CHAPTER II

DISTRIBUTION OF INORGANIC SALTS ON WOOL FIBRE

The properties of proteins must be attributed, ultimately, to their structure and the interaction of elements of this structure with the environment. Since, proteins are made up of amino-acids, the dissociation of their constituent amino acids may illuminate their mode of linkage and the nature of modifications they undergo during the formation of complex structures. Conversely, the study of dissociation of proteins may indicate the extent of acidic and basic groups in the native structure.

The dependence of the extent of dissociation on pH not only gives the numbers, but also, the equilibrium constants of various types of dissociating groups and thus helps in the investigation of protein structures. Discrepancies between the dissociation constants of proteins and those of the corresponding amino acids have been interpreted (136) in terms of -

(a) The changes in molecular polarity associated with the formation of the peptide bond,
(b) the electrostatic interactions of dissociable groups, which may alter their acidity, and

(c) the combination with ions other than hydrogen ion, which may change the electrostatic condition of the proteins and affect the hydrogen ion equilibrium, with a resultant shift in the titration curve. Such shifts afford an insight into the structure of the proteins, particularly, where the site of a highly specific combination with an ion can be inferred from them.

2.1 THE TITRATION CURVE OF WOOL

Each amino acid has its characteristic pK values of its carboxyl and amino groups. Hence, pK values of any acid on the titration curve defines the nature of the acid. In a mixture of amino acids, each one is titrated in its own pH range. If the pK values of the constituents of the mixture are far apart, the regions of titration are distinct.

Wool keratin contains in addition to carboxyl and amino groups, imidazole group (pK 6.0) of histidine, guanidyl group (pK 12.45) of arginine and phenolic group of tyrosine. The titration of these groups tend to mask the titration of carboxyl (pK 2.34) and amino group (pK 9.6), rendering an indistinct curve. Other parts of the protein molecule, such as peptide groups, also possess acid and basic properties, but they are not titrated within the range of pH 1.5 and 12.0. At
any pH value, many of these groups will be in their charged form. Since, electrostatic effects are long range effects, the course of titration of any one group will be influenced by the others.

From the pK values of various dissociating groups, it is clear that the charges on the protein will be positive at low pH values and negative at high pH values. Since, positive charges repel protons and negative charges attract them the pK values are reduced at low pH values and raised at high pH values. The electrostatic forces affect all groups alike. Hence, the difference in pK value between one group and the other at any pH, remains the same. Because of the variation in pK (maximum 1.5), the titration curve for any group will be broader than that of a monobasic acid. In wool keratin, due to the closeness of pK values of different dissociating groups, a continuous curve results, as shown in Fig. 5.

The titration curve has got three distinctive regions:

(a) Between pH 5 and 9, we find a plateau of no acid or base binding region. Though histidine is titrated in this region, the curve is parallel to the pH axis.

(b) Below pH 5, the curve sharply falls to a limiting value. The curve levels off at around pH 1.5, giving a maximum acid binding capacity. The positive charge on the protein depends on the number of hydrogen ions bound. At very low pH, protein attains maximum net positive
charge, when all dissociable groups of the protein exist in their acidic forms, either electrically neutral or positively charged as in the case of imidazolium, ammonium and guanidinium groups. Between pH 2 and 5.5, only carboxyl groups dissociate. This acidic part of titration curve is not affected by temperature.

(c) At higher pH values, the maximum base binding capacity is observed. The protein attains more and more negative charge as pH increases. At this stage, all acid groups are in ionized form. Ammonium, phenoxyl, sulphydryl and guanidinium groups dissociate above pH 8. This alkaline region of dissociation curve is temperature dependent. Proteins swell at higher pH values. Hence, the alkaline region of curve differs from theoretical and it is not possible to locate the maximum base binding capacity.

Titration curves too depend upon the ionic strength and dielectric constant of the medium. As ionic strength increases, the slope of the curve increases to a limit, which lessens the electrostatic repulsion. If one of the ions binds to protein, because of decrease in net charge, the slope increases.

Since, the amino groups are present as internally ionized ammonium like salts, the position and form of the acid titration curves are determined by the carboxyl groups of the protein.
2.2 IONIC AND ISOELECTRIC POINTS

Wool attains a maximum net positive charge at very low pH and a maximum net negative charge at very high pH. At some pH in between, protein contains an equal number of positive and negative charges. The net charge will be zero, provided only $H^+$ and $OH^-$ are bound to protein. This is called Isoelectric Point. If ions other than $H^+$ and $OH^-$ are bound, the net charge is affected. This led to the concept of Iso-ionic Point. The isoionic point involves only acidic and basic properties of the protein, whereas, iso-electric point refers to the total net charge. These two are defined as:

**Iso-Electric Point**

(i) The pH at which the net electric charge of a protein is zero\(^{(138)}\).

(ii) The pH at which no net migration of the protein is observed, when the solution is subjected to an electric field\(^{(139)}\).

**Iso-Ionic Point**\(^{(140)}\)

(i) The pH of a protein solution which does not change on addition to iso-ionic protein.

(ii) The pH of an iso-ionic protein solution in water or in a solution of mother solute, which does not produce $H^+$ or $OH^-$, when dissolved in water alone.

(iii) The hydrogen ion activity at which the specific hydrogen ionization of the ampholyte is zero.
Although, the iso-ionic and iso-electric points of some soluble proteins are the same, for insoluble proteins, they are quite far apart, e.g., for wool, pH 5.0 and 3.40 respectively.

2.3 THEORIES OF COMBINATION OF IONS WITH WOOL

2.3.1 Steinhardt-Harris Theory (133, 137, 141-144)

Steinhardt and co-workers studied the acid combination of wool as a function of pH, (i) at different ionic strength, in absence of salt, and (ii) at a constant ionic strength in presence of salt. They observed that with increase in chloride ion concentration, the acid side of the curves is shifted to higher pH values. The maximum acid binding capacity is 0.82 millimoles/gram and maximum base binding capacity is greater than 0.78 millimoles/gram.

To derive an expression for the acid sorption as a function of pH, they assumed that -

(i) both $H^+$ and $Cl^-$ combine with wool proteins according to the law of mass action,

(ii) hydrogen ion concentrations are the same inside and outside the fibre, and

(iii) the variation in the activity coefficients is insignificant in the fibre phase.
The equilibrium reactions are,

\[ \text{W}^- + \text{H}^+ \rightleftharpoons \text{WH}^+ \quad K_H = \frac{[\text{WH}^+]}{[\text{W}^-][\text{H}^+]} \quad \ldots \quad (1) \]

\[ \text{W}^- + \text{A}^- \rightleftharpoons \text{WA}^- \quad K_A' = \frac{[\text{WA}^-]}{[\text{W}^-][\text{A}^-]} \quad \ldots \quad (2) \]

\[ \text{WH}^+ + \text{A}^- \rightleftharpoons \text{WHA} \quad K_A = \frac{[\text{WHA}]}{[\text{WH}^+][\text{A}^-]} \quad \ldots \quad (3) \]

\[ \text{WA}^- + \text{H}^+ \rightleftharpoons \text{WHA} \quad K_H' = \frac{[\text{WHA}]}{[\text{WA}^-][\text{H}^+]} \quad \ldots \quad (4) \]

Therefore, \( K_H \cdot K_A = K_H' \cdot K_A' \) \ldots \quad (5)

Depending on the low and high affinity of \( \text{A}^- \) for the fibre, i.e., for (i) \( [\text{WH}^+] > [\text{WA}^-] \) and (ii) \( [\text{WH}^+] < [\text{WA}^-] \), the mole fraction \( \theta \) of the ion bound is given respectively by

\[ \theta = \frac{[\text{WHA}]}{[\text{WHA}^+] + [\text{WH}^+] + [\text{WA}^-] + [\text{A}^+]} = \frac{1}{1 + \frac{K_H' \left( \frac{a_A}{a_A + K_A} \right)}{\frac{a_A}{a_A + K_A}}} \quad \ldots \quad (6) \]

\[ \theta = \frac{[\text{WHA}]}{[\text{WHA}^+] + [\text{WH}^+] + [\text{WA}^-] + [\text{A}^+]} = \frac{1}{1 + \frac{K_A \left( \frac{a_H}{a_H + K_H} \right)}{\frac{a_H}{a_H + K_H}}} \quad \ldots \quad (7) \]
where $a_H$ and $a_A$ are the activities of $\text{H}^+$ and $A^-$ respectively in solution.

When $\Theta = 0.5$ i.e., the mid-point corresponding to half saturation, the pH is denoted by $\text{pH}_\theta$. At low ionic strength, when $\Theta = 0.5$ and $K'_A \gg a_A \gg K_A$, equation (5) simplifies to

\[ \text{pH}_\theta \approx \text{pK}_H' + \log a_A - \log K'_A \quad \ldots \quad (8) \]

This gives a relationship between $\text{pH}_\theta$ and anion concentration. From equation (5),

\[ \frac{\Theta}{1 - \Theta} = \frac{1}{K_H' \left[ a_H + a_A + K'H \right]} \quad \ldots \quad (9) \]

When $K'_A \gg a_A \gg K_A$,

\[ \frac{\Theta}{1 - \Theta} \approx \frac{a_H \cdot a_A}{K_A \cdot K'_H} \quad \ldots \quad (10) \]

\[ \therefore \log \frac{\Theta}{1 - \Theta} \approx -\text{pH} + \log a_A - \log (K'_A \cdot K_H') \quad \ldots \quad (11) \]

In absence of salt, $a_H = a_A$, therefore,

\[ \log \frac{\Theta}{1 - \Theta} \approx -2\text{pH} - \log (K'_A \cdot K_H') \quad \ldots \quad (12) \]

From equations (11) and (12), the plot of $\frac{\Theta}{1 - \Theta}$ against pH should be a straight line with slopes -1.0 and -2.0 respectively. But the experimental slopes are -0.5 and -1.0 respectively. This two-fold error in the slopes was attributed to the existence of a continuous range of $\text{pH}$ values, resulting from the electrostatic effects of ionized
carboxyl groups. The authors introduced square-roots of activities and dissociation constants without theoretical explanation, to account for this.

Peters and Speakman(145) criticized this theory on two features:

(i) The assumption that both anions and cations react at a single site, such as salt-linkage, and the rupture of salt-linkage by an ion;

(ii) In experiments involving hydrolytic and catalytic effects of hydrogen, hydroxyl and other ions, the internal solution in equilibrium with both the solid phase as well as the external solution phase, cannot be ignored, and

(iii). The theory neglects the variation in activity of water inside the fibre.

2.3.2
Gilbert-Rideal Theory(145,147)

This is a thermodynamic approach for the interpretation of acid absorption. The assumptions are:

(i) Cation and anion are bound unequally from very dilute solution of acid until the resulting potential on the fibre is such that the overall affinities of cation and anion for the fibre are equal.

(ii) The excess of hydrogen ions bound to the fibre is experimentally insignificant, i.e., $H^+ \approx A^-$. 
(iii) An anion is free to occupy any free positive site and the freedom is not affected by its position relative to charged or uncharged carboxyl groups.

(iv) The potential, $\psi$, of the fibre is assumed to be constant and uniform over the whole fibre.

According to Fowler and Guggenheim (148), the chemical potential $\mu$ of an uncharged substance distributed at random amongst a limited number of similar sites is given by,

$$
\mu = \mu^0(T,P) + RT \ln \frac{\theta}{1 - \theta} \quad \ldots \quad (13)
$$

where $\theta$ is the fraction of sites occupied and $\mu^0$ is the value of $\mu$ at constant temperature and pressure when $\theta = 0.5$. Since, the potential, $\psi$, of the fibre is uniform and constant over the whole fibre, the chemical potential of $H^+$ within the fibre is,

$$
\mu_{H}^{\text{fibre}} = \mu_{H}^0(T,P) + RT \ln \frac{\theta_H}{1 - \theta_H} + \psi_F \quad (14)
$$

and in the solution, is,

$$
\mu_{H}^{\text{sol.}} = \mu_{H}^0(T,P)_{\text{sol.}} + RT \ln a_H \quad \ldots \quad (15)
$$

At equilibrium, these two potentials are equal. Therefore, on substitution and rearrangement, we have,

$$
RT \ln \frac{\theta_H}{1 - \theta_H} = -(\mu_{H}^0_{\text{fibre}} - \mu_{H}^0_{\text{sol.}}) + RT \ln a_H - \psi_F \quad (15)
$$

Similarly, for a monovalent anion,

$$
RT \ln \frac{\theta_A}{1 - \theta_A} = -\Delta \mu^0_A + RT \ln a_A + \psi_F \quad \ldots \quad (17)
$$
On addition equations (15) and (17),
\[
RT \ln \frac{\theta_H \phi_A}{(1 - \theta_H)(1 - \phi_A)} = -(\Delta \mu_H^0 + \Delta \mu_A^0) + RT \ln \frac{a_H a_A}{a_H a_A} + RT \ln \frac{a_H a_A}{a_H a_A} (18)
\]
Assuming the total number of acidic and basic groups are equal and equivalent amount of anions and cations are sorbed by wool, we have at any time \(\theta_H = \theta_A\). Therefore, equation (18) becomes,
\[
\ln \frac{\theta_H}{1 - \theta_H} = \frac{-(\Delta \mu_H^0 + \Delta \mu_A^0)}{2RT} + \frac{1}{2} \ln \frac{a_H a_A}{a_H a_A} \ldots (19)
\]
For pure acid, \(a_H = a_A\) and hence,
\[
\log \frac{\theta_H}{1 - \theta_H} = \frac{-(\Delta \mu_H^0 + \Delta \mu_A^0)}{4.5052 RT} - \text{pH} \ldots (20)
\]
and at constant anion concentration,
\[
\log \frac{\theta_H}{1 - \theta_H} = \frac{-(\Delta \mu_H^0 + \Delta \mu_A^0)}{4.5052 RT} - \frac{1}{2} \text{pH} + \log a_A \ldots (21)
\]
For equations (20) and (21), the plot of \(\log \frac{\theta_H}{1 - \theta_H}\) against pH gives the slopes of \(-1.0\) and \(-0.5\) respectively, which are in agreement with experiments.

At low ionic strength, when \(\theta_H = 0.5\), \(\text{pH} = \text{pH}_m\) and hence, equation (21) will be,
\[
\text{pH}_m = \frac{-(\Delta \mu_H^0 + \Delta \mu_A^0)}{4.5052 \text{ RT}} + \log a_A \ldots (22)
\]
Since equation (22) is derived only for low ionic strength solutions, the plot of \(\text{pH}_m\) against salt concentration does not give any limiting value for \(\text{pH}_m\) displacement.
From equation (16) and (17), the expression for potential $\psi$ is,

$$
\psi = -\frac{(\Delta \mu_H^0 - \Delta \mu_A^0)}{2F} + \frac{RT}{2F} \ln \frac{a_H}{a_A}
$$

(23)

Since, $\psi$ is assumed to be constant, Peters(149) observed that the experimental values of \((\Delta \mu_H^0 + \Delta \mu_{Cl}^0)\) and \((2 \Delta \mu_H^0 + \Delta \mu_{SO_4}^0)\) vary with pH. That is, the data cannot be accommodated by a single dissociation constant(150). The variation of $\psi$ in different parts of the fibre may result in the variation of the affinity at different pH values. Hence, the plot of $\log \frac{\theta_H}{1 - \theta_H}$ against pH may be sigmoid in character.

Gilbert-Rideal theory too neglects the variation in the activity of water inside the fibre. Horner's(151) theoretical titration curve, based on amino acid analysis, fits better with Donnan theory than Gilbert-Rideal theory.

2.3.3
Peters-Speakman ÒR Donnan Theory(145)

This theory is based on the concept of Donnan membrane equilibrium. Peters and Speakman assume that:

(i) the external solution is in equilibrium with an internal solution,

(ii) the fibre has a constant potential $\psi$, distributed over the whole internal volume $v$. Hence preferential adsorption of $H^+$ at low concentration takes place.
(iii) equal number of anions and cations enter into the fibre, thereby maintaining the potential $\psi$ constant.

The two phases are represented as:

<table>
<thead>
<tr>
<th></th>
<th>Internal phase</th>
<th>External phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total binding capacity</td>
<td>$A$</td>
<td>$-$</td>
</tr>
<tr>
<td>COOH</td>
<td>$A\Theta$</td>
<td>$-$</td>
</tr>
<tr>
<td>COO$^-$</td>
<td>$A(1 - \Theta)$</td>
<td>$-$</td>
</tr>
<tr>
<td>NH$_3^+$</td>
<td>$A$</td>
<td>$-$</td>
</tr>
<tr>
<td>H$^+$</td>
<td>$h$</td>
<td>$H$</td>
</tr>
<tr>
<td>$X^{2-}$</td>
<td>$x$</td>
<td>$X$</td>
</tr>
<tr>
<td>Volume</td>
<td>$v$</td>
<td>$V$</td>
</tr>
</tbody>
</table>

All terms indicate the molar concentrations of respective ions and $\Theta$ is the fraction of wool combined with protons.

According to Donnan-Guggenheim(152) equation, the electrochemical potential of H$^+$ in external and internal solutions is given by,

$$\mu_H = \mu^C_H(T) + RT \ln H + RT \ln \frac{1}{b_H} + pV_H \quad \ldots \quad (24)$$

$$\mu_h = \mu^C_h(T) + RT \ln h + RT \ln \frac{1}{b_h} + p\tilde{V}_h + \psi F \quad \ldots \quad (25)$$

where, $b$ is the activity coefficient, $\tilde{V}$ is the partial molar volume at pressure equal to the mean pressure of two phases.
At equilibrium, assuming \( \mu^O_H(T) = \mu^O_h(T) \) and \( \bar{v}_H = \bar{v}_h \), we get

\[
RT \ln \frac{H}{h} \cdot \frac{\bar{v}_H}{\bar{v}_h} + \bar{v}_H (P - p) = \psi \cdot F \quad \ldots\ldots (26)
\]

If the activity of water in two phases is \( w \) and \( W \), and the partial molar volume is \( \bar{v}_w \) at a pressure equal to \( \frac{P + p}{2} \), then,

\[
RT \ln W + p\bar{v}_w = RT \ln w + p\bar{v}_w \quad \ldots\ldots (27)
\]

\[
\therefore \quad \frac{RT}{V} \ln \frac{w}{W} = (P - p) \quad \ldots\ldots (28)
\]

Hence, equation (26) will be

\[
RT \ln \frac{H}{h} \cdot \frac{\bar{v}_H}{\bar{v}_h} + RT \frac{\bar{v}_H}{\bar{v}_w} \ln \frac{w}{W} = \psi \cdot F \quad \ldots\ldots (29)
\]

Let, \( \frac{\bar{v}_H}{\bar{v}_w} = r_H \), So, equation (29) will be

\[
\frac{H}{h} \cdot \frac{\bar{v}_H}{\bar{v}_h} \left[ \frac{w}{W} \right]^{r_H} = e^{\psi F / RT} = \lambda \quad \text{(constant)} \quad \ldots\ldots (30)
\]

Similarly, for polyvalent \( X^{z-} \), the expression is

\[
\frac{x}{X} \cdot \frac{\partial X}{\partial x} \left[ \frac{w}{w} \right]^{r_X} = \lambda^z \quad \ldots\ldots (31)
\]

Or, \( \frac{x}{X} \cdot \frac{\partial z}{\partial x} = \lambda^z \) where \( \frac{\partial z}{\partial x} = \frac{\partial x}{\partial x} \left[ \frac{w}{w} \right]^{r_X} \quad \ldots\ldots (32)\]

Since both phases should be electrically neutral, we have,

in the internal phase \( \lambda^h + h = \lambda X \quad \ldots\ldots (33) \)

and in the external phase \( H = \lambda X \quad \ldots\ldots (34) \)
The amount of acid combined is determined by the difference in the original and final or equilibrium value of the external solution. Therefore, the amount of acid combined, \( a \), is given by

\[
a = v(AQ + h) + VH - (V + v)H = v(AQ + h - H)
\]

From equation (33) and (3b),

\[
a = v(zX - zX)
\]
or

\[
\frac{a}{zv} = (x - X)
\]

From equation (32),

\[
\frac{a}{zv} = X \left[ \frac{X}{2z} - 1 \right]
\]

In the absence of salt, \( H = zX \)

\[
\therefore \frac{a}{zv} = \frac{H}{z} \left[ \frac{X}{z} - 1 \right]
\]

Substituting the value of \( X \) in terms of \( H \) and \( h \) from equation (30), and on simplification we get,

\[
pH_{\text{int}} = \frac{z+1}{2} pH_{\text{ext}} + \frac{1}{z} \log \left[ \frac{a}{V} + H \right] + \frac{1}{z} \log \left[ \frac{a}{V} \right] - \log \left[ \frac{W}{V} \right] \quad (39)
\]

Since, the activity coefficients of \( H^+ \), \( X^2^- \) and water are assumed to be identical inside and outside the fibre, the last two terms of equation (39) reduce to zero.

\[
\therefore pH_{\text{int}} \approx \frac{z+1}{2} pH_{\text{ext}} + \frac{1}{z} \log \left[ \frac{a}{V} + H \right] \quad (40)
\]
In presence of salts at low ionic strength, the equation will be

\[ pH_{\text{ext}} = pH_{\text{int}} + \log X - \log \left( \frac{a}{v} + H \right) \]  \hspace{1cm} (41)

Since \( H \) is small compared to \( \frac{a}{v} \) in equations (40) and (41), \( H \) can be neglected.

At low ionic strength, when \( \Theta = 0.5 \), we have the relationship between \( pH \) and \( X \) as

\[ pH_{\%} = \log X + \text{constant} \]  \hspace{1cm} (42)

The experimental results are in good agreement with these equations. But, this theory is unable to account for the different anion affinities. It predicts zero value for \( \log k \), whereas Gilbert-Rideal theory gives various values. In fact, the value of \( \log K \) is not zero.

Though Peters was not ready to accept the different anion affinities for wool, the works of Steinhardt et al. (144), Larose-Donovan (153), and Wright (154) convinced him to accept it. This led to a modified theory called 'Generalized Theory of Peters' (155).
2.3.4
Generalized Theory of Peters(155)

The charged components of the system are:

<table>
<thead>
<tr>
<th>Internal Concentration</th>
<th>External Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-\text{NH}_3^+)</td>
<td>(\text{B}^+/\nu)</td>
</tr>
<tr>
<td>(\text{H}^+)</td>
<td>(h)</td>
</tr>
<tr>
<td>(\text{M}^{3+})</td>
<td>(m)</td>
</tr>
<tr>
<td>(\text{A}^-)</td>
<td>(x)</td>
</tr>
<tr>
<td>(\text{OH}^-)</td>
<td>(\frac{K_w}{h})</td>
</tr>
<tr>
<td>(-\text{COO}^-)</td>
<td>(\text{A}^-/\nu)</td>
</tr>
</tbody>
</table>

In a space of reasonable electric capacity, the concentration of anion is approximately equal to that of cations, without causing a large potential(155). Therefore,

\[
\frac{\text{H}^+}{\nu} + h + m = x + \frac{K_w}{h} + \frac{A^-}{\nu} \quad \ldots \quad (43)
\]

and \(H + M = X + \frac{K_w}{\text{H}} \quad \ldots \quad (44)\)

There exists a potential difference, \(\psi\), between the phases such that equations (43) and (44) are valid.

According to Donnan-Guggenheim(152), if

\[
\lambda = e^{-\frac{\psi F}{RT}} = \text{a constant} \quad \ldots \quad (45)
\]

we have,

\[
\lambda = \frac{h}{\text{H}} = \frac{m}{\text{M}} = \frac{x}{\text{X}} \quad \ldots \quad (45)
\]
Therefore, equation (43) and (44) will be

\[
\frac{B^+}{v} + (H + M) = \frac{1}{\lambda} \left[ X + \frac{K_v}{H} \right] + \frac{A^-}{v}
\]

\[
\therefore \left[ \frac{1}{\lambda} - \lambda \right] (H + M) = \frac{B^+ - A^-}{v} \quad \ldots \quad (47)
\]

On substituting the value of \( \lambda \) and simplification, we get,

\[
\text{Sinh} \left( \frac{\psi F}{RT} \right) = \frac{B^+ - A^-}{2v(H + M)} \quad \ldots \quad (48)
\]

by equating \( \frac{-\text{COO}^-}{\text{COOH}} = \frac{K_h}{h} \) and \( \frac{-\text{COO}^-}{\text{COOH}} = \frac{K_m}{m} \)

the total number of carboxyl groups \( A \) can be expressed in terms of charged carboxyl groups \( A^- \) as

\[
A = \left[ 1 + \frac{h}{K_A} + \frac{m}{K_m} \right] A^- \quad \ldots \quad (49)
\]

Similarly, from

\[
\frac{-\text{NH}_2^-}{-\text{NH}_2^+} = \frac{K_B}{h} \quad \text{and} \quad \frac{-\text{NH}_2^-}{-\text{NH}_2^+} = \frac{K_x}{x}
\]

We have, \( P = \left[ 1 + \frac{K_B}{h} + \frac{x}{K_x} \right] B^+ \quad \ldots \quad (50) \)

The amount of acid combined 'a' can be regarded as the one which has combined with -COO\(^-\) group or liberated from -NH\(_3^+\).

Therefore,

\[
a = \left( -\text{COOH} \right) = \left( -\text{NH}_2 \right)
\]

\[
= \frac{H \cdot A^-}{K_A} = \frac{K_B \cdot B^+}{\lambda \cdot H} \quad \ldots \quad (51)
\]
Hence, on acid side,

\[
\frac{1}{\lambda} = \left[ \frac{A - a}{a} \right] \frac{H}{K_A} - \frac{M}{K_m} \quad \ldots \quad (52)
\]

and on alkaline side

\[
\lambda = \left[ \frac{B + a}{-a} \right] \frac{K_B}{K_B} - \frac{X}{K_X} \quad \ldots \quad (53)
\]

With the help of these equations \( \lambda \) and hence \( v \) can be calculated.

2.4 PROTEIN-METAL COMPLEXES

Proteins have amphoteric nature due to carboxyl and amino groups of dicarboxyl and diamin acids. Nitrogen \((2s^2 2p^5)\) and oxygen \((2s^2 2p^4)\) atoms of these acids contain lone pairs of electrons and act as electron donors. Metal ions too contain lone pairs of electrons and act as electron acceptors\(^{(157)}\).

With proteins, metal forms two types of complexes – metallo-proteins and metal-protein complexes. In the former, metal serves as a cross-link between peptide chain and cannot be removed without destroying the protein structure\(^{(158)}\). On the other hand, in protein complexes, metal is bound reversibly and functions to stabilize sub-units or helical or folded configuration. This affects the biological properties and physical properties like solubility, strength etc.

There is no evidence for the combination of sodium or potassium cations with proteins\(^{(159)}\). In case of soluble proteins,
albumins, the isoionic points are not affected by chlorides of sodium, potassium and lithium(140). In case of bromides of sodium, tetramethyl ammonia and tetr.-ethyl-ammonia, the results are identical irrespective of cations. Flotz(160) observed that the precipitation of serum albumin occurs only slightly below its iso-electric pH by anions, whereas cations require three units of pH above it. That is, in ions of equal size, anions combine more strongly than cations. The binding ability of ions also favours this observation.

In case of insoluble proteins, as keratins, the acid combination of wool is not affected by cations(161). The alkali titration curves of wool with sodium or potassium hydroxides are identical(151). However, a contrary opinion is expressed by Steinhardt et al.(162). Though sodium and potassium cations are not actually bound, they affect the ionic atmosphere of proteins.

Greenberg et al.(163) have reviewed the study of alkaline earth metals, whereas Klotz et al.(164) have reviewed the binding of small ions and molecule by proteins. Bjerrum(165) is the first to review 'The Metal Ammine Complex Formation in Aqueous Solutions'. In case of transition metals, metal complexes are dependent on several factors: coordination number, hydration and hydrolysis, competition between hydrogen and metal ions, nature of electron donors, etc.(166).
2.4.1
Coordination Chemistry of Copper and Zinc

The electronic configurations of copper and zinc are:

Cu 29 = 1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 3d^{10}, 4s^1

\[
\begin{array}{cccccccc}
3d & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l \\
4s & 1 & 4p & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\
\end{array}
\]

Cu^{++} 27

\[
\begin{array}{cccccccc}
3d & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l \\
4s & 1 & 4p & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\
\end{array}
\]

Zn 30 = 1s^2, 2s^2, 2p^6, 3s^2, 3p^6, 3d^{10}, 4s^2

\[
\begin{array}{cccccccc}
3d & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l \\
4s & 1 & 4p & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\
\end{array}
\]

Zn^{++} 28

\[
\begin{array}{cccccccc}
3d & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l & 1l \\
4s & 1 & 4p & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\
\end{array}
\]

In the ground state, copper has a single unpaired 3d electron and zinc has all 3d pair-electrons. For hybridization, 3d electrons should be raised to either 4p or 4d energy levels, which requires high energy. To promote a paired electron to 4p or 4d state is improbable. So, in case of zinc only 4s and 4p states will participate in hybridization, i.e., sp^3 hybridization, resulting in tetrahedral configuration. The unpaired 3d electron of copper requires high energy for the promotion to 4p or 4d level. If sufficient energy is gained during the hybridization, the lone electron is elevated to 4p level and dsp^2 hybridization occurs, which has a square planar configuration.
All metal ions are electron acceptors and non-metals are electron donors. The vacant orbitals of metal ions share with ligand electrons to form valency bonds. The nature of the bond depends upon the position of the two ions in the electronegativity. If they are far apart, ionic bond results and if close enough covalent bond forms. The electronegativity of some elements are: Na(0.9), Zn(1.5), Cu(1.9), H(2.1), S(2.5), Cl(3.0), N(3.0), and O(3.5).

Copper and zinc form complexes with radicals and polar groups containing oxygen, nitrogen and sulphur. Since, α-amino acids contain oxygen, nitrogen and sulphur, the complex formation is of importance. In the complex formation of zinc with nitrogen and oxygen atoms, the linear sp metal orbitals are involved in the bond formation between Zn⁺ and α-amino nitrogen atoms. This results in a trans-square-planar configuration. But, O-Zn bond has an ionic character and this will not participate in sp³ hybridization. Hence, the complex has a tetrahedral configuration.

Though, the covalent bonds have equal energy in complexing with ligands, the stability of complexes differs from each other. This difference is expressed in terms of 'Successive Stability Constants'. This difference is due to (i) the statistical factor, (ii) the electrostatic effects of interaction between charged ligands and (iii) the residual effect of the complex.
Ammonia Complexes of Cu$^{++}$ and Zn$^{++}$

<table>
<thead>
<tr>
<th></th>
<th>$\log K_1$</th>
<th>$\log K_2$</th>
<th>$\log K_3$</th>
<th>$\log K_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu$^{++}$</td>
<td>4.12</td>
<td>3.48</td>
<td>2.87</td>
<td>2.11</td>
</tr>
<tr>
<td>Zn$^{++}$</td>
<td>2.27</td>
<td>2.34</td>
<td>2.40</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Statistical factor:

- Cu$^{++}$: 0.43, 0.36, 0.43
- Zn$^{++}$: 0.43, 0.36, 0.43

Residual effect:

- Cu$^{++}$: +0.21, +0.25, +0.33
- Zn$^{++}$: -0.50, -0.42, -0.08

The study of ammonia complexes reveals that Zn$^{++}$ complexes have a negative residual effect, whereas residual effect of Cu$^{++}$ is positive. From the statistical factor the consecutive stability constant of Zn$^{++}$ should decrease to a constant value. But, because of the residual charge, it increases and equals to that of Cu$^{++}$. Similar results were observed by Klotz (168) on dye uptake by pepsin.

<table>
<thead>
<tr>
<th></th>
<th>$\log K_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal + dye</td>
<td>Cu$^{++}$ 2.36 Zn$^{++}$ 3.50</td>
</tr>
<tr>
<td>Pepsin-Metal + dye</td>
<td>3.80 3.60</td>
</tr>
</tbody>
</table>

The affinity of dye for Cu$^{++}$ decreased whereas for Zn$^{++}$ increased. Both attained the same value. This indicated that Zn$^{++}$ may have a special advantage in mediating and linkage of small molecules to protein.
The entropy effect:
\[ \Delta F^0 = -RT \ln K \]
\[ = \Delta H^0 - T \Delta S^0 \]

The thermodynamic stability constant \( K \), which represents the free energy of complex formation can be subdivided into heat and entropy terms. The entropy of complex formation for zinc is less than that of copper. For zinc, the increase in stability with ring formation derives completely from the entropy factor, whereas for copper derives from both entropy and enthalpy terms equally. Hence, entropy factor is of great importance in the stability of complexes.

2.4.2 Character of Donors

In proteins there are two donors, viz. nitrogen and oxygen. Complex formation of sulphur of keratins should be considered only above pH 8.0. Oxygen atom has 8 electrons = \( 1s^2,2s^2,2p^4 \) and the ground state is \( 1s^2,2s^2,2p^2 \).

\[
\text{O} \quad 0^2s^2 \quad 2p^4 \quad \text{[1] [1] [1] [1]}
\]

Only one pair of the lone pairs of electrons is involved in coordination bond. That is, its covalency is 3. In case of nitrogen, its electronic structure is \( 1s^22s^22p^3 \) and ground state is \( 1s^22s^2 \). Its covalency is 4.

\[
\text{N} \quad 2s^2 \quad 2p^3 \quad \text{[1] [1] [1] [1]}
\]

\[
\text{N}^- \quad 2s \quad 2p^3 \quad \text{[1] [1] [1] [1]}
\]

\[
\text{N}^2- \quad 2s \quad 2p^5 \quad \text{[1] [1] [1] [1] [1]}
\]
The alkaline earth metals and Mn$^{+2}$, Fe$^{+2}$, Co$^{+2}$, form stronger bonds with oxygen than with nitrogen. Cu$^+$, Ag$^+$, Au$^+$, Ni$^{+2}$, Cd$^{++}$, Zn$^{++}$, Cu$^{++}$, Hg$^{++}$ form stronger bonds with nitrogen than with oxygen. Overall, the order of affinity of the complex formation is Hg$^{++}$ > Cu$^{++}$ > Zn$^{++}$ > Mg$^{++}$. However, the specific affinity is dependent on the spatial arrangement of ligand atoms and their normal complex forming tendencies.

2.4.3
Hydration and Hydrolysis of Metal Ions

Water molecules contain oxygen atoms, which have unshared electron pairs. The metal ion in aqueous solution combines with oxygen forming a complex, a hydrated metal-ion. Part of this water is held by van der Waals forces and part is coordinated with metal ions. When water molecules form a coordinated sphere around a positive ion, a negative charge is induced on the inner surface of the sphere, so that the outer surface bears a positive charge, just as the metal ion itself. This favours the attraction of more water molecules.

$$M^{2+} + n \text{H}_2\text{O} \rightarrow [(\text{H}_2\text{O})_{n-1} \cdot \text{M-O}_n]^{Z+}$$

When a proton of a hydrated metal ion is lost, metal ion is hydrolyzed and it either gets precipitated or will be in a complicated complex form.

$$[(\text{H}_2\text{O})_{n-1} \cdot \text{M-O}_n]^{Z+} + \text{H}_2\text{O} \rightarrow [(\text{H}_2\text{O})_{n-1} \cdot \text{M-OH}]^{(Z-1)+} + \text{H}_2\text{O}^+$$
In addition to this, if ligands are fewer in number than required by coordination number, a mixed complex may form. The general tendency of the complexes of the first transition series metals to dissociate or hydrolyse in solution indicates that the binding force is essentially electrostatic. The ease with which metallic ions form hydrates increases with increasing charge and with decreasing radius.

2.4.4 Hydrogen and Metal Ions

Of all cations, hydrogen ion has the greater affinity for proteins and forms the sole potential determining ion. Since metal ion hydrolysis is pH dependent in case of protein-metal ion interactions, hydrogen ion plays a two-fold role. At any instant, there exists a competition between metal ions and hydrogen ions for proteins. At very low pH, hydrogen ion affinity is greater. At higher pH, metal ion affinity is more. At some particular pH value both have equal affinity.

2.5 COPPER AND ZINC COMPLEXES OF SOLUBLE PROTEINS

A detailed study of the complex formation of soluble proteins with metallic cations is reviewed by Gurd and Wilcox(165). In general, the complex formation of proteins with metallic ions is different from those of amino acids and peptides in five factors:
(i) Since the chain length is long, the side chain groups are more important than the terminal groups.

(ii) Several members of different classes are present; these have different intrinsic affinity and reactivity with metal ions.

(iii) The ligand groups are not free to move and cluster around metal ion. Hence the number of groups available are fewer than the coordination valency.

(iv) Protein phase is made up of positive and negative charged groups. The net charge field either favours or hinders the approach of metal ions.

(v) The spatial arrangements of the ligands may or may not be favourable to form a complex, irrespective of specific affinities of the ligands and metal ions.

Eearing these points in mind, some equations are derived for binding of metal ions to ligands, competition of hydrogen ions with metal-ions, electrostatic effect, etc. Interactions of some specific proteins are discussed with individual metal ions (166).

Klotz and Curme(169) have studied copper-albumin complex by equilibrium dialysis and spectrophotometry. They found that at pH 4.8 and 10^{-4} M concentration, one atom of copper per mole is firmly bound. As concentration of metal ion is increased, binding also increased. Saturation, however, is not observed. Spectrophotometry showed that carboxyl groups
are the binding sites at pH 4.80. As pH increased, near to pH 6.50, the absorption maxima decreased below 700 m
, showing that one or more basic nitrogen atoms are coordinated. At pH 9.60, binding is still greater and prevents the hydrolysis of copper. At this stage, ε-amino group looses its proton and complexes with copper ion. In concentration study at no pH, maximum absorption is observed. This may be because of hydration, hydrolysis and/or precipitation of copper. The copper binding to α- and β-casein, serum albumin and β-lactoglobulin showed a decrease in binding. Hence, there is no correlation between amino-acid composition and affinity for copper.

In case of zinc-albumin only above pH 5.5 significant zinc-ion binding takes place. At 0°C zinc ion does not interact with carboxyl groups. At pH 6.0, it forms a complex with sulphydryl groups.

2.6 Metal Complexes of Keratins

The metal uptake by insoluble keratin is also known. The study of unshrinkability of wool by cross-linkage(170) has revealed that from the cross-linking agent, mercury-acetate, mercury was taken up by the fabric. But, by increasing the mercury uptake above 8% upto 19%, they did not observe any change in unshrinkability. Sikorski et al.(171) and Kossaenbeck et al.(172) observed that silver-ions were adsorbed by wool resulting in an increase of 10-16% of fibre weight. No detailed interpretation of these studies, however, has been made.
Recently, Simpson and Mason (173) have studied the absorption of silver-ions by wool as a function of pH, silver-ion concentration and time. Reaction is found to be reversible and there exists a competition between hydrogen and silver ions. The equilibrium is attained within two hours at 50°C. The study between pH 3.0 and 6.5 showed that the number of absorption sites remains constant. Donnan theory is found to be applicable. Though, there is degradation of cystine over the whole pH range and it increases with increase in pH as well as silver-ion concentration, the degradation of cystine was too small under the experimental conditions adopted. They found that the absorption of silver-ions is little affected by the conversion of large portion of degraded cystine into lanthionine or S-methyl cystine. Silver-carboxylate complex is the dominant interaction.

In case of the transition metal-ions chromium(III) complex with collagen has been reported (174). In case of wool, the effect of pH, concentration and temperature of Cr III-complex have been studied by Hartley (175). The equilibrium in chromium(III) chloride at 25°C reached after 1700 hours and at 60°C after 450 hours, whereas, chromium (VI) reaches equilibrium in 144 hours at 25°C.

Effect of concentration of chromium(III) ion uptake follows the Freundlich isotherm. There is a maximum uptake between pH 2 and 2.5, and is anion dependent. According to the dissociation of carboxyl groups, under the experimental
conditions, maximum uptake is expected at pH 3.75. Since Cr\(^{+++}\) commences to form polynuclear Cr\(^{+++}\) after pH 2, the Cr\(^{+++}\) uptake decreases after the maximum uptake. When the ratio of hydroxide to Cr\(^{+++}\) is increased, Cr\(^{+++}\) uptake decreases. This supports the above statement. As Cr\(^{+++}\) is bound, hydrogen ions are released to the external solution, and external pH decreases. This suggests that there is a competition between H\(^+\) and Cr\(^{+++}\).

When 55% amino groups are blocked, an increase in Cr\(^{+++}\) uptake is observed, whereas blocking of 82% carboxyl groups decreased the uptake. This shows that carboxyl groups are the binding sites for Cr\(^{+++}\). When amino groups are blocked the positive charge is decreased and hence Cr\(^{+++}\) approach to the sites is favoured by electric field. The mode of binding to carboxyl group is given as

![Diagram of binding](image)

and the reaction shown to be S\(_{N1}\) mechanism as:
Cr$^{+++}$ binds, normally, with carboxyl groups and not with amino groups of wool except in case of chromium(III) fluoride, where hydrogen bonding from the amino groups to the fluoride ions present in the inner-coordination sphere of Cr$^{+++}$ occurs. When Cr$^{+++}$ coordinates to wool the negative ions present in the Cr(III)-complex in solution are retained and water molecules are displaced. Hence $S_N^1$ mechanism occurs as shown above(175).

More work of metal-protein complexes is done on binding of tivalent-transition metal-ions, especially cupric ion on soluble proteins(166). In case of wool, copper and zinc salts are used to decrease the felting rate with sulphite(175).

Alkaline solutions of cupric ions cause supercontraction of wool(177). From biological point of view, wool from copper-deficient diet-fed sheep showed abnormal properties in many respects. It has an elongated sulphydryl zone(178). Most of the physical properties are shown to be dependent on the binding of the metal to the protein fibre.

Guthrie and Laurie(179) have studied the uptake of copper ions by Mohair Keratin with respect to pH and concentration of metal ions. Metal ion binding is observed from pH 2.5 onwards. As pH increased the metal uptake too increased. When fibre is esterified to block 85% carboxyl groups, the uptake decreased. Above pH 5, copper ion uptake increased sharply without attaining a maximum. They found that as copper ions are bound, hydrogen ions are released and
a competition exists between the two. But, there was no correlation between the metal ion bound to hydrogen ions released. The sharp increase in copper ion uptake is thought to be the difference between the internal and external pH values of the phases \((\text{pH}_{\text{int}} > \text{pH}_{\text{ext}})\). The study of concentration effect showed an initial sharp increase in uptake with a linear increase between 0.5 to 2.0M moles and then a levelling off. The maximum uptake is found to be 250 \(\mu\) moles/gram.

Samples treated with pH 3 to 5 showed a maximum absorption at 710 \(\text{m}\), whereas an esterified wool showed at 630 \(\text{m}\). This indicates that there are two types of sites for copper ion binding. But, the spectra in the range of 300-400 \(\text{m}\) are the same as in the case of untreated fibre. This indicates that copper ion in this range is not reacting with disulphide bonds.

These all show that carboxyl groups are the primary sites for binding. When treated fibre is immersed in alkaline solution at pH 12.9, the absorption maxima is shifted to lower wavelength without change in copper ion uptake. This shows the replacement of O-ligands by N-ligands.
EXPERIMENTAL TECHNIQUES

Materials

It has been shown in our Laboratory (180) that the behaviour of salt solutions towards different wool fibres viz., 70's Merino, 55's Corriedale and 48's New Zealand wool is the same. In the present study, throughout the work 55's Corriedale wool was used. All the reagents used were of Analar grades. Double distilled, deionized water was used for the experiments. The glasswares used were of Pyrex and cleaned thoroughly with cleansing reagents, rinsed with distilled water and finally steamed for one hour before use.

2.7.1 Purification of Wool Fibres

The tip-cut-off locks of wool fibre was hand-carded and soaked in absolute ethanol for one hour. The alcohol squeezed fibres were washed thoroughly under running tap-water. This was resoaked in ethanol for one hour and after squeezing air-dried. The dried fibres were purified by soxhlet extraction with petroleum ether (40-60) for 15 hours and then with absolute ethanol for 8 hours. Purified wool was soaked in freshly boiled out and then cooled 10^-4 M hydrochloric acid solution, for one hour. Then fibres were washed thoroughly with distilled water and finally with deionized water till the conductivity of the effluent was the same. Fibres were pressed between filter-papers and transferred to a desiccator for conditioning at 64% RH at
27°C over saturated sodium nitrite solution.

2.7.2 Equilibration of Wool with Salt Solutions

Purified-conditioned wool samples (0.250 g) were introduced (a) into 125 ml 0.05N salt solutions at different pH and (b) into 50 ml salt solution at different concentrations. The entrapped air was removed by glass rod and then by suction pump to facilitate thorough wetting. The samples were equilibrated at 50°C for 24 hours. Equilibrated samples were then removed from solutions and blotted out by pressing between pair of filter paper pads. The weights were determined by differential weighing method and samples were transferred into test-tubes for the estimations of the concentrations of ions employing techniques given below.

2.7.2.1 Determination of Chloride Ions (181)

To the equilibrated sample, in test-tubes, excess of standard silver nitrate solution was added, followed by concentrated nitric acid (5 ml). Wool was decomposed to dissolve, in water-bath at 80°C for 6 hours. When solution was cool, 2 ml water was added to form a separate layer. To this calculated amount of concentrated ammonia solution (about 6 ml) was added to neutralise excess of acid. Solution was diluted with K-H-phthalate buffer solution, distilled water and 2 drops of non-ionic detergent (Noigen CHV) to make total volume 50 ml and pH 4.0. The chloride ion concentration was indirectly determined by using a Beckman 39048 Silver electrode and a
saturated calomel electrode. Saturated potassium nitrate-Agar salt-bridge was used to measure the potential difference. The measurements were carried out at 35°C in water-bath, using Bajaj-Kaycee vernier type potentiometer. The reproducibility in the variation of potential was ± 0.5 mV. For each set, a separate calibration was taken under the identical conditions.

2.7.2.2 Determination of Sodium Ions

To the equilibrated sample, concentrated nitric acid (5 ml) and concentrated hydrochloric acid (0.5 ml) were added. The wool was decomposed to dissolve into solution in a waterbath at 80°C for one hour. After cooling, 2 ml water was added to form a layer on the solution. Then, calculated amount of concentrated ammonia solution (8 ml) was added not only to neutralise the acid, but also to bring the pH of the finally diluted solution to 9. The solution was diluted to 50 ml with distilled water. Using Vibron electrometer, with the help of Beckman 39278 Sodium ion electrode and saturated calomel electrode, the potential difference was determined at 35°C. The response of sodium ion electrode, soaked overnight in 0.1N NaCl solution is excellent. For each set, a separate calibration was taken under the identical conditions.

2.7.2.3 Determination of Cupric and Zinc Ions

To the equilibrated sample, 0.1N hydrochloric acid (5 ml) was added. The content was heated in boiling water-bath for
5-10 minutes. The extract was transferred to volumetric flask. The entrained solution was removed by pressing the sample with a clean glass rod. The extraction was repeated 5-6 times. The total extract was neutralised by adding concentrated ammonia solution and diluted to 50 ml by adding 10 ml (5M NH₄Cl + 5M NH₄OH) buffer solution, 1 ml 10% Na₂SO₃ solution, 1 ml freshly prepared 0.02% gelatin solution and distilled water. After thorough mixing, some quantity of solution was taken out and pure nitrogen gas was bubbled through to remove dissolved oxygen. The de-oxygenated solution was used to record the polarograph.

The experiments carried out on pure salt solutions and on extracts from wool samples containing known concentrations of salt solution gave reproducible results. (Fig. 6 and Table I). From the wave height, either in terms of galvanometer deflection or the current, the concentration of metal ions can be calculated.

In case of concentration study, the external solution was analysed for the estimation of cupric ions. Known amount of salt solution, after equilibration, was pipetted in to 50 ml flask, such that the final concentration of the solution lies between 10⁻³N to 10⁻²N. Then, 10 ml (5M NH₄Cl + 5M NH₄OH) buffer solution was added and the solution was diluted to 50 ml with distilled water. The cupric ions were estimated colourimetrically using VSU-2 spectrophotometer at 610 m wavelength. The calibration remained the same for all sets.
**TABLE I**

DATA FOR THE POLAROGRAPHIC CALIBRATIONS FOR Cu\(^{++}\) AND Zn\(^{++}\) IONS

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Wave Height (Divisions)</th>
<th>Blank</th>
<th>After Extraction from Wool</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^{-4} N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(^{++})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.495</td>
<td>6.20</td>
<td>5.30</td>
<td></td>
</tr>
<tr>
<td>4.992</td>
<td>12.10</td>
<td>11.50</td>
<td></td>
</tr>
<tr>
<td>7.488</td>
<td>16.00</td>
<td>15.50</td>
<td></td>
</tr>
<tr>
<td>9.984</td>
<td>22.60</td>
<td>22.00</td>
<td></td>
</tr>
<tr>
<td>12.480</td>
<td>28.00</td>
<td>27.50</td>
<td></td>
</tr>
<tr>
<td>15.000</td>
<td>34.00</td>
<td>34.50</td>
<td></td>
</tr>
<tr>
<td>17.500</td>
<td>38.50</td>
<td>39.00</td>
<td></td>
</tr>
<tr>
<td>Zn(^{++})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.035</td>
<td>4.00</td>
<td>3.80</td>
<td></td>
</tr>
<tr>
<td>2.070</td>
<td>10.00</td>
<td>9.40</td>
<td></td>
</tr>
<tr>
<td>3.105</td>
<td>13.50</td>
<td>13.00</td>
<td></td>
</tr>
<tr>
<td>4.140</td>
<td>18.30</td>
<td>17.25</td>
<td></td>
</tr>
<tr>
<td>5.175</td>
<td>21.40</td>
<td>22.00</td>
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</tr>
<tr>
<td>7.245</td>
<td>31.60</td>
<td>31.50</td>
<td></td>
</tr>
<tr>
<td>9.305</td>
<td>29.30</td>
<td>39.90</td>
<td></td>
</tr>
<tr>
<td>10.350</td>
<td>44.20</td>
<td>44.30</td>
<td></td>
</tr>
</tbody>
</table>
and is a straight line in the range of $10^{-3} \text{N}$ to $10^{-2} \text{N}$ salt solution.

2.7.3 Measurement of pH

Purified conditioned wool (0.5 g) was equilibrated with 0.05N salt solution (50 ml) at different pH values. The initial and final pH values were measured with Cambridge Bench pH meter L-267945 using glass electrode. From the difference in the pH values, the hydrogen ion absorption values were determined.
2.8
RESULTS AND DISCUSSIONS

At the outset it is essential to define the terms used to express the experimental quantities. The equilibrated wool sample is removed from the solution, pressed between filter papers and taken for the analysis. The sample pressed between filter papers, contains some amount of entrained liquor with the fibres, besides the adsorbed ions and the ions in the internal solution. The entrained liquor (w) was determined by differential weighing method. The sample analyzed, therefore, consists of the adsorbed ions \( M_a \) and \( X_a \), ions in the internal solution \( v \left[ m \right] \) and \( v \left[ x \right] \) and ions due to the entrained liquor equal to \( (w - v) \left[ M \right] \) and \( (w - v) \left[ X \right] \). If \( M_T \) and \( X_T \) are the total quantity of ions experimentally determined, we have,

\[
M_T = M_a + v \left[ m \right] + (w - v) \left[ M \right]\] ... (54)

and

\[
X_T = X_a + v \left[ x \right] + (w - v) \left[ X \right]\] ... (55)

Since \( w, M \) and \( X \) are known, \( M_T - w \left[ M \right] \) and \( X_T - w \left[ X \right] \) can be easily calculated, which are termed as \( M^* \) and \( X^* \) respectively. Therefore,

\[
M^* = M_T - w \left[ M \right] = M_a + v \left( \left[ m \right] - \left[ M \right] \right)\] ... (56)

and

\[
X^* = X_T - w \left[ X \right] = X_a + v \left( \left[ x \right] - \left[ X \right] \right)\] ... (57)
FIG. 7

![Graph showing the relationship between equilibrium pH and moles/kg dry wool for different salts.]

- **NaCl**
- **ZnCl₂**
- **CuCl₂**

**Axes:**
- Y-axis: Moles/kg dry wool
- X-axis: Equilibrium pH

---

**Legend:**
- ○ NaCl
- ▲ ZnCl₂
- □ CuCl₂
FIG. 9

H^+ ABSORPTION MOLES/KG. DRY WOOL

EQUILIBRIUM pH

- HCl
- NaCl
- ZnCl_2
- CuCl_2

EQUILIBRIUM pH
The data obtained for (i) wool:sodium chloride solution system, (ii) wool:zinc chloride solution system and (iii) wool:cupric chloride solution system are illustrated in Figs. 7 and 8. From the smooth curves, the data at different pH values are recorded in Table II. The hydrogen ion absorption of the above three salt systems was also determined from the difference in pH before and after the equilibration as described in 2.7.3. Results are plotted in Fig. 9 and tabulated in Table II. For the sake of comparison, Steinhardt and Harris(183) data of acid absorption by wool from pure hydrochloric acid is also given.

It is evident from Fig. 7 that the overall trend of the curves is the same for all salt solution systems. Though in no case the maximum binding capacity has been attained in the studied pH range, below pH 2.5, the value of Cl is same irrespective of cations in the system. Since, up to this pH, M* has not attained any positive value, the titration of wool seems to be similar to the titration with hydrochloric acid in presence of salts.

After pH 2.5, the curves of sodium chloride and cupric chloride systems are almost the same, whereas, that of zinc chloride system differs from these two. At any particular pH value the value of Cl for zinc chloride system is less than for the other systems. This may be due to the effect of cation on the value of Cl. The value of Cl in case of sodium chloride system attains zero value, but in cases of zinc chloride and
TABLE II
Sorption of metal ions and chloride ions from 0.05N salt solutions at various pH

<table>
<thead>
<tr>
<th>Material: Liquor = 1 : 500</th>
<th>Equilibrated at 50°C for 24 hours.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Equilibrium pH</th>
<th>NaCl</th>
<th>ZnCl₂</th>
<th>CuCl₂</th>
<th>HCl@</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl⁻</td>
<td>Na⁺</td>
<td>Hₐbs</td>
<td>Cl⁻</td>
</tr>
<tr>
<td>1.80</td>
<td>0.685</td>
<td>-0.0230</td>
<td>0.778</td>
<td>-</td>
</tr>
<tr>
<td>2.00</td>
<td>0.673</td>
<td>-0.0230</td>
<td>0.665</td>
<td>-87-</td>
</tr>
<tr>
<td>2.40</td>
<td>0.510</td>
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<td>0.455</td>
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</tr>
<tr>
<td>2.60</td>
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<td>-0.0228</td>
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</tr>
<tr>
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<td>0.402</td>
<td>-0.0225</td>
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<td>0.340</td>
</tr>
<tr>
<td>3.00</td>
<td>0.347</td>
<td>-0.0225</td>
<td>0.320</td>
<td>0.260</td>
</tr>
<tr>
<td>3.20</td>
<td>0.302</td>
<td>-0.0225</td>
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<td>0.207</td>
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<td>3.40</td>
<td>0.263</td>
<td>-0.0225</td>
<td>0.263</td>
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<tr>
<td>3.50</td>
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<td>-0.0220</td>
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<tr>
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<tr>
<td>4.20</td>
<td>0.155</td>
<td>-0.0215</td>
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</tr>
<tr>
<td>4.40</td>
<td>0.135</td>
<td>-0.0210</td>
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<tr>
<td>4.50</td>
<td>0.115</td>
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<td>0.072</td>
</tr>
<tr>
<td>4.80</td>
<td>0.100</td>
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<td>0.090</td>
<td>0.065</td>
</tr>
<tr>
<td>5.00</td>
<td>0.085</td>
<td>-0.0185</td>
<td>0.065</td>
<td>0.055</td>
</tr>
</tbody>
</table>

All values are expressed in terms of moles/kg dry wool.

© Steinhart et al values from Amer. Dye-Stuff Rep. 1940, 29, 125.
cupric chloride systems zero value is not at all attained. The value of Cl\(^-\) in zinc chloride system instead of tending to zero, levels off at a finite, positive value, whereas in case of cupric chloride system no levelling off occurs. In cupric chloride system near pH 2.8 a small deviation in the curve of Cl\(^-\) against equilibrated pH is observed.

From Fig. 8 it is evident that all three cations behave differently. At any particular pH value, Cu > Zn > Na. The earlier literature on the sorption of alkali and alkaline earth metals by proteins has revealed that there is no binding of these cations on to protein molecules. Moreover, it is evident from Fig. 8 that up to pH 6.3 sodium ion is not sorbed by wool. On the other hand, zinc and cupric ions attain positive values at pH 3.9 and 2.3 respectively. In case of soluble proteins, the shift in the isionic point towards acidic or basic sides is an indication of the complex formation of the cations and anions respectively with the protein molecules (160). Since, the isionic point of wool, in case of zinc chloride and cupric chloride systems, is shifted towards acidic side, the cations might have combined with wool to form a complex.

With an increase in pH, an increase in the uptake of zinc and cupric ions is observed. At all pH values the cupric ion uptake is greater than that of zinc ions. The uptake of all three cations follows a smooth curve. But, at pH 4.2 the uptake of cupric ions i.e., Cu\(^+\) increases sharply. None of these ions reveals a maximum binding capacity. In the acidic
pH region, carboxyl and amino groups of the cleaved salt-linkages are the only binding sites. At any pH, though the number and class of groups available, are the same for all the cations, the different amounts of uptake reveal a difference in the reactivity of these cations towards wool. At pH 4.4, all carboxyl groups of wool are available for combination and hence a maximum is expected. But, neither of these ions exhibit a maximum. The positive absorption of both cations, i.e. Zn$^{++}$ and Cu$^{++}$ commences at different pH values. Since, $pH_{\text{int}} > pH_{\text{ext}}$, $pH_{\text{int}}$ values corresponding to the pH at which positive absorption occurs are 4.90 and 3.70 for zinc and cupric ions respectively. This indicates that cupric ion has more affinity than zinc, which is also true in case of simple amino acids, soluble proteins and normal complexes.

The study of acid absorption (Fig. 9) shows that, though at any pH value acid absorption is greater than in case of pure acid, the amount of acid absorbed is different for the three salt systems investigated. If there were no binding of zinc and cupric ions to wool, as in case of sodium chloride system, the acid absorption would have been similar to that of sodium-chloride system. Because of the cation binding to wool, the acid absorption is affected and hence, a difference is observed. It is important, however, to note that the acid absorption curves of zinc chloride and cupric chloride systems lie on either side of the curve of sodium chloride system. That is,
zinc ion binding enhances the acid absorption, whereas cupric ion binding hinders the acid absorption. This may be due to the non-availability of the binding sites resulting from the competition between metal ions and protons or due to the residual charges of the metal ion complexes. Zinc-complexes have a residual negative charge whereas cupric-complexes have a positive charge, which in turn might affect the acid absorption in this fashion. The pH of zero acid absorption is also different for all salt solution systems and is in the order of Cu\(^{++}\) < Zn\(^{++}\) < Na\(^{+}\). The corresponding values are 4.20, 5.05 and 6.0 respectively.

It is convenient to analyse and to discuss the data of all three salt solution systems under the headings of:

1. Gilbert-Rideal Theory,
2. Donnan Theory, and

2.8.1 Gilbert-Rideal Theory

2.8.1.1 Wool: Monobasic Acid System

According to equation (20), for a pure monobasic acid,

\[
\log \left( \frac{\phi_{H}}{1 - \phi_{H}} \right) = \frac{-(\Delta f_{H}^{0} + \Delta f_{A}^{0})}{4.6052 RT} - \frac{\phi_{H}}{4.6052 RT} - pH \quad \ldots \quad (20)
\]

So, the plot of \( \log \frac{\phi_{H}}{1 - \phi_{H}} \) versus pH should give a straight line with -1.0 slope and the intercept equal to
FIG. 10

\[ \log \left( \frac{\varepsilon_H}{1 - \varepsilon_H} \right) \]

\[ \log \left( \frac{\varepsilon_N}{1 - \varepsilon_N} \right) \]

EQUILIBRIUM pH

-\[ \text{NaCl} \]
-\[ \text{HCl} \]
The data of pure hydrogen chloride system gives a straight line (Fig. 10 and Table III) with a slope of 0.8275. Since, above equation is of \( y = mx + c \) type, the intercept, \( c \), can be calculated from the plot using equation

\[
c = \frac{(y_1 + y_2)}{2} - \frac{m}{2} (x_1 + x_2)
\]

The mean value of 'c' from the hydrogen chloride system data is 1.8525. Therefore, the affinity is

\[
- \Delta \mu_{HA}^0 = 1.8525 \times 4.6052 \times 1.987 \times 323 = 5.475 \text{ kcals/mole.}
\]

### 2.8.1.2 Wool: Uni-Univalent Salt System

By rearranging equation \(18\), we have

\[
\log \frac{\Theta_H}{1 - \Theta_H} \cdot \frac{\Theta_A}{1 - \Theta_A} = -\frac{\left( \frac{\Delta \mu_{HA}^0 + \Delta \mu_{A}^0}{2.3025 RT} \right)}{1 - \Theta_H} + \log a_H - pH \quad (59)
\]

Therefore, the graph of \( \log \frac{\Theta_H}{1 - \Theta_H} \cdot \frac{\Theta_A}{1 - \Theta_A} \) against pH should be a straight line with -1.0 slope and the intercept is equal to \( -\frac{\Delta \mu_{HA}^0}{2.3025 RT} + \log a_H \). In case of sodium chloride system the plot is a straight line with slope, -1.305 (Fig. 10 and Table III). The mean intercept \( c \), using equation (58), is found to be 5.9526. Therefore, the affinity is

\[
- \Delta \mu_{HA}^0 = (5.9526 \times 2.3026 \times 1.987 \times 323) = 8.80 \text{ kcals/mole.}
\]
<table>
<thead>
<tr>
<th>Equilibrium pH</th>
<th>HCl</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\theta_H$</td>
<td>$\log(1-\theta_H)$</td>
</tr>
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<tr>
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<tr>
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<td>0.1951</td>
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</tr>
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</tr>
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<tr>
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<td>0.0074</td>
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<tr>
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<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>5.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
2.8.1.3
Wool : Bi-Univalent Salt System

Theoretical Background

In metal chelates or complexes more than one ligands are involved. Since, wool has a highly rigid and complex structure, the ligands are not free to move and cluster around the metal ions. It is very difficult for a metal ion to form complexes with more than one ligand. Hence, it is appropriate to assume that the metal ion binds on only one site.

The chemical potential, $\mu$, of an ion in solution and in the solid phase is, generally, given respectively as:

$$\mu = \mu^\circ + RT \ln a$$

and

$$\mu = \mu^\circ + RT \ln \frac{\Theta_a}{1 - \Theta_a} + n \psi_F$$

In case of bi-univalent metal salts in presence of acids, there are three different ions, viz. hydrogen ions, metal ions and anions. When these are sorbed on the sites, the chemical potentials of individual ions are given by,

$$\mu_{HA} = \mu_{HA}^\circ + RT \ln \frac{\Theta_H}{\Theta_H - \Theta_M} + \psi_F$$

$$\mu_{MA} = \mu_{MA}^\circ + RT \ln \frac{\Theta_M}{1 - \Theta_H - \Theta_M} + \psi_F$$

$$\mu_{AA} = \mu_{AA}^\circ + RT \ln \frac{\Theta_A}{1 - \Theta_A} - \psi_F$$

where suffix 'a' represents sorbed ions. The chemical potential on the fibre phase due to cations is the sum of the potentials of hydrogen and metal ions. Therefore, the chemical
potential $\mu_a$ due to cations alone is,

$$\mu_a = \mu_{H^+} + \mu_{M^+}$$

$$= (\mu_{H^+}^0 + \mu_{M^+}^0) + RT \ln \frac{\Theta_H}{1-\Theta_H - \Theta_{M^+}} \cdot \frac{\Theta_{M^+}}{1-\Theta_H - \Theta_{M^+}} + 2 \psi F$$

$$= \Delta \mu_a^0 + RT \ln \frac{\Theta_H}{1-\Theta_H - \Theta_{M^+}} \cdot \frac{\Theta_{M^+}}{1-\Theta_H - \Theta_{M^+}} + 2 \psi F \quad \ldots \quad (65)$$

Similarly, the chemical potential of cations in solution is,

$$\mu_s = \mu_{H^+}^S + \mu_{M^+}^S$$

$$= (\mu_{H^+}^0 + \mu_{M^+}^0) + RT \ln a_{H^+} \cdot a_{M^+}$$

$$= \Delta \mu_s^0 + RT \ln a_{H^+} \cdot a_{M^+} \quad \ldots \quad (66)$$

where 's' represents solution. The chemical potential of anion in solution is,

$$\mu_{A^-} = \mu_{A^-}^0 + RT \ln a_{A^-}$$

At equilibrium, $\mu_a = \mu_s$. Therefore, equating equations (65) and (66), we get,

$$RT \ln \frac{\Theta_H}{1-\Theta_H - \Theta_{M^+}} \cdot \frac{\Theta_{M^+}}{1-\Theta_H - \Theta_{M^+}} = -(\mu_a^0 - \mu_s^0) + RT \ln a_{H^+} \cdot a_{M^+} + 2 \psi F$$

$$= -\Delta \mu_{as}^0 + RT \ln a_{H^+} \cdot a_{M^+} + 2 \psi F \quad (68)$$

Similarly, for anion at equilibrium,

$$RT \ln \frac{\Theta_{A^-}}{1-\Theta_{A^-}} = -(\mu_{A^-}^0 - \mu_{A^-}^0) + RT \ln a_{A^-} - \psi F$$

$$= -\Delta \mu_{A^-}^0 + RT \ln a_{A^-} - \psi F \quad \ldots \quad (59)$$
Multiplying equation (69) by 2 and adding to equation (63), we get,

$$RT \ln \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \left[ \frac{\Theta_A}{1-\Theta_A} \right]^2$$

$$= -(\Delta \mu_{as}^o + 2 \Delta \mu_{as}^o) + RT \ln a_{Ms} a_{As}^2$$

$$= -\Delta \mu_{as}^o + RT \ln a_{Ms} a_{As}^2$$

$$\cdots \quad (70)$$

Therefore,

$$\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \left[ \frac{\Theta_A}{1-\Theta_A} \right]^2$$

$$= \frac{-\Delta \mu_{as}^o}{2.3026 RT} + \log a_{Ms} a_{As}^2 - p_{H_s}$$

$$\cdots \quad (71)$$

At constant metal ion and anion concentrations, $a_{Ms}$ and $a_{As}$ are constant and equation (71) becomes,

$$\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \left[ \frac{\Theta_A}{1-\Theta_A} \right]^2 = \text{constant} - p_{H_s}$$

$$\cdots \quad (72)$$

The plot of left hand side of equation (72) against pH should be a straight line with -1.0 slope.

When the metal ion binding is zero, the above equation reduces to,

$$\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_A}{1-\Theta_A} = \text{constant} - p_{H_s}$$

$$\cdots \quad (73)$$
According to equations (72) and (73), the plot of

\[
\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_A}{1-\Theta_A} \text{ or } \log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \left[ \frac{\Theta_A}{1-\Theta_A} \right]^2
\]

versus pH in case of zinc chloride, and cupric chloride systems (Fig. 11 and Table IV and V) shows, that though the plots are linear, two segments are observed in each case instead of a single, continuous line.

In case of cupric chloride system, the plot gives two segments of a line. The first part is linear up to pH 2.5 with a slope - 1.1667. Up to this region, there is no binding of metal ion and equation (73) should hold good. Since, the expected slope is -1.0 and hence the equation (73) holds good, and it is merely an acid titration curve of wool in presence of cupric chloride solution. Around pH 2.5, the line breaks and a separate segment is observed with a new value of intercept. This new segment has -1.081 slope which is in agreement with equation (72). That is, the equation derived holds good in case of cupric chloride. Since, the equation (72) contains a square term in anion concentration and there will be always two types of cations viz., Cu\(^{++}\) and H\(^+\), to occupy the negative sites of the wool structure, a decrease or an increase of any of these cations is a result of the replacement of the other cation. That is, there exists a competition between the two, which is governed by the affinities of the two and their concentrations. Since, protons have
### TABLE IV
DATA FOR CaCl₂ ASSUMING THE MAXIMUM ACID BINDING CAPACITY AS 0.820 EQU/ Kg. DRY WOOL

<table>
<thead>
<tr>
<th>Equilibrium pH</th>
<th>$\Theta_H$</th>
<th>$\Theta_M$</th>
<th>$1-\Theta_H-\Theta_M$</th>
<th>$\Theta_H(1-\Theta_M)^{-1}$</th>
<th>$\Theta_M(1-\Theta_H)^{-1}$</th>
<th>$\Theta_{Cl}(1-\Theta_{Cl})^{-1}$</th>
<th>$D_1$</th>
<th>$D_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.80</td>
<td>0.7522</td>
<td>-</td>
<td>0.2378</td>
<td>3.2052</td>
<td>-</td>
<td>0.8475</td>
<td>5.5600</td>
<td>1.2509</td>
</tr>
<tr>
<td>2.00</td>
<td>0.5317</td>
<td>-</td>
<td>0.4583</td>
<td>1.7153</td>
<td>-</td>
<td>0.7902</td>
<td>3.7674</td>
<td>0.8102</td>
</tr>
<tr>
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<td>-</td>
<td>0.7293</td>
<td>2.6937</td>
<td>0.5325</td>
</tr>
<tr>
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<td>0.4939</td>
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<td>0.5061</td>
<td>0.9759</td>
<td>-</td>
<td>0.5707</td>
<td>2.0370</td>
<td>0.2584</td>
</tr>
<tr>
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<td>0.0043</td>
<td>0.5528</td>
<td>0.7692</td>
<td>0.0062</td>
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<td>-</td>
</tr>
<tr>
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<td>-</td>
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<td>0.0258</td>
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<td>-</td>
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<tr>
<td>3.40</td>
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<td>0.0545</td>
<td>0.3049</td>
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<td>-</td>
</tr>
<tr>
<td>3.60</td>
<td>0.1522</td>
<td>0.0573</td>
<td>0.7805</td>
<td>0.2078</td>
<td>0.0734</td>
<td>0.2561</td>
<td>0.3443</td>
<td>-</td>
</tr>
<tr>
<td>3.80</td>
<td>0.1159</td>
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<td>0.1018</td>
<td>0.2171</td>
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<td>-</td>
</tr>
<tr>
<td>4.00</td>
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<td>0.0000</td>
<td>0.2481</td>
<td>0.1510</td>
<td>0.1919</td>
<td>-</td>
</tr>
</tbody>
</table>

\[
D_1 : \log \frac{\Theta_H}{1-\Theta_H-\Theta_M} \quad \frac{\Theta_M}{1-\Theta_H} \quad \frac{\Theta_{Cl}}{1-\Theta_{Cl}}
\]

\[
D_2 : \log \frac{\Theta_H}{1-\Theta_H-\Theta_M} \quad \frac{\Theta_M}{1-\Theta_H} \quad \left[\frac{\Theta_{Cl}}{1-\Theta_{Cl}}\right]^2
\]
TABLE V
DATA FOR ZnCl₂, ASSUMING MAXIMUM ACID BINDING CAPACITY AS 0.820 EQUI/Kg. DRY WOOL

<table>
<thead>
<tr>
<th>Equilibrium pH</th>
<th>$\Theta_H$</th>
<th>$\Theta_M$</th>
<th>$1-\Theta_H-\Theta_M$</th>
<th>$\frac{\Theta_H}{1-\Theta_H-\Theta_M}$</th>
<th>$\frac{\Theta_M}{1-\Theta_H-\Theta_M}$</th>
<th>$\Theta_{Cl}$</th>
<th>$\frac{\Theta_{Cl}}{1-\Theta_{Cl}}$</th>
<th>$D_1$</th>
<th>$D_2$</th>
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</thead>
<tbody>
<tr>
<td>2.20</td>
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<td>0.2500</td>
<td>3.0000</td>
<td>-</td>
<td>0.8842</td>
<td>7.5319</td>
<td>1.3597</td>
<td>-</td>
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</table>

$D_1 : \log \frac{\Theta_H}{1-\Theta_H-\Theta_M} \quad \frac{\Theta_M}{1-\Theta_H-\Theta_M}$

$D_2 : \log \frac{\Theta_H}{1-\Theta_H-\Theta_M} \quad \frac{\Theta_M}{1-\Theta_H-\Theta_M}$
greater affinity than any other cations, at low pH values, metal ion binding is not observed. After pH 2.4, though the affinities are unchanged, the concentration of the cupric ion overcomes the affinity difference and binds to the protein. As pH increases, the hydrogen ion concentration decreases and hence the cupric ion uptake increases.

From Fig. 7 it is evident that the values of Cl⁻ for cupric chloride system are very similar to those for sodium chloride system, hence the chloride ion binding to wool is not affected by cupric ion binding and the positive sites remain free for chloride ion binding. This also indicates that cupric ions bound are not forming chelates. Cupric ion merely replaces protons. Since, cupric ion is doubly charged ion and carboxyl group neutralizes only one, a chloride ion binding to the other charge of cupric ion is essential to maintain electrical neutrality. That is, the cupric chloride enters the site as cuprous chloride.'

So, the mechanism is

\[
\text{[--COO⁻  + H⁺N⁻]} + 2 \text{Cu}^{+} + 2\text{Cl}⁻ \rightarrow \\
\text{[--COO CuCl]} \quad \text{ClH}^+_{2N} \rightarrow
\]

This also explains the square term of equation (72) due to the presence of two chloride ions on the sites.
It is further observed that the linearity of the second segment breaks around pH 4.0. In this pH region above 4.0 not only a sharp increase in Cu is observed, but also, the Cl deviates from that of sodium chloride system, instead of approaching zero value. Above pH 4.0, almost all carboxylate ions are in carboxyl form, and the bound cupric ion has not satisfied its all coordinating valencies, cupric ion therefore tries to combine with other carboxyl groups either of the same or adjacent chain and the latter is more likely to be the case. Since, $-\text{NH}_2^+$ group dissociation into $-\text{NH}_2$ is not possible in this pH range ($\text{pK of } \text{NH}_2^+ = 9.6$), though $-\text{NH}_2^+$ groups are nearer for chelation, the combination is forbidden. During combination with another carboxyl group, cuprous chloride loses a second chloride ion. To maintain electrical neutrality, the chloride ion approaches $-\text{NH}_2^+$ group. Though, chloride ion binding remains intact, instead of decrease in the value of Cl with an increase in pH, the observed $\frac{Q_M}{1-S_H-G_M}$ will be less than expected. Hence, a deviation from equation (72) occurs. The probable mechanism is,

\[
\begin{array}{c}
\text{--COO.Cu.Cl} \\
\text{--COO}^- \\
\text{--COO}^-
\end{array}
\quad
\begin{array}{c}
\text{Cl.NH}_2^+ \rightarrow \\
\text{H}_2^N \rightarrow \\
\text{CIH}_2^N
\end{array}
\quad
\begin{array}{c}
\text{--COO.Cu} \\
\text{--COO}^- \\
\text{--COO}^-
\end{array}
\quad
\begin{array}{c}
\text{CIH}_2^N \rightarrow \\
\text{H}_2^N \rightarrow \\
\text{---NH}_2^+\text{Cl} \quad \text{--OOC}^-
\end{array}
\]
After pH 4.0, since all amino groups remain as $-NH_2\text{Cl}^-$ irrespective of pH, there should be a plateau. It is, however, not possible to study absorption beyond pH 4.4 due to hydrolysis of cupric chloride and hence, the expected plateau is not observed. Since Cl instead of tending to zero, deviates at pH 4.5, it is likely that it may lead to a plateau. In case of zinc chloride, this plateau is well defined.

The break in the line may be due to the change in the affinity of ions. Upto pH 2.3, there is no sorption of cupric ions.

The affinity of hydrogen chloride $\mu^0_{HCl}$ in presence of cupric chloride can be calculated from the first segment of the plot. The affinity determined from the second segment, is partly due to hydrogen chloride $\mu^0_{HCl}$ and partly due to cupric chloride $\mu^0_{CuCl_2}$. Hence, with the help of equation (72) and (73), the affinity of cupric chloride can be calculated.

According to equation (58), the average value of intercept 'c' of the first segment of cupric chloride is 3.14, which is equal to

$$-\frac{\Delta \mu}{2.3025 \, RT} + \log a_{HCl}$$

Therefore, the affinity, $-\Delta \mu^0_{HCl}$, is

$$-\Delta \mu^0_{HCl} = (3.14 + 1.2778) \times (2.3026 \times 1.987 \times 323)$$

$$= 6.52 \text{ kcal/mole}.$$
Similarly, for the second segment, the average value of intercept 'c' is 1.16, which is equal to 

\[ \frac{-\Delta f^0}{2.3026 R T} + \log a_{\text{Mg}} a_{\text{A}}^2 \]. Substituting the value of \( a_{\text{Mg}} \) and \( a_{\text{Cl}} \), we get,

\[ -\Delta f^0 = (1.16393 + 4.4011) (2.3026 \times 1.987 \times 323) \]

\[ = 8.22 \text{ kcals/mole}. \]

Therefore, the affinity of cupric chloride, is

\[ -\Delta f^0_{\text{CuCl}_2} = -\Delta f^0 + \Delta f^0_{\text{HCl}} \]

\[ = 8.22 - 6.52 = 1.70 \text{ kcals/mole}. \]

Since, \(-\Delta f^0_{\text{HCl}} = 2.3026 \text{ RT} \log K_{\text{HCl}}, \) we have

\[ \log K_{\text{HCl}} = \frac{-6.52598}{2.3026 \times 1.987 \times 323} = -4.415975 \]

Hence, \( K_{\text{HCl}} = 3.837 \times 10^{-5}. \)

According to Peters and Lister(150), the general formula for the proton dissociation for wool keratin is,

\[ pK_{\text{HCl}} = \frac{1912.3}{T} + 0.014956 T - 6.3969 \] \hspace{1cm} (74)

at 50°C, \( pK_{\text{HCl}} = 4.711531 \)

Therefore, \( K_{\text{HCl}} = 1.943 \times 10^{-5}. \) This shows a good agreement of the experimental results.

In case of zinc chloride system, the plot also yields two segments of a straight line (Fig. 11). The first segment
is a straight line upto pH 3.8. Since, upto this value, no metal ion is bound to the fibre, the plot is a titration curve of hydrogen chloride in presence of zinc chloride. According to equation (73) the slope is -1.083 which is near to the expected value. The average value of intercept 'c' is 2.741217, which is equal to \(-\frac{\Delta H^0}{2.3026 \text{ RT}} + \log a_{Cl} \). Therefore,

\[-\Delta H^0_{\text{HCl}} = (2.741217 + 1.1576) (2.3026 \times 1.987 \times 323).\]

\[= 5.7765 \text{ kca}l/\text{mole}.\]

This value is in close agreement with that of cupric chloride.

As soon as the zinc ion binding starts i.e. after pH 3.8, the line breaks and a separate line is observed with a slope -1.515. But, the expected value of slope is -1.0. This says that the derived equation does not hold good in case of zinc chloride system.

If \(\frac{\theta_{\text{Cl}}}{1 - \theta_{\text{Cl}}}\) is used instead of \(\left(\frac{\theta_{\text{Cl}}}{1 - \theta_{\text{Cl}}}\right)^2\) in equation (72), the graph is found to be a straight line with -1.083 slope (dotted line in Fig. 11). This second segment of the line is in continuation of the first segment. If this equation is to be valid, the reduction in chloride ion binding should be reasoned. As evidenced in case of cupric chloride, above pH 3.8, almost all carboxylate groups are in carboxyl form and are available for 1:2 binding. When zinc ion binds to two carboxyl groups of dissociated salt-linkages, two positively charged amino groups are left. These are
neutralized by two chloride ions, to maintain electrical neutrality. That is why, the chloride ion term is \( \frac{Q_{Cl}}{1 - e_{Cl}} \) instead of a square term. Similar is the case observed in case of cupric chloride above pH 3.9. Therefore, the probable mechanism is,

\[
\begin{align*}
-\text{COO}^- & + \text{H}_2\text{N}^- \rightarrow \text{COO}^- + \text{H}_2\text{N}^- + \text{Zn}^{++} + 2 \cdot \text{Cl}^- \\
-\text{COO}^- & + \text{H}_2\text{N}^- \rightarrow \\
\downarrow & \\
\text{Zn}^{++} & \quad \text{or} \\
\text{ClH}_2\text{N}^- & \quad \text{ClH}_2\text{N}^- \\
\text{Zn} & \\
\text{COO}^- & \\
\text{ClH}_2\text{N}^- & \quad \text{or} \\
\text{NH}_2\text{Cl}^- & \\
\text{COO}^- &
\end{align*}
\]

As pH increases, the \( \text{NH}_2\text{Cl}^- \) should go on dissociating. Since, there is no dissociation of \( \text{COO}^- \cdot \text{Zn} \), to maintain electrical neutrality, chloride ion remains intact. This requires a constant chloride ion concentration on the sites above pH 3.8. In case of zinc chloride system, the value of \( \text{Cl}^- \) levels off around pH 5.8 (Fig. 7). This supports the mechanism proposed above.

Since the slope and intercepts are identical to hydrogen chloride, it is very difficult to calculate the affinity of zinc chloride from our data.
FIG. 12

$H^+$ ABSORPTION MOLES/KG. DRY WOOL

$pH_{\text{internal}}$

- $HCl$
- $NaCl$
- $ZnCl_2$
- $CuCl_2$
2.8.2 
Ionnan Theory

According to equations (40) and (41), we have for hydrogen chloride system

\[ \text{pH}_{\text{int}} \approx 2 \text{pH}_{\text{ext}} + \log \frac{n}{V} \] ... (40a)

and for hydrogen chloride system in presence of salts,

\[ \text{pH}_{\text{int}} \approx \text{pH}_{\text{ext}} + \log \frac{n}{V} - \log x \] ... (40b)

Using these equations, the calculated values of pH_{int} for hydrogen chloride, sodium chloride, zinc chloride and cupric chloride systems are tabulated in Tables VI and VII and illustrated in Fig. 12.

It is seen from the graph, that in case of hydrogen chloride the linearity extends up to pH 5.0 and then a smooth curve results. In the smooth curve region, as pH_{ext} increases, pH_{int} too increases.

The plots of zinc chloride and cupric chloride systems are linear and parallel to each other upto pH_{int} = 4.9 and 4.4 respectively, which correspond to pH_{ext} = 3.8. At any particular pH value, pH_{int} for cupric chloride is always less than that for zinc chloride system. Upto pH_{ext} = 3.8, there are no zinc ions in the vicinity of carboxyl groups in the internal solution. Only hydrogen and chloride ions are present in vicinity of carboxyl and amino groups. That is, zinc chloride is not affecting the distribution of hydrogen chloride, whereas, in case of cupric chloride, cupric ions...
TABLE VI
VALUES OF pH_{int} IN CASE OF
WOOL : HYDROGEN CHLORIDE SYSTEM
WOOL : SODIUM CHLORIDE SYSTEM

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<th>Equilibrium pH</th>
<th>H_{abs}</th>
<th>H_{abs} / v</th>
<th>log H_{abs} / v</th>
<th>pH_{int}</th>
<th>H_{abs}</th>
<th>H_{abs} / v</th>
<th>log H_{abs} / v</th>
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v = 0.3 litre/kg wool

[Cl^-]_{NaCl} = 0.0748N
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<th>( \log H_{\text{abs}}/v )</th>
<th>( pH_{\text{int}} )</th>
<th>( H_{\text{abs}} )</th>
<th>( H_{\text{abs}}/v )</th>
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<td>0.158</td>
<td>0.5237</td>
<td>-0.2784</td>
<td>4.8892</td>
<td>0.045</td>
<td>0.150</td>
<td>4.3508</td>
<td></td>
</tr>
<tr>
<td>4.20</td>
<td>0.115</td>
<td>0.3833</td>
<td>-0.4155</td>
<td>4.9551</td>
<td>0.000</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.40</td>
<td>0.075</td>
<td>0.2500</td>
<td>-0.6021</td>
<td>4.9555</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.60</td>
<td>0.043</td>
<td>0.1433</td>
<td>-0.8438</td>
<td>4.9238</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.80</td>
<td>0.018</td>
<td>0.0500</td>
<td>-1.2218</td>
<td>4.7458</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>0.005</td>
<td>0.0157</td>
<td>-1.7781</td>
<td>4.3895</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

\( v = 0.3 \text{ litre/kg wool} \)

\[
[Cl^-]_{ZnCl_2} = 0.05797N \quad [Cl^-]_{CuCl_2} = 0.05536N
\]
approach the sites even before $pH_{ext} = 3.8$. When the distributed cupric ion approaches the negatively charged carboxyl group, some hydrogen ions are not permitted to approach the sites. These protons remain in the internal solution and thereby decrease the pH of the internal solution. That is why the $pH_{int}$ is lower in cupric chloride system than zinc chloride system.

After $pH_{ext} = 3.8$, the concentration of metal ions is greater than that of protons, the affinity factor is overcome and both ions approach the sites. Since, these are bivalent cations, they block two protons each, thereby the internal pH decreases by a two-fold value, and hence leads to a curve bending in a direction opposite to that of hydrogen chloride.

It appears that from $pH_{ext} = 2.4$ to 3.8 cupric chloride blocks a single proton approach and after 3.8 the second proton approach to the charged sites, hence the decrease in the internal solution pH is really not a two-fold, but is one-fold; whereas in case of zinc chloride system, after $pH_{ext} = 3.8$, the decrease is two-fold, therefore the $pH_{int}$ value manifests a sudden drop.

2.8.3 Generalized Theory of Peters

According to Delmenico and Peters (184), from the values of $M^*$ and $X^*$, the distribution coefficient, $\lambda$, and the internal solution volume, $v$, for sodium chloride system, are given by,
\[ \lambda = \frac{X^0}{X^*} - \frac{[X]}{[M]} \frac{M^*}{X^*} \]  \hspace{1cm} (75) \\
\[ v = \frac{M^*}{M \lambda (\lambda - 1)} \]  \hspace{1cm} (76) 

where, \( X^0 \) is the value of \( X^* \) at a pH when \( M^* = 0 \) and \([x], [M]\) are the concentrations of anions and cations respectively in the external solution. The calculated values of \( \lambda \) and \( v \) are tabulated in Table VIII.

In case of bi-univalent salts, according to the Donnan membrane equilibria, the distribution coefficient is given by,

\[ \lambda = \frac{[h]}{[H]^*} = \frac{[x]}{[X]^*} = \frac{[m]^{1/2}}{[M]^{1/2}} \]  \hspace{1cm} (77) 

where small letters correspond to the concentration of respective ions in internal solution and capital letters, in the external solution.

According to equations (56) and (57)

\[ M^* = M_a + v \left( \frac{[m]}{[M]} - \frac{[M]}{[m]} \right) \]  \hspace{1cm} (56) \\
\[ X^* = X_a + v \left( \frac{[x]}{[X]} - \frac{[X]}{[x]} \right) \]  \hspace{1cm} (57) 

when \( M^* \) is zero, a finite value of \( X^* \) is observed in all cases, which indicates that there is some anion binding to wool. At low pH values, \( M^* \) are less than zero, let us presume that there is no cation binding. Hence, \( M_a = 0 \) and equation (56) reduces to

\[ M^* = v \left( \frac{[m]}{[M]} - \frac{[M]}{[m]} \right) \]  \hspace{1cm} (78)
TABLE VIII
VALUES OF $\lambda$ AND $\nu$ AT 50°C FOR WOOL : NaCl SOLUTION SYSTEM

<table>
<thead>
<tr>
<th>Equilibrium pH</th>
<th>$M$ (moles/kg)</th>
<th>$X$ (moles/kg)</th>
<th>$M^*$ (moles/kg)</th>
<th>$X^*$ (moles/kg)</th>
<th>$\lambda$ (litre/kg)</th>
<th>$\nu$ (litre/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.20</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>2.40</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>2.60</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>2.80</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>3.00</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>3.20</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
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<td>0.0748</td>
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<td>0.573</td>
<td>0.0693</td>
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<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
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<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>4.00</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>4.20</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>4.40</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>4.60</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>4.80</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>5.00</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>5.20</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>5.40</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>5.60</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>5.80</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>6.00</td>
<td>0.0560</td>
<td>0.0748</td>
<td>-0.0220</td>
<td>0.573</td>
<td>0.0693</td>
<td>0.4413</td>
</tr>
<tr>
<td>7.75</td>
<td>0.0560</td>
<td>0.0748</td>
<td>+41.5000</td>
<td>8.700</td>
<td>3.5300</td>
<td>0.3870</td>
</tr>
<tr>
<td>8.00</td>
<td>0.0560</td>
<td>0.0748</td>
<td>50.0000</td>
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<td>3.8900</td>
<td>0.4240</td>
</tr>
<tr>
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<td>0.0748</td>
<td>58.5000</td>
<td>-11.400</td>
<td>4.2700</td>
<td>0.4400</td>
</tr>
<tr>
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<td>0.0748</td>
<td>67.2000</td>
<td>-12.300</td>
<td>4.6900</td>
<td>0.4490</td>
</tr>
<tr>
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<td>0.0560</td>
<td>0.0748</td>
<td>75.8000</td>
<td>-13.000</td>
<td>5.0700</td>
<td>0.4570</td>
</tr>
<tr>
<td>9.00</td>
<td>0.0560</td>
<td>0.0748</td>
<td>84.4000</td>
<td>-13.400</td>
<td>5.5700</td>
<td>0.4930</td>
</tr>
</tbody>
</table>

$x^* = 0.009$ moles/kg
Therefore, \( v = \frac{\mathbf{M}^*}{[m] - [\mathbf{M}]} \) \quad \ldots (79)

Substituting the value of \([m]\) from equation (77), we get

\[
v = \frac{\mathbf{M}^*}{[\mathbf{M}]} \left( \lambda^2 - 1 \right) \quad \ldots (80)
\]

Therefore, equation (57) will be,

\[
X^* = X_a + \frac{\mathbf{M}^* \left[ \mathbf{X} \right]}{[\mathbf{M}] \lambda (\lambda^2 - 1)} \quad \ldots (81)
\]

From equation (3),

\[
K_A = \frac{\left[ \mathbf{W}^+ \right] \left[ \mathbf{A}^- \right]}{\left[ \mathbf{WHA} \right]} = \frac{(B - X_a) \left[ \mathbf{X} \right]}{\lambda X_a} \quad \ldots (82)
\]

where \( B \) is the amount of \( \text{NH}_2^+ \) groups in moles/kg.

When \( \mathbf{M}^* = 0 \), \([m] = [\mathbf{M}]\) and hence \( \lambda = 1 \). Therefore, equation (3a) will be

\[
X^a = \frac{(B - X_a) \left[ X \right]}{X^o} \quad \ldots (83)
\]

where \( X^o \) is the value of \( \mathbf{X}^* \), when \( \mathbf{M}^* = 0 \). It is obvious from equations (82) and (83) that,

\[
X_a = \frac{X^o}{\lambda} \quad \ldots (84)
\]

Hence, equation (81) will be,

\[
X^* = X^o - \frac{\mathbf{M}^* \left[ \mathbf{X} \right]}{[\mathbf{M}] \lambda (1 + \lambda)} \quad \ldots (85)
\]
This on simplification gives,

\[ \lambda^2 X^* + \lambda (x^* - x^0) - \left( x^0 - \frac{M^* [\chi]}{[M]} \right) = 0 \quad \ldots (85) \]

Therefore,

\[ \lambda = \frac{- (x^* - x^0) \pm \left[ (x^* - x^0)^2 - \left( x^0 - \frac{M^* [\chi]}{[M]} \right) \right]^{1/2}}{2x^*} \quad \ldots (87) \]

Using equations (87) and (80), the values of \( \lambda \) and \( v \) are calculated and tabulated in Table IX.

The results show that in all the three salt systems \( \lambda \) and \( v \) have finite, positive values. The values of \( \lambda \) vary with pH continuously from lower pH values to higher pH values. When the values of \( M^* \) reach zero, the value of \( \lambda \) is found to be unity, as expected. After this pH value, \( \lambda \) increases and is more than unity.

The value of \( v \), though not constant, varies between 0.3 to 0.6 litres/kg. Since, the swelling in the acidic region of pH is minimum, \( v \) should be constant. With increase in pH or \( \lambda \), there is no regular increase in \( v \), small deviations in the values of \( v \) can be considered due to small errors in the experimental values.

On rearranging equation (75), for sodium chloride system,

\[ M^* = \lambda \cdot v \left[ M \right] - v \left[ \frac{M}{M} \right] \quad \ldots (88) \]

The plot of \( M^* \) against \( \lambda \) should be a straight line with \( v \left[ \frac{M}{M} \right] \) as slope and intercept as \(-v \left[ \frac{M}{M} \right]\). Fig. 13 shows
TABLE IX
VALUES OF $\lambda$ AND $v$ AT 50°C FOR
WOOL : ZINC CHLORIDE SOLUTION SYSTEM
WOOL : CUPRIC CHLORIDE SOLUTION SYSTEM

<table>
<thead>
<tr>
<th>Equilibrium pH</th>
<th>$M^*$ ZnCl$_2$</th>
<th>$X^*$ ZnCl$_2$</th>
<th>$\lambda$ litre/kg</th>
<th>$v$ litre/kg</th>
<th>$M^*$ CuCl$_2$</th>
<th>$X^*$ CuCl$_2$</th>
<th>$\lambda$ litre/kg</th>
<th>$v$ litre/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.0</td>
<td>-0.0125</td>
<td>0.725</td>
<td>0.177</td>
<td>0.515</td>
<td>-0.0053</td>
<td>0.695</td>
<td>0.803</td>
<td>0.598</td>
</tr>
<tr>
<td>2.4</td>
<td>-0.0123</td>
<td>0.485</td>
<td>0.260</td>
<td>0.527</td>
<td>-0.0025</td>
<td>0.598</td>
<td>0.925</td>
<td>0.696</td>
</tr>
<tr>
<td>2.6</td>
<td>-0.0120</td>
<td>0.340</td>
<td>0.354</td>
<td>0.553</td>
<td>0.0000</td>
<td>0.503</td>
<td>1.084</td>
<td>0.792</td>
</tr>
<tr>
<td>2.8</td>
<td>-0.0115</td>
<td>0.260</td>
<td>0.466</td>
<td>0.588</td>
<td>0.0035</td>
<td>0.503</td>
<td>1.201</td>
<td>0.792</td>
</tr>
<tr>
<td>3.0</td>
<td>-0.0103</td>
<td>0.207</td>
<td>0.569</td>
<td>0.609</td>
<td>0.0025</td>
<td>0.450</td>
<td>1.201</td>
<td>0.702</td>
</tr>
<tr>
<td>3.2</td>
<td>-0.0088</td>
<td>0.170</td>
<td>0.671</td>
<td>0.638</td>
<td>0.0014</td>
<td>0.355</td>
<td>1.466</td>
<td>0.487</td>
</tr>
<tr>
<td>3.4</td>
<td>-0.0070</td>
<td>0.142</td>
<td>0.779</td>
<td>0.713</td>
<td>0.0022</td>
<td>0.303</td>
<td>1.744</td>
<td>0.440</td>
</tr>
<tr>
<td>3.6</td>
<td>-0.0045</td>
<td>0.120</td>
<td>0.887</td>
<td>0.846</td>
<td>0.0033</td>
<td>0.250</td>
<td>2.088</td>
<td>0.392</td>
</tr>
<tr>
<td>3.8</td>
<td>-0.0015</td>
<td>0.105</td>
<td>0.972</td>
<td>1.089</td>
<td>0.0047</td>
<td>0.210</td>
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<td>0.376</td>
</tr>
<tr>
<td>4.0</td>
<td>0.0025</td>
<td>0.090</td>
<td>1.074</td>
<td>0.545</td>
<td>0.0059</td>
<td>0.178</td>
<td>2.833</td>
<td>0.381</td>
</tr>
<tr>
<td>4.2</td>
<td>0.0080</td>
<td>0.080</td>
<td>1.124</td>
<td>1.237</td>
<td>0.1010</td>
<td>0.153</td>
<td>3.182</td>
<td>0.442</td>
</tr>
<tr>
<td>4.4</td>
<td>0.0143</td>
<td>0.072</td>
<td>1.136</td>
<td>1.957</td>
<td>0.1630</td>
<td>0.132</td>
<td>3.439</td>
<td>0.502</td>
</tr>
<tr>
<td>4.6</td>
<td>0.0220</td>
<td>0.055</td>
<td>1.100</td>
<td>4.175</td>
<td>0.2440</td>
<td>0.115</td>
<td>3.568</td>
<td>0.831</td>
</tr>
<tr>
<td>4.8</td>
<td>0.0310</td>
<td>0.050</td>
<td>0.944</td>
<td>-11.414</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>0.0410</td>
<td>0.055</td>
<td>0.512</td>
<td>-2.522</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.2</td>
<td>0.0525</td>
<td>0.055</td>
<td>1.189</td>
<td>5.049</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.4</td>
<td>0.0640</td>
<td>0.052</td>
<td>1.551</td>
<td>1.779</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.6</td>
<td>0.0760</td>
<td>0.051</td>
<td>1.844</td>
<td>1.255</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.8</td>
<td>0.0890</td>
<td>0.050</td>
<td>2.109</td>
<td>1.032</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$[M] = 0.025 M$  $[X] = 0.06797 M$  $[M] = 0.025 M$  $[X] = 0.04535 M$
that the plot is a straight line upto $\lambda = 1$. In the acidic region since, the plot is a straight line, it indicates that the value of $v$ is constant. From the plot, the intercept $v[M] = 0.0275$ moles/kg. As $[M] = 0.05$, therefore, $v = 0.55$ litre/kg. When $K^* = 0$, $\lambda = 1$. Since, $[M]$ cannot be zero, from equation (88) $v$ should be zero. Fig. 13 shows that at $\lambda = 1$, $v$ is almost equal to zero.

The rearrangement of equation (80) for bi-univalent salts gives,

$$M^* = \chi^2 v [M] - v [M]$$ \hspace{1cm} (89)

Therefore, the plot of $M$ versus $\chi^2$ should be a straight line, with $-v [M]$ as intercept and slope equal to $v [M]$ (Fig. 14).

It is interesting to note that both the lines up to a pH where $\chi^2 = 1$ overlap each other. This indicates that the nature of distribution in both the cases is same. The intercept of these lines is $-0.025$ moles/kg, which gives a value of $0.5$ litre/kg for $v$. As $\chi$ increases, $v$ decreases to a value zero when $\chi^2 = 1$. This is as per the requirement of equation (89) when $K^* = 0$.

As soon as the value of $\chi^2$ exceeds unity, there is difference in the curves. When $\chi^2 = 1$, 2.4 and 3.9 are the corresponding equilibrium pH values for cupric chloride and zinc chloride systems respectively. At this point only, $M^*$ acquires positive values. The entry of metal ions into the internal solution shows an immediate effect in the nature of distribution.
In zinc chloride system between $\lambda^2 = 1.1$ and 1.4 an abrupt rise is observed. Then, a smooth curve is observed. In cupric chloride system the initial linearity extends up to $\lambda^2 = 8.0$ with slight change in the slope. $\lambda^2 = 8.0$ corresponds to pH 3.90. This is followed by a smooth curve. The pH of carboxylate groups in wool is 2.1. So, almost all carboxyl groups are available at pH 4.4. Since, $\text{pH}_\text{int} > \text{pH}_\text{ext}$ at $\text{pH}_\text{int} = 4.4$, $\text{pH}_\text{ext}$ is equal to 3.80. Hence, the maximum distribution or a plateau should be observed. But, this is not the case. Since, as pH increases, more and more dissociation of other groups takes place, the distribution is enhanced.

However, it is important to note that in case of cupric chloride system, the initial linearity of $M^*$ vs $\lambda^2$ extends over a larger region than that of zinc chloride system. This may be because of the more affinity of cupric ions, which facilitates the distribution of cupric ions.

When $M^*$ is plotted against $\lambda$ (Fig. 13), an interesting thing is observed. Upto $\lambda = 1$, all the three systems have a single, overlapping, straight line. That is, the nature of distribution is independent of the nature of cations. The volume term is also the same in all cases, and is not influenced by different salts. The distribution supports the assumption that $M_a = 0$. When $\lambda = 1$ exceeds, the three plots differ each other. Cupric and zinc chloride systems behave almost as in Fig. 14. Though, the plots $M^*$ against $\lambda$ and $M^*$ versus
\( X^2 \) reveal the affinity order as \( \text{Zn}^{++} > \text{Cu}^{++} > \text{Na}^+ \), the difference in the mode of interaction makes this statement insignificant.

According to equation (57),

\[
X^* = X_a + v \left( [\lambda - X] \right)
\]

This on simplification gives

\[
X^* = X_a + v \frac{[X]}{[\lambda]}
\]

The plot of \( X^* \) versus \( \frac{1}{\lambda} \) should be a straight line with \( v \frac{[X]}{[\lambda]} \) as slope and the intercept as \( X_a - v \frac{[X]}{[\lambda]} \). In case of all the three salts the plots are straight lines, but have different slopes (Fig. 15).

In case of sodium chloride system the average slope is 0.03838. Hence, the value of \( v \) is 0.7575 litre/kg. Since in case of zinc chloride and cupric chloride, the relation between \( X^* \) and \( \frac{1}{\lambda} \) is of binomial equation form, the direct determination of \( v \) is not possible. It is interesting to note that all the three lines on extrapolation meet at a point. Since, \( v \frac{[X]}{[\lambda]} \) in case sodium chloride system is 0.03838, the values of \( X_a \) is -0.0325 + 0.03838 = 0.00588 \( \approx \) 0.006 moles/kg. This value is very close to 0.009 moles/kg which is the value of \( X^* \) when \( M^* \) is zero. In case of zinc chloride system the slope is 0.13928 and hence \( X_a = 0.1059 \) moles/kg. The observed
value is 0.100 moles/kg. The slope of cupric chloride system is 0.610 moles/kg. Therefore, \( X = 0.571 \) moles/kg. The experimental value is 0.550 moles/kg.

These all show that the Donnan distribution takes place irrespective of different cations up to \( \lambda = 1 \) and the nature of distribution is the same.

2.8.4  
Effect of Concentration and Temperature

According to equation (70),

\[
\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \left[ \frac{\Theta_A}{1-\Theta_A} \right]^2 = \frac{-\Delta \mu^0}{2.3026 RT} + \log a_{Hs} \cdot a_{Ms} - a_{Cl s}
\]

At constant pH, \( a_{Hs} \) and \( a_{Cl s} \) are constants. Hence equation (70) will be,

\[
\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \left[ \frac{\Theta_A}{1-\Theta_A} \right]^2
\]

\[
= \frac{-\Delta \mu^0}{2.3026 RT} + \log a_{Hs} \cdot a_{Cl s} + \log a_{Ms}
\]

\[
= \text{constant} + \log a_{Ms}
\]

As already seen in section 2.8.1, this equation holds good up to pH 3.8. Above this pH value,

\[
\log \frac{\Theta_H}{1-\Theta_H} \cdot \frac{\Theta_M}{1-\Theta_M} \cdot \frac{\Theta_A}{1-\Theta_A}
\]

\[
= \frac{-\Delta \mu^0}{2.3026 RT} + \log a_{Hs} \cdot a_{Cl} + \log a_{Ms}
\]

\[
= \text{constant} + \log a_{Ms}
\]

... (92)
holds good. In case of unbuffered, constant pH solutions, the plot of \( \log \frac{\theta_H}{1-\theta_H-\theta_M} \cdot \frac{\theta_M}{1-\theta_H-\theta_M} \cdot \left( \frac{\phi_A}{1-\theta_A} \right)^n \) versus \( \log a_{Ms} \) should be a straight line with +1.0 slope, where \( n = 1 \) or 2.

But, in case of buffered solutions, it is very difficult to determine the value of \( \frac{\theta_H}{1-\theta_H-\theta_M} \). In the first place, as metal ion concentration increases, not only the affinity factor is overcome by metal ions, but due to excess of metal ions the approach of hydrogen ions to the binding sites is unlikely. And, as temperature increases, the dissociation of carboxylate groups is also enhanced. Hence, after certain metal ion concentration and temperature, the term \( \frac{\theta_H}{1-\theta_H-\theta_M} \) becomes insignificant. Therefore, equation (92) will be

\[
\log \frac{\theta_M}{1-\theta_M} \cdot \frac{\phi_A}{1-\theta_A} = \text{constant} + \log a_{Ms} \quad \ldots \quad (93)
\]

Since chloride ion binding is independent of the nature of cations and at constant pH, remains constant, equation (93) reduces to

\[
\log \frac{\theta_M}{1-\theta_M} = \text{constant} + \log a_{Ms} \quad \ldots \quad (94)
\]

Therefore, the plot of \( \log \frac{\theta_M}{1-\theta_M} \) against \( \log a_{Ms} \) should be a straight line with +1.0 slope.

In case of cupric chloride due to hydrolysis, the pH of the solution decreases as concentration increases. To maintain a constant pH for all solutions, either we have to choose the low
pH values or we have to use a buffer at high pH values. In the present study, since the cupric ion binding is zero below pH 2.3, and near pH 4.0 most of the carboxyl groups are available for the combination, pH 4 buffer (sodium acetate + hydrochloric acid) was used. The $M^*$ values of cupric chloride system at 30°, 40° and 50°C are obtained and the calculated data, required for equation (94), are tabulated in Tables X, XI and XII. The plot of $-\log \frac{\Theta M}{1-\Theta M}$ against $-\log a_{M^*}$ is illustrated in Fig. 16.

The plot shows that in all three cases, straight lines are obtained with slight variation in the slopes. The slopes are 0.7925, 1.122 and 0.925 for the system at 30°, 40° and 50°C respectively. The scattering of the experimental points is mainly due to the colourimetric technique, where accuracy is not satisfactory. Even then, the plots show an agreement with the derived equation (94).

If there would have been any effect of temperature on the adsorption of cupric ions, the plots should have been parallel. This is not observed in the present study. Probably in the range of 30°C to 50°C the combination of cupric chloride is independent of temperature.
**TABLE X**

**CONCENTRATION STUDY DATA FOR**

**WOOL : CUPRIC CHLORIDE SYSTEM AT 30°C**

Liquor : Wool = 100 : 1

Total number of binding sites = 0.410 moles/kg

<table>
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<tr>
<th>Cupric Ion Concentration $\times 10^{-3}$ M</th>
<th>$-\log C$</th>
<th>$10^{-3}$ moles/kg</th>
<th>$Q_M$</th>
<th>$1-Q_M$</th>
<th>$\frac{Q_M}{1-Q_M}$</th>
<th>$\log \frac{Q_M}{1-Q_M}$</th>
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Total number of binding sites = 0.910 moles/kg
CUPRIC CHLORIDE SYSTEM AT 50°C
A. CURVE OF WOOL FIBRE.

B. CURVE OF SET FIBRE.