CHAPTER

INTRODUCTION.

Collagen is one of the most widely distributed protein of the animal kingdom as it is the major constituent of skin, tendon and connective tissues. Its study is of great practical value, since the chemistry of the action of tanning agents upon collagen forms the basis for leather manufacture. Further the role played by this protein in rheumatic diseases and many disorders of the joints and connective tissues has come to be recognised so widely in medical science, that some of these diseases are now referred to as 'Collagenous diseases'. Hence, a knowledge and understanding of the physical and chemical structure of this protein, and its reactivity towards various chemical reagents are essential for a successful approach to the solution of many problems in industry, biology and medicine. A brief summary of the present state of our knowledge of collagen is given below.

Fibrous nature and solubility:

Of the two types of proteins, fibrous and globular, collagen belongs to the former category with keratin and elastin. Like keratin, it is insoluble in most of the solvents that dissolve globular proteins such as egg albumin,
β-lactoglobulin and insulin. It is, however, partly soluble in aqueous solutions of acids and alkalis and the solubilised collagens are variously called tropocollagen, procollagen, metacollagen, paracollagen etc. Anhydrous formic acid is for a long time known to dissolve it without any appreciable hydrolysis (1) and recently anhydrous mineral acids as HF are found to serve as good solvents for collagen (2).

**Amino acid composition:**

Chemically, collagen is characterised by the presence of two rare amino acids, hydroxy-lysine and hydroxy-proline. Aromatic amino acids such as tyrosine and phenylalanine form only two percent of the molecule. Glycine occurs in a rather high proportion, making up one third of the molecule, proline and hydroxy-proline are only abundant, accounting for nearly one-fourth of collagen. The prominence of the imino-acids, proline and hydroxy-proline, leads to a frequent interruption of the normal sequence of the -CONH- back bone with CON = links whose nitrogen does not contain the hydrogen atom required for the formation of hydrogen bonds (3). Therefore, collagen should normally be lower in its stability. Also, the usual sulphur containing amino acids, cystine and cystine are significantly absent in collagen, while its close analogue, keratine is rich in these amino acids and owes much of its stability to the covalent -S-S- bridges of cystine. It is rather
surprising that inspite of these two inherent shortcomings collagen maintains its stability and structure under a variety of conditions. Probably the expected loss of stability is more than made up by the hydrogen bond postulated by Gustavson (4) between the -OH of the hydroxy proline and the -CO- group in the adjacent peptide chain. Besides the hydrogen bond suggested by Gustavson, the stability of collagen as manifested by its limited swelling and insolubility in water was recently shown to be due to the lateral covalent ester crosslinks formed by the two hydroxyl of hexoses between different peptide chains of collagen (5).

Amino acid sequence studies:

The studies on the amino acid sequence of partial hydrolysates of collagen by Schroeder et al (6) and Kromer et al (7) revealed that every single tripeptide that was isolated contained a glycine residue, and that the sequence -gly-pro-hypro- is of frequent occurrence. Besides indicating the high percentage (33%) of glycine in collagen, these findings seem to indicate that while glycine is distributed rather uniformly, there is a concentration of the pyrrolidine residues in certain regions of the collagen molecule. More recently, Grassmann (5) was reported to have found that the sequence in the dark bands revealed by electron micrographs is not very regular and glycine does not
occur at every position.

End group determinations and molecular weight:

The end group determinations, which have played a large part in the elucidation of the structure of insulin by Sanger, seem to have yielded comparably little information regarding collagen. No $\alpha$-amino or $\alpha$-imino groups were detected by Bowes in native collagen, but after heating at 70°C or treating with alkali or urea a small number of $\alpha$-amino groups was found (8). These observations were interpreted to mean that collagen has a very high molecular weight, of the order of 1 to 2 million.

High angle X-ray studies and triple helical structure:

The basic features of collagen are elucidated by the pioneering X-ray studies of Ramachandran and Co-workers (9,10), Rich and Crick (11), and Cowan, McGavin and North(12). They have shown that collagen molecule is made up of three left handed helical polypeptide chains wound round one another into a right handed coiled-coil major helix. The hydrogen bonds are nearly perpendicular to the fibre axis. The wide-angle X-ray diagrams which led to the above structure show two spacings, one at 2.86Å along the fibre axis which is a distinct feature of collagen alone and of no other protein, and the other at 11Å perpendicular to the fibre
axis. The former spacing indicates the 'repeat unit' of collagen while the latter shows the distance between two sets of triple chains and is found to increase to 16-17 Å in wet collagen. In a model based on these values, the -OH group of hydroxy-proline can easily form a hydrogen bond with a suitable receptor-site in the neighbouring triple-helix.

Recent refinements in triple-helical structure:

Although there is agreement between different workers relating to the broad feature of collagen structure, there seems to be disagreement over minor details. Thus, the structure with two hydrogen bonds for every three residues proposed by Ramachandran (17) was questioned by Rich and Crick (11) because of the occurrence of some short contacts. It has been suggested by the latter that a model with only one hydrogen bond would be more satisfactory. In a recent paper (13), Ramachandran presented more evidence to show that the two bonded structure is highly specific for collagen unlike the one proposed by Rich and Crick. Ramachandran's revised structure shows that the 'repeat unit' or 'residue height' corresponds more closely to 2.95 Å rather than to 2.86 Å. Very recently Harrington et al. (14) supported the single bonded Rich-Crick type of hydrogen bonding in the collagen structure.
Low angle X-ray studies:

In addition to the above data provided by the wide angle X-ray patterns, studies of X-ray diffraction at low angles revealed a series of dark bands separated by less dense inter-bands with a well-defined spacing of about 640 Å along the fibre axis (15,16) in electron micrographs. The available data give no definite evidence as to the chemical origin of these bands and interbands. However, certain indications coming from the staining of collagen with phosphotungstic acid, polymetaphosphate and basic chromium salts point to the accumulation of diaminodicarboxylic acids in the bands (17) investigations made by &Grassmann recently (5) on the amino acid sequence of collagen seems to have confirmed this view by revealing that the dark bands contain more of polar amino acids than occur elsewhere, while the light bands are mainly made up of glycine, proline and hydroxyproline. Further, two types of much larger spacings of the order of 2000-3000 Å were observed by Schmitt et al (18,19) in electron micrographs of reconstituted collagen fibrils and these are usually referred as fibrous long spacing (FLS), and segment long spacing (SLS). They have later suggested that the acid soluble tropocollagen with a particle length of 2000-3000 Å is the common building unit of all three structural forms of collagen observed with the electron microscope (20).
Schmidt et al(5) seem to have obtained evidence to show
that the long spacing segments are built up of parallel
bundles of tropocollagen molecules, whereas, in the fibrils
of native collagen, neighboring molecules are displaced
relative to one another by one quarter of their length.

Physical studies in solution:

Evidence of a three-chain structure for collagen
molecules in solution has been obtained by Boodtker and
Doty(21), who have studied ichthyocol of carp-swim bladder
by sedimentation, viscosity, light-scattering and flow-
birefringence. The molecules of native ichthyocol have
a molecular weight of about 345,000 and are very long thin
structures of 13.6 Å diameter and 3000 Å long. On heat
denaturation, they break up into three sub-units apparently
of unequal length, a finding which fits well with the three-
chain structure proposed from X-ray studies. Further, these
findings indicate the suggestion made by Highton et al(22)
that the protofibrillar particle with a mean length of about
2500 Å may constitute the basic building unit of the collagen
molecule. Recent work of Grassmann(5) on the denaturation
of tropocollagen gave results which are not quite similar to
those found by Boodtker and Doty. These results indicated
that the denaturation of tropocollagen (M.n.360,000) results
in the production of β-collagen of mol.wt.110,000 and
β-collagen of mol.wt.215,000 in equal amounts by causing
multiple breaks in the rod-like tropocollagen.

**Polar groups and chemical reactivity:**

Apart from the limitation imposed by its helical structure the chemical reactivity of a protein is mainly governed by the number and nature of its polar groups, particularly the side-chain amino, carboxyl and hydroxyl groups. Collagen seems to be an ideal protein in this respect as it contains roughly equal proportions of the acidic and basic groups amounting to 0.77 and 0.95 m moles/gm respectively, while the acid-amide and the hydroxyl groups amount to 0.47 and 1.66 m mole/gm respectively (23).

**Techniques for evaluation of reactivity:**

The reactivity of these groups is usually followed in solution by titration-curves which show the amount of acid or base bound at different equilibrium values of pH, or by the dye binding studies. In the case of collagen, different tanning reagents are also used to find out the effect on its reactivity by various chemical modifications.

**Titration-curve:**

The titration curves (24) and dye binding studies (25) show that only about half the number of carboxyl and amino groups become available in the pH range 9-12, and higher
acidity or alkalinity is required for their complete discharge. Various studies (25, 26) have revealed that these groups would become more available in the above pH range if subjected to prior hydrothermal or alkali treatment, but treatments like the latter would often produce drastic effects in collagen (26).

Reversal of titration regions in deaminated collagen:

Since the cationic anionic groups in untreated collagen usually react in the alkaline region of pH, it is to be expected that deaminated collagen will bind correspondingly a smaller number of hydroxyl ions but actually it binds the same amount of alkali as collagen. The amount of acid bound is, however, decreased to the extent that in deamination is effected (27). Therefore, it has to be inferred that in deaminated collagen, the amino groups get titrated with acid. Thus, there seems to be a reversal in the titration regions of the amino and carboxyl groups of deaminated collagen. Such a reversal could have been brought about in the process of deamination. All the same, the changed character of the titration curves remains rather confusing.

Limitations of titration curves:

The effect of other chemical modifications, such as esterification and acetylation cannot be studied in
solution because such modified collagens are likely to undergo hydrolysis under acidic or alkaline conditions. Therefore, the influence of the hydroxyl or carboxyl groups on other groups cannot be evaluated by either titration curves or dye-binding studies. Furthermore, these techniques do not bring out the reactivity of the peptide groups constituting the major fraction of the collagen molecule. The peptide groups being only slightly basic, require much higher concentrations of the acid than those normally used in the titration curves, and even then a correct estimate of the acid binding cannot be made because of the enormous hydration of the protein, the rupture of hydrogen bonds and the possible hydrolysis of the peptide bond itself.

Need for a complimentary technique:

Therefore a technique complimentary to that of titration in solution would be helpful in furnishing the information regarding the reactivity of the peptide bonds and the basic and acidic groups without involving the complications introduced by the presence of water (solvent). The adsorption and desorption curves of volatile acids and bases would provide useful data in this respect, besides verifying the stoichiometry generally assumed in the acid or alkali binding in solution. Moreover the gas and protein
interaction can be studied over a wider temperature range than is possible in solution.

Earlier studies by gas-adsorption technique:

Such a technique was first applied extensively to a number of proteins by Bancroft et al (28) to verify whether compound formation occurred between proteins and HCl or NH₃. They were able to show from phase-rule considerations of the curves representing the gas bound as a function of pressure that in most cases compound formation occurred, whereas it did not occur with NH₃. Consequently, it is to be inferred that stoichiometric reaction of the protein in solution occurs in only one region of the titration curves but not in both acidic and alkaline conditions. A similar study with HCl and NH₃ was made by Belden (29) on gelatin which closely resembles collagen chemically. He concluded that 110 mg or about 3 mole of HCl was bound to 1 gm of gelatin through compound formation while the reaction with NH₃ was only adsorptive. Since the recently available data on the amino acid composition of collagen and gelatin shows that only 0.9 m mole/gm of the basic amino groups is present, the binding of HCl to the extent of 3 m mole does not appear reasonable. Later experiments of Parks and Melavan (30,31) with HCl and NH₃ and hide powder not only showed that the amount of bound HCl at about the same pressure differed, but that compound formation did not occur in both the cases.
Most recently Rao (32) reported the absence of compound formation from his detailed investigation on collagen HCl system.

Studies on protein-gas interaction:

Benson and Coworkers (33-38) working with purified proteins and HCl, NH₃, BF₃ and CH₃NH₂ gases and employing very low pressures (10⁻⁵ mm Hg) for desorption have demonstrated the full potentialities of the gas adsorption technique in elucidating the structural features of the protein molecule from sorption behaviour. They have shown that the sorption of polar gases is characterised by (i) the large up-take of the gas at relatively low pressures which cannot be accounted for by the BET surface area of the protein and therefore involving bulk sorption, (ii) the long intervals of time (from hours, days to weeks depending upon the state of dispersion of the protein) needed for the attainment of equilibrium, (iii) the rapid irreversible sorption by the cationic or anionic groups present on the surface followed by a slow diffusion of the gas into the interior of the protein molecule, (iv) large amounts of heat evolution estimated to be in the neighbourhood of 6-20 kcal/mole, of gas adsorbed and resulting in sudden increases in temperature on sorption (about 12°C for H₂O and 40°C for HCl on egg albumin), (v) a considerable
hysteresis in the desorption isotherm amounting to 10-50% of the sorbed material and (vi) the formation of protein-gas complexes of reproducible composition with NH₃, HCl and BF₃.

**Structural data provided by protein-polar gas interaction:**

The binding of polar gases was also shown to reveal the zwitter ion nature of the protein and the internal hydrogen bonding between different polar groups of the protein(35). Unlike the previous researches, Benson et al showed that in a number of cases, the HCl remaining firmly bound on the protein at pressures of 10⁻⁵ mm (permanently bound HCl) corresponded excellently with the analytical data of the sum of arginine, lysine and histidine contents of the protein.

**Desorption isotherms and stoichiometric compound formation:**

This correlation between the permanently bound HCl and the free amino groups of the proteins seemed to suggest stoichiometric compound formation. But, it was soon found by Rayerson(39) and by Benson(38) that the firmly bound HCl depended markedly upon the temperature in the case of insulin, egg albumin and β-lactoglobulin, the latter two containing almost no HCl at 87°C. Therefore, it appeared quite essential to test the formation of a stoichiometric compound by a different technique which is
capable of giving unequivocal evidence. This was done by Benson and Srinivasan (36) in the egg albumin-HCl system by following the desorption isotherms. This system showed an isobar in the isotherm at about $10^{-2}$ cm Hg at 52°C, thus removing a serious doubt regarding the reality of compound formation posed by the temperature dependence of the permanently bound HCl. Very recently Rao (32) reported the absence of compound formation from a detailed investigation on collagen with HCl. It may be noted here that egg albumin-HCl system seems to be the only one in which stoichiometry is demonstrated by a rigorous procedure.

Some sources of error in protein-polar gas interaction on:

Further, the studies of Benson et al revealed a number of subtle points which often lead to abnormal results and wrong conclusions as was probably the case with earlier workers. For example, if low sorption pressures are employed, the gas may not be able to react with the groups lying in the interior of the molecule and therefore may yield lower values of permanently bound HCl than required for the titration of all the basic groups. In such a case, it would be necessary to demonstrate that the permanently bound HCl did not vary with the sorption pressure before any correlation with the reactive groups of the protein
could be made (34,35). Misleading results may also be obtained if the protein sample under study is not sufficiently well dispersed. In the first case, the sorption itself may be very low and subsequently the gas left on the sample after desorption, also very low. In the second instance, although the total sorption is low, the permanently bound HCl may have a high value and may not show any correlation with the composition of the protein. All these experimental pitfalls have to be carefully guarded against before one can place reliance on the results.

Previous investigations of hide-powder-HCl system and t.-limit data available:

In spite of such detailed investigations on a number of proteins, very little was reported by Benson on collagen. A fairly recent investigation by Green(47,41) also on hide powder and HCl does not give much information about collagen except that it bound 1.5 mole/gm permanently. In fact, the original (i), deaminated (ii), and hypochlorite treated and then deaminated (iii) hide powders were reported to have bound practically the same amount of HCl. This is rather surprising, because hide powder with almost no amino groups left, should not bind any HCl strongly. Green has not explained this discrepancy but made a new suggestion that the hydroxyl groups of serine and threonine residues of hide powder react with HCl to eliminate water.
Most recent investigations (32, 32 A) in our laboratory have shown that the sorption of HCl (pressure is 47 cm Hg for all experiments) by various physically and chemically modified collagens occurs essentially on side chain amino, hydroxyl, carboxyl and amide groups and the imide residues of proline and hydroxyproline in collagen. At 80°C, however, only the polar side chains are active in sorbing HCl. Evidence was presented in the case of hydrothermally shrunk collagen to indicate the presence of activated sorption at 80°C. Unlyophilised samples of collagen have been demonstrated to possess low sorption and desorption rates, and larger amounts of firmly bound HCl in comparison with the behaviour of lyophilised samples. Furthermore, the stoichiometric compound formation between HCl and collagen was found to be absent from the desorption isotherms at different temperatures (29 to 80°C).

Need for further investigation of collagen-HCl sys m:

However, in the above investigations, the effect of lyophilisation on the sorption of HCl over deaminated collagen has not been examined. Furthermore, it is important to note that no work has so far been reported on the finely dispersed, spray frozen samples of collagen.
in order to understand the reactivity of this protein towards HCl is affected by the dispersion of the collagen sample. A detailed study of the various aspects of HCl interaction with collagen has therefore been undertaken.

Scope of the thesis:

The thesis consists of two parts. The first part deals with the interaction of HCl with unmodified and modified collagens and the second part is devoted to the reaction of water vapour with several collagens and polyimino acids, poly-L-proline and poly-L-hydroxyproline. These polyimino acids have essentially the same conformational pattern as that of collagen.

This part of the investigation concerning the interaction of HCl with collagen is primarily intended at elucidating the effect of the dispersion of the protein sample on (i) the kinetics of gas uptake by deaminated collagen, (ii) the quantity of firmly bound HCl by the deaminated collagen, (iii) the influence of temperature on the reversibly and irreversibly bound HCl by unmodified collagen at different temperatures 29°, 40°, 60°, and 80°C, (iv) the formation of a stoichiometric compound between collagen and HCl (through the determination of the desorption isotherms in the temperature interval 29-80°C). As mentioned
earlier, in all of these studies, finely dispersed spray frozen protein samples were used. Whereas in the studies of earlier workers the collagen or hide powder was at best, only lyophilised. Moreover, for the first time sorption isotherms of H₂O on collagen were determined at several temperatures, 29⁰, 40⁰, 60⁰ and 80⁰C in the pressure range, 0.5 to 40 cm Hg and the differential heats of sorption evaluated.

Studies on collagen-water vapour interaction:

The studies on the adsorption of water vapour on collagen are of great value to the leather industry as collagen is the basic material for leather. The results obtained in various laboratories on water vapour sorption by proteins are summarised below.

Kanagy (42) studied the water vapour adsorption on collagen and leathers. The results were analysed by means of the BET equation. The heats of adsorption for collagen as well as for leathers were also determined and found to be higher for leathers than for collagen, presumably due to the interaction of water with the tanning agents. On the other hand, Nekryach (43) states that tanned hide powder exhibits a lower heat of wetting.
water sorption by modified collagen:

In general, materials that have a high percentage of hydrophylic groups give the highest heats of wetting. The experiments of Kanag and Cassel (44) show that the heats of wetting are lower for modified collagens than those for original collagen. The observation by Green and Ang (45) that the \( v_\text{m} \) (monolayer adsorption) value decreases when collagen is acetylated also proves this point.

water vapour sorpt on on polar side chains of amino acids and pro elin:

Mollon et al. (46) correlated the water vapour sorption on glycine peptides to the water vapour sorption on casein and zein. According to them the peptide groups appear to be responsible for about 45% of water vapour absorption by Casein and 77% of the absorption by Zein. Sorption of water vapour on amino acids like glycine and leucine suggested Frey and Moore (47) that the adsorption of water appeared to occur primarily on the ionic COO\(^-\) and NH\(_3^+\) groups. Investigations by Grassmann et al. (48) on the water vapour sorption by collagen and silk have shown that the absorption occurs on polar groups of the skin, such as peptide bonds, free carboxyl and amino groups.

App ication of NFT theory:

The experimental results obtained by Bull (49) on a series of proteins like collagen and salamin were
satisfactorily explained by the Brunauea, Emmet and Teller equation. He believed that the data obtained are consistent with the view that protein molecules in the solid state are linked together to form coherent planes whose exposed surfaces are hydrophilic and water is adsorbed in between these planes. He has also reported the thermodynamic changes of the adsorption of water vapour by proteins. From Bull's data the number of molecular layers formed were calculated to be 2 by Dunford and Morrison (50).

**Studies of water vapour sorption by using X-ray techniques:**

With the help of sophisticated X-ray diffraction techniques, Zlides (51) finds from his studies of water vapour sorption on collagen that the kink at 70% humidity corresponds to an uptake of 27 gm of water vapour for 100 gm of collagen. This is explained with the assumption that each peptide bond and each CO group takes up a molecule of water. Further information is given by the investigations of Esipova et al.(52) using the same technique on the water vapour sorption on collagen and procollagen. They suggest that water can form a bridge between CO groups giving rise to continuous chains of structurally incorporated water along the fiber axis in the diffracting regions.

The X-ray work of Hougview and Bear (53) on water vapour sorption by kangaroo tail tendon reveals that the
equatorial spacing and the macroperiod of collagen increase with increasing water sorption from 17.6 to 14.6 Å and from 603 to about 670 Å respectively. The initial water sorption by dry collagen involves primarily the fibrillar bands, at which the axes of the roughly parallel helical polypeptide chains straighten. Next the polar groups located in the interbands become hydrated and the chains begin to separate laterally in these regions. These processes continue with multilayer sorption of water and further chain straightening and separation.

Several years later, Burge et al. (54) reconsidered some of the hydration data of Rougvie and Bear and pointed out that since only one-third of the carboxyl oxygens and peptide nitrogens of this structure are involved in hydrogen bonding within the three-chain collagen unit, a considerable number of polar groups are left unbonded. However, they pointed out that the experimental value is close to that calculated for one water molecule per polar side chain plus one for every two carboxyl groups.

Bradbury et al. (55) calculated the diffraction pattern expected from collagen II with water molecules singly bonded in every possible systematic position along the chains and found that this amounts to approximately 25 gm of water per 100 gm of collagen.
Additional informations are provided by the infrared measurements about hydration and hydrogen bonding in collagen. The rate of deuteration of ratskin collagen suggested Bradbury (55) that there are three groups of labile protons differing in rate of exchange. The most rapidly exchangeable group was attributed to partially degraded portions of the specimen, the second group to labile protons on side chains and N-H groups not involved in N-H --- O = C - hydrogen bonding. Very slowly exchanging protons were assigned to N-H groups involved in interchain hydrogen bonding.

The investigation of hydrated collagen was conducted by Fraser and Machae (56) who concluded on the basis of infrared dichroic measurements that the bound water molecules are singly bonded to the -C = O groups which project radially outward from the collagen molecule.

Work on the isothermal adsorption of water vapour on proteins:

Isothermal work on water vapour adsorption has been done on number of proteins like wool keratin (57-59), egg albumin (60-62) and gelatin (63). Interesting information was gathered from the above studies for instance, the water vapour absorption isotherms on wool keratin (57) after various chemical modifications indicate that the disruption of the
fibers has little effect at low and intermediate humidities, thus the lower limit of the solution region has been set at 80% relative humidity.

In the studies of wool water sorption by Jeffries (58), the amount of sorption was found to decrease with the rise of temperature, in agreement with the findings of Nicholls and Speakman (59). He reports that the relative hysteresis for wool decreases markedly with the temperature.

Benson et al (67) have investigated the water vapour sorption on globular proteins by studying the isotherms at different temperatures. They also found that \( \text{H}_2\text{O} \) sorption decreases with temperature as was observed for wool by Jeffries (58). The hysteresis loops in these isotherms were shown to be a function of the total amount of water sorption.

Altman and Benson (61) have demonstrated from the water vapour isotherms of denatured egg albumin that the size of the hysteresis loop decreases with increasing temperature disappearing almost completely at 100°C. Also, they showed that between 70 and 100°C, denatured albumins exhibit no hysteresis above a relative pressure of 0.7. Since the isotherms obtained on steam, heat and alcohol denatured egg albumins were significantly different both at 25°C and 40°C, they concluded that the denaturation cannot be unique but
it is dependent on the procedure employed.

Seehof et al (62) proposed that the hysteresis is associated with binding on the free basic groups of the protein, since an excellent correlation between the number of basic groups and the maximum amount of hysteresis was observed. On the other hand, with all the knowledge on the reproducibility of hysteresis loops, Hao et al (63) find that the hysteresis on gelatin varies with the number of adsorption-desorption runs.

Previous studies of H$_2$O sorption on collagen have provided information regarding the heats of sorption, hysteresis etc. However, the interpretations have not been supported by studies on model peptides or polypeptides related to collagen.

Need for investigation on the collagen-water system:

Although the general features of the interaction of water vapour with collagen are known from the previous studies of other workers, interpretation of the results has been based largely on assumptions due to the paucity of the much needed information on model polypeptides. Since collagen contains an unusually large percentage (about 20%) of proline and hydroxyproline residues, poly-L-proline-II and poly-L-hydroxyproline have been
chosen as the model polypeptides in the present study.

Furthermore, no systematic study of the effect of dispersion of collagen or its hydration properties has been made previously. Therefore, native, lyophilised and spray frozen samples of collagen are used in this investigation.

Scope of the thesis:

This part of the work deals in understanding the nature of water vapour sorption on the protein by studying (i) the water vapour sorption on poly-L-proline-II, (ii) the strength of water molecule the bonding to hydroxyl group and the neighbouring carboxyl group of poly-L-hydroxyproline, (iii) the isotherms of water vapour on native collagen at 30°, 40° and 60°C, (iv) the isotherms of water vapour on lyophilised collagen at various temperatures, (v) the effect of spray freezing the sample on water sorption at 30°, 40° and 60°C. It should be mentioned here that the emphasis is given to the initial adsorption of water vapour on the proteins and polypeptides to relate the water of hydration of the polypeptides with that of collagen.
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