
0.75
0.50
0.25
Coeff. of Compressibility (recipr. s.bar)
A/W
O/W
pH 6.8
μ 0.1
1
2
3
4
5
Area (m²/mg)

0.02 μ (inset, Fig. 58a) the pressure on decomposition would rapidly fall to a minimum value attained at about the area of close-packing. No further decrease occurred on continued decompression. On recompressing the initial pressures were gradually regained. The minimum decompression pressure was well reproducible on recycling but the reproducibility was less satisfactory between different films of the same dispersion. No improvement in this condition could be obtained by compressing and decompressing at a uniform rate with the aid of the electric motor.

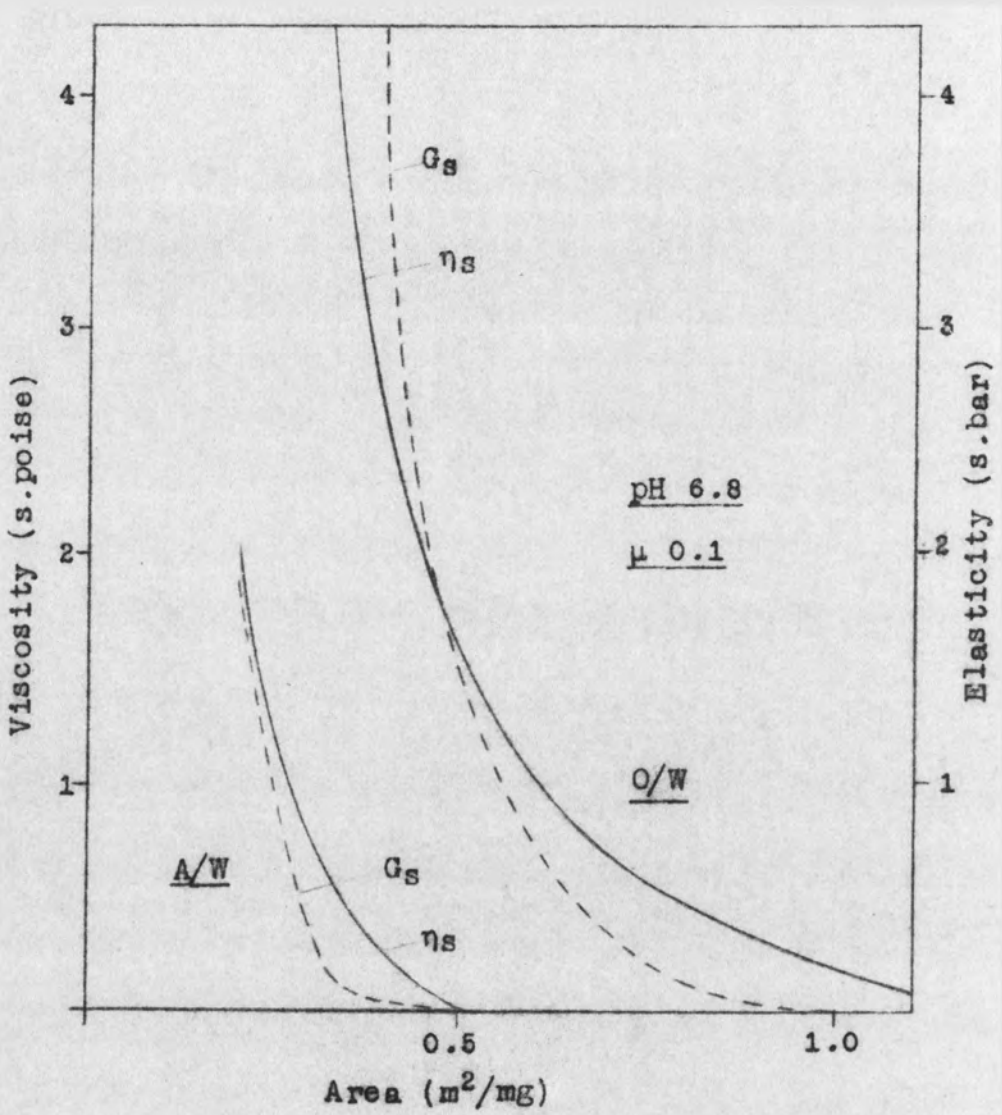
At the same pH but the higher ionic strength of 0.1 μ, the hysteresis loops were the same but the minimum pressure was only very slightly higher than the initial pressure.

The effect of pH on the minimum pressure is interesting. At pH 2.1, about the same minimum pressure was observed at both ionic strengths. At pH 12.0 no minimum pressure was noticeable even at the lower ionic strength.

Fig. 60a shows the coefficient of compressibility, obtained by graphical differentiation of the isotherm, as a function of the area at pH 6.8 and 0.1 μ. The minimum compressibility is about 0.05 reciprocal bars (cm/dyn) and is the same at both interfaces. The minimum is rather broad but the lowest point seems to be at about 1.1 m^2/mg, i.e. below the area of close-packing. The film is highly compressible before it is close-packed, but less so at low areas.

The pH had no influence on either the magnitude or the location of the minimum compressibility. However, at

Fig.6la. Surface and Interfacial Viscosity-Area and Elasticity-Area Curves (Gluten).


Viscosity (s.poise)
Elasticity (s.bar)
Area (m^2/mg)
4
3
2
1
0.5
1.0
G_s
η_s
pH 6.8
μ 0.1
O/W
A/W
G_s
η_s

the extreme pH values (pH 2.1 and 12.0) the film was less compressible at the higher areas. The kink in the O/W curve was present in all curves obtained and occurred between 2.5 and 3.5 m^2/mg.

2. The Effect of pH and Ionic Strength on the Interfacial Viscoelastic Properties.

Typical viscosity-area and elasticity-area curves are shown in Fig. 61a for pH 6.8, μ 0.1, at both interfaces. Changing the interface from A/W to O/W results in a tremendous increase in the viscoelasticity of the film. While viscosity appears at about 1.5 m^2/mg at the O/W interface, the film must be compressed to about 0.5 m^2/mg at the A/W interface before the surface viscosity becomes noticeable. Elasticity at both interfaces appears when the viscosity has reached about 0.3 surface poises. Measurements carried out at the A/W interface showed that there was very little hysteresis, the viscosities obtained on decompression being only very slightly lower.

Since the preliminary studies indicated that the interfacial viscosity and elasticity of gluten films might depend to a considerable degree on the pH of the substrate, the influence of pH and ionic strength was investigated in detail. Figs. 62a and b show the interfacial viscosity and elasticity at the ionic strengths of 0.1 and 0.2 μ as functions of the pH at different areas. The dependence on pH is indeed marked. All curves showed a very strong maximum at about pH 7.5. The curves are asymetrical, the fall being much more pronounced on the acid than on the alkaline side of

Fig.62a. Interfacial Viscosity-pH Curves (Gluten).

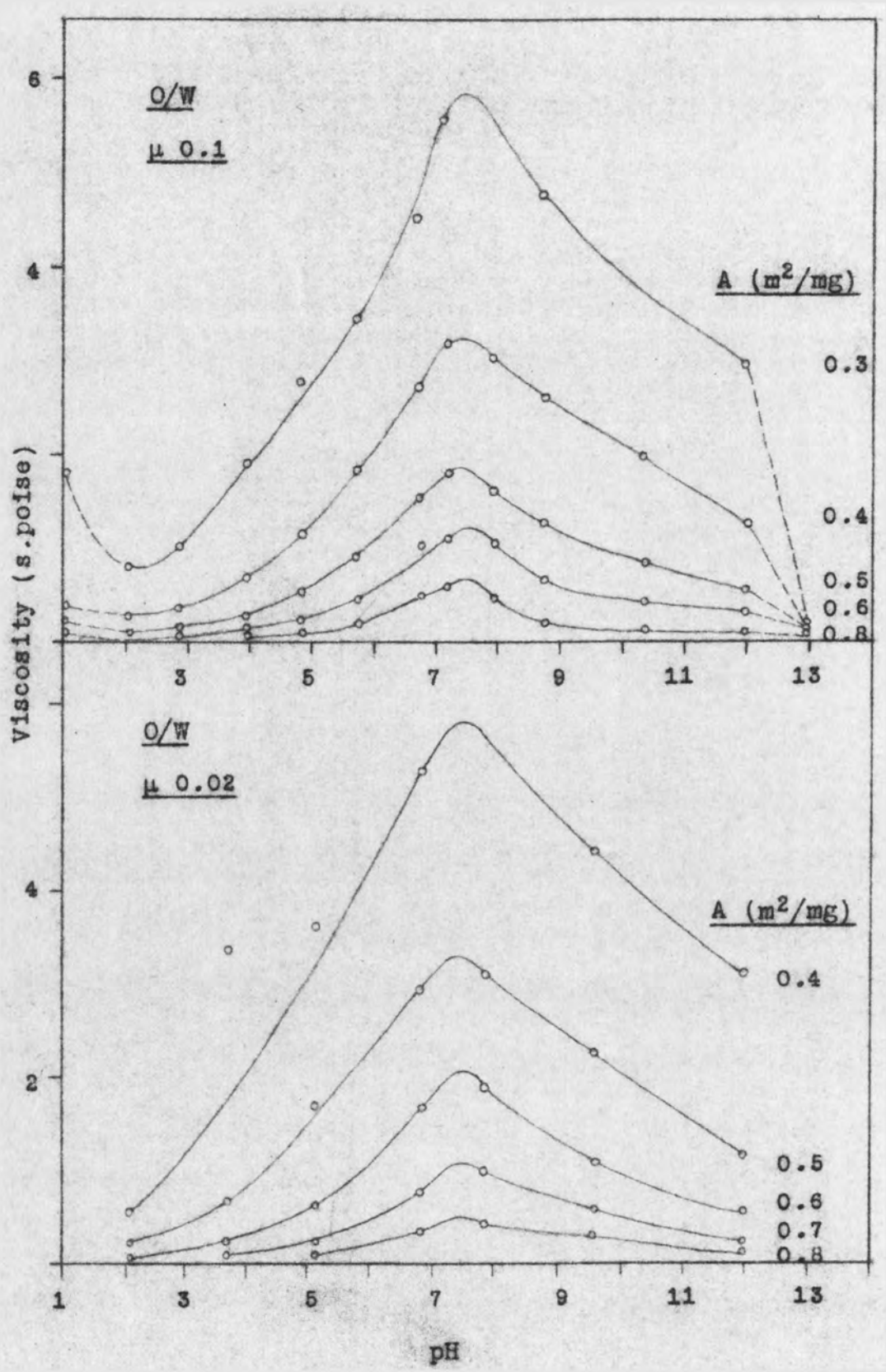

O/W
μ 0.1
A (m²/mg)
0.3
0.4
0.5
0.6
0.8
O/W
μ 0.02
A (m²/mg)
0.4
0.5
0.6
0.7
0.8
Viscosity (s.poise)
pH
1
3
5
7
9
11
13
2
4
6

Fig.62b. Interfacial Elasticity-pH Curves (Gluten).

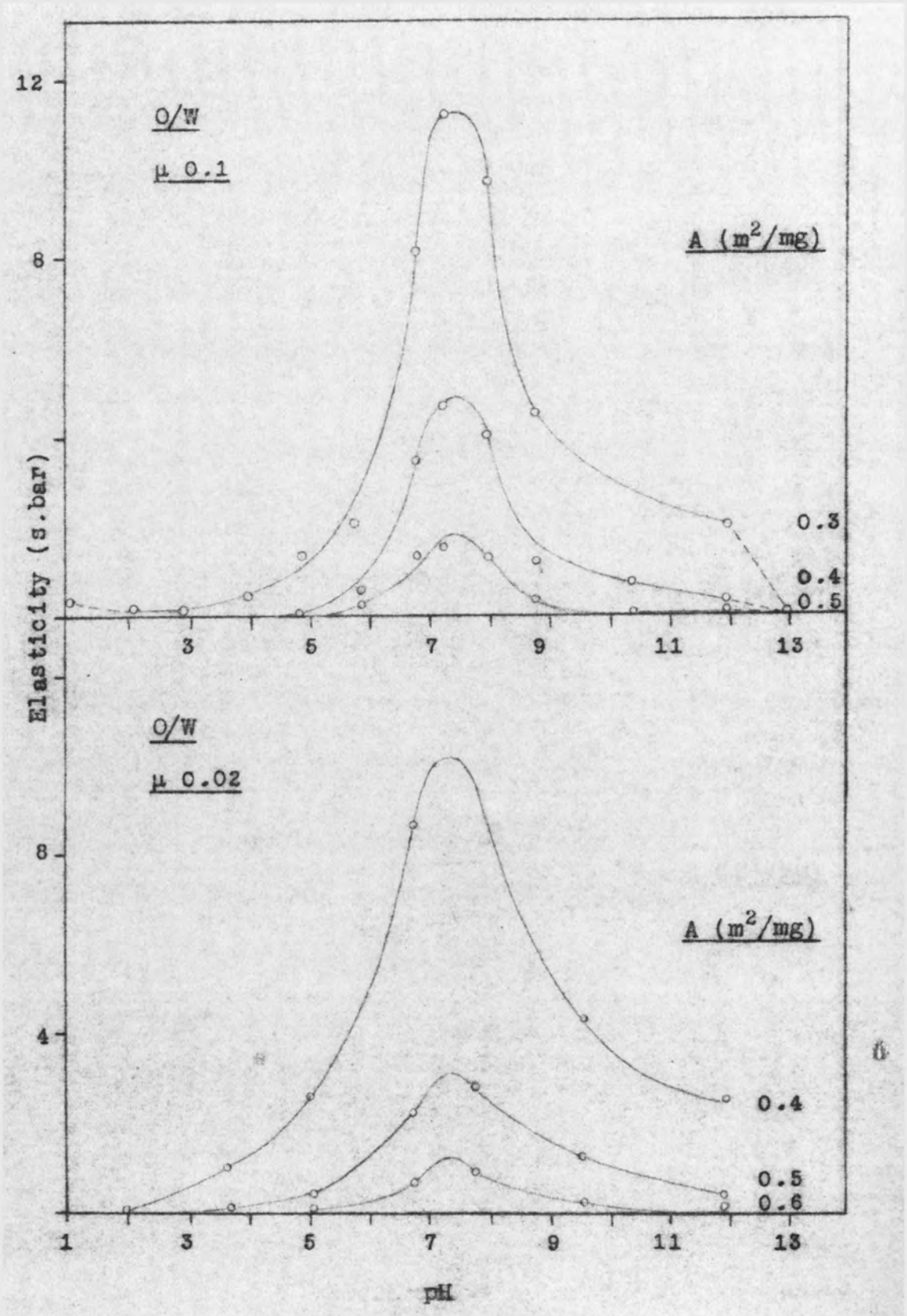
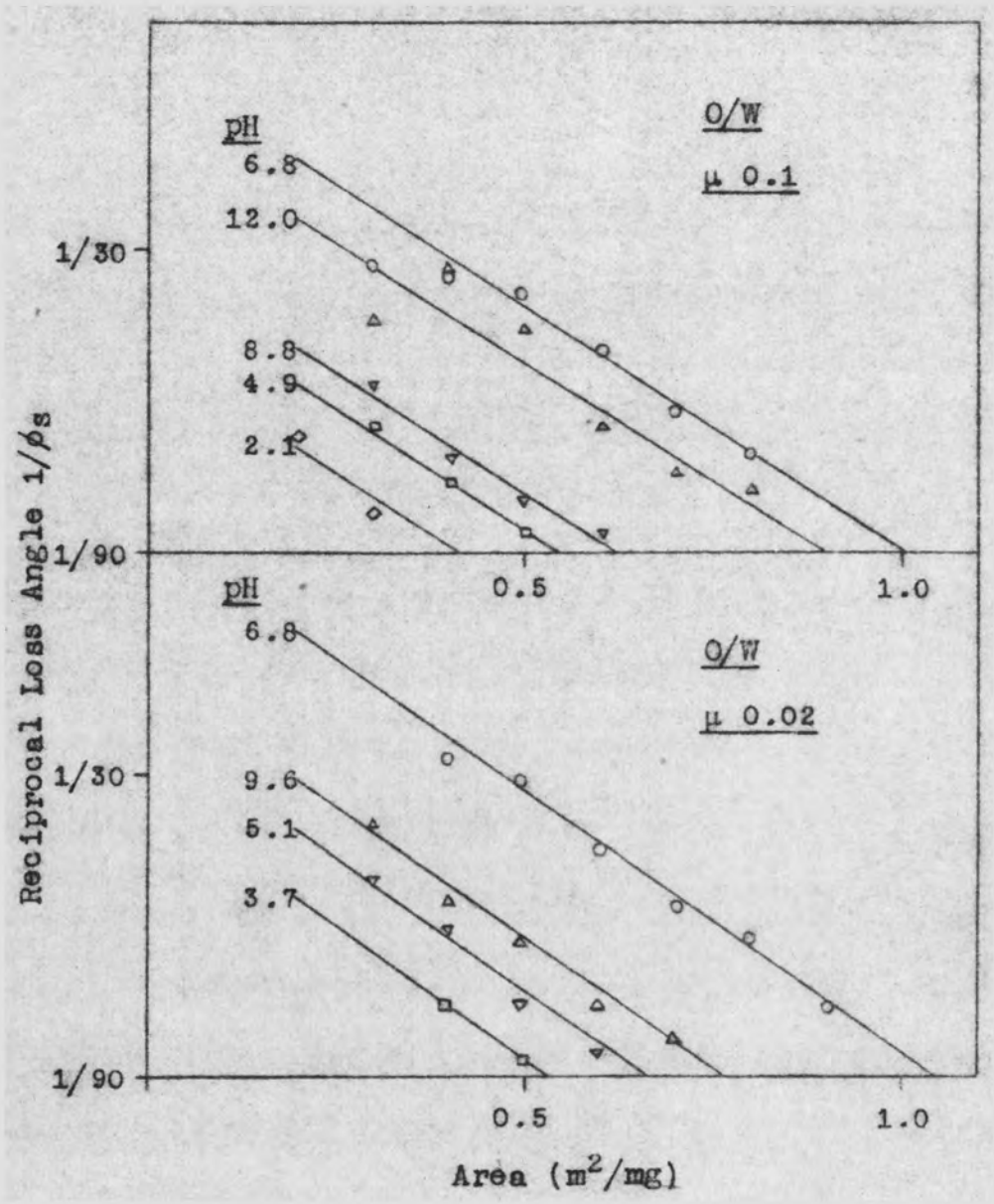

O/W
μ 0.1
A (m²/mg)
0.3
0.4
0.5
O/W
μ 0.02
A (m²/mg)
0.4
0.5
0.6
Elasticity (s.bar)
pH
12
8
4
1
3
5
7
9
11
13

Fig.62c. Reciprocal Loss Angle-Area Curves (Gluten).


pH
6.8
12.0
8.8
4.9
2.1
O/W
μ 0.1
1/30
1/90
0.5
1.0
pH
6.8
9.6
5.1
3.7
O/W
μ 0.02
1/30
1/90
0.5
1.0
Reciprocal Loss Angle 1/φs
Area (m²/mg)

the pH scale. At pH 1.0 a small rise was apparent. Between pH 12 and 13, η_S and G_S both showed a sudden fall, thus paralleling the abnormal pressure–area curve at pH 13.

As may be seen from Figs. 62a and b the ionic strength also had a decided influence on η_S and G_S at the same pH. Both were always definitely higher at the lower ionic strength.

An attempt was made to characterize the interfacial viscoelastic behaviour of gluten films in terms of the absolute surface modulus $\overline{G}_S$ and the surface loss angle or dissipation factor ϕ_S (see Appendix) instead of η_S and G_S.

The absolute modulus $\overline{G}_S$ is the ratio of the peak stress to peak strain while ϕ_S is the phase angle between strain and stress. A purely viscous film would have a loss angle of $\pi/2$ or 90°, while a purely elastic film would have one of zero. This mode of representing dynamic behaviour has certain advantages when the system is treated from a rheological point of view.

The $\overline{G}_S$ – pH curves at various areas were very similar to the η_S – pH and the G_S – pH curves shown in Figs. 62a and b. Similar plots were also obtained by plotting the loss angles ϕ_S as a function of pH. In these plots, minima appeared instead of maxima around pH 7.5, indicating that the proportion of the energy stored elastically is greatest at this pH.

These curves are not reproduced here. A more useful representation is obtained by plotting the reciprocal loss angles as a function of the area at various pH's. These plots seem to lie on parallel straight lines as shown in

Fig63a. Interfacial Viscosity-Time Curves (Gluten).

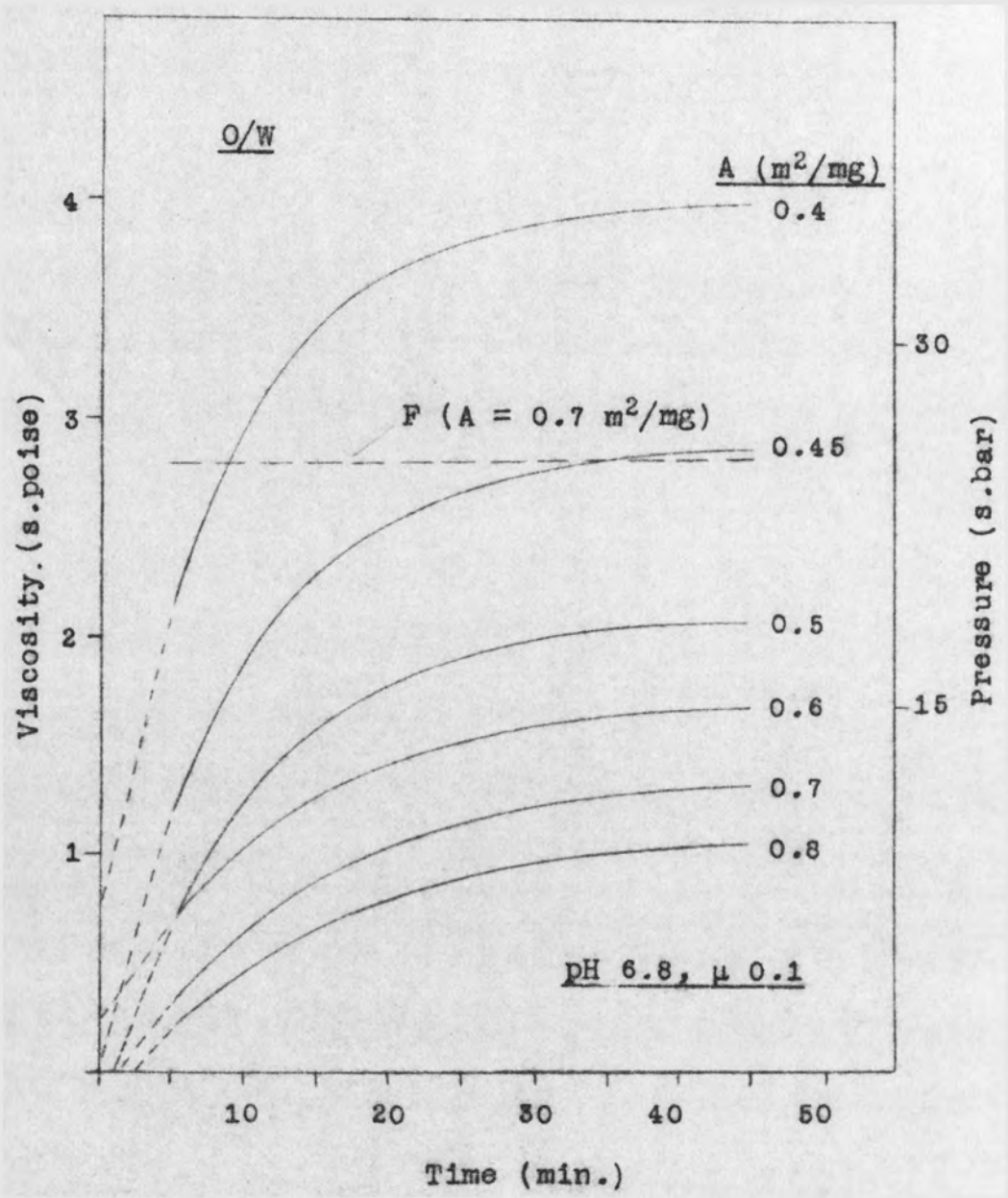
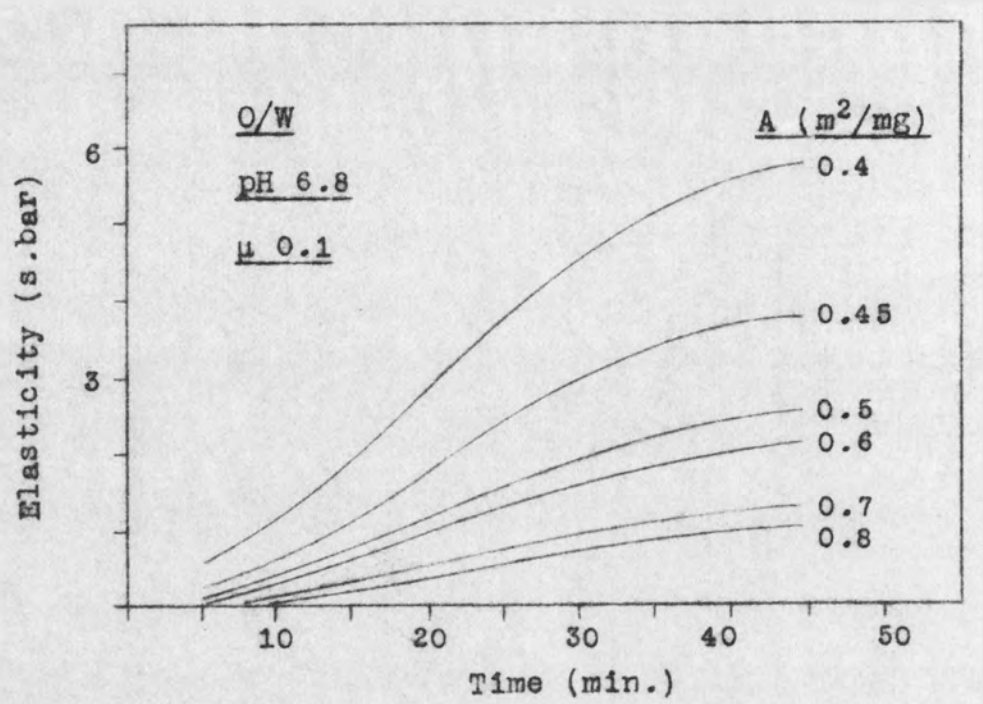

O/W
A (m²/mg)
0.4
0.45
0.5
0.6
0.7
0.8
F (A = 0.7 m²/mg)
pH 6.8, μ 0.1
Viscosity (s.poise)
Pressure (s.bar)
Time (min.)
1
2
3
4
15
30
10
20
30
40
50

Fig.63b. Interfacial Elasticity-Time Curves (Gluten).

Fig.63c. Rate Constant(Viscosity)-Area Curve (Gluten).


O/W
pH 6.8
μ 0.1
A (m²/mg)
0.4
0.45
0.5
0.6
0.7
0.8
Elasticity (s.bar)
6
3
10
20
30
40
50
Time (min.)

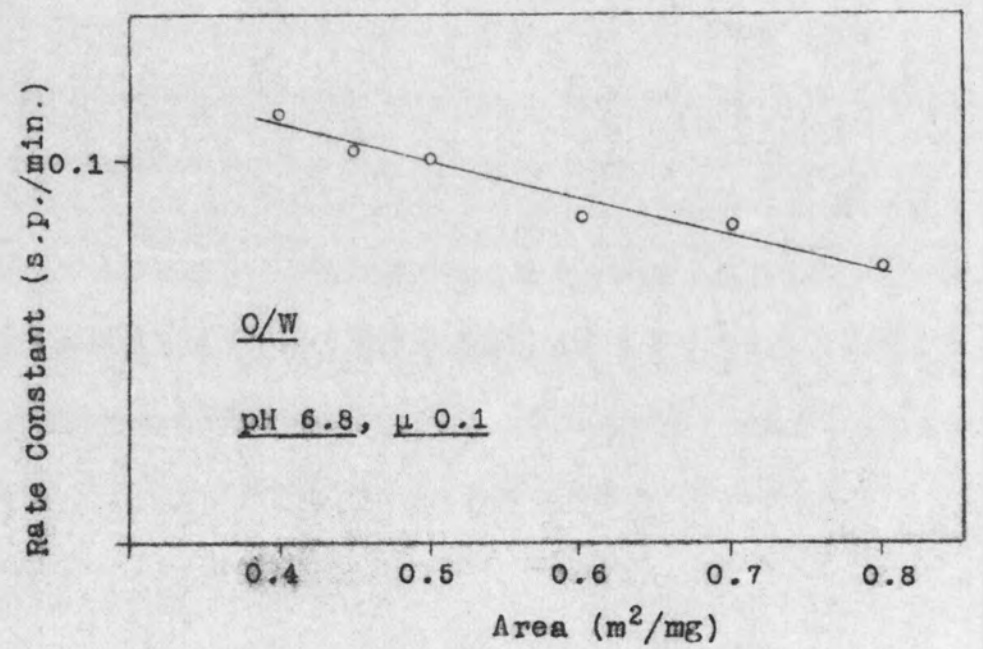

Rate Constant (s.p./min.)
0.1
O/W
pH 6.8, μ 0.1
0.4
0.5
0.6
0.7
0.8
Area (m²/mg)

Fig. 62c for some pH values at 0.1 and 0.02 μ. Extrapolation of the $1/\phi_S$–A curves to $1/90^o$ should indicate the area at which the elastic contribution becomes apparent in the film. At pH 6.8 this area was found to be about 1 m^2/mg.

3. The Effect of Time on the Viscoelastic Properties.

The effect of time on the interfacial elasticity and viscosity was examined in detail at pH 6.8, μ 0.1. The plots of η_S as a function of time at different areas are shown in Fig. 63a. The corresponding plots of G_S versus time are shown in Fig. 63b.

The increase of η_S with time was found to obey the relation

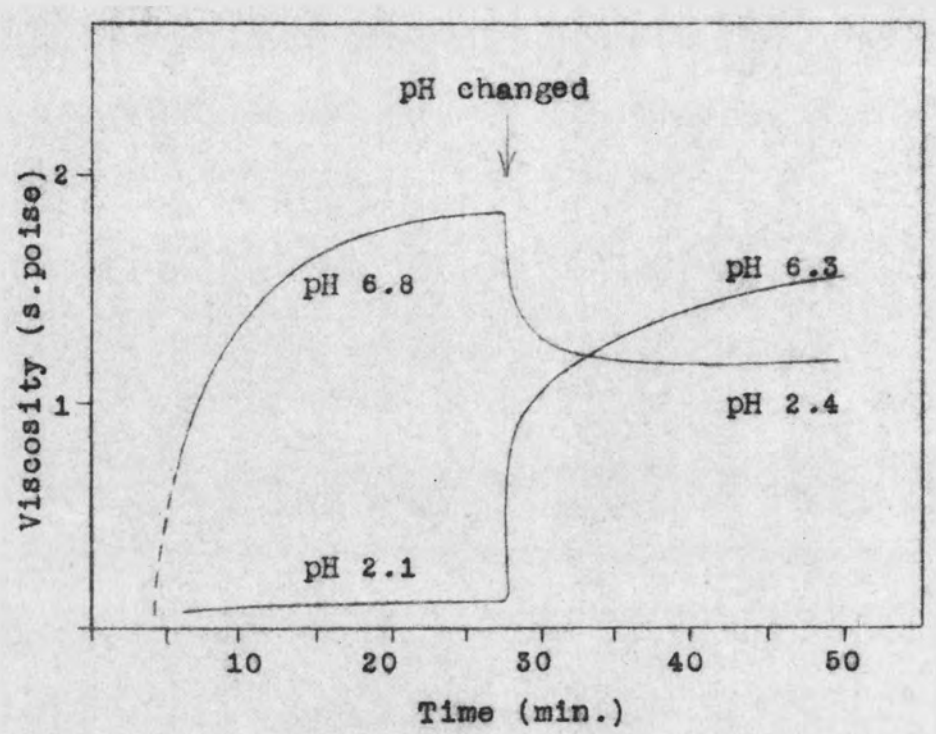
$$\ln (\eta_L - \eta_t)/\eta_L = - K(t-t_o) \qquad (7)$$

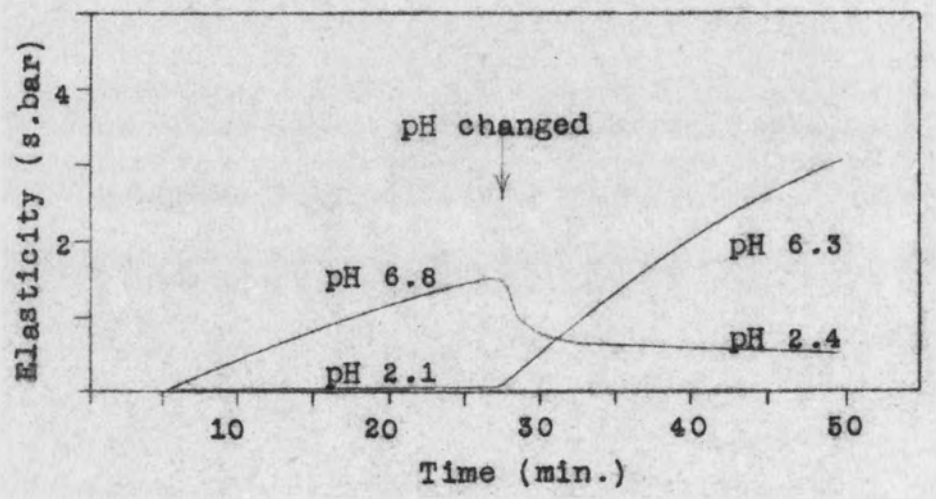
where η_L is the limiting viscosity at infinite time, η_t is the instantaneous viscosity, K is the rate constant, t the time and t_o the time at which the viscosity is zero. The curves in Fig. 63a were fitted to the above relation by Hartley's[113] method of "internal least squares". The different t_o values arise from the choice of the beginning of spreading as the origin of the time scale. The time required for spreading was several minutes according to the amount to be deposited.

The G_S curves (Fig. 63b) differ in shape from the η_S curves. They are sigmoid rather than exponential.

It is important to note that during these rather considerable changes in η_S and G_S the interfacial pressure

Fig.64a. Effect of Change of pH on Viscosity-Time and Elasticity-Time Curves (Gluten).


pH changed
Viscosity (s.poise)
2
1
pH 6.8
pH 6.3
pH 2.4
pH 2.1
10
20
30
40
50
Time (min.)


pH changed
Elasticity (s.bar)
4
2
pH 6.8
pH 6.3
pH 2.4
pH 2.1
10
20
30
40
50
Time (min.)

remained quite steady. The pressure recorded simultaneously with the viscoelastic measurements at 0.7 m^2/mg is shown by the broken line in Fig. 63a.

As may be seen from Fig. 63c the rate constants K lie on a straight line when plotted against the area. This line extrapolates to 1.54 m^2/mg and might be taken as the area at which viscosity begins to appear.

The shape of the η_S–t and G_S–t curves was the same at lower (2.1) and higher (10.6) pH and also at the A/W interface.

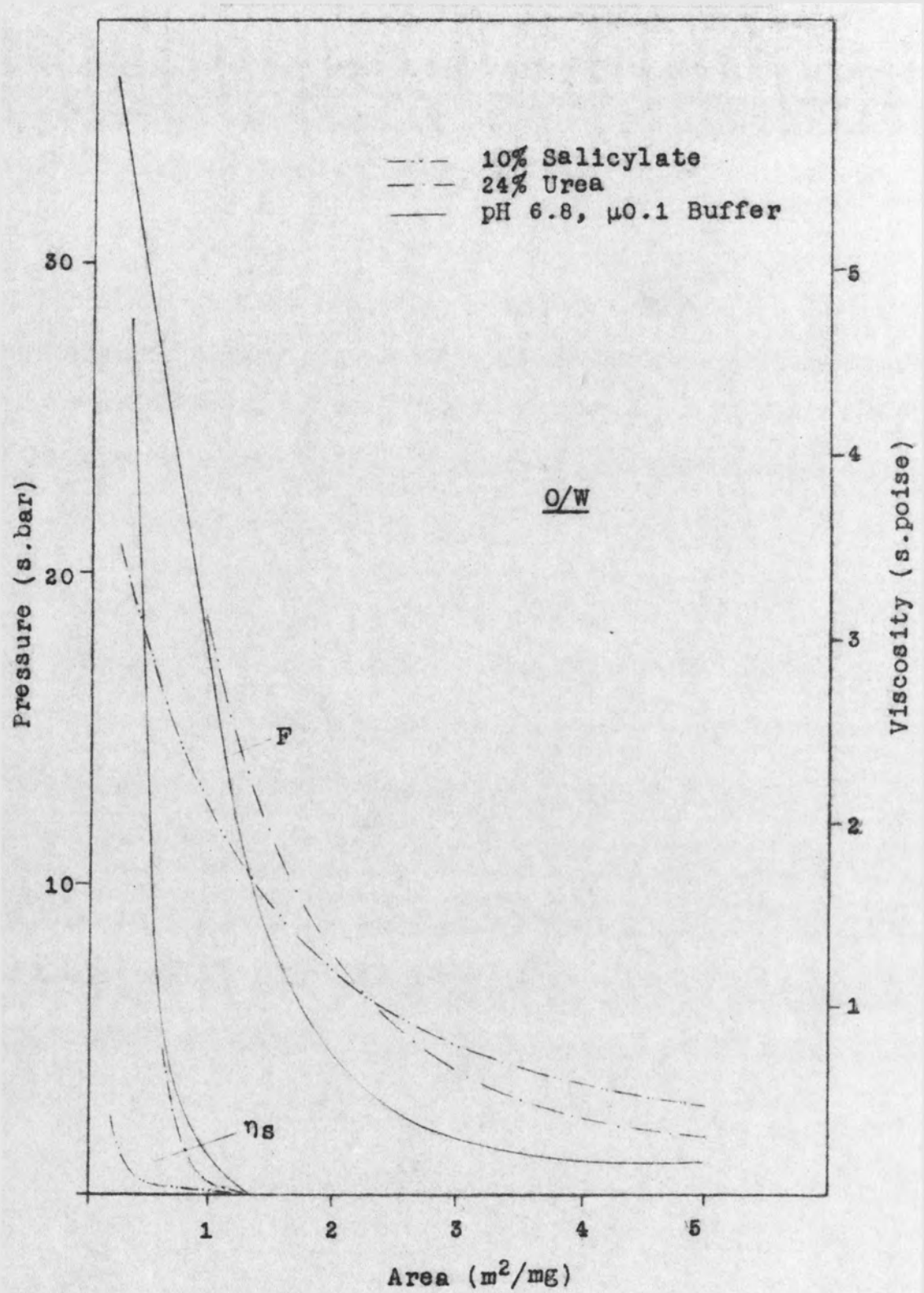
That the movement of the oscillating needle had no effect on the rise of η_S was shown by spreading a film at 0.5 m^2/mg and commencing the measurements 30 minutes after deposition. The viscosities obtained in this way were in complete agreement with those obtained by taking measurements every 5 minutes.

4. The Reversibility of the Effect of pH.

In order to see whether the effect of pH on η_S and G_S is reversible, a film was spread at 0.5 m^2/mg under a dilute buffer of pH 6.8 and measurements were taken every 5 minutes. At 27.5 minutes, the pH of the aqueous epiphase was changed by running in a calculated amount of acid. The resulting pH was determined on completion of the run. The experiment was also carried out in the reverse way, by starting under dilute acid of pH 2.1 and changing to near neutral. The results are shown in Fig. 64a.

It appears that when the pH is changed from acid to neutral, the film rapidly attains the viscosity and elasticity

Fig.65a. Pressure-Area and Viscosity-Area Curves of Gluten Spread under Urea and Salicylate.


10% Salicylate
24% Urea
pH 6.8, μ0.1 Buffer
O/W
F
ηs
Pressure (s.bar)
Viscosity (s.poise)
Area (m²/mg)
30
20
10
5
4
3
2
1
1
2
3
4
5

it would have had, had it been spread initially under that pH. When however, the change is from neutral to lower pH, η_S and G_S do not drop to the lower value they would have attained if spread initially at that pH.

5. Spreading under Urea and Sodium Salicylate.

Some experiments were carried out by spreading under 10% sodium salicylate and 24% urea. The results, shown in Fig. 65a are rather surprising. Under salicylate the gluten film showed hardly any viscosity even at the lowest areas. The pressure-area curve is similar to that at pH 13 (fig. 58a) indicating a breakdown of the film. Under urea, however, although the film is considerably expanded at the higher areas, the isotherm joins that under pH 6.8 buffer at the usual area of 1 m^2/mg and the viscosity is hardly affected at all.

Chapter VIII.

Studies on Proteins Extracted from Various Flours.

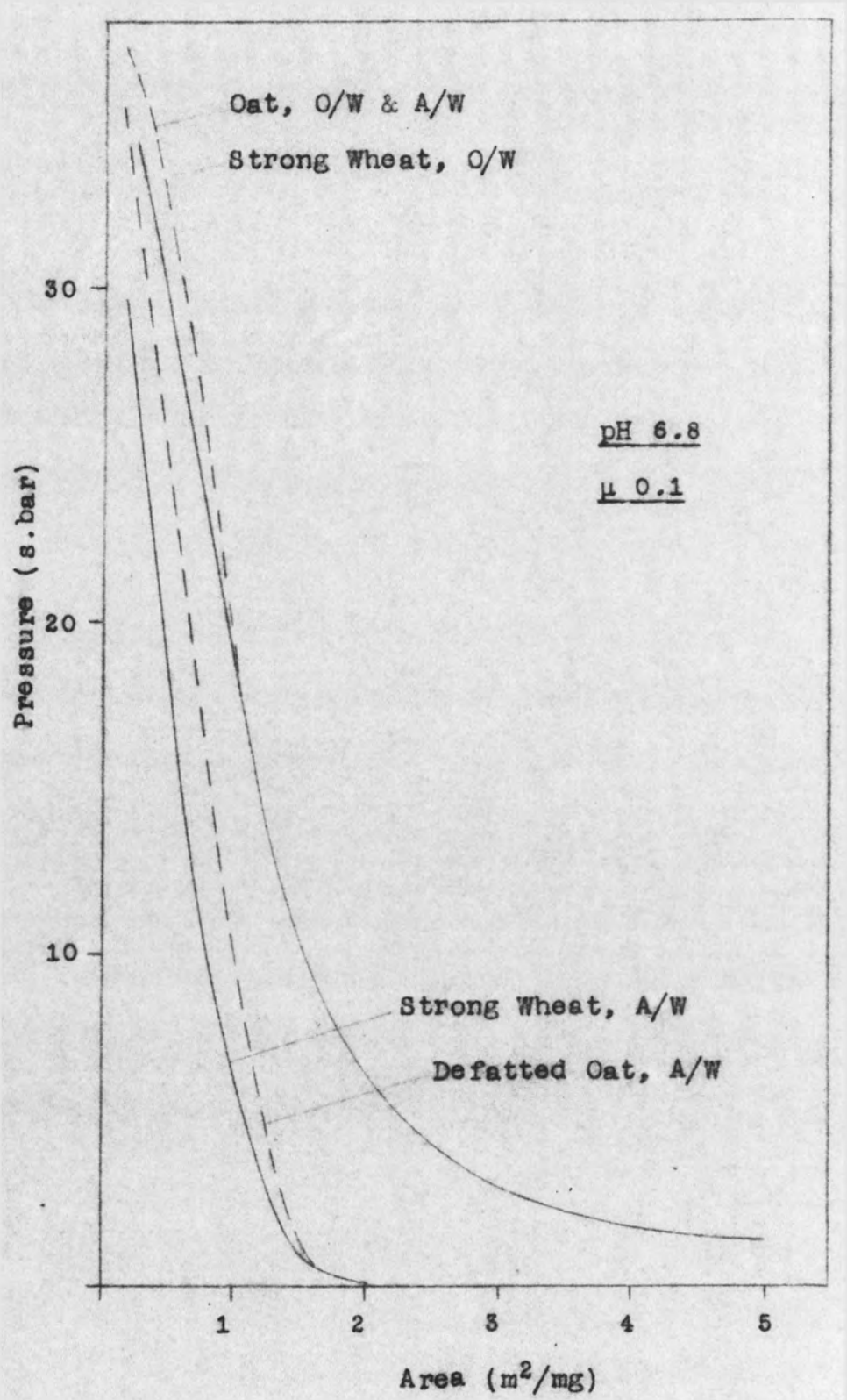
In the first study of flour extracts, films were spread of the proteins extracted with acid chlorhydrin from a strong and a weak wheat flour, from rye, barley and oats flour and from the flour milled from the germ of the carob bean.

The object of this study was to see whether any difference in flour strength could be picked up in the behaviour of the films. The other cereal flours were added on the assumption that pointers to differences in flour strength might be obtained from contrasting the film properties of flours possessing "baking strength" (wheat and rye) with those having none (barley and oats). The carob bean germ flour was included as the only known non-cereal seed flour from which apparently a coherent gluten resembling wheat gluten can be obtained.

The analytical data of the flours are contained in Tables 22a and b. The extracts were prepared as detailed in Section III-2. The films were spread in the usual way at the A/W interface (at pH 6.8 and 0.1 and 0.02 μ) and at the O/W interface (at pH 6.8 and 2.1, both at 0.1 μ).

A second study was carried out with a view to see whether dough extensibility would be reflected in film properties. Extracts were prepared from the extensible, medium (well-balanced) and harsh (short) wheat flour. Films were spread and examined in the same way as were the others.

Fig.67a. Pressure-Area Curves (Strong Wheat, Oat, and Defatted Oat Flour Protein).


Oat, O/W & A/W
Strong Wheat, O/W
pH 6.8
μ 0.1
Strong Wheat, A/W
Defatted Oat, A/W
Pressure (s.bar)
30
20
10
1
2
3
4
5
Area (m²/mg)

1. Pressure-Area Curves.

The pressure area isotherms of the strong wheat and of the oat extracts spread at pH 6.8 are shown in Fig. 67a. At the O/W interface the corresponding curves for the weak wheat, rye, barley and carob were practically identical with the strong wheat curve.

The differences were somewhat greater at the A/W interface. Here the barley and carob curves were slightly to the right of the oat curve, the weak wheat and rye curves were between those shown. At the very lowest areas, the pressures obtained with the wheat films were always lower than those exhibited by the rye, barley and oat and carob films.

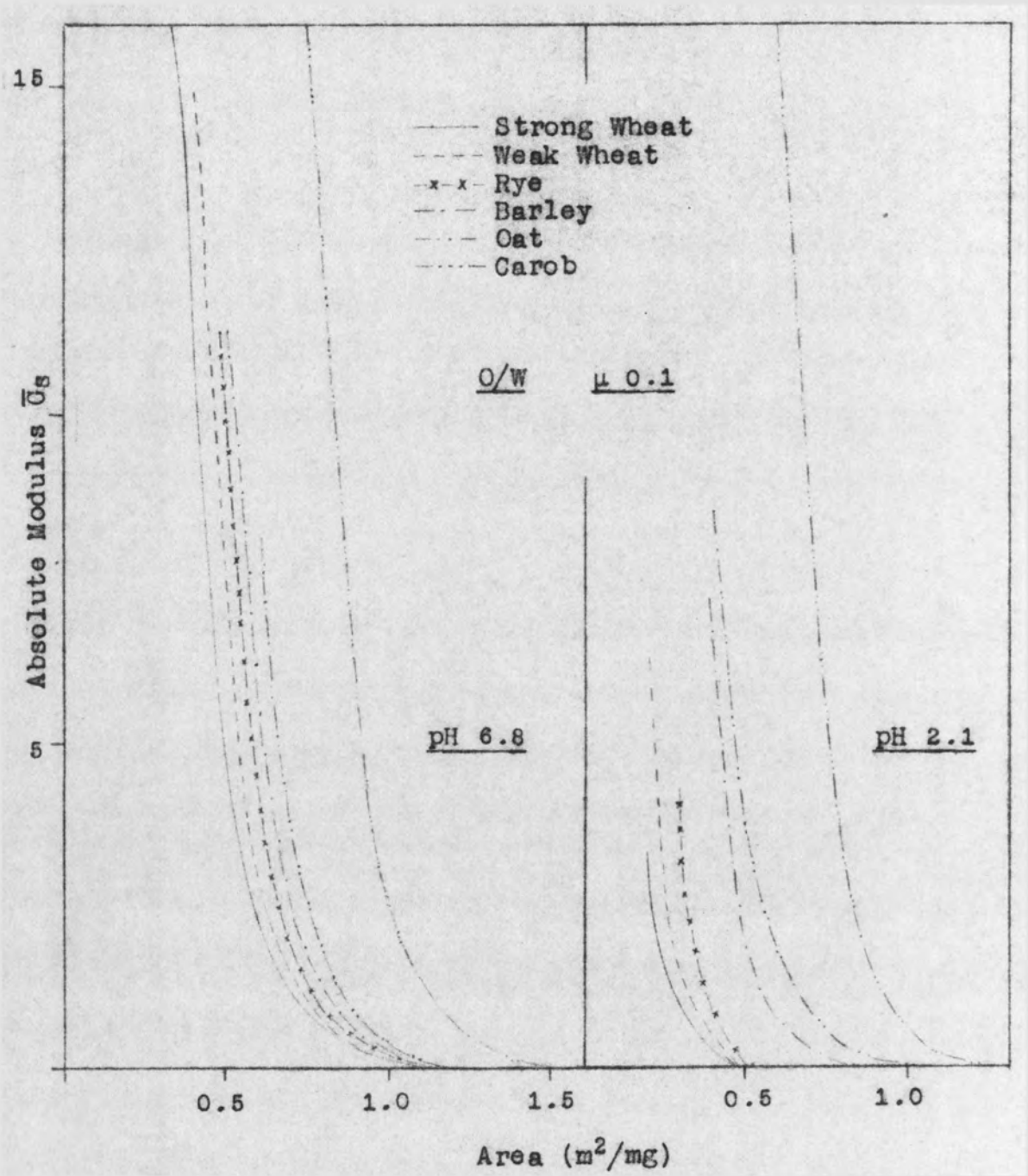
At pH 2.1, at the O/W interface, the curves were again identical but slightly lower than the pH 6.8 curve at areas above 1 m^2/mg and slightly higher at the very lowest areas.

The pressure-area curves obtained in the second study were identical among themselves but seemed to be displaced slightly towards lower areas compared with the strong wheat curve in Fig. 67a. This is thought to be due to some difference in the preparation or analysis of the extracts.

Ionic strength, (at the A/W), had no influence on the compression isotherms. The hysteresis effects (at pH 6.8) were quite similar to those experienced with the gluten films. At the lower ionic strength all curves showed a minimum pressure on decompression and this was again practically nil at the ionic strength of 0.1 μ.

The oat extract showed a rather peculiar behaviour at the A/W interface where the pressure-area isotherm obtained

Fig.68a. Absolute Modulus-Area Curves (Wheat, Rye, Barley, Oat and Carob Germ Flour Protein).


15
5
Absolute Modulus $\bar{G}_s$
Strong Wheat
Weak Wheat
Rye
Barley
Oat
Carob
O/W
μ 0.1
pH 6.8
pH 2.1
0.5
1.0
1.5
0.5
1.0
Area (m^2/mg)

in the trough was identical with that obtained at the interface between carbon tetrachloride and water in the dish. Now as may be seen from Table 22b, the oat flour had a fat content several times higher than the other samples. Softening point determination showed this to be an oil rather than a fat. This oil would be extracted along with the protein by the chlorhydrin. It therefore seemed likely that the great expansion of the oat extract curve observed at the A/W interface was due to the fact that the film was effectively spread at an interface between water and flour oil instead of an A/W interface. To check this assumption, the oat flour was extracted with petroleum ether prior to the extraction with acid chlorhydrin. When the defatted oat flour extract was spread again at the A/W interface, the pressure—area curve became perfectly normal.

The compressibility curves showed only little variation and were quite similar to the gluten curve in Fig. 60a. Again kinks appeared at the O/W interface between 2.5 and 3.5 m^2/mg.

2. Viscoelastic Properties.

Fig. 68a shows the variation of the absolute modulus $\bar{G}_S$ as a function of the area for the flour extracts of the first study. The η_S—A and G_S—A plots are not shown as they presented much the same picture. The wheat flours examined in the second study gave $\bar{G}_S$—A curves very similar to those of the strong and weak wheat flour curves in Fig. 68a.

Fig. 69a shows the plot of the reciprocal loss angle

Fig.69a. Reciprocal Loss Angle-Area Curves.
(Wheat Flour Proteins).

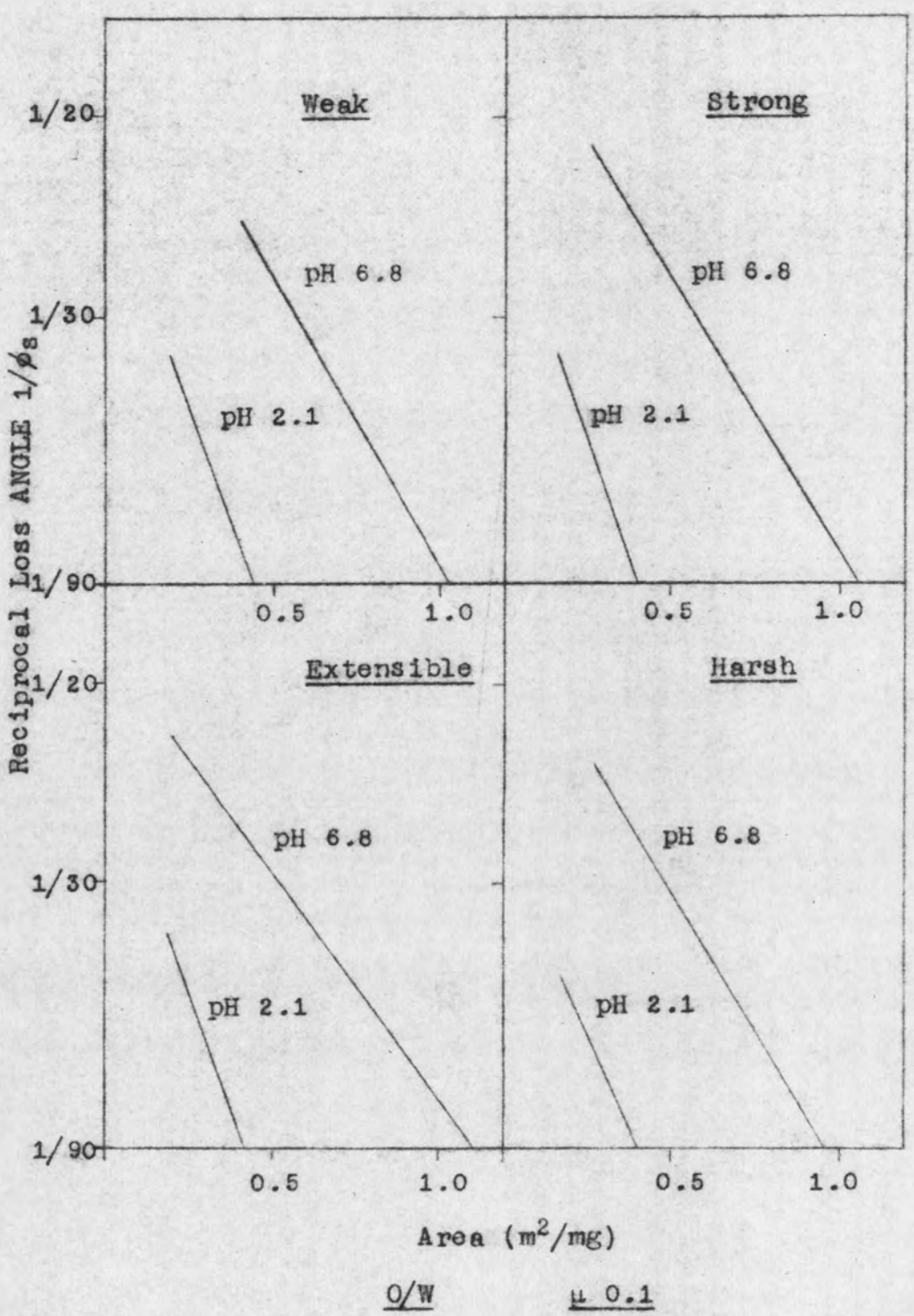
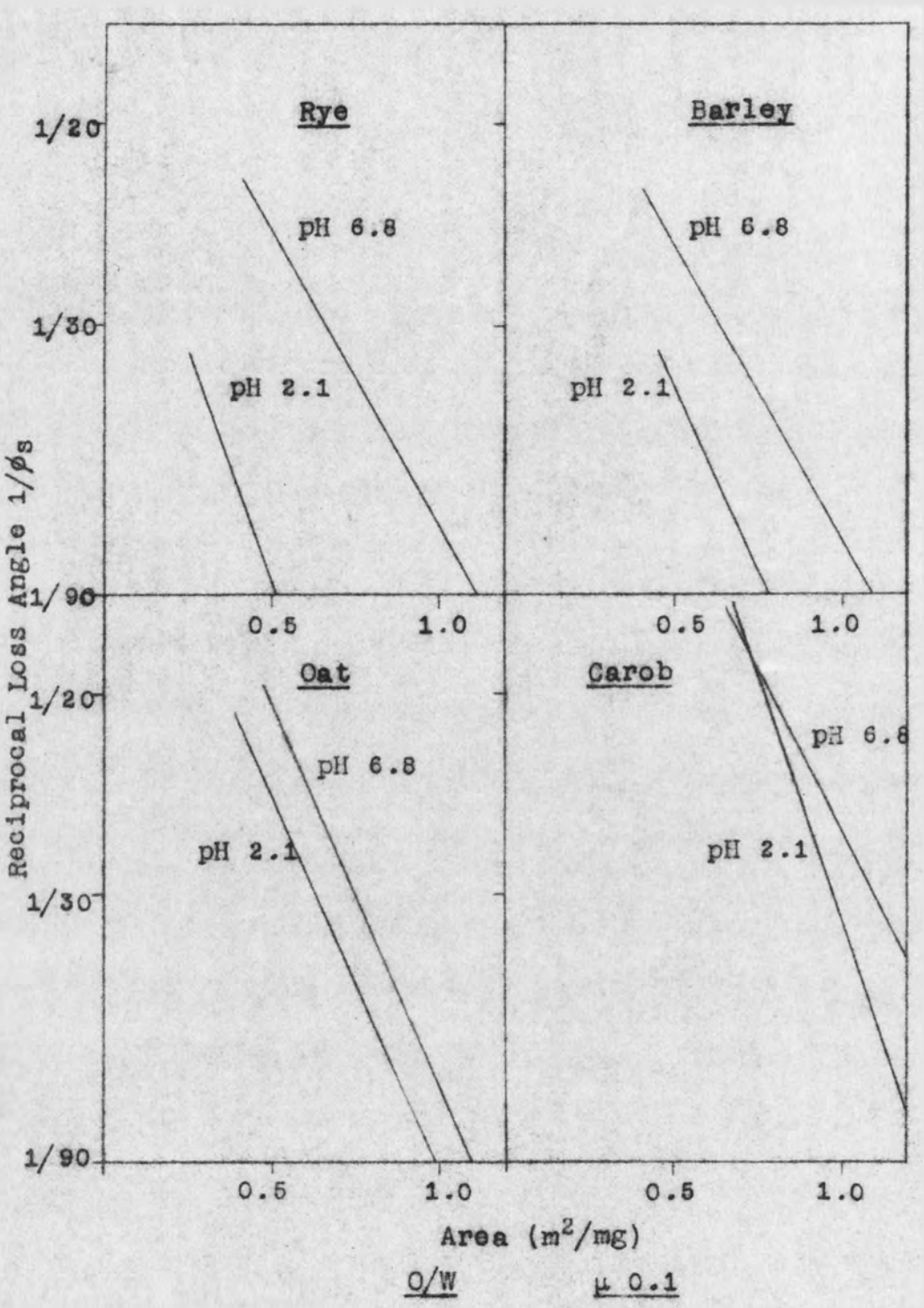

Weak
Strong
Extensible
Harsh
pH 6.8
pH 2.1
1/20
1/30
1/90
0.5
1.0
Reciprocal Loss ANGLE 1/ϕ_s
Area (m^2/mg)
O/W
μ 0.1

Fig.69b. Reciprocal Loss Angle-Area Curves.
(Other Cereal Flour Proteins).


Rye
Barley
Oat
Carob
pH 6.8
pH 2.1
1/20
1/30
1/90
0.5
1.0
Reciprocal Loss Angle 1/φs
Area (m²/mg)
O/W
μ 0.1

$1/\phi_S$ as a function of the area at pH 6.8 and 2.1 for the weak, strong, extensible and harsh flours. The corresponding plot for the medium flour was quite similar. The same relations are shown for the other cereal and the carob germ flour in Fig. 69b.

Both the $\overline{G}_S$–A and the $1/\phi_S$–A plots reveal striking differences in the effect of pH on the viscoelasticity of the films.

The influence of time on the viscoelastic properties was checked in the case of the strong wheat and the defatted oat flour. The wheat extract behaved exactly like gluten but the η_S–t curves of the defatted oat film were much flatter, while the elasticity rose more rapidly.

Chapter IX.

Discussion of Results.

In the following, the general behaviour of proteins at liquid interfaces is discussed briefly and the peculiar features of the cereal flour proteins are then considered in some detail against this background.

1. Protein Films at Liquid Interfaces.

The general behaviour of protein monolayers at liquid interfaces has been discussed in several textbooks[3,7] and reviews[32,38,50,177].

When a globular protein is spread at a liquid interface, the molecule is unfolded (surface denaturation) and the peptide backbone becomes anchored in the interface. As many of the polar (hydrophilic) side chains as is sterically possible, are oriented towards the water phase while the non-polar (hydrophobic) side chains similarly tend to turn towards the air or oil phase. The question whether at large areas, the side chains lie flat in the interface and become immersed in the respective phases only on compression, does not appear to be settled unambiguously at the moment and may well depend on the type of protein. As the film is compressed, the interfacial or surface pressure begins to rise steeply at an area of about 1 m^2/mg. This is fairly uniform for most proteins studied and corresponds roughly to the area calculated from X-ray data at which the protein molecules might be expected to be close-packed, i.e. having all side chains oriented vertically while the peptide back-

bones approach each other as closely as possible.

Surface viscosity becomes detectable at about or below the same area, while an elastic contribution usually appears as compression is continued. These phenomena are taken to indicate intermolecular secondary bond formation (salt-links, electrostatic dipole interaction, hydrogen-bonds and Van der Waals interaction) resulting in the formation of a surface gel or coagulum. During this stage there is a steep increase in the viscoelasticity.

While the pressure-area curves of proteins are relatively similar, considerable differences are found in the viscoelastic properties.

A characteristic feature of protein films at interfaces is their low stability. Around about 20 surface bars pressure the film collapses, i.e. the pressure-area curve flattens out.

Provided the proper spreading technique is used, pH and ionic strength seem to have little influence on either pressure or viscoelastic properties[50], although at pH values sufficiently removed from the isoelectric point, an increase in pressure is generally observed. This is attributed to electrostatic repulsion due to the net charge on the molecules.

The behaviour of protein films is essentially similar at both the A/W and the O/W interface, except that the pressure-area[8,50] as well as the viscoelastic curves are considerably more expanded at the O/W interface[56] due to the elimination of Van der Waals attraction between non-

polar side chains.

Considering the experimental results presented in the previous chapter, it is clear that while the influence of the interface on gluten films (cf. Figs 58a and 61a) and on films of the other cereal flour extracts (Fig. 67a) is similar to that of the water soluble globular proteins, gluten films show striking differences in a number of points. The most important of these are the great stability of these films and the marked effect of pH and ionic strength.

2. The Stability of Gluten Films.

The fact that the high stability of these films is independent of the type of the interface and of the pH and ionic strength of the substrate within considerable limits (Fig. 58a) would indicate that it is due to hydrogen bonding rather than salt bridges or non-polar attraction of the Van der Waals type. This view seems to be supported by the completely different type of pressure-area curve obtained under 10% sodium salicylate (Fig. 65a) which is known to break hydrogen bonds. The failure of 24% urea to produce a similar curve might be due to its relatively smaller efficiency as a hydrogen bond breaker[48]. As urea is not capable of disrupting salt-links, it is also possible that the effect of salicylate is due to its ability to break these as well as hydrogen bonds.

About a third of the amino acids in gluten and in the other cereal proteins studied here, are glutamine residues.

It is tempting to correlate this unusually high amount of potentially strong hydrogen bond forming amide groups[230] with the stability of these films. Evidence of interaction existing between materials containing large numbers of primary amide groups, interpreted as being due to intermolecular hydrogen bonding, was obtained by Holms[121]. It should be noted, however, that the stability of gliadin films does not seem to be different from that of the globular proteins[8,22], although gliadin contains an even larger amount of amide groups[27b].

The fact that the interfacial pressure of gluten films increases even at areas where the film must be several times thicker than a monolayer (the film thickness calculated at 0.2 m^2/mg, assuming a protein density of 1.33, is about 38 A, i.e. about four times that of the usual thickness of 10 A for a protein monolayer[50]) is difficult to explain unless one assumes that on compression below about 1 m^2/mg the less surface active anchoring points of the strongly bonded coherent gluten films are pushed out of the interface, segments of the film thus forming loose loops or bridges, while more and more strongly active groups are brought into the interface per unit area. At least some support of this view may be found in the work on mixed gliadin–cholesterol films[143,204] in which it was shown that gliadin films could be reversibly displaced from the interface[142a].

The considerable compressibility (Fig. 60a) of gluten films is probably also best explained by the assumption

that chain segments of gluten films are relatively free to leave the interface. The minimum compressibility of gluten films was found to be of the order of 0.05 reciprocal bars, which is in excellent agreement with the minimum compressibility of gliadin films as reported by Gorter and Blokker[87]. This is, on the average, about twice as high as the minimum compressibilities reported for other proteins[32,177,190].

At areas lower than the point of minimum compressibility (Fig. 60a) the coefficient of compressibility increases more rapidly at the O/W interface than at the A/W interface. While the surface (A/W) and interfacial (O/W) pressures of other proteins tend to attain the same value at low areas[8], the interfacial pressures of gluten films were always higher, even at low areas, than the surface pressures observed at the same areas. These facts seem to indicate that segments of the film are lifted out of the O/W interface with greater ease.

The unusual stability of gluten films is also reflected in the hysteresis phenomena they exhibit. Decompression and recompression curves of some proteins (serum albumin, ovalbumin and hemoglobin) were studied by Dervichian[63]. In all cases, decided hysteresis loops were observed but the surface pressure on decompression always returned to the initial value. Gluten films at pH 2.1 as well as pH 6.8, when the ionic strength of the substrate was low (0.02 μ), exhibited a minimum pressure which corresponded

to the surface pressure attained, on decompressing, around the area of 1 m^2/mg.

It is believed that this minimum pressure arises in the following way. When the film is compressed below about 1 m^2/mg, strong intermolecular bonds are established linking the film into a two-dimensional network. Segments of the film, as has been explained earlier, leave the interface as compression continues. On reexpansion, the displaced segments again enter the interface. When all folds have been deposited, the film, now held firmly together, cannot expand any further, although the available area is increased as the movable barrier continues to travel in the direction of decompression. The mica plate, being completely surrounded by the film, registers the surface pressure corresponding to the area the film actually occupies rather than that which were available to it if it had freedom to expand.

The good reproducibility obtained on recycling the same film, and the less satisfactory reproducibility between films is clearly explained by this assumption. The initial formation of the network would be difficult to control. However, once the network is formed, its characteristics would be largely fixed. Hence, the reproducibility on recycling.

The effect of ionic strength on this phenomenon is interesting. Network formation seems to be aided by low ionic strength. This is in accordance with the effect of

ionic strength on the viscoelasticity of the gluten films (cf. Figs.62a and b). Inasmuch as this network may be regarded as a two-dimensional gel, the tendency of an increase in the ionic strength to prevent network formation is analogous to the effect of salts on three-diemnsional gels, of, for instance, gelatin. Spreading factors, however, must also be taken into consideration. When the film is reexpanded, there is no spreading agent present to assist it. Any factor which generally helps spreading, such as higher ionic strength, would conceivably have a bearing on these hysteresis phenomena.

The influence of pH is difficult to interpret and should be investigated in more detail.

The question whether other proteins would show a similar behaviour, is an interesting one. It does not seem likely that a film balance of the horizontal float type would be able to pick up this phenomenon and this may be responsible for Dervichian's[63] failure to observe it. No minimum decompression pressure was observed in several studies of the hysteresis of synthetic high polymer films in which a float type balance was used[171,172,205].

The observed hysteresis effects may, of course, be a peculiar feature of gluten films. Surface potential measurements might contribute to a clarification of this phenomenon.

3. The Influence of pH and Ionic Strength on the Viscoelastic Properties of Gluten Films.

The second remarkable characteristic of gluten films

is their dependence on the pH and the ionic strength of the substrate. This is particularly prominent in the visco-elastic properties. There is relatively little information on this point in the literature, most surface viscosity measurements having been carried out at selected pH values rather than a complete range.

Joly[130] noted that the surface viscosity of the proteins investigated by him was highest near the isoelectric point, lower at pH 2 and intermediate at pH 12. His films, however, were spread from solid particles and the influence of various factors affecting spreading cannot be assessed. Pouradier[191] without presenting any data, reported a slight maximum in the viscosity of gelatin films at the A/W interface. Cumper and Alexander[49] found no appreciable effect of pH on the interfacial viscosity and elasticity of pepsin, insulin and β-bovulin at the O/W interface. Tachibana, Inokuchi and Inokuchi[219] reported surface viscosity and elasticity curves of ovalbumin at the A/W interface. These showed a maximum at pH 0.2, a plateau between pH 2 and 7, and again lower values between pH 7 and 10. These results are at variance with the earlier findings of Joly[130] who also investigated ovalbumin. They are also very different from those reported here on gluten films.

Of particular interest are the results of Gorter and Blokker[87] on gliadin films. These workers obtained no evidence of an effect of pH on the surface viscosity of gliadin films over the pH range 1–11. They state, however, that their viscosity measurements were rather poorly re-

producible, sometimes differences from 100% more to 50% less having been observed. Their results cannot therefore, be taken as evidence that the viscoelasticity of gliadin films would not show a pH dependence similar to that of gluten films.

Some light on the pH dependence of the viscoelasticity of gluten films is shed from the recent work of Isemura, Hamaguchi[2,8] and Ikeda[126] on the surface chemistry of the synthetic copolypeptides 1:2:1-(poly)lysine/leucine/glutamic acid and 1:3:1-(poly)lysine/phenylalanine/glutamic acid. These copolypeptides, particularly the former, may be regarded as simplified gluten models in two important respects. They resemble gluten in having about an equal number of basic and acidic side chains and a similar polar-nonpolar side chain ratio.

Both copolypeptides were found to exhibit a maximum in surface viscosity at constant area at the isoelectric point of pH 7, while the surface viscosity decreased regularly on either side of this point. An increase in the ionic strength of the substrate resulted in a lowering of the surface viscosity values measured at the same areas. The Japanese workers attribute the increase in surface viscosity at the isoelectric point to maximum salt link formation and the decrease with increasing ionic strength to the screening effect of the added electrolyte.

It seems reasonable to adopt the same explanation for gluten films although a certain difficulty arises from the fact that the overall number of acidic and basic

side chains in gluten films must be considerably less than the number of such groups in the synthetic copolypeptides[225]. It is however, a well-known fact that even a small number of crosslinks will have a profound influence on the physical properties of a polymeric material.

The asymmetrical shape of the curves in Figs. 62a and b seems to be explicable by a consideration of the ionogenic groups in the chain. On the acid side there are only α-carboxyl, glutamyl and aspartyl groups with nearly the same pK. On the alkaline side there are histidyl, lysyl, arginyl and α-amine groups dissociationg at different pK's. The amine groups of arginine is still ionised[40b] even above pH 12. This would appear to account for the fact that the influence of pH on either interfacial viscosity or elasticity is greater on the acid side of the maximum than on the alkaline side. If all ionogenic groups would dissociate within narrow pK ranges one would expect a stepped curve showing discontinuities at the corresponding pH values. However, the pH's of the various groups are influenced by adjacent groups[5] and this results in the continuous curves observed.

On the grounds that maximum viscoelasticity occurs at the pH of maximum salt-link formation, the iscelectric point of gluten films must be considered to be around pH 7.5, although other data (cf. Chapter I) would have prepared one to expect the isoelectric point to be somewhat lower, perhaps around 6.0 to 6.8. Work on simple compounds has shown that surface dissociation constants are not

identical with bulk pK's[85,114,116]. These investigations have not, however, been extended so far to charged high polymers and no information seems to be available at present concerning the influence of the interface on the isoelectric point of such compounds.

A word of explanation seems indicated concerning the fact that the surface viscosity shows a maximum in the isoelectric range while charged high polymers in bulk solution exhibit a minimum in the same region. This difference is easily explained if one considers the factors producing viscosity in bulk and in the interface. In the former, the viscosity increases with a progressive uncoiling of the protein chain. The viscosity is therefore lowest at the isoelectric point when the molecule is coiled up and intermolecular electrostatic interaction is at its lowest[10,11,29]. In the interface, however, the chain is already extended and the viscosity is greatest at the isoelectric point where resistance to shear is greatest because of maximum salt-link formation[125]. The effect of conditions promoting or hindering salt-link formation between protein chains in bulk solution is therefore opposite to the effect in the interface.

4. The Influence of pH and Ionic Strength on the Pressure-Area Isotherms of Gluten Films.

The influence of the pH and ionic strength of the substrate on the pressure-area curves of gluten films is more difficult to explain. W-shaped curves similar to those

shown in Fig. 59a were frequently reported in earlier surface chemical studies on proteins. However, it was soon realised that factors affecting the spreading of the film had to be taken into account. The maximum around the isoelectric point decreased when sufficient time was allowed for the film to reach equilibrium[91,177], or when spreading was facilitated either by increasing the ionic strength of the substrate[73,145], or by spreading at the O/W interface[8,15], or by the use of a spreading agent[8,61,62,98,213]. Indeed, it is difficult to see why a protein should spread most readily at the isoelectric point. One would rather expect the reverse to be true.

In fact, Guastalla[98] showed that the $(A)_F$ – pH curve of hemoglobin had a minimum at the isoelectric point when the proper precautions were observed. Cumper and Alexander[49,50] found no appreciable influence of the pH (3–10) on pepsin, insulin and β–bovulin monolayers and of the ionic strength (0.003 – 0.3 μ) on pepsin films at the O/W interface. Alexander and Teorell[8], spreading gliadin from aqueous alcohol dispersion at the O/W interface found no effect of pH over the range of 2–9 units.

On the other hand, Gorter and Blokkar[87] found minima in the $(A)_F$ – pH curve of gliadin at the A/W interface within rather narrow pH limits around pH 3.4 and 9.2. The curve had no clear maximum but a flat plateau between about pH 4.5 and 8.5. Ellis and Pankhurst[73] found a clear maximum at pH 2.2 in films of collagen spread at the A/W interface from anhydrous formic acid dispersion. This maximum

disappeared as the ionic strength of the substrate was increased. Similarly, the 1:2:1-(poly)lys/leu/glu copolypeptide of Isemura and his coworkers[126] exhibited a maximum at pH 7. Their 1:3:1-(poly)lys/phe/glu copolypeptide showed no maximum. This compound, however, was spread on salt solutions because of its solubility in the substrate.

It must, therefore, be concluded that certain proteins and polypeptides do give W-shaped $(A)_F$ – pH curves as a genuine phenomenon and not as a result of faulty spreading. Isemura and coworkers[126] attempt to explain this in terms of a competition between the expanding effect of a net charge on the molecule and the condensing effect of salt links. However, it is difficult to see why $(A)_F$ should be at a maximum at the isoelectric point when the film is least expanded because of the absence of a net charge and most condensed because of maximum salt bridge formation. Miller[166] considers the effect of pH on the rigidity of the chain network. According to his view, the film would have the most rigid configuration at the isoelectric point. Increasing or decreasing pH causes rupture of the rigid structure and segments of the chain tend to dissolve, causing a fall in the area. At still higher or lower pH values, apart from the effect of electrostatic repulsion, the number of water soluble ionised groups decreases and the area at constant pressure again rises.

Although an increase in the ionic strength of the substrate should tend to depress any maxima in the $(A)_F$ – pH curve[73,126], the reverse was observed on gluten

films. This is difficult to explain since the nature of the interface and the spreading from chlorhydrin should ensure perfect spreading. This question therefore seems to invite further investigation.

5. The Effect of Time on the Viscoelastic Properties.

No effect of the age of the film on surface viscosity was noticed by Ellis and Pankhurst[72] on collagen monolayers and by Isemura and coworkers[126] on the synthetic polypeptides examined by them. Cumper and Alexander[49] also did not seem to have observed any time effect on the viscoelasticity of pepsin, insulin and β-bovulin monolayers at the O/W interface.

All gluten dispersions showed a considerable increase in their surface viscoelastic properties with time. (Fig. 50a). The same effect was observed by Joly[130] on a number of other proteins. However, as mentioned earlier, Joly's films were spread from solid particles without the help of a spreading agent. They showed not only a rise in viscosity but also a simultaneous rise in surface pressure, indicating slow spreading or gradual unfolding of the molecules.

The rise in the viscosity and elasticity of gluten films when spread at the O/W interface from chlorhydrin dispersions, was not accompanied by any rise in the interfacial tension (cf. Fig. 63a). This rise must therefore represent a genuine change in the rheological properties of these films, and is thought to reflect intermolecular

bonding. Pouradier[191] observed the same phenomenon on gelatin films spread at the A/W interface and also attributed the increase in surface viscosity to a gradual, spontaneous linking of the molecules.

The nature of the interface at which the gluten films were spread would seem to exclude Van der Waals attraction between non-polar side chains as a contributing factor. The immediate response witnessed on changing the pH of the substrates (Fig. 64a), indicates again that electrostatic attraction must be involved. On the other hand, the fact that bonding occurs even at pH 2.1 where only a few salt bridges would be likely, points to a participation of hydrogen bonds. This is supported by the observation that the bonds, once established, are much less susceptible to a change in pH.

It would seem that the surface gelation of gluten films at the O/W interface is due to an interplay between hydrogen bonds and salt links, to which the behaviour under urea and sodium salicylate also provides a pointer.

Some evidence that the stability of hydrogen-bonded structures in proteins is dependent on favourable side-chain interaction has been obtained by Harrington and Schellman[106] and by Kauzmann and Douglas[134].

6. The Areas of Close-packing and Minimum Compressibility.

The extrapolation of the steep portion of a pressure-area curve to zero pressure is often used as a means of obtaining an estimate of the area of close packing of the

molecule in a monolayer at the A/W interface. This procedure is often difficult with proteins since their low stability generally prevents the development of a sufficiently long straight portion of the curve. Such protein films are usually better characterised by the co-area or "gaseous area" (cf. p.31) corresponding to the co-volume of the three-dimensional Van der Waals equation.

At the O/W interface extrapolation to zero pressure is even more difficult because of the expansion of the film. At the same time Eq.(2) is not applicable, as the plot of FA against F does not result in a straight line. The area of close packing at the O/W interface is therefore difficult to estimate with most proteins.

The great stability of gluten films results in an extended straight portion in the pressure-area curves which makes an extrapolation to zero-pressure feasible at both interfaces. In fact, the extrapolated area at the A/W interface was found to be 1.33 m^2/mg (cf. Inset Fig. 58a), i.e. in excellent agreement (Table 56) with the "gaseous area" calculated from Eq. (2).

At the O/W interface extrapolation to zero-pressure gives an area of close packing of 1.54 m^2/mg (cf. Fig. 58a) This coincides with the intercept on the area axis (for K = 0), of the regression of K on A (Fig. 63c) where K is the rate constant of the increase of the interfacial viscosity with time, calculated using Eq. (7).

Although this striking agreement is certainly fortuitous, it does seem to lend support to the validity of the

extrapolation to zero-pressure of the O/W pressure-area isotherms of gluten films as an estimate of the area of close packing at this interface.

On the other hand, it also supports the view that the increase of the viscoelasticity of these films with time reflects intermolecular bonding.

It is interesting to speculate on the difference in the areas of close packing of gluten films at the two interfaces. Gluten films are likely to contain micelles of greater regularity (closer packing) within less oriented regions. Any factor removing intermolecular cohesion would be likely to increase the disoriented areas in which the molecules cannot be packed as closely as in the "crystalline" regions. On this assumption one would expect the area of close packing to be larger at the O/W interface where the penetration of the oil molecules between the nonpolar side chains would weaken the organised structure of the film.

It is reasonable to assume that the area of minimum compressibility of 1.1 m^2/mg is the area at which portions of the film must leave the interface. This area coincides with the area found from the extrapolation of the reciprocal loss angles (Fig. 62c) at pH 6.8. This is thought to indicate the point where elasticity appears in the film. As explained above, gluten films probably form loops or folds when compressed below about 1 m^2/mg, and it seems likely that elasticity is linked with the appearance of these loops. The fact that elasticity appears at the

O/W interface at higher areas than at the A/W interface might then be connected with the greater ease with which segments of the chain seem to be able to leave the O/W interface.

The fact that both the area of close packing and the area of minimum compressibility are greater in gluten films than those of most other proteins, merits some comments. In this respect, it is interesting to note that Ellis and Pankhurst[73] found the area of close packing of collagen films at the A/W interface to be 1.3 m^2/mg, in good agreement with the 1.33 m^2/mg for gluten films. Ellis and Pankhurst[73] attribute this to the high content (25%) of proline in collagen. Proline is known to interfere with the usual regular arrangement of amino-acids, resulting in an increase in the observed spacings[184,218].

Isemura, Ikeda and Yamashita[127] have recently investigated the monolayers of (poly)-L-proline and (poly)-L-proline -L-leucyglycine. The area of close packing of the former was found to be greater than 20 Å/residue, that of the latter 57 A^2/residue. This must be compared with the value of 15.7 A^2/residue for (poly)-alanine and (poly)-phenylalanine at the A/W interface.[49]

Finally, it might be useful to translate areas in square meter per unit weight into areas in square Angstroms per average amino acid residues for gluten films. Using the data (moles per 100.000 g) of Table 8, one arrives at an average residue weight of 128 for gluten. One m^2/mg

is then equivalent to 21.2 A^2/residue. Thus, at the O/W interface, the area of close packing becomes 32.7 A^2/residue and the area of minimum compressibility 23.5 A^2/res. At the A/W interface, the area of close packing is found to be 28.2 A^2/res. It is interesting, to note that this latter agrees exactly with the area calculated by Ellis and Pankhurst[73] at which the molecules are in closest contact with the side chains assumed to lie flat on the surface.

7. The Film Properties of the Proteins Extracted from Various Flours.

The results obtained in this study are of particular interest as no similar information has previously been published. As is clear from Fig. 67a the great stability of the wheat flour protein monolayers is a common feature of the cereal proteins in general, including the carob germ protein. The expansion of the film at the O/W interface is the same for all of the extracted proteins and suggests that Van der Waals type of cohesion is about the same for all of them.

One of the most important findings of this study is the demonstration of a clear difference between the bread baking cereals, wheat and rye, and the non-bread cereals, barley and oats, in the role coulombic attraction plays in the intermolecular cohesion. This is clearly brought out in the effect of pH on the viscoelastic properties of the films (Figs. 68a, 69a and b).

The cereal proteins exhibit a clear gradation from strong to weak wheat to rye, barley and oat, i.e. in the same sense in which they are suitable, by and large, for baking purposes. The effect of the pH of the substrate is most striking. The bread flour proteins, both wheat and rye, form a distinct group while barley and oat protein form another with respect to the effect of pH on the viscoelasticity of their films. The more suitable the cereal is for bread making the greater is the effect of the pH.

The carob germ protein does not fit into this picture. In view of the fact that a coherent gluten can apparently be washed from this material (cf. p.10) one would have expected it to fall into the wheat—rye group rather than the barley—oat group although it does represent, of course, a different type of protein. It is therefore relevant to note that the author did not succeed in washing gluten from the material prepared by him. Although the carob germ flour doughed up relatively well, it completely crumbled away when an attempt was made to wash gluten from it.

The author's method of preparation of carob germ flour differed in two essential points from that used by Rice and Ramstad[19], who were able to obtain a gluten from their material. To remove the tough seed coats, the American authors soaked their seeds in concentrated sulphuric acid instead of boiling them in water for 30 minutes, and they also extracted the ground carob germ flour with petroleum ether prior to the washing process. Extraction was omitted in this study for reasons of comparability. This point,

however, merits attention as has been demonstrated in the case of ~~wheat~~ oat flour protein.

The order into which the various proteins fall according to the magnitude of their film viscoelasticity at the same area (Fig. 68a) would suggest that a comparatively low film viscoelasticity might be pertinent to good baking performance. Measurements of the bulk viscosity of gluten dispersions[84,112,198] indicated that the dispersions of stronger glutens were more viscous than weaker glutens and this was regarded[112] as an indication that the stronger gluten consisted of longer and more flexible molecules. The discrepancy is, however, easily resolved if one considers that the film viscosities at the areas at which they were measured correspond to solid viscosities rather than solution viscosities in bulk. The same holds for the elasticities (rigidities). These would be dependent more on the number of cross links determining the gel structure, than the length and flexibility of the molecules.

While the protein from the different plant species seem to be well differentiated in their film characteristics, the same cannot be said with any degree of certainity concerning the different wheat flour proteins.

The indication is that a weaker flour should have a higher absolute surface modulus than a stronger flour at the same area. There is also some indication (Fig. 69a) that the elastic contribution is greater in the film of an extensible flour than in that of a harsh, short one. However, further more detailed work is clearly necessary before it

might become possible to compare the rheological behaviour of doughs with the viscoelastic monolayer properties of the proteins from the same flour.

It should be noted here, that no significant difference existed between the film characteristics of the native wheat flour and the gluten washed from it. The pressure area isotherms as well as the viscoelastic curves of films of gluten and of the native flour protein extracts from the same flour (medium) were identical within the experimental error.

Chapter X.

Conclusions and Suggestions for Future Work.

In this final chapter an attempt is made to arrive at some broad conclusions from the work presented in this thesis and to outline further lines of attack.

1. Acid Chlorhydrin.

Ethylene chlorhydrin containing hydrochloric acid was shown to be a useful dispersant for gluten and other cereal proteins in surface chemical studies. Further work seems indicated to obtain more information on the optimum conditions and the mode of action of this dispersant. Similar dispersing systems should be investigated for their potential usefulness.

Acid chlorhydrin should also prove of value for investigations in bulk solution by the standard techniques of colloid chemistry such as viscometry, osmometry, the ultracentrifuge and light scattering. Particularly the latter method should be likely to contribute materially to our present knowledge of the physical chemistry of the gluten molecule. These colloid chemical studies would be needed to integrate the results obtained by surface chemical methods into a comprehensive picture.

2. Surface Chemical Studies:

The studies reported in this thesis have furnished some insight into the forces responsible for molecular cohesion in gluten and in the cereal proteins as exhibited in their

film properties.

The unusually strong cohesion found to be a common feature of all these films may be regarded as a reflection of the poor solubility characteristics of this class of proteins. Evidence was obtained that attraction of the Van der Waals type between nonpolar side chains is considerable and that this is largely of the same magnitude in all of the proteins studied here. The great stability of the films would seem to be due principally to hydrogen bonding and a possible link with the exceptionally high amide content of the cereal proteins is suggested.

The most important result of these studies is the clear differentiation of the wheat and rye proteins from those of barley and oat in the role of coulombic interaction in the viscoelasticity of these films. Some evidence was also obtained of a possible interplay between hydrogen bonds and ionic linkages.

These results suggest that the bread baking value of a cereal flour may be linked in some way with the number and reactivity of free charged groups in the protein chain. This point must be further elucidated by a comparative study of these groups in the various proteins.

With regard to monolayer studies, the techniques of measuring surface potentials should be useful in this respect. An attempt could be made to study chemically modified cereal proteins. Deamidation and blocking or elimination of reactive groups might provide much interesting information. Such studies should be combined with a re-

examination of the surface chemistry of gliadin and with the study of synthetic model polypeptides.

Further information on the role and interaction of hydrogen bonds and ionic linkages could be obtained from spreading on substrates containing various salts together with ionic and nonionic reagents known to break hydrogen bonds.

Molecular weight (and perhaps axial ratio) distribution studies on fractions obtained in chlorhydrin containing various amounts of hydrochloric acid could be likely to furnish information on the size and shape of the molecule. Another interesting possibility here would be the use of these techniques in studying the mechanical degradation of gluten in over- mixing by examining glutens washed out from doughs at various stages of mixing.

An attempt should be made to obtain conclusive evidence whether the spontaneous increase in the viscoelasticity of the films does in fact reflect intermolecular bonding. The factors influencing this phenomenon should than be studied in detail. This could then lead to a differentation of the proteins from various types of wheat and would also allow a renewed attack on the mode of action of redox reagents.

The influence of various additives should be studied not only by incorporating them in the substrate, but also by examining dispersions containing such additives and by the investigation of gluten washed out from doughs to which they were added.

Finally, monolayer studies could possibly assist in the elucidation of the role of lipids and carbohydrates. Here studies on mixed films and spreading on substrates containing various specific enzymes might be useful.

The suggestions outlined here, must, of necessity, be tentative. However, the author feels convinced of the potential usefulness of monolayer techniques in the study of the insoluble cereal proteins and is certain that they will contribute to the solution of the problem of baking strength and its variations.

Appendix.

The Mathematics of the Damped Oscillating Surface Torsion Pendulum.

If an angular displacement θ is induced in a surface torsion pendulum such as that described in SectionIV-3, some of the imparted kinetic energy is stored elastically while the rest is dissipated as heat through frictional losses. Upon release the potential energy is reconverted into kinetic energy and heat. Eventually the pendulum will come to rest in its original position provided there is no permanent deformation, the case of which will not be considered here.

The equation governing the motion of the pendulum is

$$M = -(M' + M'') \qquad (1)$$

i.e. the imparted torque M is opposed by the restoring torque M' and the resisting torque M''. The imparted torque will be equal to $I(d^2\theta/dt^2)$ where I is the moment of inertia of the system and $d^2\theta/dt^2$ is the angular acceleration. If the pendulum is in contact with the clean surface, the restoring torque is $E_\sigma\theta$ where E_σ is the torsional constant of the wire and θ is the angular displacement. The resisting torque is $R_\sigma(d\theta/dt)$ where $d\theta/dt$ is the angular velocity while R_σ is the damping factor due to the frictional losses in the pendulum and in the surface.

Eq. (1) is then equivalent to the differential equation

$$I(d^2\theta/dt^2) + R_\sigma(d\theta/dt) + E_\sigma = 0. \qquad (2)$$

This gives the angular displacement θ as a function of time in terms of I, R_o and E_o. I is assumed to be known. If

$$R_o^2 < 4IE_o \tag{3}$$

the solution of Eq. (2) for the boundary conditions $\theta = \theta_{max}$, $d\theta/dt = 0$, when $t = 0$, is[214]

$$\theta = \theta_{max} \exp a_o t \cos w_o t. \tag{4}$$

The pendulum is seen to perform damped oscillations with attenuation a_o and circular frequency w_o. a_o and w_o are the real and imaginary parts respectively of the roots of the auxiliary equation

$$Im^2 + R_o m + E_o = 0. \tag{5}$$

We find

$$R_o = 2Ia_o \tag{6a}$$

$$E_o = I(a_o^2 + w_o^2). \tag{6b}$$

For the surface covered with a viscoelastic film, we have

$$I(d^2\theta/dt^2) + R(d\theta/dt) + E\theta = 0 \tag{7}$$

where

$$R = R_o + R_s \tag{8a}$$

$$E = E_o + E_s. \tag{8b}$$

R_s and E_s represent the damping factor and the torsional constant of the film. The additivity expressed by Eqs. (8a,b) is analogous to the additivity of impedances in series in the equivalent electrical circuit[9]. The viscous and elastic elements of the mechanical system may be regarded

as resistances and reciprocal capacitances in series.

Eq. (8a) assumes that R_O does not change when the surface is covered with the film, or in other words, that the monolayer slides freely on the substrate. Although this does not appear to be true, the error will be negligible for small angular displacements.

Solving Eq.(7) and using Eqs. (8a) and (8b) we find

$$R_S = 2Ia - 2Ia_O \qquad (9a)$$

$$E_S = I(a^2 + w^2) - I(a_O^2 + w_O^2). \qquad (9b)$$

R_S and E_S were derived considering torques. To convert them into the coefficient of surface viscosity η'_S and the modulus of surface rigidity G'_S respectively, R_S and E_S must be multiplied by a conversion factor H, translating torque (dyn cm) into surface stress (dyn/cm). H, often called the "apparatus constant" since it depends on the experimental arrangement, therefore must have the dimensions cm^{-2}. (The reason for the primes in η'_S and G'_S will become apparent later).

We have then

$$\eta'_S = 2IH(a - a_O) \qquad (10a)$$

$$G'_S = IH(a^2 + w^2 - a_O^2 - w_O^2). \qquad (10b)$$

Now w, by definition, is $2\pi/T$, where T is the periodic time or period of the oscillations and we have

$$w = 2\pi/T \qquad w_O = 2\pi/T_O \quad . \qquad (11a,b)$$

The ratio of two successive peak amplitudes is readily

shown to be

$$\Theta_1/\Theta_2 = \exp \alpha T_o \qquad (12)$$

Introducing the (decadic) logarithm of this ratio as the (decadic) logarithmic decrement λ, we find

$$\alpha = 2{,}303\lambda/T \qquad \alpha_o = 2{,}303\lambda_o/T_o. \qquad (13a,b)$$

The period T and the logarithmic decrement λ of the oscillations are the observed variables. Substituting Eqs. (11a,b) and (13a,b) into Eqs. (10a,b) we obtain

$$\eta'_S = 4{,}605IH(\lambda/T - \lambda_o/T_o) \qquad (14a)$$

$$G'_S = IH(4\pi^2 + 5{,}304\lambda^2)/T^2 - IH(4\pi^2 + 5{,}304\lambda_o^2)/T_o^2. \qquad (14b)$$

If the logarithmic decrements are sufficiently small, Eq. (14b) may be simplified to

$$G'_S = 4\pi^2 HI(1/T^2 - 1/T_o^2). \qquad (14b')$$

For a ring or disc oscillating in the centre of a circular surface the apparatus constant H is easily derived[146] as

$$H = (1/r_1^2 - 1/r_2^2)/4\pi \qquad (15)$$

where r_1 and r_2 are the radii of the ring or disc and the film respectively.

The case of an oscillating needle or vane has been discussed elsewhere by the author[222] who derived the apparatus constant for this case from an equation proposed by Fourt[80] as

$$H = 2/l^2 \qquad (15')$$

where l is the length of the needle or vane. This, however,

can only be regarded as an approximation and derivation of a more exact expression is needed.

Substituting Eqs. (15) or (15') into (14a) and (14b) it is seen that η'_S has the dimensions g/sec while G'_S has g/sec^2 (dyn/cm). The former corresponds to a two-dimensional (surface) viscosity and the latter to a two-dimensional (surface) rigidity or elasticity. The units are called "surface poise" and "surface bar" respectively. The second is, of course, identical with the unit of surface pressure.

Both η'_S and G'_S depend upon the frequency at which they are measured. At a single frequency, two independent quantities will suffice to specify the linear viscoelastic behaviour in shear of a monolayer. G'_S and η'_S are the two most readily obtained with an oscillating surface torsion pendulum. However, when it is desired to characterize linear viscoelasticity from a rheological point of view, another choice of quantities is often more advantageous.

In dynamical testing, where a sinusoidally varying stress is applied to a viscoelastic body as to the monolayer in our case, the stress, which is generally out of phase with the strain, may be decomposed into two components. One of these is in phase and the other 90° out of phase with the strain. G'_S then represents the in-phase component divided by the strain and is proportional to the energy stored elastically. It is therefore also called the (surface) storage modulus[149]. G''_S, the out-of-phase component divided by the strain, is termed the (surface) loss modulus[149] and is proportional to the energy dissipated as heat.

These two moduli may be combined vectorially in the complex plane into a complex surface modulus

$$G^*_S = G'_S + jG''_S. \tag{16}$$

Instead of the above cartesian form, G^*_S may be expressed in polar form as

$$G^*_S = \bar{G}_S \exp j\phi_S = \bar{G}_S(\cos\phi_S + j \sin\phi_S). \tag{16'}$$

$\bar{G}_S$, the absolute surface modulus, is the ratio of peak stress to peak strain. ϕ_S, the suface loss angle or dissipation factor, is the phase angle between stress and strain.

It follows from the rules of elementary complex algebra that

$$\phi_S = \tan^{-1} G''_S/G'_S \tag{17}$$

while

$$\bar{G}_S = (G'^2_S + G''^2_S)^{1/2} \tag{18}$$

$$\bar{G}_S = G'_S/\cos\phi_S = G''_S/\sin\phi_S \tag{18'}$$

The reciprocal of the complex surface modulus

$$J^*_S = G^{*-1}_S \tag{19}$$

is termed the complex surface compliance[149]. We have

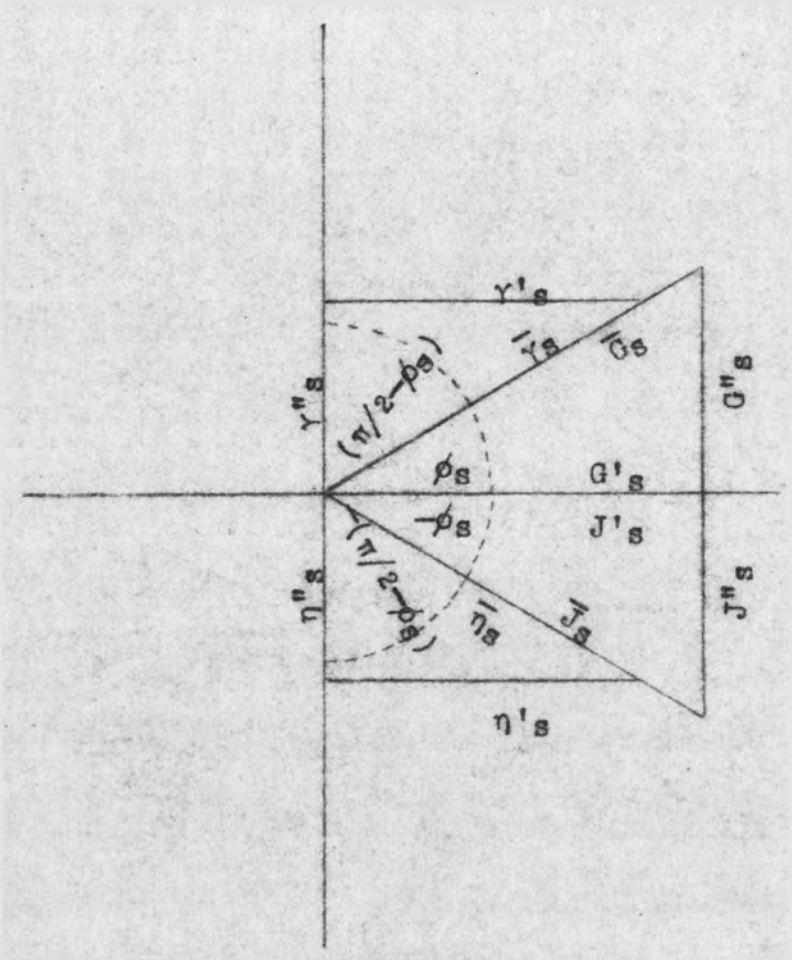
$$J^*_S = (G'_S + jG''_S)^{-1} = \bar{G}_S^{-2}(G'_S - jG''_S) = J'_S - jJ''_S \tag{20}$$

$$J^*_S = \bar{G}_S^{-1} \exp -j\phi_S . \tag{20'}$$

The complex surface modulus G^*_S connects the sinusoidal stress s(w) with the sinusoidal strain e(w)

$$s(w) = G^*_S e(w). \tag{21}$$

Fig.102a. Representation of Complex Surface Modulus, Compliance, Viscosity and Fluidity in the Complex Plane.


γ's
γ̄s
Ḡs
γ"s
G"s
(π/2−φs)
φs
G's
−φs
J's
η"s
(π/2−φs)
η̄s
J̄s
J"s
η's

Similarly, the complex surface viscosity η^*_s is defined as the proportionality constant between the sinusoidal stress $s(w)$ and the sinusoidal rate of strain $\dot{e}(w)$

$$s(w) = \eta^*_s \dot{e}(w) . \tag{22}$$

Since

$$e(w) = e_{max} \exp(jwt + \phi_s) \tag{23}$$

$$\dot{e}(W) = jwe_{max} \exp(jwt + \phi_s) \tag{24}$$

$$\dot{e}(w) = jwe(w) . \tag{25}$$

It is seen that the rate of strain is 90° out of phase with the strain itself.

It follows that

$$G^*_s e(w) = \eta^*_s \dot{e}(w) = jw\eta^*_s e(w) \tag{26}$$

and

$$G^*_s = jw\eta^*_s . \tag{27}$$

From this we find

$$\eta^*_s = w^{-1}(G''_s - jG'_s) = \eta'_s - j\eta''_s \tag{28}$$

$$\eta^*_s = w^{-1}\bar{G}_s \exp -j(\pi/2 - \phi_s) . \tag{28'}$$

The complex surface fluidity γ^*_s follows from

$$\gamma^*_s = \eta^{*-1}_s = jwG^{*-1}_s \tag{29}$$

as

$$\gamma^*_s = w\bar{G}_s^{-2}(G''_s + jG'_s) = \gamma'_s + j\gamma''_s \tag{30}$$

$$\gamma^*_s = w\bar{G}_s^{-1} \exp j(\pi/2 - \phi_s) \tag{30'}$$

Finally we find from Eq. (28)

$$G''_s = w\eta'_s = 2\pi\eta'_s/T . \tag{31}$$

Fig. 102a shows the representation of these various quantities in the complex plane.

All quantities measured in dynamical tests depend on the frequency at which the measurements are carried out. Consequently, G_S, G'_S, G''_S, etc., should properly be written $G_S(w)$, $G'_S(w)$, $G''_S(w)$, etc. In tests with damped oscillating surface torsion pendulum the

$$w^2 = (E_S + E_O)/I - a^2. \qquad (32)$$

The frequency therefore depends on the viscoelasticity of the monolayer and can only be changed by altering either the moment of inertia or the torsional constant of the pendulum.

The damped pendulum has the further drawback that it does not permit control of the applied stress. It is therefore not possible to obtain any but qualitative information on non-Newtonian behaviour.

The advantage of the damped pendulum, simplicity of construction and operation, is therefore obtained at the sacrifice of strict comparability of the measurements.

Application of forced instead of damped oscillations would allow control of both frequency and stress. Such an apparatus should be useful in the investigation of the frequency and stress dependence of monolayers. No such study has been reported so far.