Modification and characterisation by ESCA, of starch and related polymers

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MODIFICATION AND CHARACTERISATION BY ESCA, OF STARCH AND RELATED POLYMERS

A thesis submitted for the degree of Master of Science

by

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To Mum and Dad

Abstract.

Starch was isolated from soft wheat cultivar (Galahad), by wet fractionation. Electron Spectroscopy for Chemical Analysis (ESCA) was used to examine the surface composition of native and chlorine treated starches, before and after secondary treatments with Pronase enzyme and methanol, which remove respectively, lipid and protein from the starch granule surface. Analysis showed the presence of carbon, oxygen, nitrogen, sulphur and phosphorus in native starch, with a lower C/O elemental ratio than the theoretical value. This was attributed to the presence of lipid, protein and extraneous hydrocarbon, and confirmed by the analysis of native starch modified by the secondary treatments.

Chlorine treatment was found to induce surface oxidation of native starch samples. Covalently bound chlorine was detected which was thought to bind to the lipid and protein residues in preference to the carbohydrate component of starch. Results from chlorinations performed on ovalbumin, cellulose and nylon model polymers supported this postulate.

The effects upon native starch and ovalbumin of radio-frequency oxygen plasma, corona discharge and ozonolysis have been examined by ESCA. The oxygen plasma and corona discharge treatments achieved more extensive modifications than chlorine. Ozonolysis produced a similar level of oxidation to that of chlorine treatment, but at a much slower rate.

The effects of these novel surface treatments on the performance of starches in cake baking have not been examined.

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MEMORANDUM

The work of this thesis was carried out in the Chemistry Laboratories of the University of Durham between October 1988 and September 1989. This work has not been submitted for any other degree and is the original work of the author, except where acknowledged by reference.

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CHAPTER 1
INTRODUCTION.

1.1 Background to Project.

The work presented in this thesis was originally proposed by the Flour Milling and Baking Research Association (FMBRA), who deal with the research and development problems for an important sector of the food industry, namely milling and baking.

The problem concerns the industrial mass production of cakes and sponges in the U.K. in which a high-ratio cake formulation is used. High-ratio cakes differ from traditional recipes in that they contain higher levels of sugar and water in proportion to that of flour. This formulation is extremely versatile, gives greater volume with finer texture and offers good shelf-life characteristics. In order to obtain well-risen cakes, that do not collapse as they cool, it has been found that the flour requires a previous treatment with chlorine or heat[1]. Cakes made using untreated flour are characterised by a collapsed cellular structure and pudding-like texture. The production of a satisfactory structure relies upon a delicate balance between the flowing of aerated batter and the expansion of air bubbles during baking. This balance is disturbed by the increased sugar levels used in high-ratio cakes, but is restored by flour which has been modified by chlorination.

Chlorination of cake flours has been practised in this country and the U.S.A. for over 50 years[2,3]. However, the use of chlorinated flour is not permitted in most of the E.C. despite rigorous testing that has not revealed identifiable toxicological problems. At present in the U.K., the use of chlorine treated cake flours is allowed, but there is increasing pressure to find an alternative (more toxicologically acceptable), treatment with the approach of the Single European Market in 1992.

Much work has been done on the fate of chlorine in wheat flour[1,4], to determine the technologically significant target of the chlorine and the mechanism of the chlorine's action. Many studies[5,6] have implicated the starch fraction of wheat flour to be the main site of chlorine attack. However, an acceptable mechanism for the action of chlorine still remains to be elucidated. The FMBRA hoped, through this collaboration, that an elucidation of the action of chlorine on wheat flours would be achieved, and on the basis of this knowledge, that an alternative treatment might be found.

In order to understand the problem it is intended to present a detailed discussion of the structure of wheat starch, it's properties and function in baked products. However, before commencing this, brief consideration will be given to the other constituents of wheat flour, from which the starch is derived.

1.2 Wheat flour - it's constituents.

In Western European and North American markets, wheat flour is the major cereal component of baked products. It

consists of a mixture of carbohydrates, proteins, lipids, mineral salts, water and vitamins (see below).

1.2.1 Carbohydrates[7]

This constituent is quantitatively the most significant forming about 80% of the total dry matter of wheat grain (Table 1.1).

Table 1.1 The Approximate Composition of Wheat Grain[7]

Constituent	% Composition
Protein	15.0
Fat	2.1
carbohydrate	78.6
Crude fibre	2.4
Mineral matter	1.9

Those carbohydrates found in wheat flour include starch, cellulose, hemi-cellulose, pentosans, dextrins and sugars.

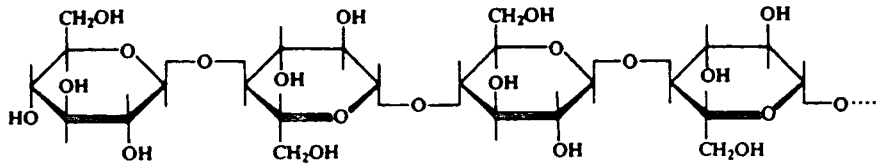
a) **Starch.**

See section 1.4 for a detailed description of starch.

b) Cellulose and Non-starch Polysaccharides

These substances are the main constituents of the cell wall of cereal grain and function as support tissue. The cellulose molecule is a polymer of β-(1,4)-linked D-glucose residues (1). The cellulose chain has a repeat distance of 10.3 Å and contains an approximate two-fold screw axis. At least four polymorphic forms of cellulose (derived by

chemical treatment of native cellulose), have been identified on the basis of their x-ray diffraction patterns[8].



(1)

'The non-starch polysaccharides are a heterogeneous group of polysaccharide types. The most important of these from a functional and nutritional point of view are the so-called pentosans, of which the arabinoxylans are the most important. Both the arabinoxylans and the quantitatively less important arabinogalactans occur as glycoprotein complexes'.

d) Sugars.

Wheat has a free sugar content of about 25%. The oligosaccharides common to all wheats are maltotriose, tetraose and pentaose, which produce glucose upon hydrolysis. Raffinose (0.07%) and melibiose (0.2%) have also been found in wheat flour.

Oligomeric substances called dextrins are also present in flour.

1.2.2 Proteins.[10]

The bulk of the proteins in wheat flour are derived from the endosperm of wheat caryopsis i.e. grain that has

been threshed to remove the chaff. These proteins are crucial in determining a flour's bread making quality, which varies between different wheats.

Wheat proteins have been classified traditionally according to their extractabilities in various solvents. Five fractions are produced when the sequential extraction scheme of Osborne[11] is used, which are shown in Table 1.2.

Table 1.2 Proteins in Wheat.

Type of protein	Approximate % of total protein	Extracted by
Albumin	2.5	Dilute salt solution
Globulin	5.0	Dilute salt solution
Proteose	2.5	Water
Prolamin	40-50	70% alcohol
Glutelin	40-50	Dilute acids/alkalis

Although research, using modern protein extraction techniques such as electrophoresis, over the last 30 years has shown this to be an over-simplified classification, it is still generally used in food science research.

The albumins and globulins in flour are commonly referred to as the soluble proteins. The albumin proteins are thought to be responsible for differences in baking characteristics among flours, and the globulins also may be essential for proper baking performance.

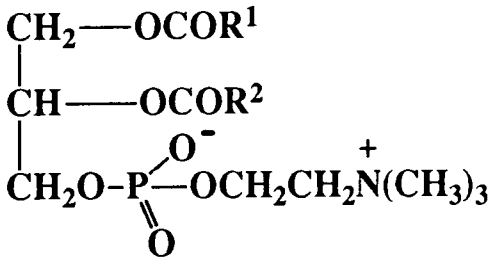
The most technologically significant proteins in bread making are the glutenins and gliadins, collectively known as

gluten, which account for 80-90% of the total protein in wheat flour. This substance, produced when a flour/water dough is washed with excess water, has unique viscoelastic properties, important in bread making, and which arise from interactions between the gliadin and glutenin components. The gliadin fraction is comprised of monomeric protein, and where present, disulphide bonds are intra-chain. The glutenin fraction, on the other hand, is comprised of polymeric proteins, with the component sub -units linked by inter-chain disulphide bonds[10,12]. It is differences in the glutenin fraction which are thought to account for the differences in baking quality between varieties. The detailed aspects of bread making are not of relevance to this work; and for this, readers are directed to reference 10.

1.2.3 Lipids.[7]

The lipid content of wheat is about 1-2%, the majority of this occurs in the germ (6-11%) with smaller proportions present in the bran (3-5%) and endosperm (0.8-1.5%). The lipids of cereals consist of the glycerides of fatty acids, and those common to wheat are given in Table 1.3.

'Phospholipids and glycolipids also occur in wheat flour, and these polar lipids, particularly the glycolipids, are thought to have an important effect on breadmaking quality'.



R^1 and R^2 are fatty acid residues (2)

Table 1.3 Composition of Fatty acids in Wheat.

Fatty Acids	Grain %	Germ %	Endosperm %
Saturated			
Myristic	0.1	-	-
Palmitic	24.5	18.5	18.0
Stearic	1.0	0.4	1.2
Unsaturated			
Palmitoleic	0.8	0.7	1.0
Oleic	11.5	17.3	19.4
Linoleic	56.3	57.0	56.2
Linolenic	3.7	5.2	3.1
Others	1.9	0.8	1.1

Once milled, the lipids in wheat are liable to deterioration due to their interaction with enzymes present in flour (originally remote from the lipid), such as lipases and lipoxides, giving rise to hydrolysis and oxidation respectively. Such processes promote the development of unpleasant flavours and odours.

1.3 Starch-it's structure and function in baked products.

1.3.1 Introduction.

Starch is the most predominant carbohydrate in all cereals. In wheat it constitutes about 57% of the entire wheat grain and about 70% of the endosperm[7,13].

It is laid down inside small vacoules called amyloplasts in the form of particles known as starch granules, by a process of "apposition". The size of these granules varies considerably, ranging from 2μm to 175μm in diameter, and depends on their source.

1.3.2 Isolation of starch.

On a laboratory scale a number of methods for the recovery of starch from wheat flour have been reported in the literature. A dry milling process, using screening and air classification has been used by some[14,15,16], whereby components are separated according to particle size. However, dry milling physically damages the starch and fractionation is not particularly efficient.

A wet fractionation technique, of which there are a number, is preferable. One method which has been employed[17], is that developed by Banks and Greenwood[18]. It consists of a wet milling stage whereby the wheat grains are steeped in an acetate buffer (pH 6.5) (0.01M in HgCl) for 30h, to soften the grain and inactivate the starch-damaging enzymes present. The softened grains are reduced to a porridge-like consistency in a mincer and recombined with water to form a slurry. This is repeatedly sieved, in succession, through 150μm and 75μm vibrational screens, followed by differential centrifugation and a shaking process with toluene/water, to produce a starch of low protein content.

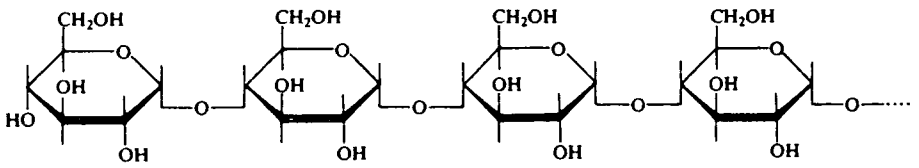
An alternative fractionation procedure frequently used is that described by Johnson[19]. Starch is washed out of flour using an excess of water and purified by a series of

centrifugations and re-suspensions in water. The material finally recovered is brought to equilibrium water content (13% water) in a current of air at ambient temperature.

Other procedures include a water/acetic acid fractionation employed by Sollars[20] and Seguchi[21], and the ultracentrifugation of a flour slurry in 50% w/w sucrose solution, used by Guy[22].

1.3.3 Composition and structure of granular starch.

The starch granule consists of two main components. Amylose, the minor component, is an essentially linear poly-α-(1,4)-D-glucose (3), with a DP in the range 200 to 1000. Amylopectin is also poly-α-(1,4)-D-glucose with branches via 1,6-linkages (4), and an average separation between the branch points of 20 units. Early light scattering studies[23] showed that the average molecular weight of amylopectin was of the order of 10^7 to 10^8, making it one of the largest natural polymers. These high values have subsequently been confirmed by Erlander and French[24].



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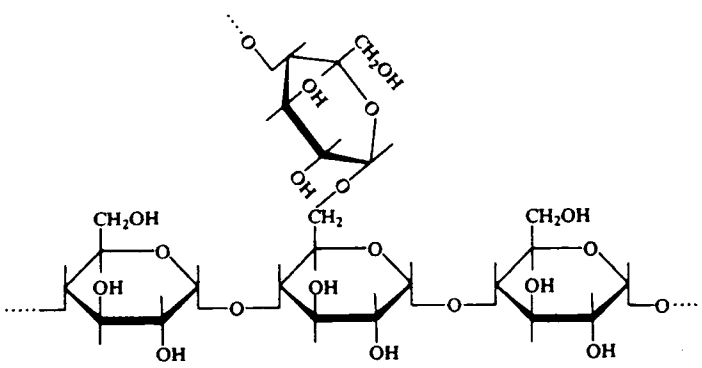


(4)

A variety of physical techniques have been used to explore the architecture (supermolecular structure) of starch. The classic work of Katz and co-workers[25] using wide angle x-ray diffraction (WAXS) showed that starch is a semi-crystalline material. They distinguished two principal types of polymer crystallinity in intact starch granules, by differences in the diffraction patterns. These were designated as A- and B- patterns. The structural type depends on the botanical source of the starch: the A-pattern is given by cereal starches, usually composed of lenticular granules; and the B-pattern, by starches of tuberous origin, characterised by spherical granules. A third type, the so-called C-pattern, has been observed, which is intermediate in form between the A- and B- types, and found in leguminous starches.

The amylopectin is responsible for the crystallinity[26,27], (absolute value of crystallinity of 25-30% using WAXS) and the remainder of the granule is an amorphous form of the amylose.

Various other physical techniques have provided additional structural information. Positive birefringence under cross-polars with a single wavelength retardation plate in the incident beam, suggests that the amylopectin chains are probably orientated with their long axis along the radius of the granules[27]. In addition, the use of both small-angle x-ray scattering (SAXS)[18] and small-angle neutron scattering (SANS)[28] show that the amylopectin chains exhibit regular periodicity of 9-10 nm.

An extension of the SANS technique, that of contrast matching, allows the masking out of one component so that a

second can be observed more clearly. This method has been used to mask out the major polysaccharide component of the starch granule, and has revealed the presence of lipid[13]. Starch granules are also known to contain small amounts of other non-carbohydrate species, namely proteins, phosphate and inorganic material.

Much of the information that has been described can be summarised in the hypothetical model for starch granule crystallites proposed by Blanschard[28] and shown in Figure 1.1.

Until recently, little was known of any technological importance about the non-carbohydrate species associated with the starch granule, in particular the protein.

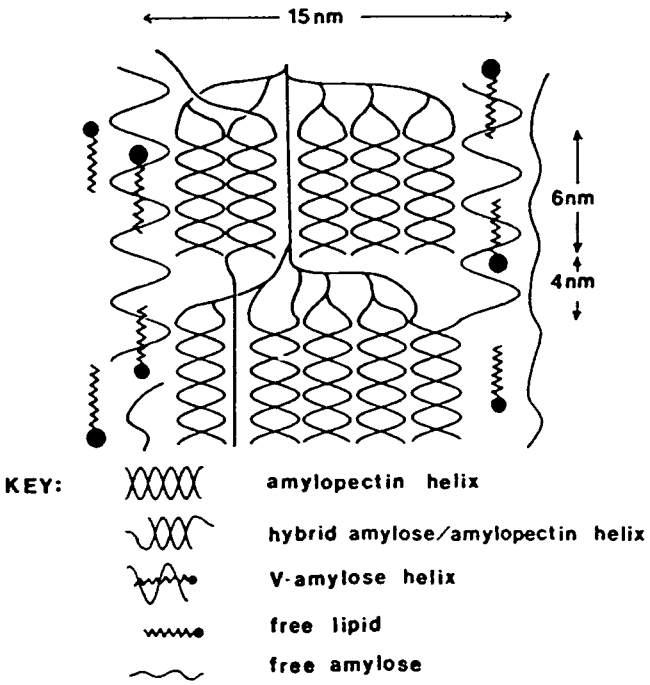


Figure 1.1 A hypothetical model of crystallites in the starch granule

Purified wheat starch granules contain a series of proteins that are quite distinct from the bulk of flour proteins, and are usually referred to as "starch granule proteins" (SGP). SGP comprise about 1% of the total flour proteins and about 0.2% of the total starch granule mass (Fig. 1.2)[29]. Most of the SGP (about 10%) is desorbed readily with dilute NaCl solution[30]. Due to the ease of extraction this group of proteins was assumed to be associated with the starch granule surface. Other proteins are also associated with starch granules, but they require more vigorous conditions for their extraction and are

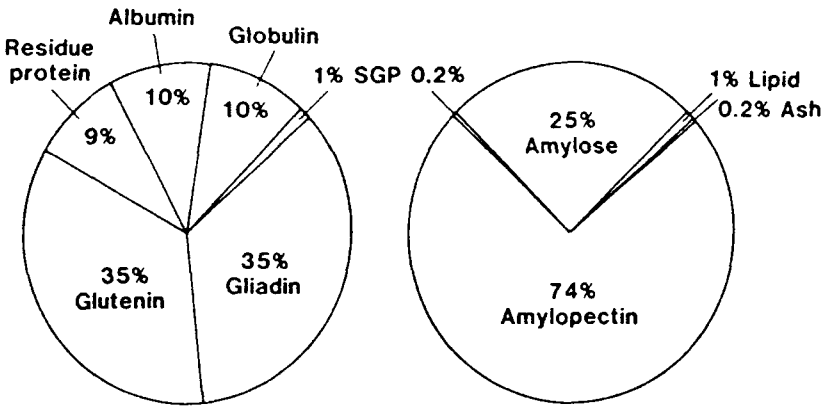


Figure 1.2 Pie charts showing the approximate proportion of starch granule protein in relation to total flour proteins (left) or total starch granule components (right).

thought to be integral granule components[30,31]. Characterisation of starch granule proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate,

has shown that the surface and integral proteins each comprise a mixture of 5 different polypeptides. Those proteins associated with the surface range in molecular mass from 5,000-30,000 and those with bulk have masses in the range 59,000-149,000. Despite being such a minor component of the starch granule, the SGP are believed to be of considerable importance in baking technology. There is circumstantial evidence that one or more of these starch granule proteins may be involved in the improving effects of chlorine on cake flours[1].

1.3.4 Function of starch in baked products.

The wheat flour used for cake baking has certain specifications which are determined by the wheat variety. Wheat varieties differ in their endosperm texture and protein content and are classified accordingly as being hard or soft and strong or weak[7]. Hardness and softness are milling characteristics relating to the way the endosperm breaks down. Strong and weak wheats are characterised by high and low protein contents respectively, and determine a flour's baking properties. The production of high-ratio cakes uses so called high-ratio flour, a medium strength, soft flour (finest particle size fraction) which is treated with chlorine, typically 3oz and 5oz per sack (280lb) for low and high protein flours respectively[7].

The purpose of flour in cakes is to allow an aerated structure to be retained after the cake has been prepared and baked. The process of baking relies upon the gelatinisation of intact granular starch (found in flour

milled from soft wheat). This is a phenomenon which occurs when starch, almost insoluble in cold water, absorbs water, swells and may eventually disperse. Amylose is released from the swollen granules[32] and this combines with the quantity of gluten present, pentosans, lipids and other proteins, forming a semi-continuous filamentous network. Entangled in this network are the gelatinised starch granules which progressively firm with "amylose-exudation". This behaviour is due to increasing crystillinity which developes with the accumulation of the residual amylopectin component[13]. It is this solid matrix or filled network of material that provides support to the incorporated air cells and hence to the baked cake structure.

1.4 The Chlorination process.

1.4.1 Introduction.

The effectiveness of chlorine treatment of flour as a method of avoiding structural collapse in high-ratio cakes was first observed by Montzheimer in 1931[2]. He reported that chlorine-bleached flours produced cakes of a finer, more even crumb texture and increased volume. These observations were confirmed by Smith[3]. Since then, the chlorination process has been widely adopted throughout the U.K. and the U.S.A..

The normal commercial range of chlorine treatment for a cake flour is between 5 and 10 oz/sack (280lb) or 1100 to 2300 ppm[1]. Industrially, a continuous process is used,

achieved by agitating the flour in a stream of gas. The reduction in pH of flour observed[33], affords a convenient means of monitoring the addition of gas. Adequate treatment of most flours is found when the treated flour exhibits a pH between 4.5 and 5.2 units in a slurry with water. The exact volume of chlorine required to achieve this varies with the sample, and is particularly dependent upon the protein content[5].

On a laboratory scale, chlorination is carried out by the procedure adopted by Cauvain and Gough[17]. Measured volumes of chlorine are added to a partially evacuated flask containing small aliquots of flour, which is then agitated to ensure thorough mixing. At normal levels of treatment the gas is absorbed very rapidly, with all traces disappearing within minutes of it's addition to the flour.

1.4.2 Mechanism of Chlorination.

The chlorination treatment has been of considerable academic as well as technological interest, and there have been many investigations. Studies have included the distribution of chlorine amongst the major flour components; the chemical reactions involved and the indentification of the factor(s) which influence(s) the baking performance. Much of the published literature has been reviewed by Gough et al[1] and Varriano-Marston[4].

Investigations of the chemical reactions occuring during the chlorination process are hampered by the complexity of the chemical nature of the flour. The flour components, i.e. the starch, protein, lipids and other minor constituents, vary in their nature and properties with

variety, growing conditions, milling conditions and age. In addition, chlorine can interact with all of these constituents. Early workers made assumptions regarding the components which received the chlorine preferentially. James and Huber[33] assumed the main recipients to be the gluten and starch, although Hanson[34] suggested that the unsaturated fatty acids were favoured.

More recently, the distribution of the chlorine among the major flour components has been determined by the fractionation of treated samples followed by elemental analysis[20,35]. There was some variation in results, but it was agreed that the starch (the major component) received very little chlorine. The approach used, that of wet fractionation, was the focus of much criticism because of the risk of chlorine redistribution during fractionation. This risk was minimized by Chamberlain[14] who used the method of air classification to fractionate, and confirmed that the starch component received only 15-20% of the chlorine. However, the flour fractionation and reconstitution studies of Sollars[5,36,37], Sollars and Rubenthaler[38] and Johnson and Hoseney[6], indicate that the starch fraction is responsible for the improved properties of chlorinated flours.

As regards the nature of the chemical reactions involved during chlorination. Whistler and co-workers[38-41] reported that the major effect upon semi-dry wheat starch was an oxidative de-polymerisation (fragmentation) induced by cleavage of glycosidic bonds, following the attack of chlorine at the glycosidic oxygen atom (5) (Fig. 1.3).

The mechanism they proposed occurs via nucleophilic displacement of the chloro-oxonium complex (6) by hydroxyl anions, either from the water giving normal sugar end units (8), or with the hydroxyl group at the C6 position, generating a 1,6-anhydro ring (7).

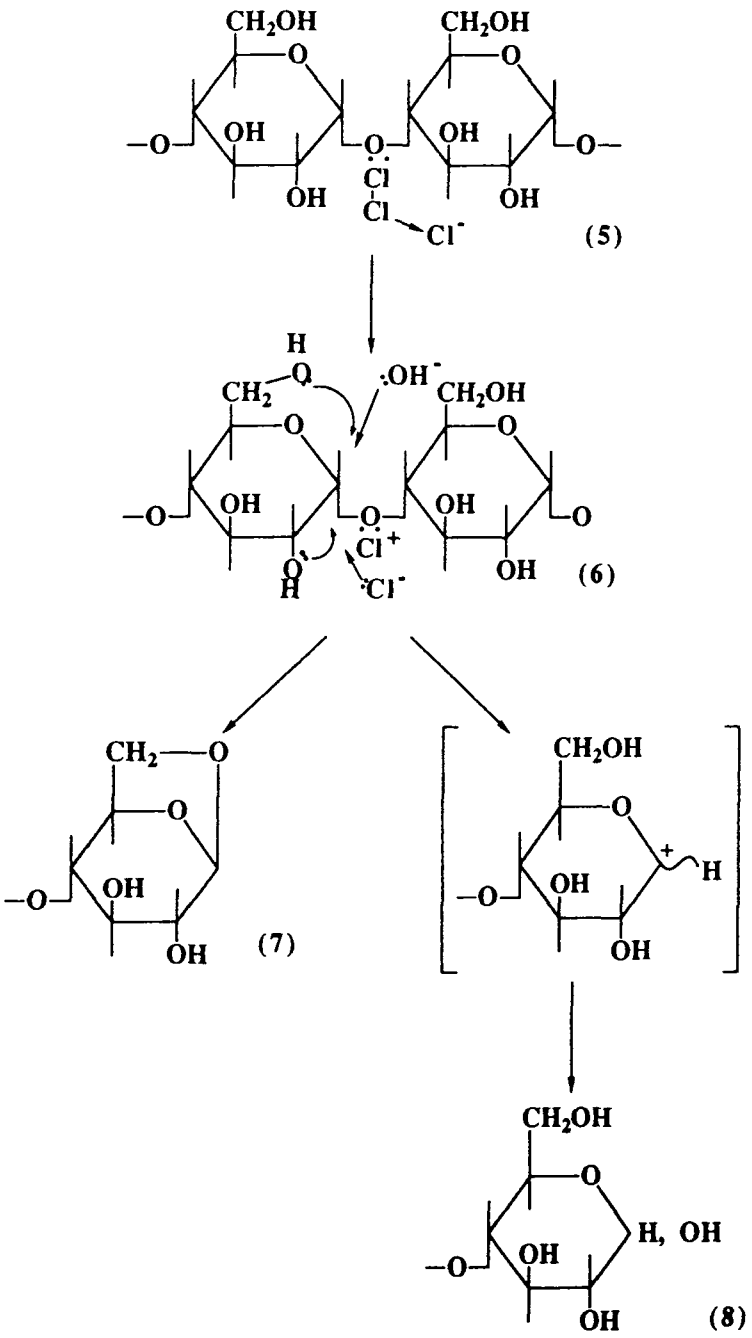


Figure 1.3 Oxidative de-polymerisation of a starch chain.

The proposed mechanism of oxidative depolymerisation is supported by the studies of Kulp et al[42] and Huang et al[43]. Both groups found that chlorinated starch imbibes more free water, swells to form pastes which increase in viscosity at a faster rate than for unchlorinated starch, observations which are consistent with a fragmented and hence more open structured starch granule. Telloke[44] observed a similar increase in paste viscosity with chlorination during a study of chlorine's influence on starch gelatinisation behaviour. An increase in amylose exudation was also found and this was considered, via an increase in gel strength, to be largely responsible for the prevention of high-ratio cake collapse. Thus, from the evidence that now exists, it appears that oxidation and/or oxidative de-polymerisation of starch granules, promoting increased amylose exudation[41], is at least partly responsible for the improvement in the baking properties of high-ratio cake flour.

However, alternative hypotheses exist, which implicate the involvement of the minor components associated with the starch granule surface, mainly lipid and protein, [31] in the improved baking performance of chlorinated flour and starches. Clements and Donelson[45] reported that the improving effect of chlorination on cake expansion observed in the baking tests using reconstituted flour, was brought about by modification of the lipid fraction. During work aimed at developing a "starch cake" as a model system for studying the response to chlorine, it was found that the cake baking properties of starch were diminished if it was purified by shaking with toluene and water, prior to baking[17]. The toluene extraction removed the lipid and

protein trace components associated with the starch granule surface. Seguchi and co-workers[46,47] observed an increase in the hydrophobicity of chlorine-treated starch, as evidenced by it's ability to bind more oil than untreated starch. Seguchi postulated that this was due to a modification of the protein on the surface of starch, which improved the interaction between the starch granules and other flour components e.g. protein. Such an interaction would encourage a greater transfer of water to the starch from these other components, and hence promote earlier gelatinisation. The effect has been shown to be reversed by treatments designed to remove protein from the granule surfaces (e.g proteinase or alkali treatment)[48]. Other workers[1,49] have correlated chlorine induced modifications of the starch granule protein (SGP) with the improved flour performance. Research performed at the FMBRA has shown that chlorine treatment causes SGP degradation (P. Greenwell, unpublished results). Similar effects upon SGP can also be achieved by heat treatment[50-52].

As long ago as 1934 Mangels[53] showed that dry treatment (i.e. heating) of starch increased it's rate of swelling in dilute sodium hydroxide solution. In 1968 a British Patent was published describing the work of Russo and Doe[54] for an alternative flour treatment, as a replacement for chlorination. However, this treatment has not been widely adopted owing to a tendency for "off-flavours" (i.e. bad tastes) developing in the flour.

The review presented here indicates that chlorine affects all the components of flour. However, it would appear that it is the reaction of chlorine with the starch

fraction, and in particular the minor components associated with the granule surface, is responsible for the improved baking characteristics of wheat flour.

The work performed and reported in this thesis, has concentrated upon determining the underlying surface chemistry of starch granules and how this might be affected by the chlorination process. In addition, using the premise that one effect of chlorine upon starch is oxidation, a variety of novel dry oxidative treatments (radio frequency and corona discharges, and ozonolysis) have been investigated, in an attempt to discover an alternative treatment. The effects of some of these upon the surface structures of: native starch; it's analogue cellulose; starch exposed to a number of surface treatments and various model systems including ovalbumin, poly(phenylene sulphide) and poly(ether sulphone) have been examined using the technique of Electron Spectroscopy for Chemical Analysis (ECSA), otherwise known as X-Ray Photoelectron Spectroscopy (XPS). Apart from some work using microanalysis[42] and the measurement of zeta potentials, no other surface analysis techniques have been applied to starch.

In the field of cereal science ESCA has not been widely used. It has been employed to investigate the protein content of grain meals[55], and is briefly mentioned in the review by Varriano-Marston[4]. However, a preliminary collaboration between the FMBRA and Durham University employed ESCA and showed that protein and phospholipid can be detected at the starch granule surface[56]. The removal of these species altered the cake baking quality (P.L Russel, unpublished results).

1.5 ESCA - General Description of the technique.

Electron Spectroscopy for Chemical Analysis (ESCA) is based on the photoelectric effect and provides information on chemical composition of surfaces.

The bombardment of matter by x-ray photons with energies in the region of 10^3 ev, causes electrons to be emitted, by a process called photoionisation. If the sample is irradiated with monochromatic radiation (fixed energy photons) of frequency ν, the energetics of the process are defined by the Einstein relation:

$$h\nu = \text{K.E.} + \text{B.E.}$$

where K.E. is the kinetic energy of the emitted photoelectron

$h\nu$ is the energy of the photon

B.E. is the binding energy of the emitted photoelectron.

The ESCA experiment consists of measuring the numbers of photo-electrons emitted at specific kinetic energies; $h\nu$ is known and hence B.E. can be calculated, yielding the energy with which the electron was held in it's atomic or molecular environment. For photoemission from solids, a work function φ must be added to this equation. This term expresses the additional energy required after the ionization process, to get the electron away from the surface.

For each atomic core level, each element has a characteristic binding energy, so by comparing the number of electrons observed at various binding energies, a quantitative elemental analysis of the surface can be obtained.

This can be achieved for all elements except hydrogen, since this does not possess any core level electrons.

The net electronic charge on an atom in a given environment, has an influence on the precise energies of the core levels. Hence, the presence of charged species and electron withdrawing or donating substituents on an atom, alters the binding energy of the core levels by an amount referred to as the chemical shift. This enables functional group analysis to be performed, particularly by examination of the C_{1s} core level, although substituent groups that are not electron withdrawing may not be detected (e.g. C_{1s} signals from all hydrocarbon groups occur at the same binding energy).

A schematic diagram of an ESCA instrument is shown in Figure 1.4 . ESCA analysis is performed in an ultra-high vacuum environment, typically less than 10^{-8} Torr. A high vacuum is essential to avoid the electrons undergoing inelastic collisions before being detected.

A sample is exposed to a low energy mono-chromatic x-ray source, the most common being the Al $K_{\alpha\ 1,2}$ ($h\nu$ = 1487 ev) and Mg $K_{\alpha\ 1,2}$ ($h\nu$ = 1253 ev) lines, emitted by aluminium or magnesium when bombarded with high energy electrons. (The designation $K_{\alpha\ 1,2}$ indicates the emission process 2p -> 1s, subsequent to creation of a 1s or K hole by electron impact.) The photo-electrons emitted from the core and valence orbitals of the elements present within the sampling depth are detected and counted according to the energy they possess.

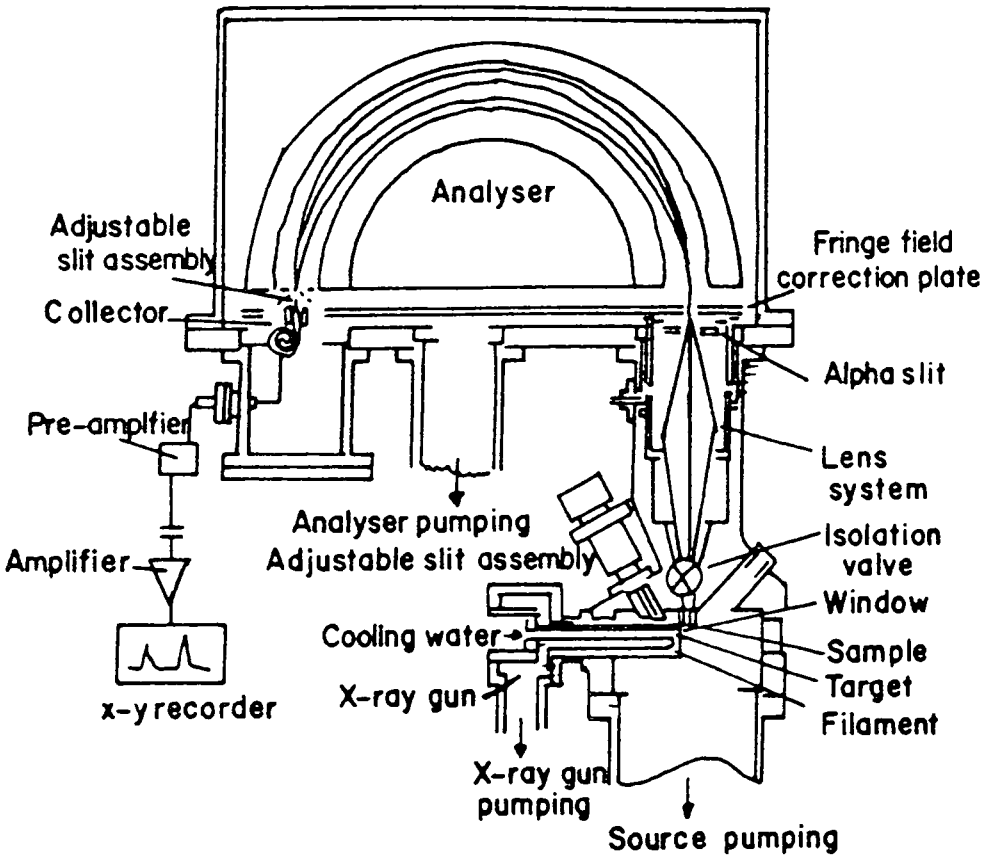


Figure 1.4 Schematic diagram of the components of an ESCA spectrometer.

The spectrum consists of a plot of the number of electrons N(e) with energy E, as a function of this energy. The area under each peak is a measure of the relative amount of each element present, while the shape and position of the peaks reflect the chemical environment of each element.

ESCA is a surface sensitive technique because only those electrons that leave the surface without energy loss,

will contribute to a spectral feature characteristic of an element. Those electrons originating from far below the surface (100 Å) have a high probability of suffering energy loss through inelastic collisions, and so either fail to escape, or do so, contributing to the background and the noise.

In principal, a depth profile of elemental composition in a material up to depths of about 100 Å can be achieved. This is done by a combination of analysing spectra recorded for different take-off angles for the sample relative to the detector, and also by using different energy x-ray sources with the sample position unchanged. The relationship between the depth of analysis and the take-off angle is given below:

$$d = 3\lambda \cos\theta$$

where d is the depth of analysis

λ is the mean free path of the electron

θ is the take-off angle between the sample normal and the detector.

The mean free path of an electron is proportional to $K.E.^{1/2}$. By using x-rays of differing energies e.g. Mg, Al, Ti, the photo-electrons ejected from a given level will have differing energies and thus differing mean free paths; hence signals from varying depths can be detected.

The specific types of information that can be obtained from this surface analysis technique are listed in Table 1.4. There are several books[57,58] available, which provide an introduction to photo-electron spectroscopy, it's theory, instrumentation and applications.

Table 1.4[58] Information derived from an ESCA experiment.

All elements present (except H)
Approximate surface concentration of elements
Partial functional group analysis
"Fingerprinting" materials using valence band spectra
Information on unsaturated and aromatic structures from shake-up ($\pi^* \rightarrow \pi$) transitions
Information on surface electrical properties
Non destructive depth profiling using i) angular depend ence ESCA and ii) photo-electrons of differing escape depth

1.6 Objectives of this project.

i) To elucidate the overall chemical and physical consequences of chlorine treatment on starch granules and flour varieties of known milling and baking quality. The proposed method of tackling these problems involves the surface characterisation technique of ESCA of the material before and after the treatment.

ii) To investigate the effects of novel dry treatments e.g. corona, microwave or radio-frequency electric discharge and ozonolysis, on the surface of starch granules.

iii) To compare the effects of these dry treatments with those achieved by chlorination treatments.

iv) To simulate the desired technological qualities using such novel dry treatments.

CHAPTER 2
OXIDATIVE SURFACE TREATMENTS.

2.1 Radio-Frequency Plasma Discharge.

2.1.1 Definition of a Plasma.

Classically the term plasma is defined as a partially ionised gas consisting of a complex mixture of highly reactive species. Two types of plasma exist. A "low temperature" plasma in which the free electrons are at a higher temperature than the rest of the gas, and a "hot" plasma, in which the free electrons are in thermal equilibrium with the gas.

2.1.2 Reactive species in a Plasma.

Generation of an ionised gas can be achieved by using microwave radiation, heat or electrical discharge. For the purpose of this study a radio-frequency inductively coupled, electrical discharge has been employed.

A plasma, excited by an electrical discharge in a gas at low pressure, contains a variety of species arising from collisions between electrons accelerated by the electric field and molecules in the gas phase. Many of the species produced by these collisions are energetic enough to cause chemical reactions.

A typical plasma may contain electrons, ions, metastables, neutral molecules and free radicals, in ground and excited states, and photons of various energies. Typical energies of these species are given in Table 2.1 together with some typical bond energies for organic systems[59].

Table 2.1 Energies of species present in a plasma and some typical bond energies.

Plasma	Energies (ev)
Electron	0-20
Ions	0-2
Metastables	0-20
UV/Vis radiation	3-40
Bond Energies (ev)	
C-H 4.3	C-C 3.4
C-N 2.9	C=C 6.1
C=O 8.0	C≡C 8.4

2.1.3 Applications.

The use of plasmas formed by radio-frequency electrical discharges in gases at low pressures, is an important industrial technique for the formation and treatment of polymer surface coatings. The process has been used for surface treatment of small articles[60] , though some work has been done on fabrics[61,62] and also on the continuous treatment of mono-filaments and fibres[63] . The nature of the surface modification produced, depends on the type of gas used in the discharge and on the energy density of the plasma.

It has been known for many years that certain organic monomers polymerise in a plasma, e.g. acetylene, benzene and ethylene[64] , including some that do not polymerise by conventional molecular polymerisation e.g. methane. The process is known as plasma polymerisation, and is a unique

gas phase method of polymerisation. The mechanism of reaction is different from that of conventional molecular polymerisation. It is usually extremely complex in nature, and the polymer so formed does not retain the structure of the monomer. A material exposed to the plasma becomes coated in a thin, "pin-hole" free, highly cross-linked polymer film.

Plasmas involving non-polymer forming gases such as argon, oxygen, nitrogen and hydrogen can be used. They modify a surface by processes such as oxidation, with subsequent cross-linking, and ablation or chemical etching. The plasma discharge experiments for the work in this thesis were carried out using oxygen gas.

The extent of any surface modification by a plasma depends on a number of operational parameters. These include the power coupled to the plasma, the pressure within the reactor, the gas flow-rate, the temperature and the exposure time of a sample to the plasma.

The advantage the plasma treatment technique over other surface treatment techniques,is that it enables the surface properties of a material to be selectively modified, without interfering with the bulk properties.

2.2 Corona Discharge.

2.2.1 General Aspects.

The type of discharge in a gas between two electrodes is determined by their potential difference, the gas pressure and the current density that is obtained.

Three basic types of electrical discharge may be distinguished: silent, glow and arc discharge. The conditions required to obtain each particular type are shown in Figure 2.1

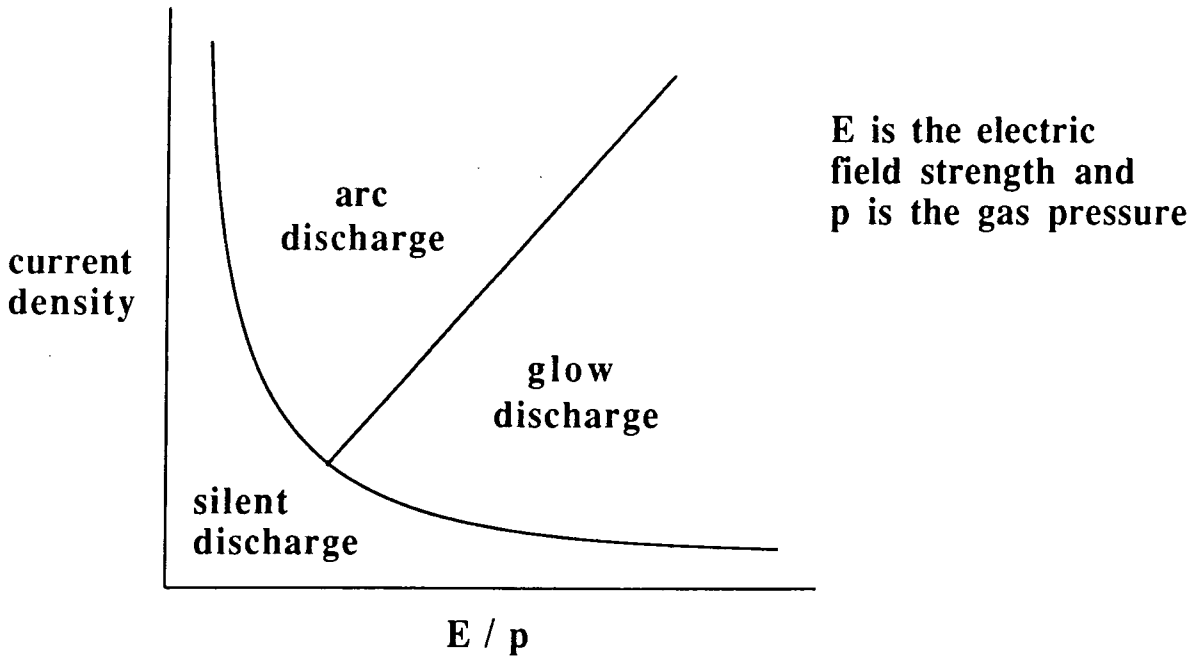


Figure 2.1 Basic Types of Electric Discharge.

At atmospheric pressure as the voltage between two electrodes is increased a silent discharge is initially observed. Conduction arises due to the ionisation of the gas by background cosmic rays. A further increase in voltage generates a particular type of silent discharge called a corona discharge.

2.2.2 Applications.

The commercial treatment of polymer surfaces by corona discharges to modify their structure and composition has been used for over three decades[65,66]. In particular, it has been widely used for the treatment of thin film material, such as that used in the packaging industry.

Treatment may be carried out by either a dynamic process or a static process and these are illustrated schematically in Figure 2.2[67].

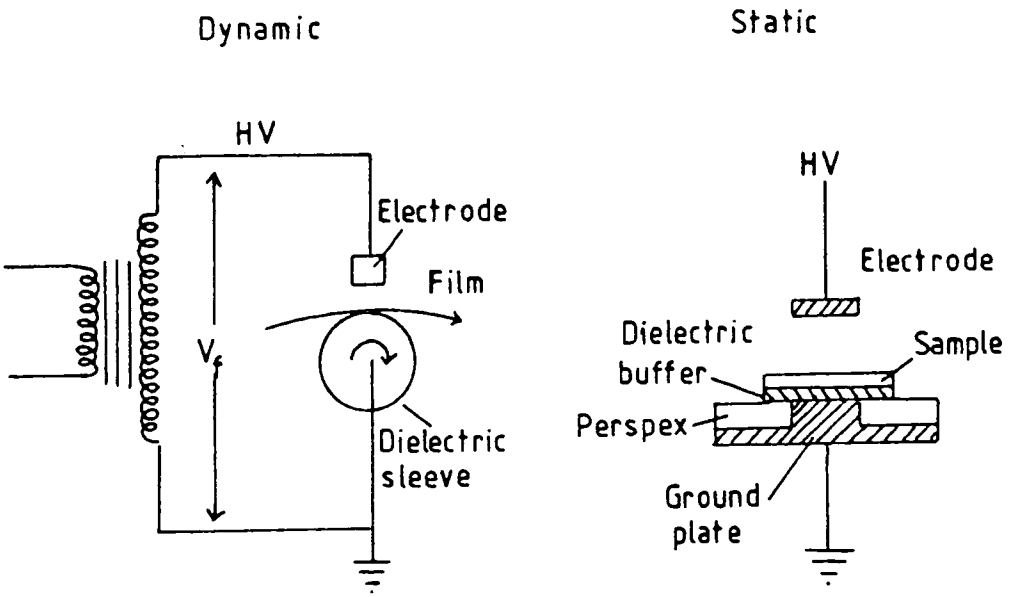


Figure 2.2 Corona Discharge Apparatus

The static process is normally used for small scale investigations and is the method employed for the work presented in this thesis (see below). Industrially where larger scale continuous treatment of polymer film is

required, the dynamic process is used.

The basic principles of operation are the same in both cases. The film to be treated is in contact with a buffer dielectric which is fixed to one electrode, and this is separated from a second electrode, normally of bare metal, by a gap of between 1 and 3 millimetres. The function of the buffer dielectric is primarily to prevent "burn-through" arising from pin-holes or weak patches in the film. The upper electrode is usually a bar or knife edge arranged parallel to a lower roll, although a curved metal "shoe" electrode (see Fig. 2.3) is sometimes used.

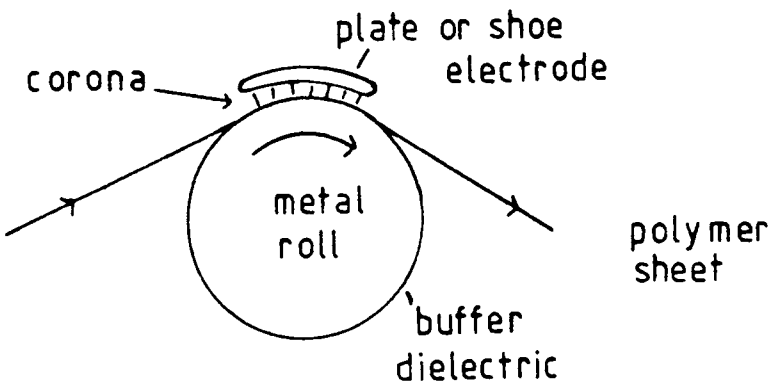


Figure 2.3 Metal Shoe Electrode Arrangement.

The shoe electrode arrangement enables films to be exposed to longer treatments than other designs, due to the larger area covered by the corona. In the static process the upper electrode is usually a flat plate, which has to be parallel to the lower electrode to avoid discharge occuring across the narrowest gap.

Corona treatment has many advantages over chemical and physical treatments. The reaction is inherently "clean" and does not involve any hazardous chemicals, apart from ozone generated by the discharge. In addition, considerable changes in the surface energy of a material can be achieved, whilst the bulk properties remain unchanged.

2.2.3 Ions and Molecules formed in a Corona.

Commercially, treatments of polymer surfaces usually use air, containing variable amounts of water vapour, at atmospheric pressure. Under such conditions, a complex series of reactions may occur in the gas phase, leading to the formation of various ions, including hydrated species.

Air has been used in the work of this thesis and the principle species to be expected in this discharge are O_2^+, O_4^+, $O^+ (H_2O)_n$, NO^+, and NO[68]. In addition various other activated molecules and ions may be formed as precursors to these species. They include singlet oxygen, O_2^-, atomic oxygen and atomic nitrogen.

In view of the complex nature of the discharge, workers employing the process have not been able to identify the components responsible for the surface modifications obtained. The thickness of the modified layer is usually of the order of 10^{-8}m, and as a consequence, cannot be examined by conventional analytical techniques such as infra red and ultra-violet spectroscopy. However analysis can be achieved by using ESCA.

2.3 Ozonolysis.

2.3.1 Introduction.

The use of ozonolysis for the modification of the surface properties of a material, particularly polymers, has been the subject of a number of investigations. Ozonolysis of polyethylene[69-72] , nylon 66[74] and polystyrene[71,75-77] , have been studied. It has been shown that the reaction between ozone and solid polymers takes place at the polymer surface[71,75] . In view of this, the technique of ESCA has often been used to monitor the changes which occur[73,74,77] .

Ozone is a much more powerful oxidant than oxygen and reacts with most substances at room temperature, with the exception of water, in which it is only slightly soluble[78] . Organic polymers undergo changes in both composition, by oxidation, and molecular weight by chain scission and cross-linking[79] .

2.3.2 Ozone Generation.

In general, ozone is formed whenever oxygen is subjected to high energy radiation or electrical discharge. The latter is most commonly employed and has been used in the work presented in this thesis. A simple form of the apparatus is shown in Figure 2.4 .

It consists of a double tube immersed in a dilute electrolyte ($CuSO_4$) with which the inner tube is also filled. Oxygen flows through the annular space, across which a high voltage is applied, generating ozone. The electrodes are continuously cooled by the weak electrolyte surrounding the annular space, thereby reducing ozone decomposition.

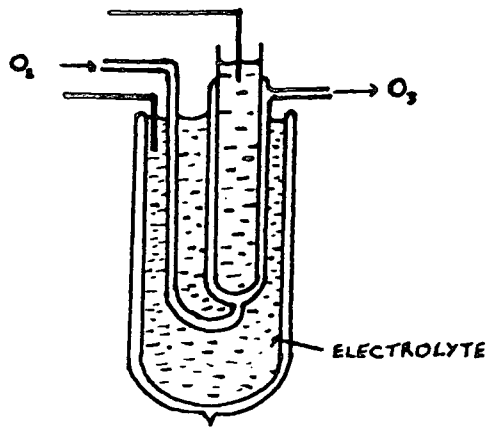


Figure 2.4

CHAPTER 3
EXPERIMENTAL.

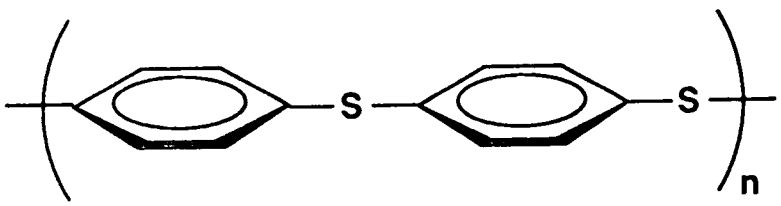
3.1 Materials employed.

Starch has been extracted from white flour, prepared from a type of grain called Galahad (1988 harvest). It is a soft milling wheat that produces a weak gluten flour, suitable for biscuit and cake-making, but not bread making. The milling was performed on a Buhler experimental mill (MLU-202) at the FMBRA.

Ovalbumin (Crude Grade II) was purchased from Sigma Chemicals and used as a model for the protein occuring in flour.

Cellulose film, prepared by the viscose process was supplied by BCL Cellophane and contained impurities of sodium (<10 ppm), chloride (<10 ppm) and sulphur (50 ppm).

Poly (acrylic acid) powder, nylon film, polyethylene film, poly (phenylene sulphide) (9) and poly (ether sulphone) (10) have been studied, to facilitate the unambiguous characterisation of untreated and chlorine treated starches, cellulose and ovalbumin.



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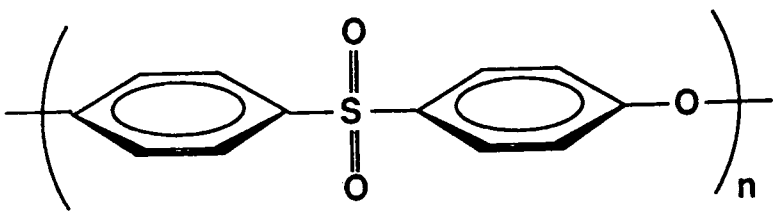


(10)

3.2 Sample preparations.

3.2.1 Starch.

Starch has been isolated from flour and samples of it treated, to selectively remove lipid or protein from the starch granule surface. The procedures used are outlined below.

a) Starch Isolation.

Starch was extracted from flour using a method based on that described by Johnson[6]. Flour was mixed with distilled water (215ml) in a Hobart mixer, initially at a slow speed (2 mins.) to start gluten development, and then at a medium speed (3 mins.) to work the gluten. Distilled water (650ml) was added and mixing continued at a slow speed (5 mins.). The resulting starch suspension was decanted and the gluten discarded. The suspension was then centrifuged (2000 *g*, 10 mins.) and the supernatant liquid discarded. The starch, located below a pale gelatinous "squeegy" layer (mainly pentosans and endosperm cell wall) was retrieved using a spatula. Resuspension in distilled water, centrifugation and scraping was repeated a further three times. The wet pellets of starch recovered, were crumbled and brought to equilibrium water content (≈ 13%) in a current of air at ambient temperature, and then lightly rolled using a wooden roll.

b) Starch treated with "Pronase".

"Pronase", the tradename for a proteinase enzyme i.e. one that digests protein, has been used to remove protein from the surface of starch granules. In addition to the removal of protein, lipid may also be removed due to the

presence of "lipo-protein" complexes which are thought to exist at the starch granule surface[4].

Portions of starch (25g) were digested with pronase (75mg) in a stirred 0.01M $(NH_4)_2CO_3$ (pH 7.85, 37^oC, 4h) according to the method reported by Greenwell and co-workers[80]. The centrifuged starch pellets were washed, recovered, air-dried and hand rolled as described in 3.2.1.

c) Starch treated with methanol.

Methanol washing of starch has been used to selectively remove lipid from starch granule surfaces. Starch (50g) was dispersed in methanol (GPR, 125ml) and shaken vigorously (1h, 22^oC) in a stoppered flask. The resultant slurry was centrifuged (300g, 10 mins.) and the supernatant decanted. The pellets remaining were resuspended in and centrifuged from methanol, air-dried and hand rolled as above.

3.2.2 Ovalbumin.

This was used both in it's received form, that of a powder, and also as thin films cast onto aluminium foil substrates. Prior to casting, the aluminium was thoroughly cleaned by exposing it to an intense oxygen plasma, typically established at 100 W power, 0.2 mbar pressure for approximately 30 minutes. During this cleaning procedure a solution of ovalbumin was prepared (2% w/v), by gradual addition of ovalbumin powder to distilled water with continuous stirring, to ensure complete solvation. The cleaned aluminium was dipped repeatedly into the ovalbumin solution for a period of 30 seconds; shaken to remove excess surface liquid and allowed to dry overnight ($\approx$ 18 h) in a

vacuum dessicator over phosphorus pentoxide.

3.2.3 Poly (phenylene sulphide) Powder.

Pre-treatment of the powder was not necessary.

3.2.4 Cellulose and model Polymer Films.

Samples were washed with methanol and allowed to dry before any surface modification or ESCA analysis was performed.

3.2.5 Sample Preparations for Plasma and Corona treatment.

The low pressure condition required for establishing a plasma presents problems of sample handling when treating powders. As a consequence, powders are often exposed to a plasma in a modified physical form that is representative of the sample. Thus, starch was treated as a disc, compressed using a KBr pellet press (maximum force of 15 tons), and ovalbumin as thin films supported by aluminium (see above).

To facilitate sample handling in the corona discharge treatments, discs of starch and thin films of ovalbumin were also used.

The procedures for improving sample handling also improved the ESCA analysis. The samples prepared had smoother and flatter surfaces which enhances the signal/noise ratio of spectra obtained by ESCA.

For the ozonolysis and chlorine treatments all samples were used in their native forms and as described in section 3.2.1.

3.3 Procedures used for oxidative surface treatments.

3.3.1 Oxygen Plasma.

Oxygen plasma treatments have been performed in a capacitatively coupled radio-frequency plasma asher (tradename Poloron, with an operating frequency of 13.56 MHz), connected to a grease-free vacuum system. Prior to treating a sample, the equipment was adjusted to the chosen treatment conditions according to the following procedure.

The reaction chamber was pumped to a base pressure of about 4×10^{-2} mbar using an "Alcatel" two stage rotary pump. The oxygen reaction gas was admitted and the system flushed out for approximately 1 minute, before adjusting to the required gas pressure of 0.2 mbar. The plasma was ignited and balanced at a power of 5 W by alteration of the power and tuning controls to maximise the percentage of power which is coupled to the plasma. Once the correct treatment conditions were obtained, the gas flow was stopped and the system let up to atmosphere. A sample was then introduced into the centre of the reaction chamber on a glass stage, and pumping down commenced as above. The gas was admitted at the pre-set flow rate and a 5 minute interval was allowed for equilibriation before the plasma was ignited. Samples (starch discs, films of cellulose, ovalbumin, poly (ether sulphone) and poly (phenylene sulphide powder) were plasma treated at fixed power (5W), pressure (0.2 mbar) and flow-rate (0.67 cm^3/ min.) for variable periods of time up to 1 minute.

3.3.2 Corona Discharge.

The corona reactor used is illustrated schematically in Figure 3.1. It essentially consists of two plane parallel aluminium discs, one attached to an insulated movable rod, and the other to a grounded aluminium plate.

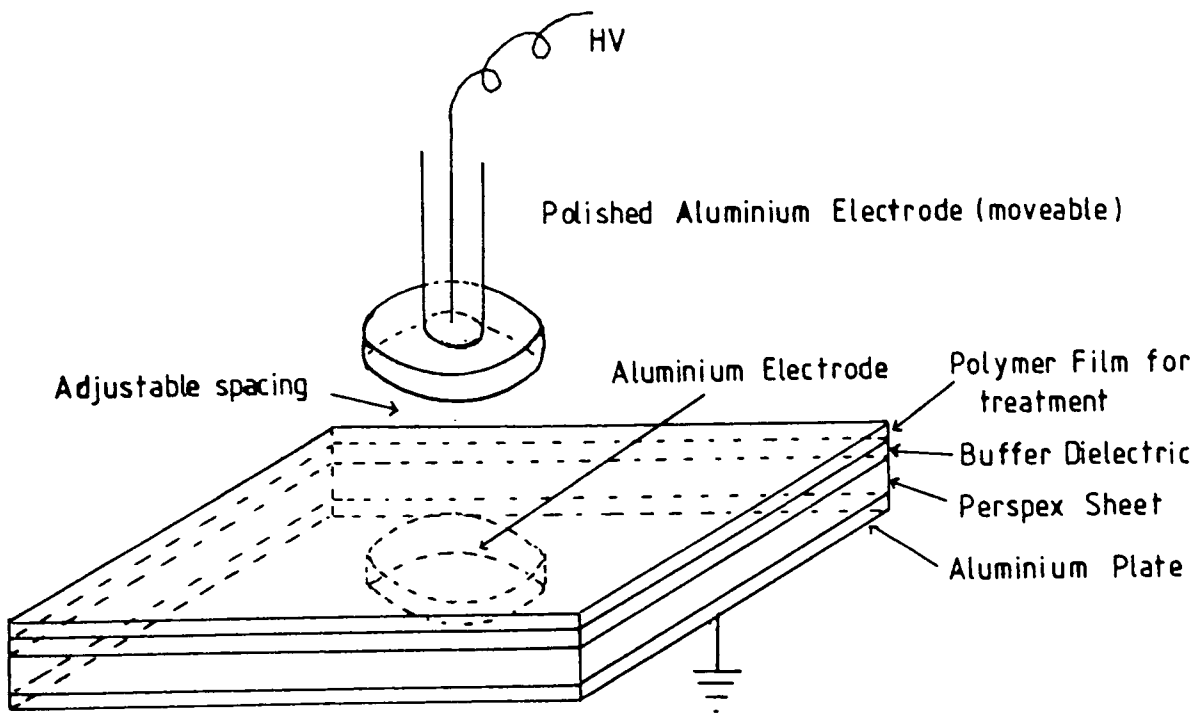


Figure 3.1 Schematic Diagram of a Corona Reactor using disc electrodes

A thick perspex sheet covers the fixed aluminium plate to prevent arc-ing, and a buffer dielectric is placed over this to prevent burn-through, via pinholes or weak points in the materials treated. The electrode separation can be varied by a rack and pinion system connected to the upper electrode, and this was adjusted and fixed at 2 mm using a set of feeler gauges. The reactor is enclosed in a perspex box, fitted with safety switches, and this was mounted on top of the power supply. A simplified diagram of the high voltage supply is shown in Figure 3.2.

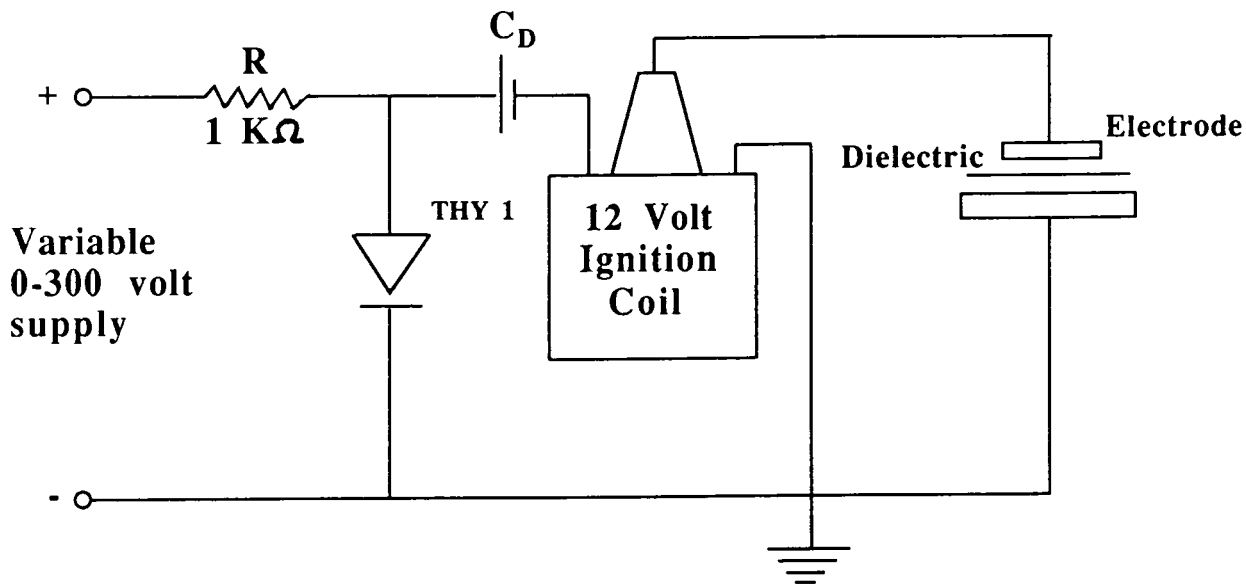


Figure 3.2 High voltage Power supply.

The condenser, C_D, is charged up to a pre-selected voltage from the rectified mains supply and the thyristor, THY1, is then switched on. This allows the condenser to discharge through the primary of an induction coil which produces a damped oscillation in the secondary, with a frequency of approximately 3 KHz. The output voltage that is applied

across the disc electrodes is controlled by altering the charging voltage supplied to C_D; for the work performed, the maximum charging voltage was used.

Samples of starch, ovalbumin and low density polyethylene were treated for variable periods of time, by placing them, in turn, on the dielectric buffer directly beneath the upper electrode. The length of exposure was controlled by a pre-set timing device within the high voltage supply unit.

3.3.3 Ozonolysis.

An instrument known as an ozonizer (Tower Ozone Apparatus GE-150) has been used. The apparatus (Fig. 3.3) consists of ten ozone units, each with it's own effective annular space, bridged in parallel across an inlet and outlet manifold. The units are suspended in a lead-lined wooden tank containing $CuSO_4$ solution (0.2% w/v). A ten multiple high voltage electrode completes the circuit. A transformer supplies the required 7500V across the electrode terminals.

The equipment generates ozonized oxygen consisting of 6-7% O_3. This was introduced into a 3-necked round bottom flask containing the stirred sample, thus ensuring an even distribution of the gas.

A preliminary investigation has been performed upon native starch and ovalbumin powders, exposing them to a maximum of 8h ozone treatment. Samples were taken at regular intervals for characterisation by ESCA.

Primarily, this initial study was aimed at obtaining a response to ozone treatment; hence detailed flow-rate and O_3 concentration measurements were not recorded.

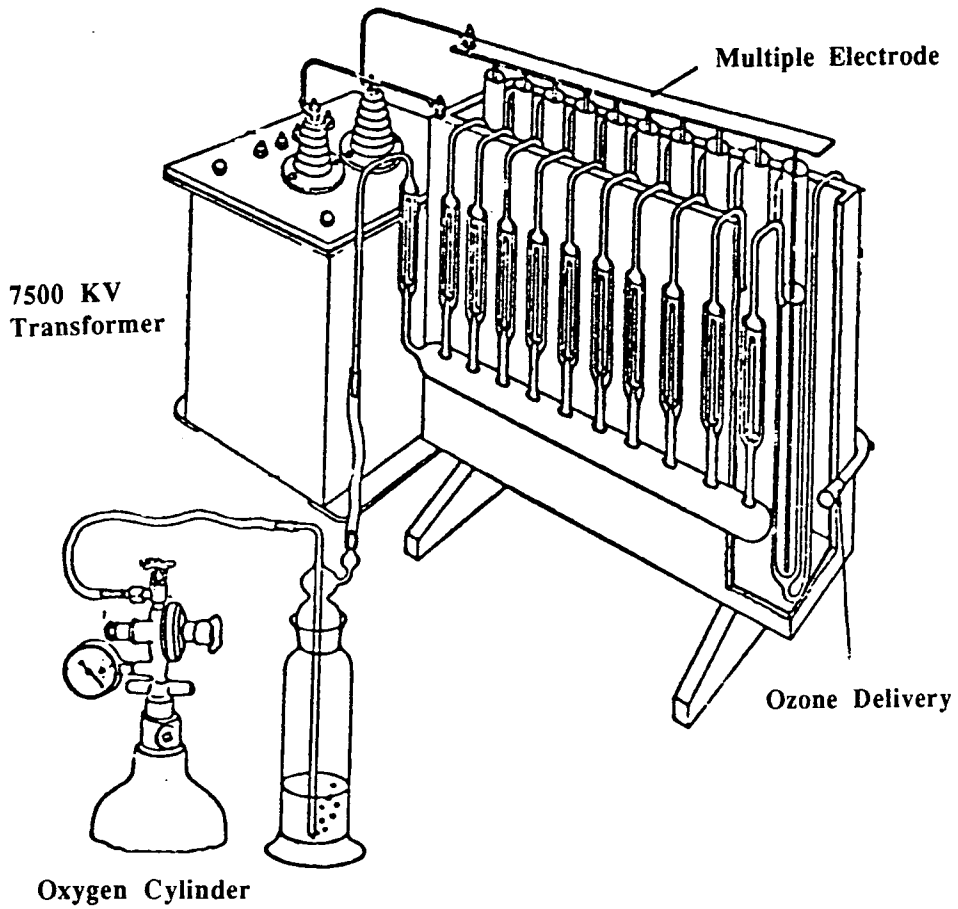


Figure 3.3 Diagram of a Towers Ozone Apparatus.

3.3.4 Chlorine treatments.

Chlorinations were performed at a treatment level of ≈ 200 ppm, using the apparatus illustrated in Figure 3.4. Chlorine gas was admitted into the gas burette and the required volume for treatment was obtained by adjusting the height of the reservoir. The measured volume of gas was introduced into a partially evacuated flask (achieved by a water pump) containing the sample, and retained in there by means of a stopper. The stoppered flask was shaken

intermittently over a period of 24h, to ensure thorough mixing. Chlorination was performed on samples of: native, methanol and pronase treated starches; ovalbumin; cellulose; nylon and poly(ether sulphone) films.

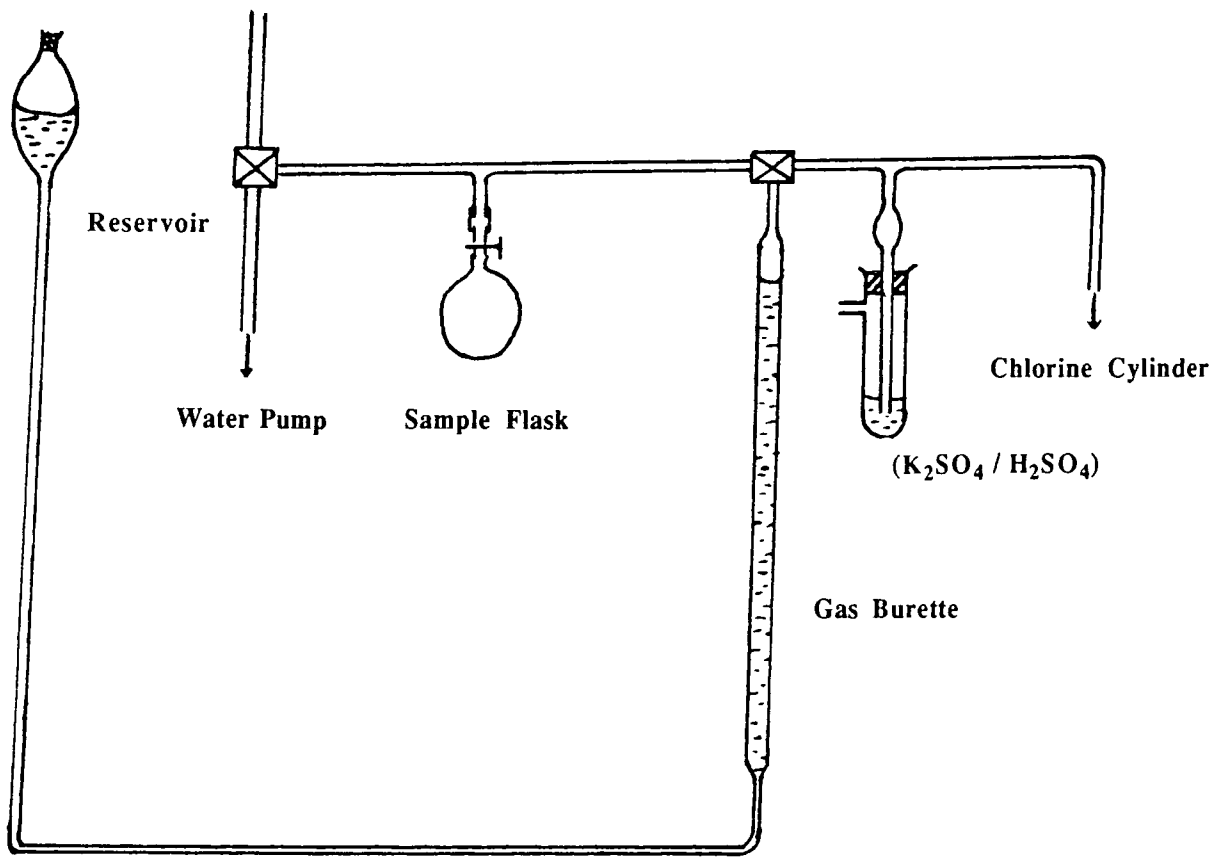


Figure 3.5 Experimental arrangement for chlorination.

3.4 ESCA Measurements.

ESCA spectra have been obtained on Kratos ES200 and ES300 spectrometers, both using a Mg $K_{\alpha\ 1,2}$ X-ray source ($h\nu$=1253 ev). Powder and film samples were mounted onto the sample probe tip using double-sided scotch tape, a material commonly used which is known not to cause contamination. Core binding energies have been measured relative to the C_{1s} peak, which arises from extraneous hydrocarbon, at 285 ev. Peak fitting of unresolved core energy levels and area measurements were achieved on the Kratos ES300 and ES200 data systems.

CHAPTER 4

DISCUSSION OF RESULTS AND CONCLUSIONS.

4.1 Introduction.

Primarily the investigation undertaken and presented in this thesis, has been concerned with the examination, by ESCA, of native starch and of starch exposed to a variety of wet and dry surface treatments. The chlorination study examined chlorine's interaction with the lipid and protein species that are located at the surface of starch granules. In addition, the effects of the dry oxidative treatments described in Chapter 2 on the native starch have been investigated. The results have been compared with the effects of the treatments on ovalbumin, and on some other model compounds.

This chapter presents a detailed account of the ESCA results from samples of starch and the related model compounds.

4.2 Starch Investigation.

4.2.1 Atomic Composition of the Granule Surface.

The high resolution ESCA spectra of native starch are dominated by signals from carbon and oxygen of the carbohydrate. Nitrogen and trace amounts of phosphorus and sulphur are detected (Fig. 4.1). The atomic stoichiometries calculated from these spectra for native starch and modified starches are given in Table 4.1.

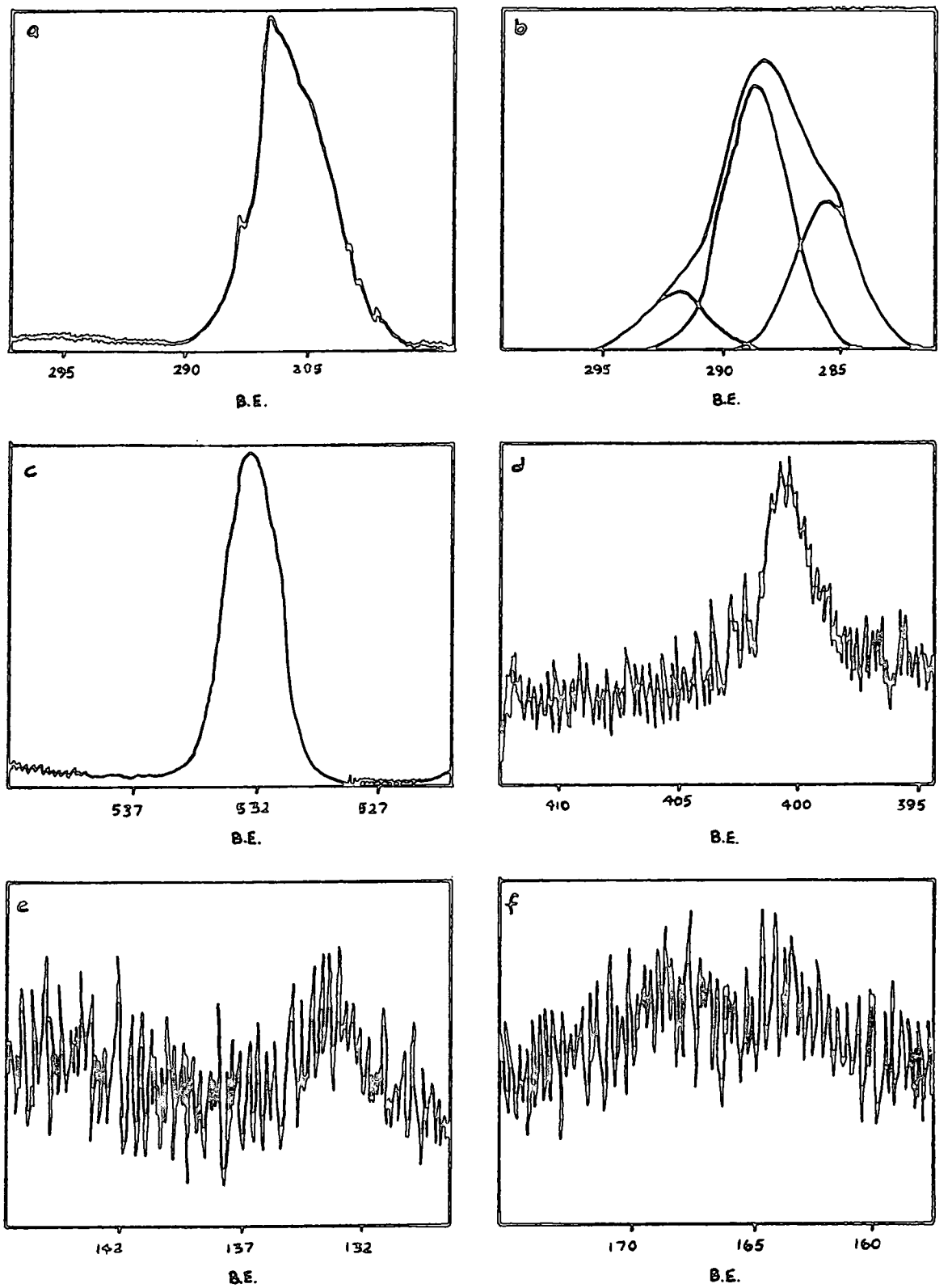


Figure 4.1 Core level spectra of native starch (a)C_{1s};(b) deconvoluted C_{1s}; (c)O_{1s}; (d)N_{1s}; (e)P_{2p};(f)S_{2p}.

Table 4.1 ESCA results from starch samples subjected to surface modification techniques.

Starch Treatment	Atomic Stoichiometry (relative to C=100)					
	C	O	N	S	P	Cl
Native	100	55	2.6	0.5	0.3	0.0
Chlorine	100	82	8.0	nd	nd	4.0
Pronase	100	55	0.0	nd	nd	0.0
Pronase/Cl_2	100	89	0.0	nd	nd	2.0
Methanol	100	74	3.0	nd	nd	0.0
Methanol/Cl_2	100	92	3.0	nd	nd	0.0
Cl_2/methanol	100	72	3.0	nd	nd	1.2

Note: nd - not detected .

Curve fitting of the C_{1s} envelope of native starch suggests there are three component peaks at binding energies of 285, 286.64 and 288.58 ev (Fig. 4.1 b), with intensities in the ratio of 30:54:11.8 (Table 4.2). The binding energy shifts of 1.64 and 3.58 ev from the reference position of 285 ev, for hydrocarbon species (C-H), correspond to carbon directly bonded to oxygen in C-O and O-C-O environments, respectively.

Table 4.2 C_{1s} photoelectron line parameters of starch.

Peak	Binding energy (ev)	Relative area	Assignment
1	285.0	30.3	C-H
2	286.64	53.6	C-O
3	288.58	11.8	O-C-O

The measured intensity ratio of 11.8:54 (1:4.7) for the O-C-O and C-O environments, is in reasonable agreement with the expected 1:5, attributable to the C-1 and the remaining 5 carbons of the glucose residues.

As the starch polymer is composed of carbon bonded to at least one oxygen, the presence of a hydrocarbon component at 285 ev in the C_{1s} spectrum indicates that carbon is present in a non-starch form. In principle this signal may arise from extraneous hydrocarbon contamination; or from hydrocarbon structures in components naturally present at the starch granule surface such as lipids and proteins. The data presented in Figure 4.1 and later from the methanol and pronase treated starches, shows that lipids and proteins are present at the starch granule surface. They give rise to the ESCA signals from phosphorus, sulphur and nitrogen atoms. However, the intensity of these signals ,relative to that at 285 ev, cannot account totally for the hydrocarbon present. It is unlikely that the excess hydrocarbon arises from within the analysis chamber of the spectrometer, as a build up of hydrocarbon has not been observed over data acquisition times of a few hours. There is some variation in the size of the hydrocarbon contribution between different native starch samples, which suggests that the discrepancy is due to the presence of hydrocarbon contamination in the native starch prior to analysis.

The O_{1s} binding energy for starch is observed to be 532.7 ev. This is consistent with both the alcohol and acetal groups present in starch (Fig 4.1 c). The C/O atomic ratio of 100/55 is lower than the expected ratio of 100/83

for pure starch, and is thought to be reduced by the presence of non carbohydrate species at the surface of the starch granules i.e. lipids and proteins and also extraneous hydrocarbon. This reasoning is supported by the observed increase in C/O ratio to 100/74 when starch is treated with methanol, which removes lipid species from the surface of the starch granules, and is closer to the expected value of 100/83.

A N_{1s} signal (Fig. 4.1 d), with a C/N atomic ratio of 0.02 is observed at a binding energy of 400 ev. This is consistent with amide linked nitrogen, and suggests the presence of protein at the starch granule surface. Spectra collected from pronase treated starch further supports this assignment, as a complete disappearance of the N_{1s} core level signal occurs. This is indicative of enzymatic protein degradation at the surface.

Sulphur is observed in minute quantities (Table 4.1) after long data acquisition times (Fig 4.1g). The main S_{2p} core level signal occurs at a binding energy of 163.8 ev, corresponding to sulphur in a sulphide environment, which is consistent with the sulphydryl, disulphide or thioether groups present in some proteins[82]. In addition, there is also a small contribution to the peak at a binding energy of 168.3 ev which corresponds to a sulphone functionality. This can probably be attributed to a small amount of surface oxidation in air.

Phosphorus is also detected (Fig. 4.1e) at very low concentration with an approximate P_{2p} binding energy of 134 ev. This binding energy corresponds to that of phosphorus in phosphate. The phosphate could be present as

diester functionalities in phospholipid species. Owing to the long data acquisition times required, and the poor signal to noise ratio of the spectra produced, analysis for this element was not often made.

4.2.2 Atomic Composition of the Granule Surface after Chlorine Treatment.

The overall spectral line profile of the C_{1s} envelope, from chlorine treated starch, is very similar to that of native starch, although there appears to be a slight decrease in C-H concentration coinciding with an increase in the amount of C-O environments present (Fig. 4.2a). This is consistent with the oxidation by chlorine of hydrocarbon species at the surface of starch. Similar behaviour is observed upon chlorinating cellulose. Comparison of curve fitted spectra for untreated and chlorine treated samples of cellulose show decreases in numbers of C-H and C-O functionalities, with a definite increase in the signal from the O-C-O functionality and the appearance of O-C=O species following chlorine treatment (Fig. 4.3). Several mechanisms may be proposed for this result based on those postulated by other workers[1,4,40].

Chlorine gas may react directly with the hydroxyl groups of the carbohydrate, with the formation of HCl (probably removed under vacuum), and carbonyl groups. Such a reaction may be assisted by surface bound water on the starch and would cause ring cleavage and depolymerisation of the type proposed by Whistler[40].

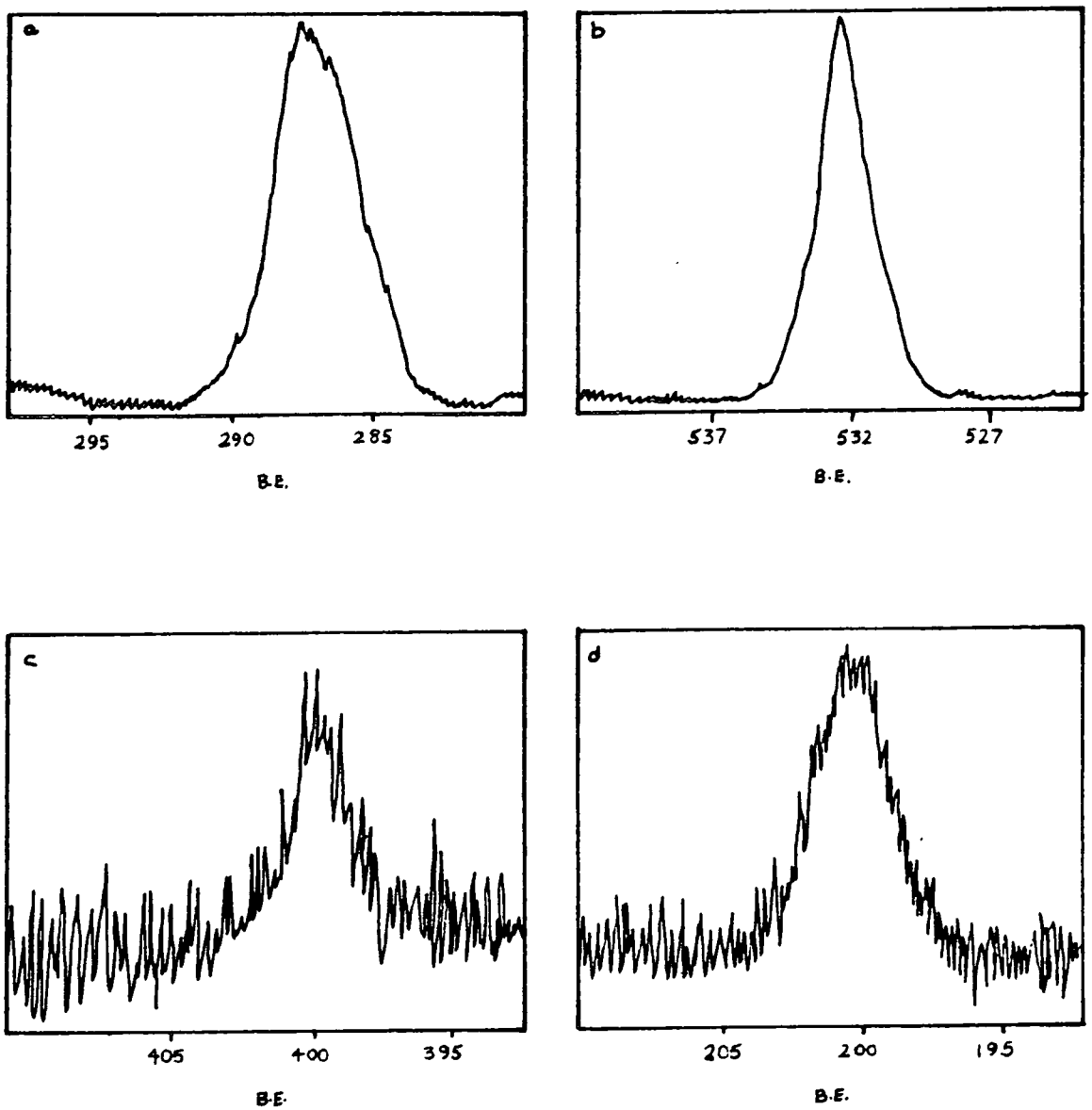
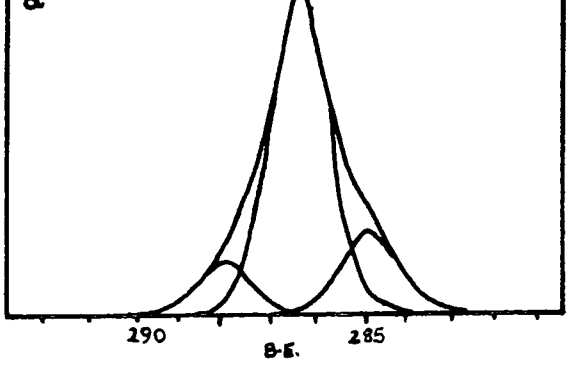
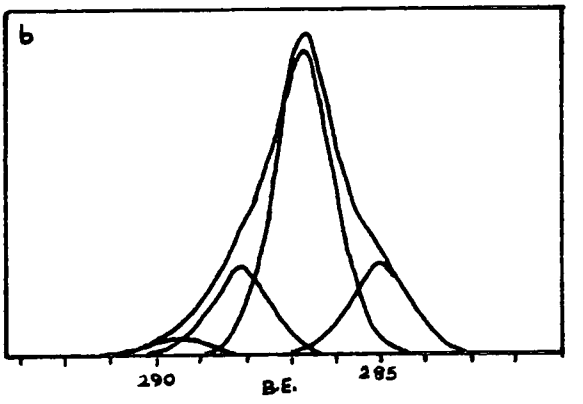


Figure 4.2 Core level spectra of chlorine treated starch: (a) C_{1s}; (b) O_{1s}; (c) N_{1s} (d) Cl_{2p}



Peak	Binding energy (ev)	Relative area	Assignment
1	285.0	18.42	C-H
2	286.6	68.36	C-O
3	288.25	12.00	O-C-O



Peak	Binding energy (ev)	Relative area	Assignment
1	285.0	17.92	C-H
2	286.65	60.13	C-O
3	288.15	17.92	O-C-O
4	289.4	3.38	O-C=O

Figure 4.3 Deconvoluted C_{1s} core level spectra and photoelectron line parameters of (a) untreated and (b) chlorine treated cellulose.

Alternatively, since the prepared starch has a bound water content of ≈ 13%[1], HOCl species might be formed which react with the surface, leading to the incorporation of oxygen observed and also chlorine (see later). In this case chlorine might be present as either chloride ion, from solvated HCl retained at the surface; or as organo chlorine, from reaction with the organic material present.

The binding energies and intensities of the O_{1s} and N_{1s} signals of chlorine treated starch are not significantly changed compared to those measured for native starch (Fig. 4.2b,c).

It was not possible to detect the effects of chlorination upon the phosphorus and sulphur components owing to very poor signal to noise ratio of their spectra. However, it is expected that the sulphide species present at the surface of starch granules also undergo oxidation. This expectation is based on the results of the chlorination of ovalbumin (presented later), which shows complete oxidation of it's sulphide component.

Chlorine is detected at the surface of chlorine treated starch granules in a low concentration (Table 4.1). The Cl_{2p} binding energy is 201.5 ev, which is typical of a covalently bound organo chlorine species (Fig. 4.2d). This result is similar to that reported for starch washed from chlorine-treated flour[4] and also coincides with the chlorine signal detected in chlorine treated cellulose. However, the concentration of chlorine bound to cellulose was almost three times that found in the starch. This suggests that the chlorination of starch is hindered in some way, by species residing at it's surface which is not the case for

cellulose.

The goal of identifying the site or sites where chlorine binds to the surface of starch granules has eluded workers in the field for many years. Conflicting results have been presented. Some identify the lipid and protein as the main sites of attack[30,49], whilst others suggest attack on the starch it-self is more important[5,36,37]. It is not possible from the analysis of the C_{1s} spectrum alone to assign the precise molecular location of the chlorine. However, chlorine treatments of pronase and methanol treated starches suggests that lipids and proteins associated with the granule's surface are involved in the chlorination. ESCA analysis (Table 4.1) reveals that approximately 50% of the chlorine found in chlorine treated starch binds to pronase digested material; 70% of chlorine bound to native starch is removed upon methanol treatment; chlorine does not bind to methanol pre-treated starch. It appears that the removal of lipid or protein from the surface of starch granules significantly reduces the incorporation of chlorine. This agrees with the findings stated earlier regarding the chlorination of cellulose, and suggests that chlorine is incorporated into the surface SGP and/or lipid in preference to the carbohydrate starch polymer. Results from chlorination studies performed on the model polymers also support the postulated preferential attack on non-carbohydrate material.

Poly(acrylic acid) and poly(ether sulphone) were not affected by chlorine treatment, but nylon, a polyamide used as a model polypeptide back-bone of a protein is substantially oxidised. A comparison of the C_{1s} spectra of

native and chlorine treated nylon is given in Figure 4.4, showing a definite increase in C-O and the formation of

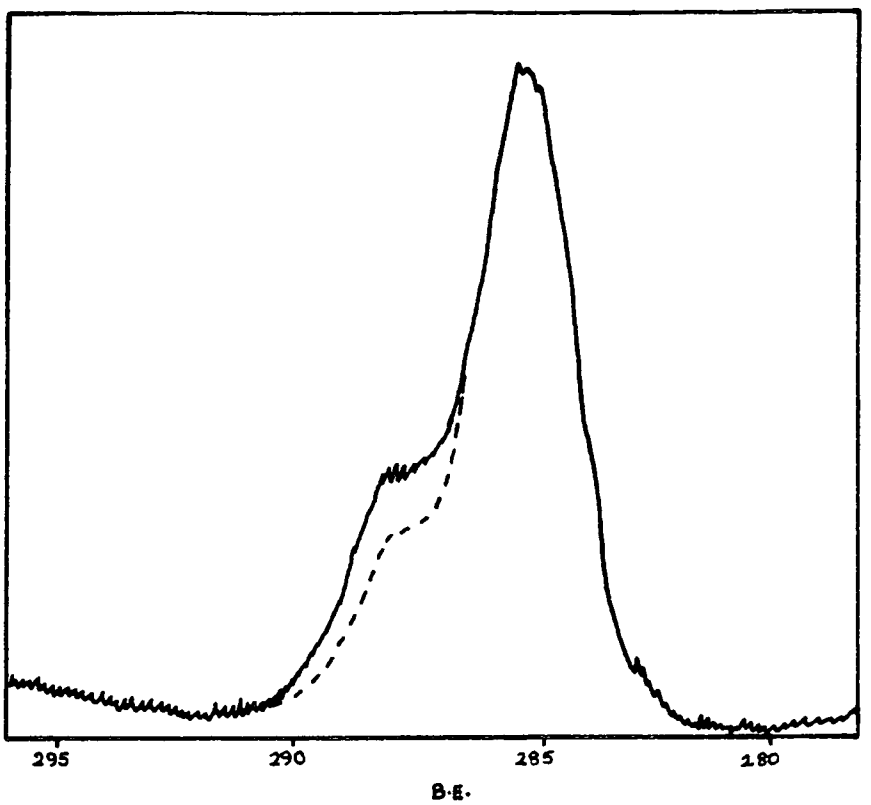


Figure 4.4 C_{1s} core level spectra of native (---) and chlorine treated (-) nylon.

O-C=O species. The Cl_{2p} spectrum indicates that chlorine exists in a single covalently bound environment.

The results reported here, which suggest a preferential attack of chlorine on lipid and protein components of starch, support earlier proposals which identify attack on the surface protein[1,31,49], as the important factor in the improving effects of chlorine on cake flour.

4.2.3 Oxygen Plasma Treatment.

The results that have been obtained show that starch discs exposed to an oxygen plasma suffer considerable chemical disruption at their surfaces. However, this is not entirely reflected in the C_{1s} spectra shown in Figure 4.5. These indicate a gradual growth of a signal at 285 ev during a 30 second plasma treatment, which is consistent with an accumulation of hydrocarbon at the surface of the discs. The fact that the concentration of hydrocarbon is seen to only increase at the surface following treatment, means that it cannot arise from contamination within the spectrometer. However, the signal at 285 ev is also representative of quarternary carbon species. It is likely that these species appear as a result of plasma induced rearrangements e.g. cross-linking of C-O and O-C-O functionalities involving dehydration, accompanied by some re-orientation.

A similar effect is observed upon exposing cellulose to an oxygen plasma. Data from the peak synthesis of C_{1s} spectra of untreated and 1 minute plasma treated cellulose are given in Figure 4.6, and indicate the occurence of such processes within the surface of cellulose.

Calculations of % composition from data recorded (Table 4.3) show that the total carbon concentration decreases with time (ignoring data for the 30 second treatment). This can be attributed to the increased level of oxygen at the surface, incorporated by the impact of excited species from the plasma, which reduces the total carbon :oxygen ratio.

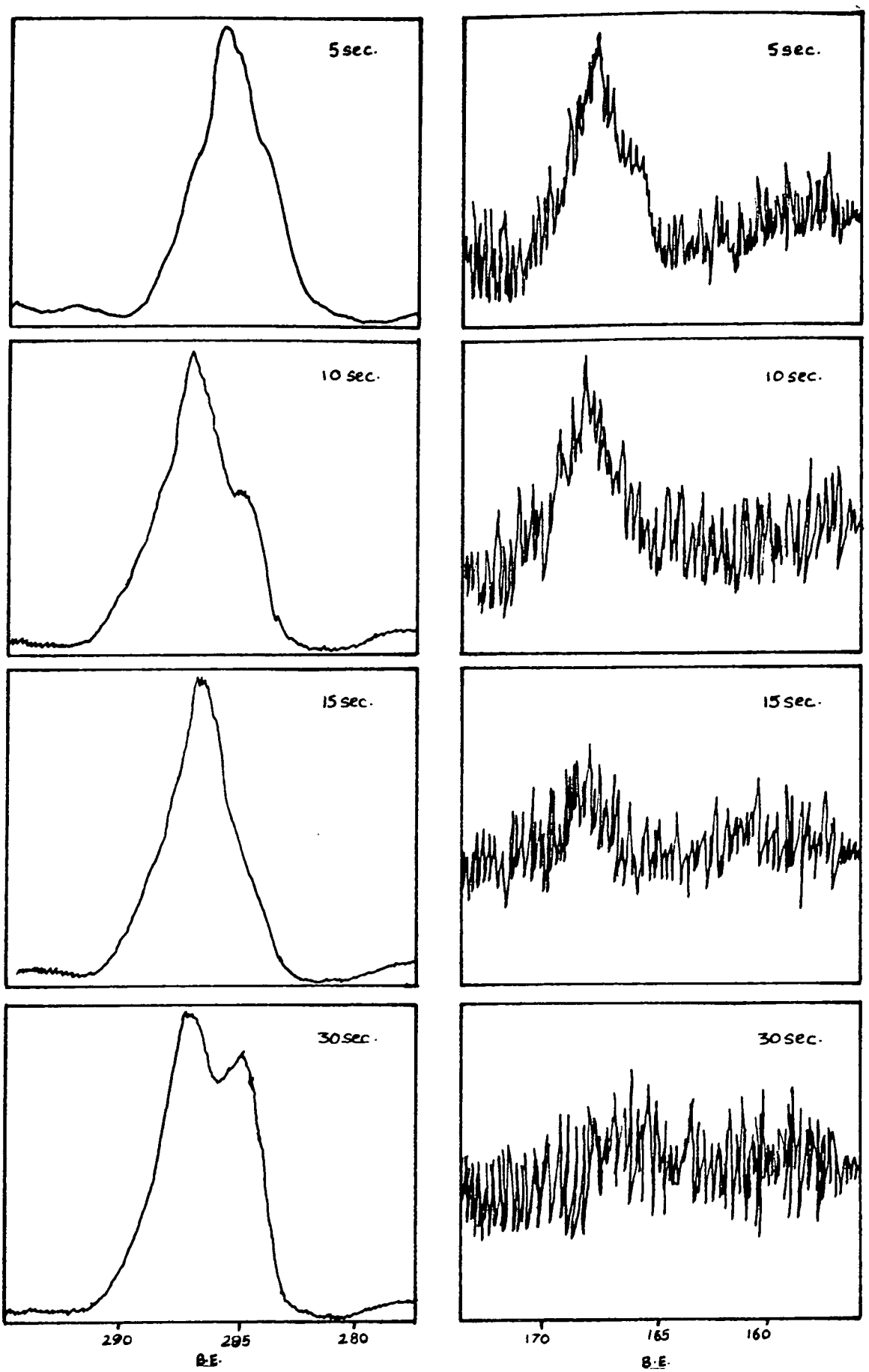
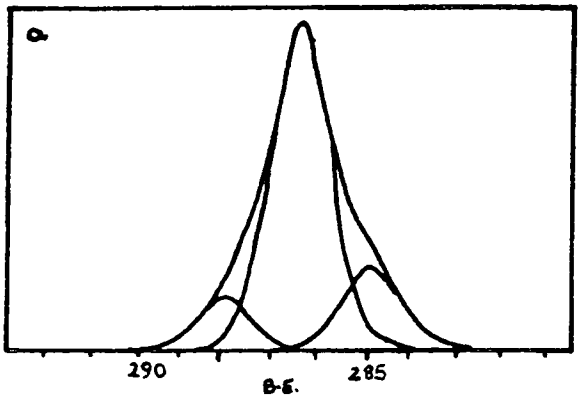
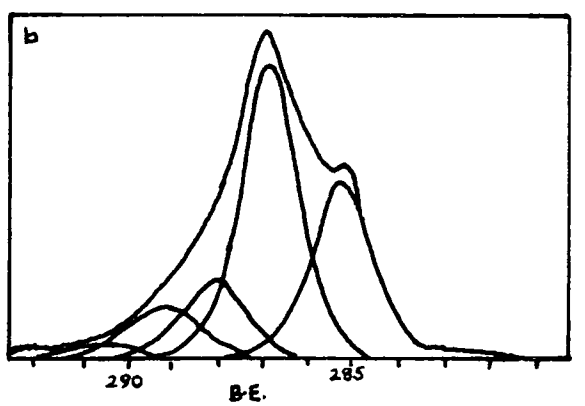


Figure 4.5 C_{1s} and S_{2p} core level spectra from a time dependent oxygen plasma treatment of native starch.



Peak	Binding energy (ev)	Relative area	Assignment
1	285.0	18.42	C-H
2	286.6	68.36	C-O
3	288.25	12.00	O-C-O



Peak	Binding energy (ev)	Relative area	Assignment
1	285.0	28.87	C-H
2	286.64	48.78	C-O
3	287.86	12.73	O-C-O
4	289.0	9.00	C=O; O-C=O
5	290.4	1.67	$O-CO_2$

Figure 4.6 Deconvoluted C_{1s} spectra and photoelectron line parameters of cellulose: (a) native; (b) oxygen plasma treated.

Table 4.3 Data from a time dependent oxygen plasma treatment study on starch.

Treatment Time(sec.)	Atomic % Composition from Peak Areas.				
	C	O	N	S	P
0	66	33	0.74	0.02	.02
5	61	36	1.90	0.30	nd
10	62	36	1.30	0.30	nd
15	61	38	1.00	0.19	nd
30	68	31	0.60	0.10	nd

Note: nd - not detected for.

Oxidised sulphur containing species are observed following a 5 second plasma treatment. Although very low concentrations of sulphur species were detected in the native starch this was in the unoxidised form associated with protein (Fig. 4.5). Bulk elemental analysis of native starch (Table 4.4) shows the presence of sulphur. It is not known if any of this bulk sulphur is in the oxidised form, or if oxidation of the sulphur containing species is caused by oxygen plasma treatment. The latter is probable.

Table 4.4 Bulk elemental analysis data of native starch.

Element	% Composition by weight
C	39.69
H	6.69
N	-
S	0.22
P	0.037

Further plasma treatment leads to a gradual decrease in the S_{2p} signal intensity. One possible explanation for this behaviour involves the postulate that sulphur groups, associated with protein components, are concentrated just below the analysis depth (40 Å) of the native starch. Plasma treatment etches the surface leading to the detection of these groups. Further etching removes this layer resulting in the observed loss of sulphur. This is, of course, only a provisional hypothesis, and further work is required before any firm conclusions can be made.

4.2.4 Corona Discharge Treatment (CDT).

The spectra of all the core levels analysed (i.e. C_{1s}, O_{1s}, N_{1s} and S_{2p}) indicate that, with the exception of sulphur, all components of the starch disc surface are modified upon CDT. The most noticeable changes occur in the C_{1s} and N_{1s} spectra.

The C_{1s} spectra show a gradual reduction in the level of hydrocarbon with time and this is accompanied by an increase in the level of oxidised carbon species (Fig. 4.7). The binding energy shifts indicate that a variety of such species are formed and probably include: $\underline{C}$-O; $\underline{C}$=O; O-$\underline{C}$-O. A small quantity of O-$\underline{C}$=O is also observed after 20 seconds treatment. A broadening of the O_{1s} peak width is also seen which is consistent with the formation of a variety of oxidised carbon species. Atomic stoichiometry data reveals a large amount of oxygen is incorporated into the disc with time (Table 4.5).

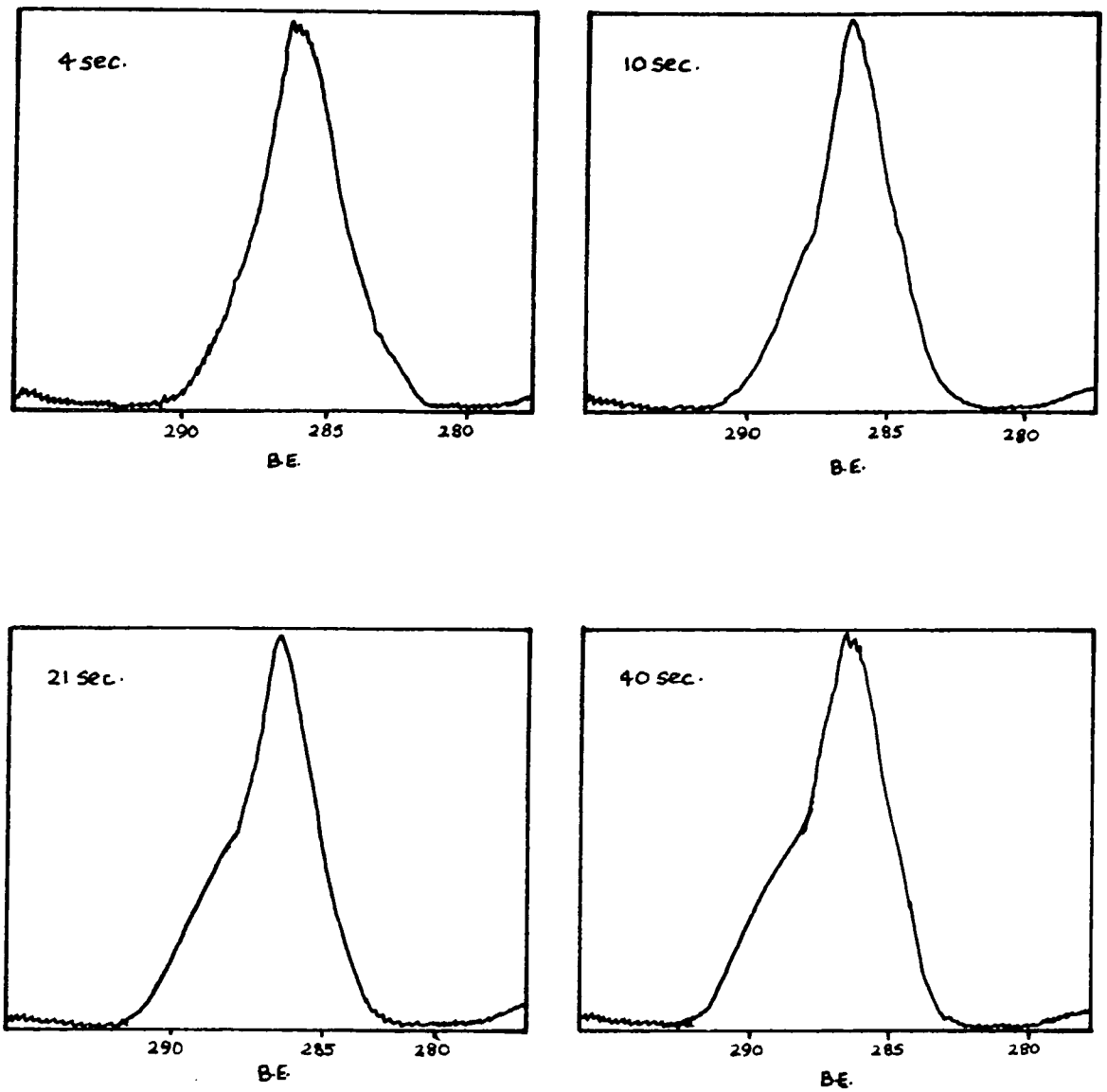


Figure 4.7 C_{1s} core level spectra from a time dependent CDT study on starch.

Table 4.5 Time dependent CDT of Starch Discs.

Treatment time(sec.)	Atomic stoichiometry (relative to C=100)			
	C	O	N	S
0	100	58.0	3.0	0.0
2	100	71.0	1.4	0.0
5	100	65.0	2.0	0.0
10	100	67.0	2.0	0.0
21	100	72.0	2.3	0.0
40	100	75.0	2.0	0.0

As a result of the poor signal:noise for the S_{2p} feature it has not been possible to characterise any sulphur species which might be present following the treatment. Some of the spectra do appear to show a trace of oxidised sulphur but this is not certain.

More interesting behaviour is seen in the N_{1s} core level spectra (Fig. 4.8). A second N_{1s} peak appears at a binding energy of 407 ev following the discharge. This has a binding energy shift of 6 ev from the amide N_{1s} signal and corresponds to "nitrite" type species. The intensity of this new signal increases with time. However, there is no simultaneous decrease in the amide N_{1s} peak intensity, expected if oxidation of the amide produced the nitrite. These observations indicate that the nitrite species do not originate from the oxidation of protein or other components, in the starch. This is supported by results of the CDT of low density poly (ethylene) (LDPE). Nitrogen is not present in untreated LDPE but after exposure to a 10 second discharge a N_{1s} signal appears at a binding energy corresponding to nitrite. It is thought that oxides of nitrogen generated in the air upon discharge, become deposited on the surface of the disc. The nitrite is gradually decomposed by the x-rays over a 12 minute period of ESCA analysis. This was the only material of this study which showed evidence of decomposition by the x-ray beam used in ESCA.

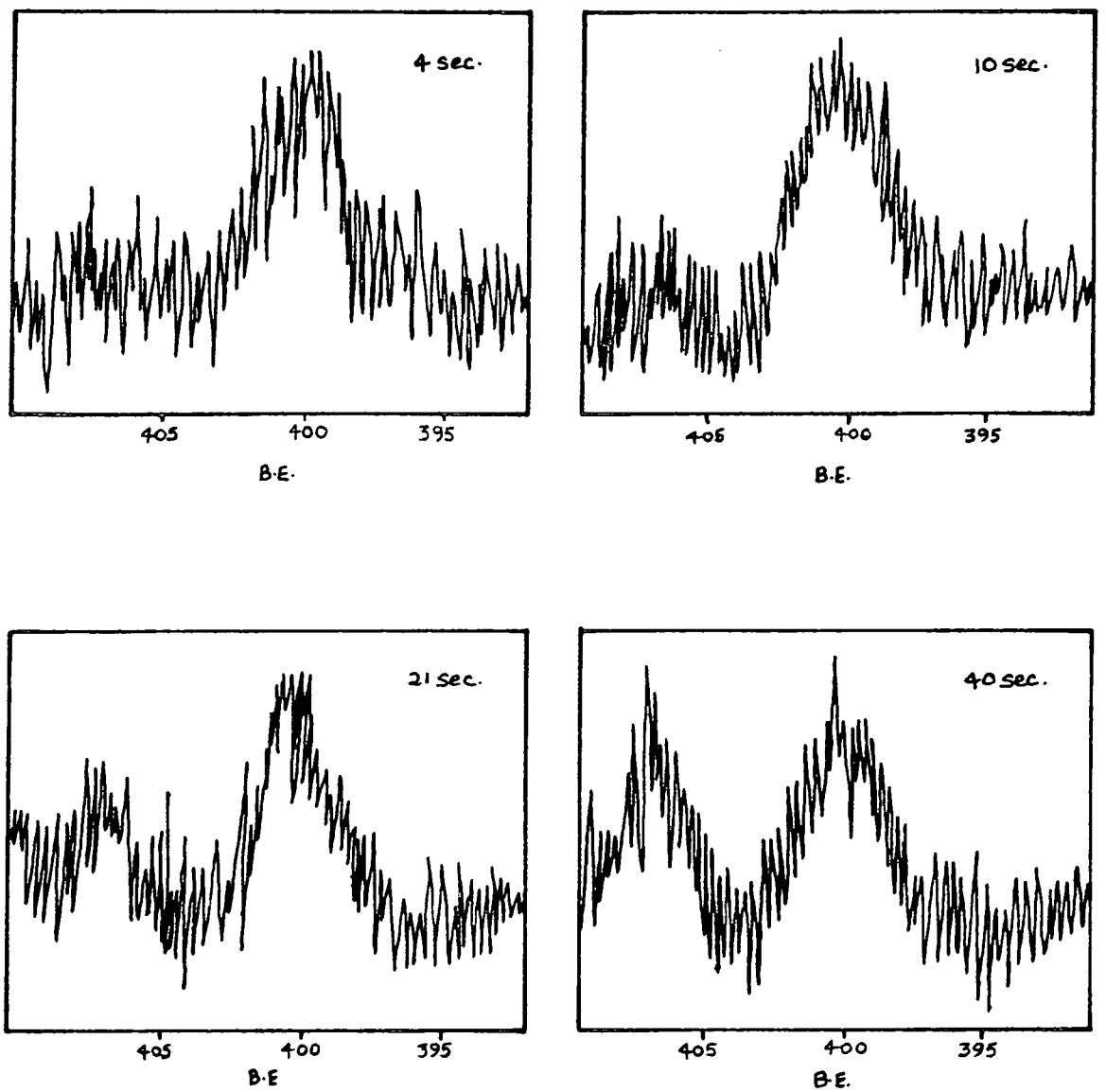


Figure 4.8 N_{1s} core level spectra of a time dependent CDT study on starch.

4.2.5 Ozonolysis Treatment.

A time dependent study of ozone treatment upon native starch powder revealed little change in the C_{1s}, O_{1s} and N_{1s} spectra over a 4h period. More extended treatment, however, is accompanied by the appearance of a shoulder on the high binding energy side of the N_{1s} line (Fig. 4.9), and a feature at 292.8 ev assigned to K_{2p} in the C_{1s} spectra. This feature increases in intensity with ozone treatment (Fig 4.10).

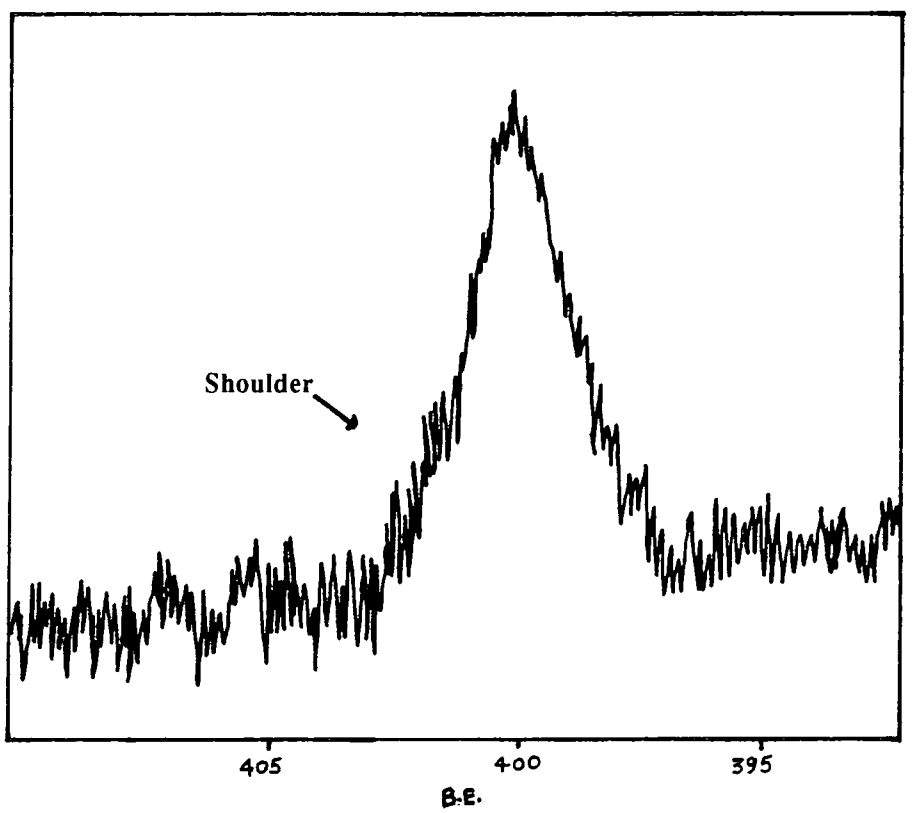


Figure 4.9 N_{1s} core level spectrum of native starch after 6h ozone treatment.

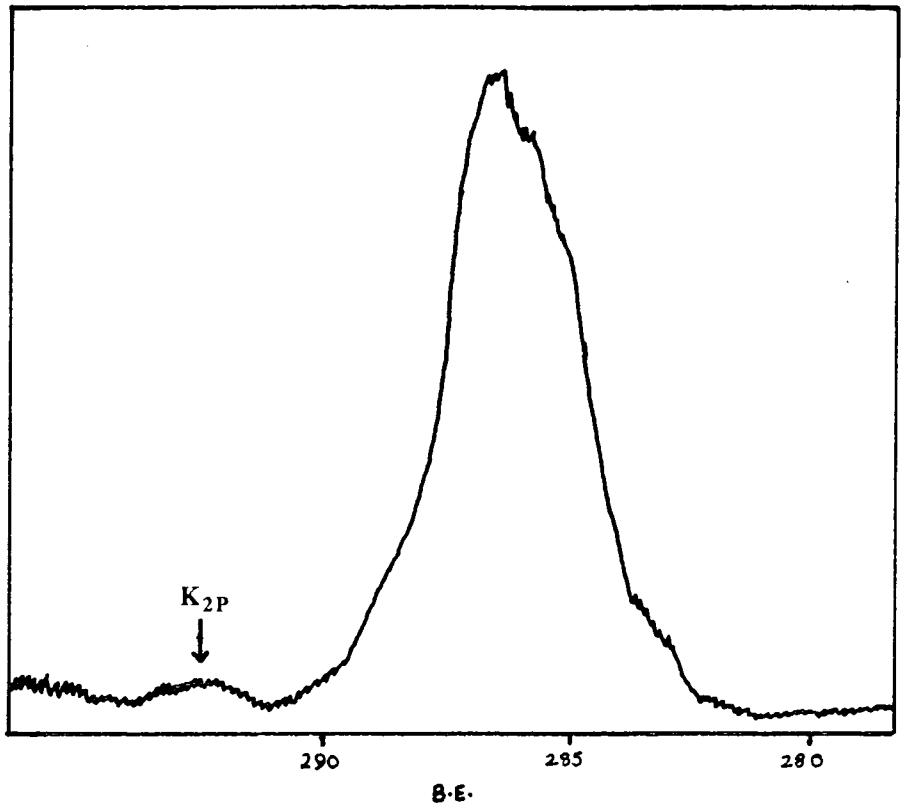


Figure 4.10 C_{1s} core level spectrum of native starch after 5h ozone treatment.

The potassium detected at the starch granule surface is thought to originate from the bulk and arise by one of two mechanisms. One involves the diffusion of potassium to the surface region of starch, facilitated by the disruption of the surface caused by the action of ozone. Alternatively, oxidative chemical sputtering might occur, by which volatile species, formed upon oxidation by ozone, are released from the surface of starch granules, revealing involatile species e.g. potassium, below.

Oxidation appears to be responsible for the feature in the N_{1S} spectrum with a binding energy of 401.3 ev, which corresponds to an oxide of nitrogen.

These observations suggest that an oxidation of the surface of starch granules occurs after prolonged periods of treatment.

4.3 Ovalbumin Investigation.

4.3.1 Atomic Composition of the surface of an ovalbumin Film.

ESCA spectra from the surface of ovalbumin films show the presence of carbon, oxygen, nitrogen and a small quantity of sulphur (Fig 4.11). The atomic stoichiometries for the surface region, calculated from the measured intensity data are given in Table 4.6.

Table 4.6 ESCA results from surface modified ovalbumin.

	Atomic stoichiometry (relative to C=100)				
Ovalbumin treatment	C	O	N	S	K
Native	100	24	21	1.7	0.0
Chlorine	100	27	24	2.0	0.0
O_2 plasma	100	36	20	2.5	0.9

Predicted[82] bulk values are:C 100; O 31.6; N 25.1; S 1.3 .

Figure 4.12 shows the curve fitted C_{1s} core level spectrum, composed of 8 components characteristic of environments which are consistent with protein characterisation (Table 4.7). The relative areas given here agree with those calculated from the known composition of ovalbumin[81].

Curve fitting of this nature was not persued as it proved difficult to assign individual binding energies and relative intensities to the functional groups with any degree of confidence, owing to the great overlap in this spectral region.

The O_{1s} core level spectrum (Fig. 4.11b) at a binding energy of ≈ 532.3 ev, is quite broad and reflects the contribution to it from the alcohol, amide and acid functionalities found in proteins.

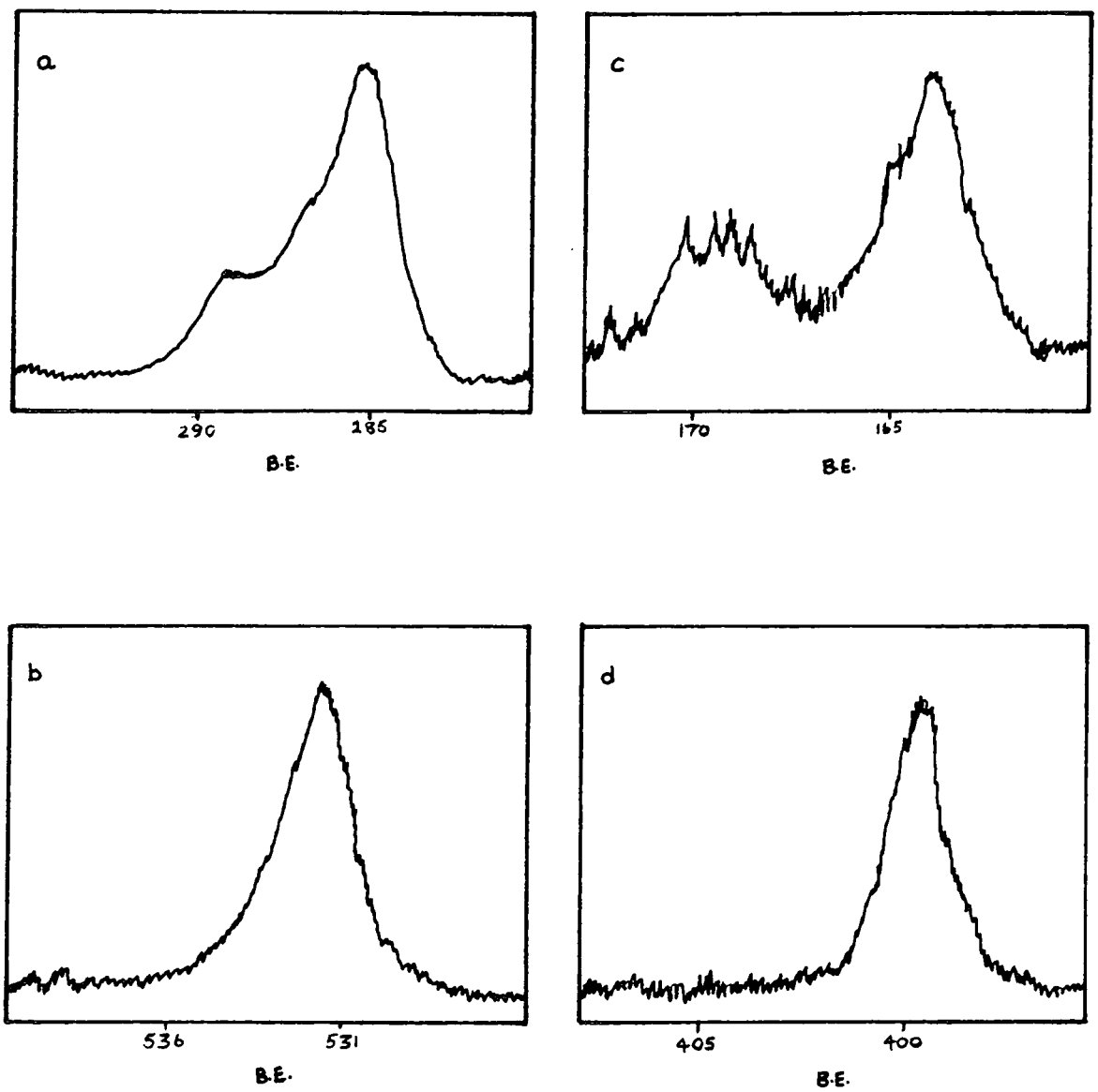


Figure 4.11 Core level spectra of native ovalbumin (a)C_{1s}; (b)O_{1s}; (c)S_{2p}; (d)N_{1s}.

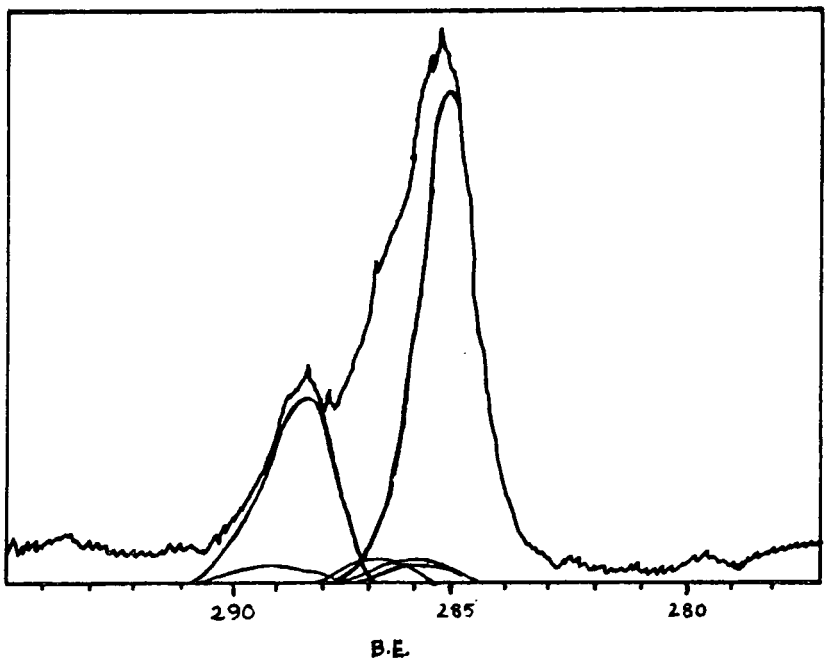


Figure 4.12 Deconvoluted C_{1s} core level spectrum of ovalbumin.

Table 4.7 C_{1s} photoelectron line parameters of ovalbumin.

Peak	Binding energy (ev)	Relative area	Assignment
1	285.0	52.1(55)	C-H
2	285.67	1.8	C-CO_2
3	285.8	0.6	C-S
4	286.2	3.7	C-N
5	286.4	18.4(20)	C(R)-NH-C=O
6	286.6	3.7(1)	C-O
7	288.2	18.4(20)	N-C=O
8	289.1	1.8(4)	O-C=O

Figures in brackets are the theoretical values[81].

The nitrogen N_{1s} signal is observed (Fig. 4.11c) with a binding energy of 399.9 ev which is assigned to the amide-linked nitrogen present in the protein. The nitrogen and oxygen concentration in the protein are very similar (C/N and C/O stoichiometry of 100/21 and 100/24 respectively) which suggests that there are either few, or a similar number of acidic and basic side groups attached to the polypeptide back bone.

Inspection of the S_{2p} core level spectrum (Fig. 4.11d) reveals that sulphur exists in at least two distinct environments. A S_{2p} signal is observed at a binding energy of 164.2 ev with a smaller signal at 168.9 ev, corresponding to sulphide and sulphone type species respectively. The presence of oxidised sulphur is likely to arise from a small amount of surface oxidation in air.

A comparison of the S_{2p} and O_{1s} core level spectra (Fig. 4.11b,d) with spectra of the model polymers, poly (phenylene sulphide) and poly (ether sulphone) (Fig. 4.13) and published data[83] of S_{2p} binding energy shifts, suggests that ovalbumin contains Cys-S-S-Cys type species. The small quantity of oxidised species found appears to be Cys-S-SO_2-Cys groups.

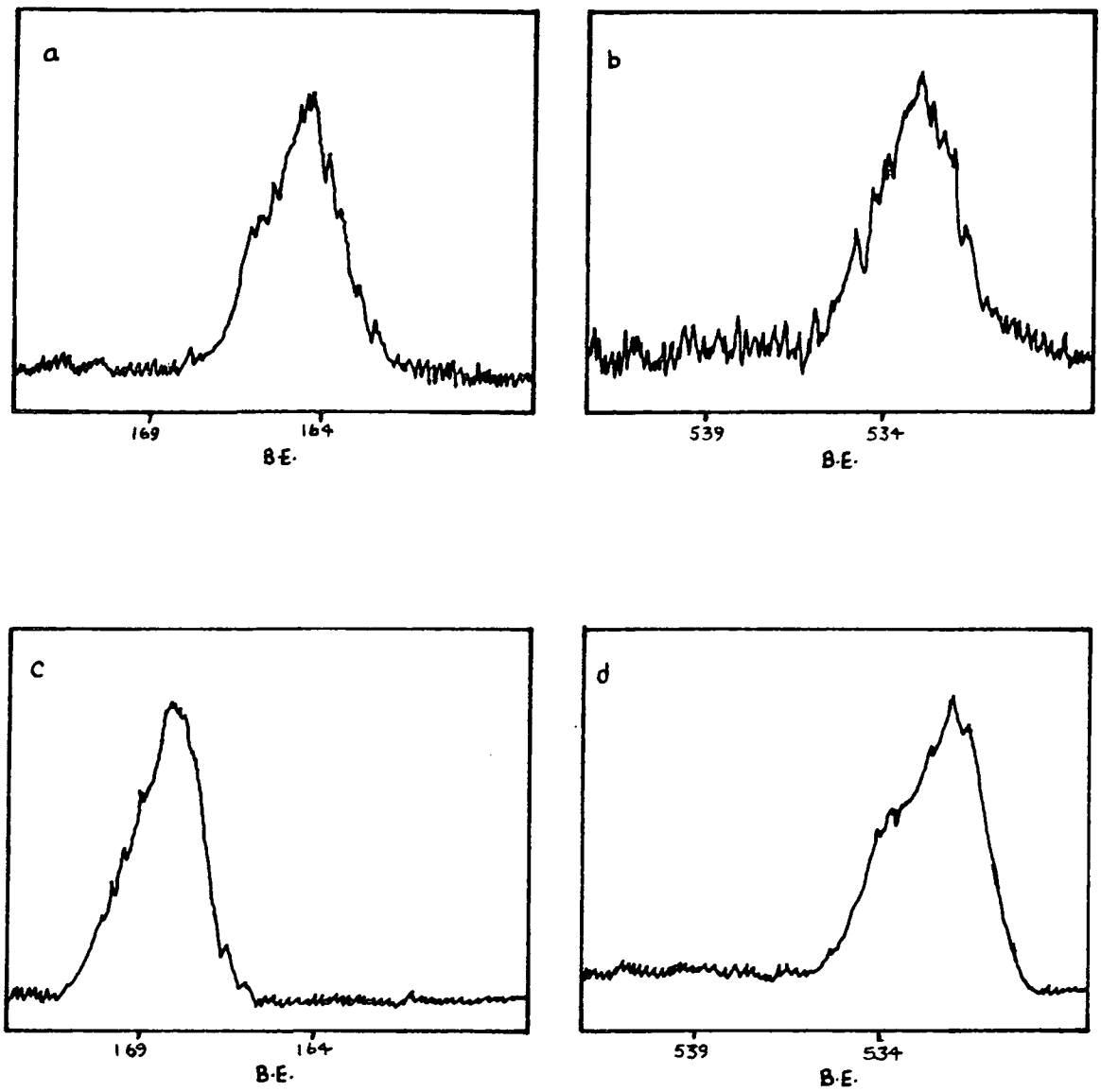


Figure 4.13 S_{2p} and O_{1s} core level spectra of model polymers: (a,b) poly (phenylene sulphide); (c,d) poly (ether sulphone).

4.3.2 Atomic Composition of the surface of ovalbumin powder after Chlorine Treatment.

The data collected after chlorine treatment reveal that ovalbumin is modified quite extensively by the treatment. Comparison of the C_{1s} core level spectra of treated (Fig. 4.14) and untreated ovalbumin (Fig. 4.11a), show a decrease in the signal assigned to O-C=O environments concurrent with an increase in C-O environments upon chlorination. This

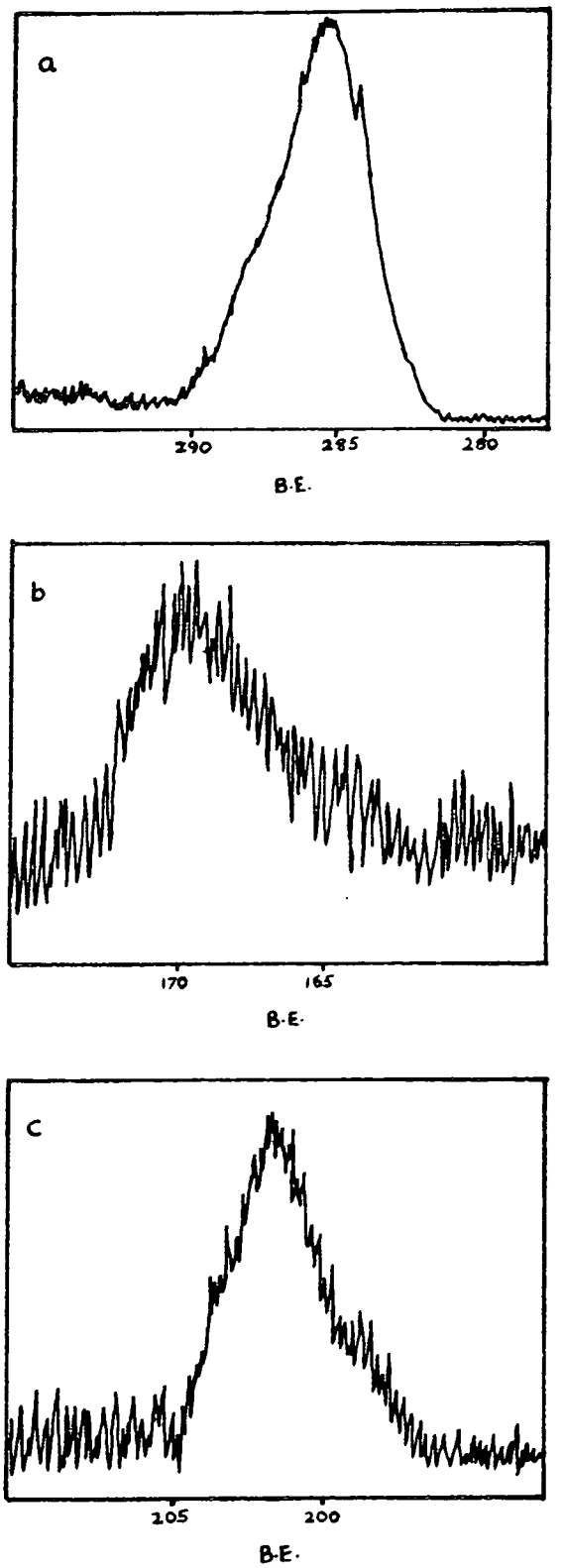


Figure 4.14 Core level spectra of chlorine treated ovalbumin: (a) C_{1s}; (b) S_{2p}; (c) Cl_{2p}.

behaviour is probably due to some form of sputtering, in which the O-C=O type species present at the surface of ovalbumin are oxidised, becoming volatile and being lost.

The N_{1s} spectrum is very similar to that for untreated ovalbumin which suggests that the peptide bonds have not been disrupted by chlorine.

The sulphide type residues of untreated ovalbumin are almost completely oxidised to the sulphone during chlorination. This can be seen in the S_{2p} spectrum shown in Figure 4.14b, which is composed of a broad peak at 168.3 ev with a "tail" at 164 ev.

The chlorine spectrum shows two components, at binding energies of 201 ev and 199.2 ev. These correspond to covalently bound chlorine and ionic chloride respectively (Fig. 4.14c). The chloride is probably associated with potassium ions that are known, from bulk analysis , to reside in the film (Table 4.8).

Table 4.8 Bulk elemental analysis data of ovalbumin.

Element	%Composition by weight
C	46.14
H	6.89
N	13.21
S	1.48
K	0.54

4.3.3 Oxygen Plasma Treatment.

The spectra of the C_{1s}, O_{1s} and N_{1s} regions following plasma treatment are very similar to the spectra of native ovalbumin; although the proportions of oxidised carbon species (C-O; C=O; O-C=O and N-C=O) are found to increase (Fig. 4.15).

A comparison of the S_{2p} core level spectrum (Fig. 4.15b) with that of native ovalbumin reveals that a large proportion of the Cys-S-S-Cys component is oxidised to Cys-S-SO_2-Cys. In addition, data presented in Table 4.6 shows that there is an increase in the level of sulphur at the film's surface following plasma treatment. This is possibly caused by a sputtering process.

Oxygen plasmas are capable of powerful chemical sputtering, containing highly reactive oxidising species. The latter might react with the protein with the resultant generation and loss of volatile species from the surface, e.g. CO, CO_2, NO, NO_2. This sputtering process would cause an apparent continuous increase in the level of oxidised sulphur species , which tend to be involatile. The process has also revealed potassium , which is involatile and known to reside in the film (Table 4.8), but is not usually detected. A K_{2p} peak is observed at $\approx$ 293 ev on the C_{1s} core level spectrum, following a 1 minute treatment (Fig.4.15a) and constitutes a C/K ratio of 100/0.9 (Table 4.6).

Results from a time dependent study, indicate that the oxidation caused by the plasma is most significant during a 10 second treatment. This is indicated by large intensity increases of the N-C=O, O-C=O and Cys-S-SO_2-Cys

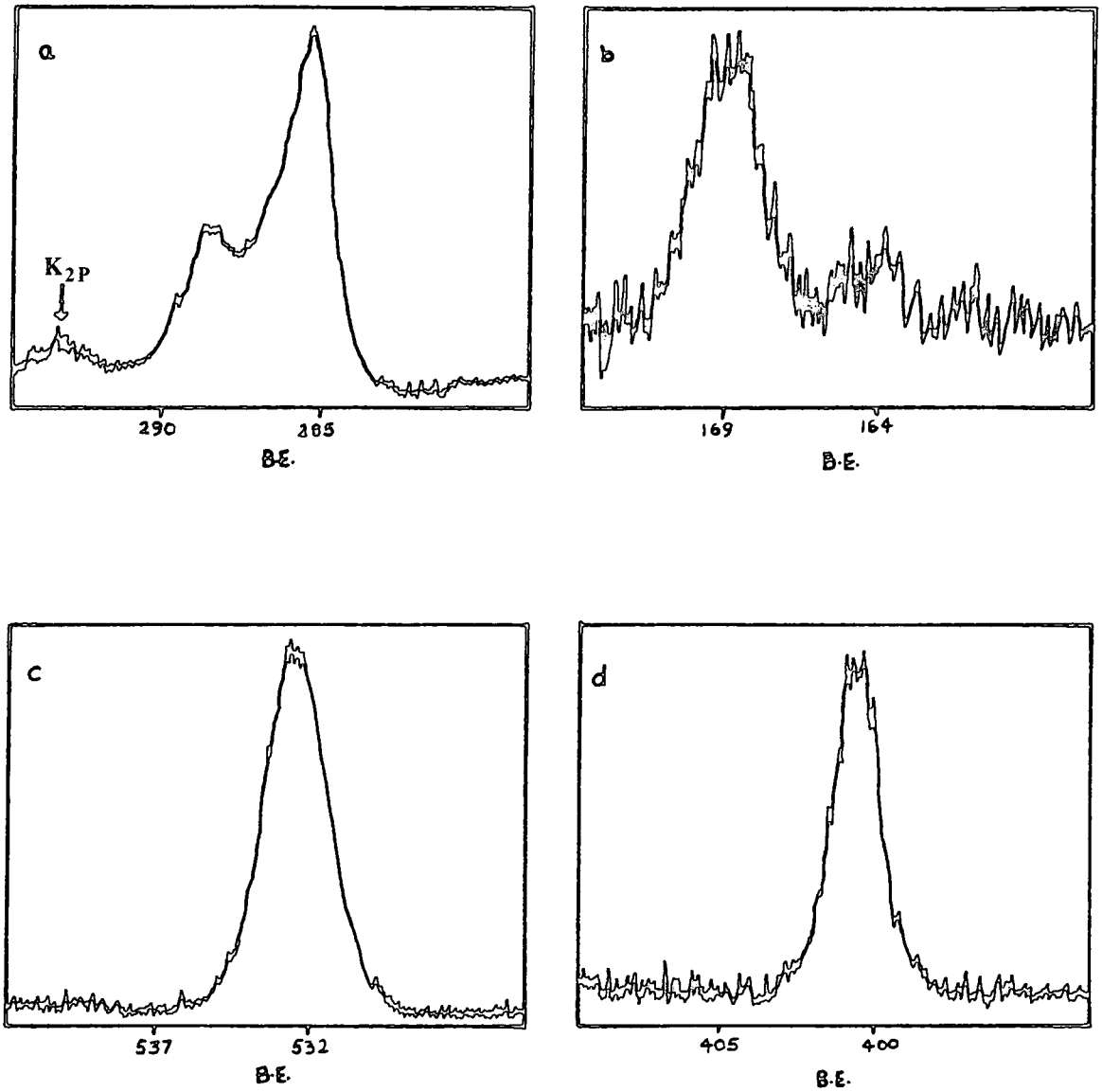


Figure 4.15 Core level spectra of O_2 plasma treated ovalbumin: (a) C_{1s}; (b) S_{2p}; (c) O_{1s}; (d) N_{1s}.

functionalities (Fig. 4.16b). There is also evidence to suggest that an oxygen plasma has a limited "depth of penetration". Comparison of the S_{2p} core level spectra (Fig. 4.16) shows that the rate of oxidation (Cys-S-S-Cys → Cys-S-SO_2-Cys) decreases with time. The spectra for treatment times greater than 15 seconds show very little change, even though Cys-S-S-Cys species are still detected in the surface region after this time, implying that they are inaccessible to the oxygen plasma.

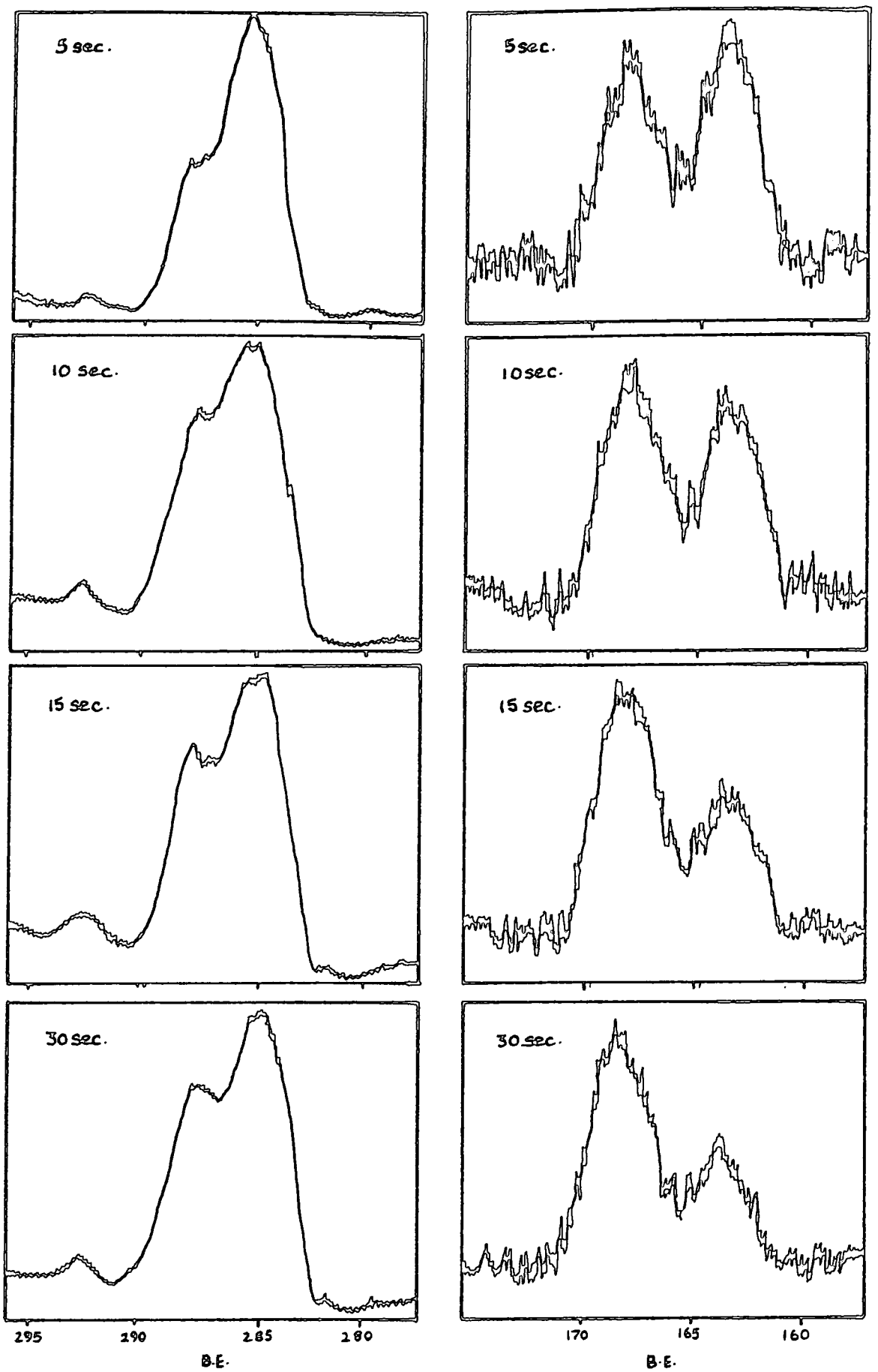


Figure 4.16 C_{1s} and S_{2p} core level spectra from time dependent O_2 plasma treatment of ovalbumin

4.3.4 Corona Discharge Treatment (CDT).

The results from a time dependent study performed on ovalbumin film, show that the surface is significantly disrupted by a corona discharge. Extensive oxidation occurs after a 1 second treatment, the oxidation continues with time and the O_{1s} intensity reaches saturation after ≈ 6 seconds. This can be seen from the C_{1s} core level spectra (Fig. 4.17). These spectra show a gradual increase in the proportions of N-C=O and O-C=O; and a reduction in C-O environments with treatment time. In addition a K_{2p} signal emerges after 3 seconds treatment with an intensity which also increases with time. These observations suggest that a chemical sputtering process is occuring similar in severity to that caused by an oxygen plasma.

The C_{1s}/O_{1s} peak ratio measurements presented in Table 4.9 reflect a continual increase in the level of oxygen incorporation with time, which is not consistent with the saturation time of 6 seconds. This anomaly is attributed to the oxidation of sulphide species which continues beyond 6 seconds. Figure 4.18 shows that just a 1 second treatment oxidises 50% of the sulphide component to sulphone.

N_{1s} core level spectra show a second peak at a binding energy of ≈ 407 ev, similar to that observed with the treatment of starch. Again this peak probably arises from the deposition of oxides of nitrogen generated in the air by the discharge.

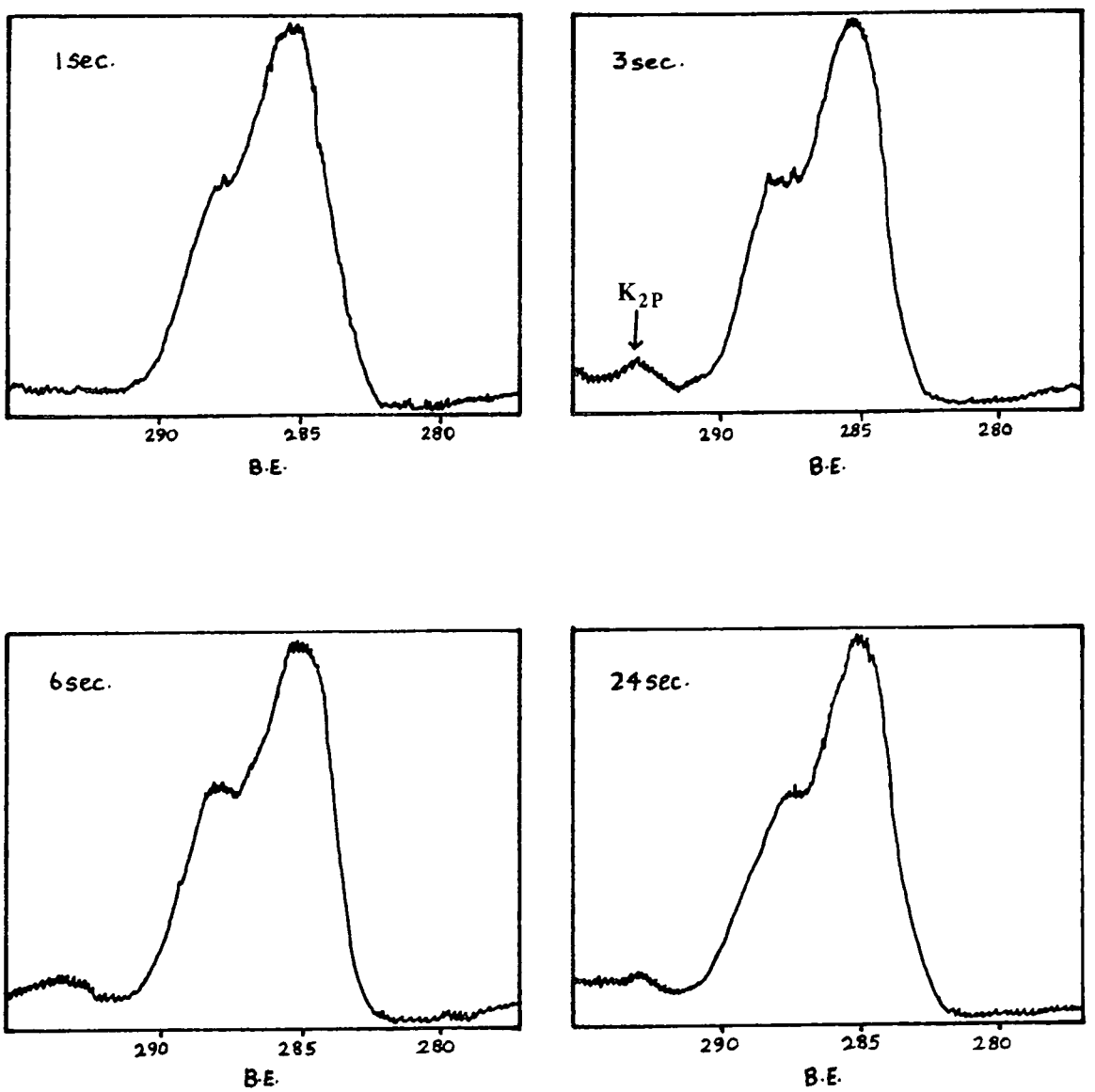


Figure 4.17 C_{1s} core level spectra from a time dependent CDT study on ovalbumin.

Table 4.9 Data form a time dependent CDT study of ovalbumin.

Treatment time(sec.)	Atomic stoichiometry (relative to C=100)				
	C	O	N	S	K
0	100	25	22	2.0	0.0
1	100	35	23	1.3	0.0
2	100	39	24	2.0	0.0
3	100	41	23	1.5	0.3
6	100	45	23	1.6	0.5
10	100	42	25	1.5	0.3
24	100	44	24	2.0	0.4
34	100	46	24	1.7	0.35

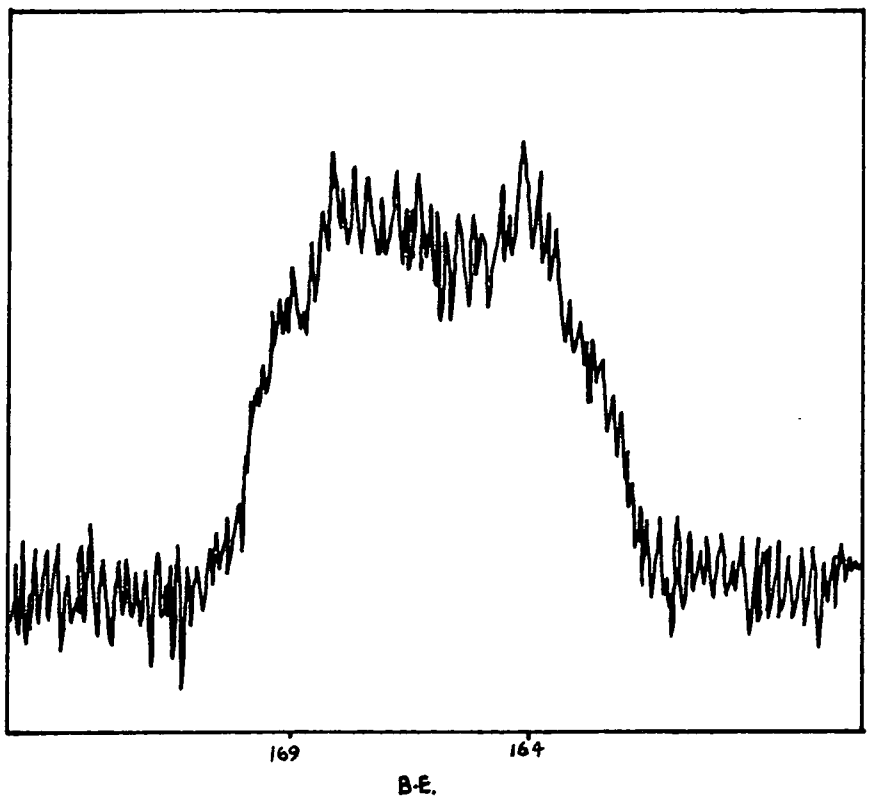


Figure 4.18 S_{2p} core level spectrum of ovalbumin after a 1 second CDT.

4.3.5 Ozonolysis.

C_{1s} core level spectra collected at intervals during ozonolysis, reveal a gradual increase in the proportion of C-O and O-C=O environments, and a concurrent decrease in C(R)-NH-C=O and N-C=O (Fig. 4.19). This implies cleavage of the peptide back-bone and subsequent rearrangement to form acid functionalities and is consistent with peak area ratio measurements given in Table 4.10.

Table 4.10 Peak area measurement data of ozone treated ovalbumin.

Treatment time (h)	Elemental Ratio (relative to C=100)			
	C	O	N	S
0	100	24	19	1.6
2.25	100	27	17	1.5
3.75	100	28	24	1.8
5.00	100	31	21	1.6
6.25	100	31	19	1.3
7.25	100	31	21	2.0
8.50	100	33	25	1.6

The C/N elemental ratio for nitrogen remains constant whilst that for oxygen is observed to increase. Analysis of the data reveals that oxidation of carbon alone cannot account for the total oxygen uptake observed. Oxidation of Cys-S-S-Cys type species also occurs which accounts for this discrepancy.

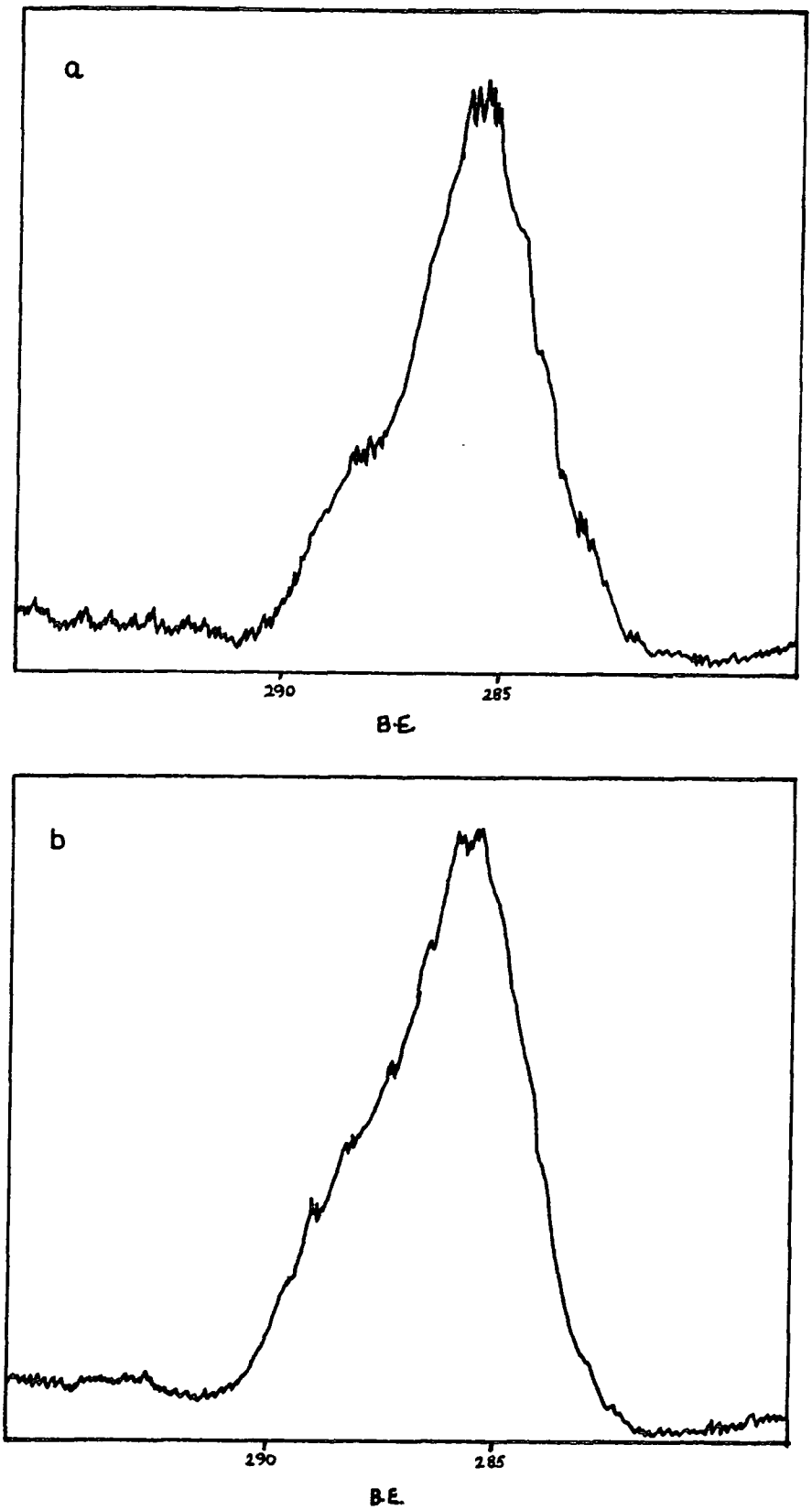


Figure 4.19 C_{1s} core level spectra of (a) native and (b) 8.5h ozone treated ovalbumin.

Figure 4.20 shows that a 2h treatment is sufficient to reverse the 2:1 composition ratio of Cys-S-S-Cys: Cys-S-SO_2-Cys species, which exists in native ovalbumin. The rate of oxidation of the sulphur groups decreases with time and a residual number are not oxidised, similar to that observed with the plasma treatment.

The appearance of a K_{2p} peak on the C_{1s} core level spectra after 4h treatment (Fig. 4.19), illustrates that ozone is capable of disrupting the surface. Once again a chemical sputtering process appears to be taking place, of a similar nature to that observed in the oxygen plasma and corona discharge treatments.

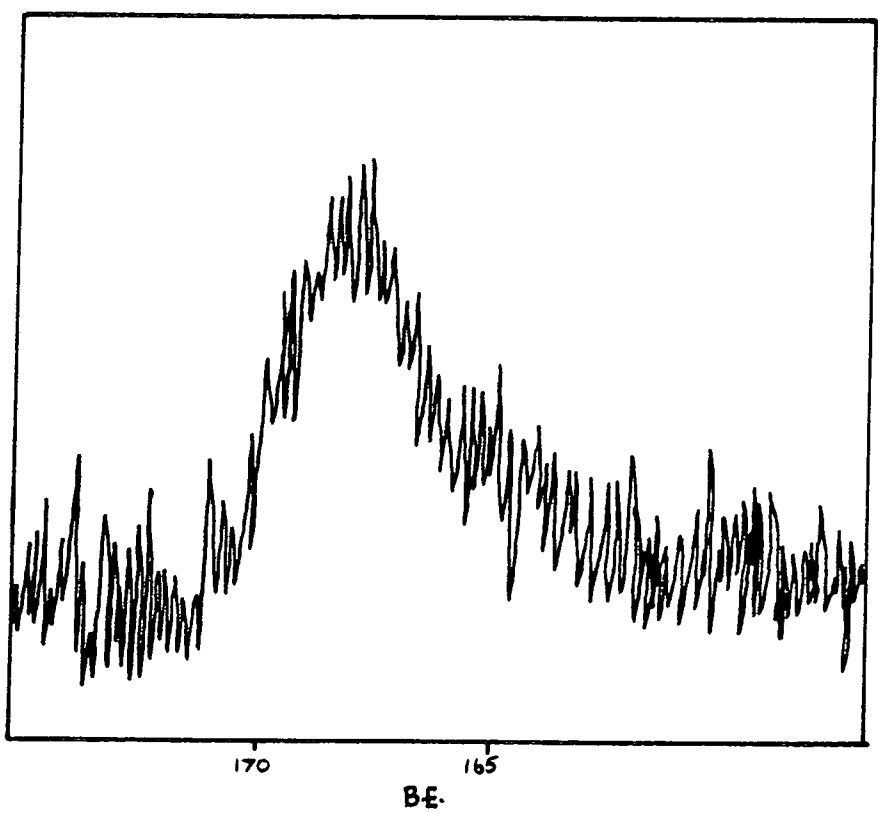


Figure 4.20 S_{2p} core level spectra of 2h ozone treated ovalbumin.

4.4 Conclusions and suggestions for further work.

The investigation of starch using ESCA has revealed some of the complex nature of the structure of the surface of starch granules. The elemental composition of the surface shows a C-O:O-C-O ratio consistent with the theoretical value predicted for the carbohydrate. The protein, lipid and phospholipids, known to be associated with the starch granule surfaces, have been detected, but required long data accumulation times.

ESCA has given information regarding the dry oxidative effects of: chlorination; radio-frequency oxygen plasma; corona discharge and ozonolysis upon starch, ovalbumin and a variety of model polymers.

Chlorine is found to covalently bind to native starch probably attacking the lipid and protein. The results reported concerning the removal of lipid and protein from the starch granule surface, suggest that chlorine preferentially binds to these species rather than the carbohydrate component of pure starch. These findings were supported by the incorporation of chlorine in ovalbumin (a protein) and nylon (a polypeptide back-bone equivalent) in contrast to poly (acrylic acid) powder and poly (ether sulphone).

There is a similarity in chlorine's disruptive action upon starch and ovalbumin. The observed modifications to the surface compositions of these materials, upon exposure to chlorine are consistent with an oxidative chemical process.

Oxygen plasma, corona discharge and to a lesser extent ozone treatments inflict more damage upon starch and ovalbumin, achieving similar but more extensive modifications than chlorine. The disruptions are most severe with an oxygen plasma, (being a highly energetic and powerful medium) and are consistent with surface rearrangements e.g. cross-linking; loss of volatile species uncovering non-volatile bulk components e.g. potassium which accumulate on the surface. This type of behaviour is also characteristic of corona discharge and ozone treatments and has been attributed to chemical sputtering processes.

If this project is investigated further it would seem obvious as a first step to ascertain if any of the novel oxidative surface treatments reported have a desirable effect on starch in baked cakes. The performance of these modified starches in cakes (baked according to a "starch cake" formulation) should be assessed against cakes baked using chlorine treated starches. In this way, if any of the treatments proved to be favourable conditions could be optimised for use in industry. However, the conversion of the ozonolysis and oxygen plasma treatments to an industrial scale could be difficult. Ozonolysis is also not thought to be suitable because of the long treatment times required to generate small changes to the starch. Although corona discharge enables powders to be modified in a controlled fashion, with the possibility of continuous treatment, the generation of nitrite, a carcinogen, at the surface of treated samples is undesirable.

Academically in any continuation, it would seem appropriate to extend the chlorination treatments studied to

include a more detailed examination of the individual starch granule surface components e.g. the isolation of: SGP (by SDS-PAGE$_i$); lipid/phospholipid (solvent extraction) and amylose (wet fractionation of starch). A study of this nature would require the use of additional surface characterisation techniques, e.g. Secondary Ion Mass Spectroscopy (SIMS) and Fourier Transform Infra red Spectroscopy (FTIR), to compliment that of ESCA. Other more conventional characterisation techniques should also be employed such as n.m.r spectroscopy, Differential Scanning Calorimetry and mass spectroscopy. By this approach more light may be shed on the species that are targetted by chlorine.

$_i$Note SDS-PAGE:
Polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulphate.

APPENDIX A.
INSTRUMENTATION.

Carbon, Nitrogen and Hydrogen Analysis were carried out on a Carlo ERBA Elemental Analyser-1106.

Potassium and Sulphur Analysis were carried out on a Perkin Elmer 5000 Atomic Absorption Spectrometer.

APPENDIX B.
RESEARCH COLLOQUIA, SEMINARS, LECTURES AND CONFERENCES

The Board of Studies in Chemistry requires that each postgraduate research thesis contains an appendix listing:

(A) all research colloquia, seminars and lectures arranged by the Department of Chemistry during the period of the author's residence as a postgraduate student;

(B) lectures organised by Durham University Chemical Society;

(C) all research conferences attended and papers presented by the author during the period when research for the thesis was carried out;

(D) details of the postgraduate induction course.

(A) Research colloquia, seminars and lectures organised by Durham University Chemistry Department, 1988-1989

(those attended are marked *)

18.10.88 Dr. J. Dingwall (Ciba Geigy), 'Phosphorus-containing Amino Acids: Biologically Active Natural and Unnatural Products'.

18.10.88 Mr. F. Bollen (Durham Chemistry Teachers' Centre), 'The Use of SATIS in the classroom'.

18.10.88 Dr. C.J. Ludman (Durham University), 'The Energetics of Explosives'. (*)

9.11.88 Dr. G. Singh (Teesside Polytechnic), 'Towards Third Generation Anti-Leukaemics'. (*)

16.11.88 Dr. K.A. McLauchlan (University of Oxford), 'The Effect of Magnetic Fields on Chemical Reactions'.

2.12.88 Dr. G. Hardgrove (St. Olaf College, U.S.A.), 'Polymers in the Physical Chemistry Laboratory'. (*)

9.12.88 Dr. C. Jaeger (Friedrich-Schiller University GDR), 'NMR investigations of Fast Ion Conductors of the NASICON Type'.

14.12.88 Dr. C. Mortimer (Durham University Teachers' Centre), 'The Hindenberg Disaster - An Excuse for Some Experiments'.

25.1.89 Dr. L. Harwood (University of Oxford), 'Synthetic Approaches to Phorbols Via Intramolecular Furan Diels-Alder Reactions: Chemistry Under Pressure'.

1.2.89 Mr. T. Cressey and Mr. D. Waters (Durham Chemistry Teachers' Centre), 'GCSE Chemistry 1988: A Coroner's Report'.

13.2.89 Prof. R.R. Schrock (M.I.T.), 'Recent Advances in Living Metathesis' .

15.2.89 Dr. A.R. Butler (St. Andrews University), 'Cancer in Linxiam: The Chemical Dimension'. (*)

22.2.89 Dr. G. MacDougall (Edinburgh University), 'Vibrational Spectroscopy of Model Catalytic Systems'.

1.3.89 Dr. R.J. Errington (University of Newcastle-upon-Tyne), 'Polymetalate Assembly in Organic Solvents'.

9.3.89 Dr. I. Marko (Sheffield University), 'Catalytic Asymmetric Osmylation of Olefins'.

14.3.89 Mr. P. Revell (Durham Chemistry Teachers' Centre), 'Implementing Broad and Balanced Science 11-16'.

15.3.89 Dr. R. Aveyard (University of Hull), 'Surfactants at your Surface'.

20.4.89 Dr. M. Casey (University of Salford), 'Sulphoxides in Stereoselective Synthesis'.

27.4.89 Dr. D. Crich (University College London), 'Some Novel Uses of Free Radicals in Organic Synthesis' (*).

3.5.89 Mr. A. Ashman (Durham Chemistry Teachers' Centre), 'The Chemical Aspects of the National Curriculum'.

3.5.89 Dr. P.C.B. Page (University of Liverpool), 'Stereocontrol of Organic Reactions Using 1,3-dithiane-1-oxides'.

10.5.89 Prof. P.B. Wells (Hull University), 'Catalyst Characterisation and Activity'.

11.5.89 Dr. J. Frey (Southampton University),'Spectroscopy of the Reaction Path: Photodissociation Raman Spectra of NOCl'.

16.5.89 Dr. R. Stibr (Czechoslovak Academy of Sciences), 'Recent Developments in the Chemistry of Intermediate-Sited Carboranes'.

17.5.89 Dr. C.J. Moody (Imperial College), 'Reactive Intermediates in Heterocyclic Synthesis' .

23.5.89 Prof. P. Paetzold (Aachen), 'Iminoboranes XB≡≡NR: Inorganic Acetylenes ?'.

14.6.89 Dr. M.E. Jones (Durham Chemistry Teachers' Centre), 'GCSE and A-level Chemistry 1989'.

15.6.89 Prof. J. Pola (Czechoslovak Academy of Sciences), 'Carbon Dioxide Laser Induced Chemical Reactions - New Pathways in Gas-Phase Chemistry'.

28.6.89 Dr. M.E. Jones (Durham Chemistry Teachers' Centre), 'GCSE and A-level Chemistry 1989'.

11.7.89 Dr. D. Nicholls (Durham Chemistry Teachers' Centre), 'Liquid Air Demonstration'.

(B) Lectures organised by Durham University Chemical Society 1988-1989

(those attended are marked *)

6.10.88 Prof. R. Schmutzler (University of Braunschweig), 'Fluorophosphines Revisited - New Contributions to an Old Theme'.

21.10.88 Prof. P. von Rague Schleyer (University of Erlangen), 'The Fruitful Interplay Between Calculational and Experimental Chemistry'.

27.10.88 Prof. W.C. Rees (Imperial College), 'Some Very Heterocyclic Compounds'.

10.11.88 Prof. J.I.G. Cadogan (B.P. Research), 'From Pure Science to Profit'. (*)

24.11.88 Dr. R.W. Walker and Dr. R.R. Baldwin (University of Hull), 'Combustion - Some Burning Problems'. (*)

1.12.88 Dr. R. Snaith (University of Cambridge), 'Egyptian Mummies - What, Where, Why and How ?' (*)

26.1.89 Prof. K.R. Jennings (University of Warwick), 'Chemistry of the Masses'.

2.2.89 Prof. L.D. Hall (Addenbrookes' Hospital), 'NMR - A Window to the Human Body'. (*)

9.2.89 Prof. J. Baldwin (University of Oxford), 'Recent Advances in the Bioorganic Chemistry of Penicillin'. (*)

16.2.89 Prof. J.B. Aylett (Queen Mary College), 'Silicon-based Chips: The Chemists Contribution'. (*)

23.2.89 Dr. B.F.G. Johnson (University of Cambridge), 'The Binary Carbonyls'.

(C) Conferences attended

(D) First year induction course, October 1988

This course consists of a series of one hour lectures on the services available in the department.

1. Departmental organisation.
2. Safety matters.
3. Electrical appliances and infra-red spectroscopy.
4. Chromatography and microanalysis.
5. Atomic absorptiometry and inorganic analysis.
6. Library facilities.
7. Mass spectroscopy.
8. Nuclear magnetic resonance spectroscopy.
9. Glassblowing technique.

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