CHAPTER IV

RESULTS AND DISCUSSION

4.1. Determination of Stability constant 2-aminoethane sulfonic acid in binary solvent medium (70% DMF-30% Water (v/v)) and (50%DMF-50% Water (v/v)) with metal ions

Normally, amino acids develop their acid and basic function through the carboxylic groups and the amine, respectively. Contrarily, taurine contains an aminic and a SO$_3^-$ group an analogue of ß-alanine. However, the properties of its ligand depend on the aminic nitrogen atom so that it majorly behaves as a monodentate ligand. Taurine contains the amino group which is expected to bind with the metal ions even though the affinity of sulfonate groups on their own for metal ions is weak. Hence, the amino groups in taurine might play as an “anchor”, admitting the formation of stable six-membered chelate rings. In this manner, taurine could coordinate to metal ions as a monodentate or bidentate ligand (Figure. 7).

Even though taurine was a simple compound, only a few studies were found in the literature on its role as a ligand towards cations. This was apparently because of the poor stability of its metal complexes. The only formerly reported solid metal complex of taurine was by Ford and Nolan [207] who isolated the complex cis-
[Co(en)₂{NH₂(CH₂)SO₃⁻}Cl]Cl (en = 1,2-diaminoethane) in which taurine behaves as a monodentate ligand through its amino group. Datta et al. [208] examined the complex formation between taurine and silver (I) at room temperature. Albert studied the formation of taurine complex at 20°C with Cobalt and Copper metal ions. However, these values were obtained from log K' (at low values of n) by adding (log K' - 1), a method which works well with α-amino-acids but which may not be valid in taurine complex because at higher values of n, precipitation occurred [209].

These values of n from the former reported works were calculated from e.m.f measurements in solutions at an ionic strength which is low (about 0.01 mol dm⁻³) in order to determine the taurine protonation and complex formation constants. van Poucke et al. [210] studied the equilibria going on between silver(I) and taurine at 25°C from e.m.f. measurements using glass electrode, presuming the presence of two complexes, 1:1 and 1:2, with stability constants (log K) 2.97 and 6.15, respectively. Lately, the equilibrium reaction between taurine (L) and metal ions of Ca(II) and Ni(II) were studied [211]. In the case of Ni(II) complex [212], e.m.f. measurements carried out by using a glass electrode at 25°C in 0.1 mol dm⁻³ NaClO₄ or LiClO₄, were clarified by presuming the existence of the only species, NiL, with overall stability constant log β=3.62.

As a consequence, an alternate solution was provided by introducing binary solvent mixture for determining better stability constants. DMF-Water mixture was used in this study at two different compositions. Over the various organic solvents, N,N-Dimethylformamide (DMF) was a very suitable co-solvent, since it was completely miscible with water and aprotic in nature. Aqueous- DMF mixtures were widely used as solubilizing agents for sparingly water soluble drugs and reagents. The permittivity values of aqueous-DMF mixtures in the range of 10 % DMF (v/v) - 80%
DMF (v/v) were greater than 51 and the logarithmic autoprotolysis (dissociation) constants of the mixtures in the range within 13.73±17.05. Concerning the differentiating power for levelling, the usage of the resolution approach was desirable. The constancy (resolution) of the acid strength in a particular solvent, especially for acids fitting to the similar chemical family, is the slope obtained from the straight line plotting between the pKₐ of the acids in the particular solvent against the pKₐ in the water [213]). On these terms in aqueous-DMF mixtures, the resolution range of the solvents existed in the range from 1.1 to 1.3 (20% DMF to 80% DMF) [214]. By this means, it can be concluded that aqueous-DMF mixtures are more differentiating i.e. less levelling systems than water. Respectively, the aqueous-DMF mixtures accomplished the aforementioned necessities and can be well-considered as an apt solvent mixture for acid-base titrations.

**Protonation constants**

In pharmacological studies, pKₐ is one of the fundamental properties that were used to determine the absorption, distribution, metabolism and excretion of compounds in biological systems and the surrounding environment. These properties are crucial to the development of new drugs for human and veterinary usage [215]. The acid dissociation constant values could give an overview about the pH at which the ligand is 50% ionized.

**Formation constant for complex in binary system**

To determine the metal complex formation constant, the prepared solutions consisting of metal and ligand in 1:5 molar ratios were titrated against sodium hydroxide solution free from carbonate. The representative pH-potentiometric titration curves between metal ions (Mn²⁺, Cu²⁺ and Zn²⁺) and the ligand (2-aminoethane...
sulfonic acid) were drawn. It was observed that at a particular addition of titrant, there was a shift to higher pH indicating the metal ion-ligand complex formation.

The pH where complexation started to occur, and the splitting between the curve of ligand separately and the curve of metal-ligand were taken into account and the figures provide proof that complexation with divalent metal ions involves different behaviors. For the divalent ions (Mn, Cu, Zn) all the ligands started to form complexes with them under basic/less acidic conditions.

The obtained pH-metric titration curves then were processed by ORIGIN 8 program software. The refined proton-ligand dissociation constant and metal-ligand stability constants (Log K) of taurine and their complexes with Mn(II), Cu(II) and Zn(II) metal ions determined in 50% DMF-water mixture (v/v) and 70% DMF-water mixture (v/v) at 300 K and ionic strength 0.1 mol dm$^{-3}$ KNO$_3$. The extent of deviation observed may be due to the complete dissociation of the OH group (Figure. 8 & 9). The proton-ligand formation number $n_A$ was calculated by the Irving-Rossotti expression. The pK$_a$ values of the ligand and formation constants of the complexes were calculated by the algebraic method point wise calculation and also determined from formation curves $n_A$ Vs pH (half integral method) by noted pH at which $n_A = 0.5$ (Bjerrum 1941) [216]. The accurate values of pK$_a$ were determined at different solvent media by point wise calculations which were represented in Table. 4.
Figure. 8 pH metric titration curves of Free Acid + Ligand (2-Aminoethane sulfonic acid) + metal ions (0.01 mol.dm$^{-3}$) in 70% DMF-30% Water mixture (v/v)

Figure. 9 pH metric titration curves of Free Acid + Ligand (2-Aminoethane sulfonic acid) + metal ions (0.01 mol.dm$^{-3}$) in 50% DMF-50% Water mixture (v/v)
Figure. 10 Half integral method for determining pKₐ of 2-Aminoethane sulfonic acid at 70% DMF-30% Water mixture (v/v)

Figure. 11 Half integral method for determining pKₐ of 2-Aminoethane sulfonic acid at 50% DMF-50% Water mixture (v/v)
**Figure. 12** Plot of n Vs pH of ligand 2-Aminoethane sulfonic acid with metal ions a) Mn(II), b) Cu(II), c) Zn(II) in 70% DMF-30% Water mixture (v/v) d) Mn(II) e) Cu(II), f) Zn(II) in 50% DMF-50% Water mixture (v/v)
Table. 4 Proton-ligand formation number ($n_A$) of 2-Aminoethane sulfonic acid at 300K and the ionic strength $I = 0.1\text{mol dm}^{-3} \text{KNO}_3$ in binary solvent mixture at different compositions

<table>
<thead>
<tr>
<th>pH</th>
<th>$V_1$ (ml)</th>
<th>$V_2$ (ml)</th>
<th>$V_2-V_1$ (ml)</th>
<th>$n_A$</th>
<th>$pK_a$</th>
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</thead>
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<th>$V_2-V_1$ (ml)</th>
<th>$n_A$</th>
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<td>9.4</td>
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<td>3.35</td>
<td>0.29</td>
<td>0.410</td>
<td>9.165</td>
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</table>

Table. 5 Proton-ligand dissociation constant of 2-Aminoethane sulfonic acid in binary solvent mixture at 0.1mol dm$^{-3}$ ionic strength

<table>
<thead>
<tr>
<th>Ratio of DMF-water mixture</th>
<th>$pK_a$ (Half integral method)</th>
<th>$pK_a$ (Pointwise calculation)</th>
<th>$pK_a$ value in water (literature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% DMF-30% Water (v/v)</td>
<td>9.03</td>
<td>8.96</td>
<td>8.90-9.01 [217-219]</td>
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<tr>
<td>50% DMF-50% Water (v/v)</td>
<td>9.00</td>
<td>8.93</td>
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Table. 6 Variation of $n$ values with pH in 70% DMF - 30% Water mixture (v/v)
The formation curves were constructed by plotting the values of $n_A$ against pH of the solution. The dissociation constant $pK_a$ of ligand was deduced from the

<table>
<thead>
<tr>
<th>2-aminoethane sulfonic acid + Mn (II)</th>
<th>2-aminoethane sulfonic acid + Cu (II)</th>
<th>2-aminoethane sulfonic acid + Zn (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>$V_3 - V_2$ (ml)</td>
<td>$n$</td>
</tr>
<tr>
<td>6.0</td>
<td>0.05</td>
<td>0.502</td>
</tr>
<tr>
<td>6.2</td>
<td>0.08</td>
<td>0.802</td>
</tr>
<tr>
<td>6.4</td>
<td>0.1</td>
<td>1.002</td>
</tr>
<tr>
<td>6.6</td>
<td>0.12</td>
<td>1.201</td>
</tr>
<tr>
<td>6.8</td>
<td>0.13</td>
<td>1.301</td>
</tr>
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<td>7.0</td>
<td>0.15</td>
<td>1.500</td>
</tr>
<tr>
<td>7.2</td>
<td>0.16</td>
<td>1.597</td>
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<tr>
<td>7.4</td>
<td>0.17</td>
<td>1.695</td>
</tr>
<tr>
<td>7.6</td>
<td>0.18</td>
<td>1.792</td>
</tr>
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</table>

Table. 7 Variation of $n$ values with pH in 50% DMF - 50% Water mixture (v/v)
formation curves. The values of pKₐ obtained were given in Table. 5. The accurate pKₐ values of ligand were deduced by the pointwise calculation method and the results were in good concordance with those obtained by the half integral method (Figure. 10 & 11). The increase in pKₐ in binary solvent medium than pure water showed that DMF played a major role to enhance the basicity. It was well known that the addition of DMF to pure water breaks the three dimensional network of water and it forms the H-bonded complexes between DMF-nH₂O [220].

**Table. 8** Stability constant of 2-Aminoethane sulfonic acid with Mn (II), Cu (II) and Zn (II) metal ions at 300K and the ionic strength I = 0.1mol dm⁻³ KNO₃ in different binary solvent mixture

<table>
<thead>
<tr>
<th>Metal complexes of 2-aminoethane sulfonic acid</th>
<th>log K₁</th>
<th>log K₂</th>
<th>log K₁ - log K₂</th>
<th>log K₁/ log K₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>70% DMF - 30% Water mixture (v/v)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn(II)</td>
<td>3.29</td>
<td>2.62</td>
<td>0.67</td>
<td>1.256</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>3.99</td>
<td>3.11</td>
<td>0.88</td>
<td>1.283</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>3.29</td>
<td>2.71</td>
<td>0.58</td>
<td>1.214</td>
</tr>
<tr>
<td><strong>50% DMF - 50% Water mixture (v/v)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn(II)</td>
<td>3.19</td>
<td>2.81</td>
<td>0.38</td>
<td>1.135</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>2.99</td>
<td>2.52</td>
<td>0.47</td>
<td>1.186</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>2.90</td>
<td>2.43</td>
<td>0.47</td>
<td>1.193</td>
</tr>
</tbody>
</table>

These H bonded complexes were presumably more basic than pure water by considering the +I effect of the methyl group which tends to increase the mesomeric shift of the lone pair of electrons on Nitrogen to the more electronegative Oxygen of DMF. It was further enhanced when a molecule capable of delocalizing this excess charge on Oxygen of DMF was present. Since water molecules were capable of forming H-bonds with such sites the excess negative charge on Oxygen of DMF (arising due to mesomeric shift) is now partially transferred on to the Oxygen of water.
Hence, the co-operative nature of H-bonding in all DMF-nH₂O molecule complexes was expected to be more basic than the water molecule in pure solvent and thereby reduces its protonation constant. In addition, dielectric constant also plays a major role in dissociation constant. The solvent which has a lower dielectric constant increases the electrostatic forces between the ions and enables the formation of a molecular species. Therefore, while increasing the DMF concentration, the dielectric constant of solvent decreases resulting in the increase in pKₐ values. The metal-ligand stability constants were calculated by the half integral method by plotting n Vs pL.

The stability constants of complexes have been calculated from n values (Figure. 12) and are represented in Table. 6 & 7. It was seen that the stability of the Metal- Taurine complex was slightly more in the DMF-water concentration of 70%DMF-30%Water mixture. The present study has great significance in understanding co-ordination chemistry. The metal-ligand stability constant of 2-aminoethane sulfonic acid was given in Table. 8. The deviation of the metal titration curves from the ligand curve indicates the formation of binary complex. The larger value of stability constant indicated that the formed complex was more stable. The larger the stability constant value, the greater will be the driving force to push the reaction to the right side. The binding of metal ions used in the cancer treatment is suggested by Furst [221]. The difference between LogK₁ and LogK₂ was small. Therefore, it seems that both the 1:1 and 1:2 complexes were formed simultaneously and not in a stepwise process. The change in the color (colorless to dusty white for Manganese complex, pale blue color to dark blue for copper complex and colorless to
milky white for zinc complex) with respect to pH value of solution and deviation of ligand curve from metal ion curve also shows the origination of complex formation. The value of stability constants was found to be higher in 70% DMF-30% water (v/v) than 50% DMF-50% water (v/v) because the value of the dielectric constant of DMF is lower than that of water which was responsible for the less solvation of metal ion in DMF, which in turn, makes the approach of the ligand to occupy a coordination site in the coordination sphere of metal ion easier and hence greater the stability of the complex in higher solvent (DMF) composition was achieved.

4.2. Determination of Stability constant Tetradecanoic acid in binary solvent medium (50%DMF-50% water (v/v)) and (70% DMF-30% water (v/v)) with metal ions

Myristic acid was highly ample in milk fat (7-12%) and coconut and palm oils where its level can reach up to 23% of total fatty acids [222]. For binding the metal ions with myristic acid, pH metric method was applied to determine the stability constant for the formation of a complex in solution to obtain the accurate results. Myristic acid was insoluble in water therefore DMF was used as co-solvent, since the properties of DMF as co-solvent have already been discussed in stability constant of 2-aminoethane sulfonic acid. The effect of metal ions such as Mn(II), Cu(II) and Zn(II) on the properties of complexes of myristic acid in binary solvent mixture at 303 K has been studied.

From the pH metric titration curves, the proton ligand dissociation constant and metal ligand stability constant were obtained at different solvent ratio. The extent of deviation observed may be due to the complete dissociation of the OH group (Figure. 13 & 14). From the Table. 10, it was seen from the point wise and the half-integral
method (Figure. 15 &16), the pKₐ of myristic acid increased from the literature value [223-224]. In binary solvent medium, the dielectric constant of the DMF solvent decreased resulting in decrease in the acidity constant which in turn found to increase the pKₐ values [225]. The deviation occurred in pKₐ values should never exceed than 0.2 units which was well compromised for myristic acid [226-227].

From the graphical data (Figure. 17), it was observed that the formation of strong metal complexes due to highest pH values of ligand titration curves as compared to the metal titration curves in both DMF-Water mixture. The data in Table. 13 clearly implies the effect of solvent properties on the stability of complexes. The stability of a complex formed in a solution intensely depends on the nature of the solvent medium. During complexation, the metal ion should have the capacity to replace the solvent molecule with the ligand or the ligand should have the capacity to replace the solvent molecules in the first solvation shell of the cation. Since, the different solvent ratio yields a critical change in their binding properties and their selectivity of ligand for certain metal ion over the others. The results showed that the stability constants of all metal complexes were higher in 70% DMF-30% water mixture (v/v). The donor number of DMF (DN 26.2) was less than that of water (DN 33) which makes the weak solvating nature of DMF [228]. Hence, the solvation of metal ions and ligand were expected to be less than water.

The higher co-ordination was achieved by the larger metal ion, hence Cu (II) shows higher stability and comparatively Mn (II) shows lesser stability in 70% DMF and 50% DMF mixture. Apart from ionic radii, Jahn–Teller stabilization contributed in copper complex which attained higher stability [229]. The Irving-Williams order of stability constant was followed for the metal complexes [230]. The difference between log K₁ and log K₂ values showed the complex formation between metal ion and ligand. The less difference between these two values indicated that the formation of complex
between metal ion and myristic acid occurred simultaneously. The ratio between the two values (log $K_1$ / log $K_2$) revealed that stability of the complex formed between the metal ions and ligand.

Figure. 13 pH metric titration curve of Free Acid + Ligand (Tetradecanoic acid) + metal ions in 70% DMF-30% Water mixture (v/v)
**Figure. 14** pH metric titration curve of Free Acid + Ligand (Tetradecanoic acid) + metal ions in 50% DMF-50% Water mixture (v/v)

![Graph of pH metric titration curve](image1)

**Figure. 15** Formation of $n_A$ Vs pH in 70% DMF medium -30% Water mixture (v/v)

![Graph of $n_A$ Vs pH](image2)

**Figure. 16** Formation of $n_A$ Vs pH in 50%- 50% Water mixture DMF medium (v/v)

![Graph of $n_A$ Vs pH](image3)
Figure. 17 Plot of n Vs pH of ligand Tetradecanoic acid with metal ions

a) Mn(II), b) Cu(II), c) Zn(II) in 70% DMF-30% Water mixture (v/v)
d) Mn(II) e) Cu(II), f) Zn(II) in 50% DMF-50% Water mixture (v/v)

Table. 9 Proton-ligand formation number (nA) of Tetradecanoic acid at 303 ± 0.1 K and the ionic strength I = 0.1 mol dm⁻³ KNO₃ in different binary solvent mixture

<table>
<thead>
<tr>
<th>pH</th>
<th>V₁ (ml)</th>
<th>V₂ (ml)</th>
<th>V₂-V₁ (ml)</th>
<th>n_A</th>
<th>pKₐ</th>
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<td>0.34</td>
<td>0.325</td>
<td>5.082</td>
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**Table. 10** Proton-ligand dissociation constant of Tetradecanoic acid in binary solvent mixture at 0.1mol dm\(^{-3}\) ionic strength

<table>
<thead>
<tr>
<th>Solvent mixture ratio (v/v)</th>
<th>(pK_a) (Half- integral method)</th>
<th>(pK_a) (Pointwise calculation)</th>
<th>(pK_a) value (literature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% DMF - 30% Water</td>
<td>5.02</td>
<td>5.015</td>
<td>4.90</td>
</tr>
<tr>
<td>50% DMF - 50% Water</td>
<td>4.94</td>
<td>4.86</td>
<td>4.00</td>
</tr>
</tbody>
</table>

**Table. 11** Variation of n values with pH in 70% DMF - 30% Water mixture (v/v)

<table>
<thead>
<tr>
<th>Myristic acid + Mn (II)</th>
<th>Myristic acid + Cu (II)</th>
<th>Myristic acid + Zn (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>(V_3 - V_2) (ml)</td>
<td>n</td>
</tr>
<tr>
<td>6.2</td>
<td>0.04</td>
<td>0.410</td>
</tr>
<tr>
<td>6.4</td>
<td>0.05</td>
<td>0.512</td>
</tr>
<tr>
<td>6.6</td>
<td>0.07</td>
<td>0.716</td>
</tr>
<tr>
<td>6.8</td>
<td>0.09</td>
<td>0.818</td>
</tr>
<tr>
<td>7.0</td>
<td>0.10</td>
<td>1.022</td>
</tr>
<tr>
<td>7.2</td>
<td>0.12</td>
<td>1.229</td>
</tr>
<tr>
<td>7.4</td>
<td>0.14</td>
<td>1.429</td>
</tr>
<tr>
<td>7.6</td>
<td>0.15</td>
<td>1.530</td>
</tr>
<tr>
<td>7.8</td>
<td>0.16</td>
<td>1.631</td>
</tr>
</tbody>
</table>

**Table. 12** Variation of n values with pH in 50% DMF – 50% Water mixture (v/v)
### Table 13 Stability constant of Tetradecanoic acid with Mn (II), Cu (II) and Zn (II) metal ions at 303 ±0.1 K and the ionic strength I = 0.1 mol dm$^{-3}$ KNO$_3$ in different binary solvent mixture

<table>
<thead>
<tr>
<th>Metal complexes of myristic acid</th>
<th>log $K_1$</th>
<th>log $K_2$</th>
<th>log $K_1$ - log $K_2$</th>
<th>log $K_1$ / log $K_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>70% DMF - 30% Water mixture (v/v)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn(II)</td>
<td>3.19</td>
<td>2.91</td>
<td>0.28</td>
<td>1.096</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>3.29</td>
<td>2.81</td>
<td>0.48</td>
<td>1.171</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>3.30</td>
<td>2.82</td>
<td>0.48</td>
<td>1.170</td>
</tr>
<tr>
<td><strong>50% DMF - 50% Water mixture (v/v)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn(II)</td>
<td>2.79</td>
<td>2.41</td>
<td>0.38</td>
<td>1.158</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>2.99</td>
<td>2.31</td>
<td>0.68</td>
<td>1.294</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>2.89</td>
<td>2.41</td>
<td>0.48</td>
<td>1.199</td>
</tr>
</tbody>
</table>
4.3. Determination of Stability constant Acetylsalicylic acid in binary solvent medium (50% DMF-50% water (v/v)) and (70% DMF-30% water (v/v)) with metal ions

From the pH metric titration curves (Figure. 18 & 19), the proton-ligand formation number $n_A$ was calculated by the Irving- Rossotti expression mentioned in Table. 14. The value of dissociation constant of acetylsalicylic acid in binary solvent medium was slightly greater than the literature value ($pK_a = 3.49$) in water [231-232] shown in Table. 15, where the pointwise calculation method and half integral method were well agreed (Figure. 20 & 21). The reason for the binary solvent and its significance have been already discussed in stability of taurine.

The metal-ligand stability constants of binary complexes were estimated in different solvent compositions. Examination of the titration curves indicated that complex formation took place in the solution. The metal titration curves showed a marked shift with respect to the ligand titration curves along the volume axis. This indicated the affinity of the ligand with metal ions which released protons and the difference in volume was obtained from ($V_3 - V_2$) (Table. 16 & 17). In addition, the color change of the ligand in the presence of metal ions was observed (colorless to white for Mn complex, colorless to dark blue Cu complex and colorless to white for zinc complex) after the required volume of NaOH showing the formation of new species.

From the ligand and metal titration curves the value of $n$ was calculated (Figure. 22). From $n$ value the pH values are obtained.

The variation of $n$ is found be 0-0.8 for 1:1 complexes and 1.2-1.8 for 1:2 complexes. The data in Table. 18 indicates the stability constant of acetylsalicylic acid metal complexes. The stability of a complex formed in a solution tends to strongly depend upon the nature of the solvent medium. During complexation, the metal ion
should be able to replace the solvent molecule with the ligand or the ligand should be able to replace the solvent molecules in the first solvation shell of the cation.

**Table. 14** Proton-ligand formation number \( (n_A) \) of Acetylsalicylic acid at \( 300 \pm 0.1 \) K and the ionic strength \( I = 0.1 \) mol dm\(^{-3}\) KNO\(_3\) in different binary solvent mixture

<table>
<thead>
<tr>
<th>pH</th>
<th>( V_1 ) (ml)</th>
<th>( V_2 ) (ml)</th>
<th>( V_2-V_1 ) (ml)</th>
<th>( n_A )</th>
<th>( pK_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>3.04</td>
<td>2.70</td>
<td>-0.34</td>
<td>1.667</td>
<td>3.498</td>
</tr>
<tr>
<td>3.4</td>
<td>3.05</td>
<td>2.73</td>
<td>-0.32</td>
<td>1.627</td>
<td>3.614</td>
</tr>
<tr>
<td>3.6</td>
<td>3.06</td>
<td>2.76</td>
<td>-0.30</td>
<td>1.588</td>
<td>3.714</td>
</tr>
<tr>
<td>3.8</td>
<td>3.07</td>
<td>2.80</td>
<td>-0.27</td>
<td>1.529</td>
<td>3.798</td>
</tr>
<tr>
<td>4.0</td>
<td>3.08</td>
<td>2.84</td>
<td>-0.24</td>
<td>1.47</td>
<td>3.803</td>
</tr>
<tr>
<td>4.2</td>
<td>3.09</td>
<td>2.88</td>
<td>-0.21</td>
<td>1.411</td>
<td>3.845</td>
</tr>
<tr>
<td>4.4</td>
<td>3.10</td>
<td>2.92</td>
<td>-0.18</td>
<td>1.352</td>
<td>3.874</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>( V_1 ) (ml)</th>
<th>( V_2 ) (ml)</th>
<th>( V_2-V_1 ) (ml)</th>
<th>( n_A )</th>
<th>( pK_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>3.88</td>
<td>3.54</td>
<td>-0.34</td>
<td>1.648</td>
<td>3.606</td>
</tr>
<tr>
<td>3.4</td>
<td>3.89</td>
<td>3.57</td>
<td>-0.32</td>
<td>1.609</td>
<td>3.822</td>
</tr>
<tr>
<td>3.6</td>
<td>3.90</td>
<td>3.60</td>
<td>-0.30</td>
<td>1.571</td>
<td>4.039</td>
</tr>
<tr>
<td>3.8</td>
<td>3.91</td>
<td>3.64</td>
<td>-0.27</td>
<td>1.514</td>
<td>4.269</td>
</tr>
<tr>
<td>4.0</td>
<td>3.92</td>
<td>3.68</td>
<td>-0.24</td>
<td>1.456</td>
<td>4.504</td>
</tr>
<tr>
<td>4.2</td>
<td>3.93</td>
<td>3.72</td>
<td>-0.21</td>
<td>1.399</td>
<td>4.745</td>
</tr>
<tr>
<td>4.4</td>
<td>3.94</td>
<td>3.76</td>
<td>-0.18</td>
<td>1.342</td>
<td>4.994</td>
</tr>
</tbody>
</table>

**Table. 15** Proton-ligand dissociation constant of Acetylsalicylic acid in binary solvent mixture at 0.1 mol dm\(^{-3}\) ionic strength
<table>
<thead>
<tr>
<th>Solvent mixture ratio (v/v)</th>
<th>$pK_a$ (Half-integral method)</th>
<th>$pK_a$ (Pointwise calculation)</th>
<th>$pK_a$ value (literature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% DMF - 30% Water</td>
<td>3.70</td>
<td>3.90</td>
<td>3.49 (in water)</td>
</tr>
<tr>
<td>50% DMF - 50% Water</td>
<td>3.60</td>
<td>3.86</td>
<td></td>
</tr>
</tbody>
</table>

**Table. 16** Variation of $n$ values with pH in 70% DMF - 30% Water mixture (v/v)

<table>
<thead>
<tr>
<th>Acetylsalicylic acid + Mn (II)</th>
<th>Acetylsalicylic acid + Cu (II)</th>
<th>Acetylsalicylic acid + Zn (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>$V_3 - V_2$ (ml)</td>
<td>$n$</td>
</tr>
<tr>
<td>----</td>
<td>----------------</td>
<td>-----</td>
</tr>
<tr>
<td>9.8</td>
<td>0.03</td>
<td>0.293</td>
</tr>
<tr>
<td>10</td>
<td>0.04</td>
<td>0.389</td>
</tr>
<tr>
<td>10.2</td>
<td>0.06</td>
<td>0.584</td>
</tr>
<tr>
<td>10.4</td>
<td>0.09</td>
<td>0.875</td>
</tr>
<tr>
<td>10.6</td>
<td>0.12</td>
<td>1.165</td>
</tr>
<tr>
<td>10.8</td>
<td>0.14</td>
<td>1.357</td>
</tr>
<tr>
<td>11</td>
<td>0.16</td>
<td>1.549</td>
</tr>
<tr>
<td>11.2</td>
<td>0.17</td>
<td>1.644</td>
</tr>
<tr>
<td>11.4</td>
<td>0.18</td>
<td>1.739</td>
</tr>
</tbody>
</table>

**Table. 17** Variation of $n$ values with pH in 50% DMF – 50% Water mixture (v/v)

<table>
<thead>
<tr>
<th>Acetylsalicylic acid + Mn (II)</th>
<th>Acetylsalicylic acid + Cu (II)</th>
<th>Acetylsalicylic acid + Zn (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>$V_3 - V_2$</td>
<td>$n$</td>
</tr>
<tr>
<td>----</td>
<td>-------------</td>
<td>-----</td>
</tr>
<tr>
<td>10.4</td>
<td>0.04</td>
<td>0.390</td>
</tr>
<tr>
<td>10.2</td>
<td>0.06</td>
<td>0.584</td>
</tr>
<tr>
<td>10.4</td>
<td>0.09</td>
<td>0.875</td>
</tr>
<tr>
<td>10.6</td>
<td>0.12</td>
<td>1.165</td>
</tr>
<tr>
<td>10.8</td>
<td>0.14</td>
<td>1.357</td>
</tr>
<tr>
<td>11</td>
<td>0.16</td>
<td>1.549</td>
</tr>
<tr>
<td>11.2</td>
<td>0.17</td>
<td>1.644</td>
</tr>
<tr>
<td>11.4</td>
<td>0.18</td>
<td>1.739</td>
</tr>
</tbody>
</table>
The different solvent ratio yields a compelling change in their binding properties and the tendency to select a ligand for certain cation over the others. The stability constants of all complexes increase with increase in DMF concentration in the binary mixture. The solvating of the metal ions and the ligand in DMF mixture should be less than water. The donor number of DMF (26.2) is less than the water (33) which makes the weak solvating nature of DMF [233]. The difference between log $K_1$ and log $K_2$ values are smaller which shows the simultaneous complex formation. The value of log $K_1$ for the formation of Cu (C$_9$H$_7$O$_4$)$^+$ appear to be fairly good while log $K_2$ is small due to low metal concentration. The deviations may be due to the difficulty caused by the hydroxide formation at higher pH values. The Irving-Williams order of stability constant was followed for the Cu(II) and Zn(II) complexes.

Table 18 Stability constant of Acetylsalicylic acid with Mn (II), Cu (II) and Zn (II) metal ions at 300 ± 0.1 K and the ionic strength $I = 0.1$ mol dm$^{-3}$ KNO$_3$ in different binary solvent mixture

<table>
<thead>
<tr>
<th>Metal complexes of</th>
<th>log $K_1$</th>
<th>log $K_2$</th>
<th>log $K_1$ - log $K_2$</th>
<th>log $K_1$ / log $K_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn (II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70% DMF - 30% Water mixture (v/v)</td>
<td>50% DMF - 50% Water mixture (v/v)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn(II)</td>
<td>3.29 2.81 0.48 1.170</td>
<td>Mn(II) 3.19 2.72 0.47 1.173</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu(II)</td>
<td>3.19 2.71 0.48 1.177</td>
<td>Cu(II) 2.99 2.81 0.18 1.064</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn(II)</td>
<td>3.01 2.71 0.30 1.111</td>
<td>Zn(II) 2.89 2.51 0.38 1.151</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure. 18 pH metric titration curve of Free Acid + Ligand (Acetylsalicylic acid) + metal ions in 70% DMF-30% Water mixture (v/v)
Figure. 19 pH metric titration curve of Free Acid + Ligand (Acetylsalicylic acid) + metal ions in 50% DMF-50% Water mixture (v/v)

Figure. 20 Half integral method for determining pK of Acetylsalicylic acid at 70% DMF-30% Water mixture (v/v)
Figure 21 Half integral method for determining pK of Acetylsalicylic acid at 50% DMF-50% Water mixture (v/v)
Figure. 22  Plot of n Vs pH of ligand Acetylsalicylic acid-with metal ions a) Mn(II), b) Cu(II), c) Zn(II) in 70% DMF-30% Water mixture (v/v) d) Mn(II) e) Cu(II), f) Zn(II) in 50% DMF-50% Water mixture (v/v)

4.4. Metal ligand complex synthesis

Based on the pH-metric study of the complexes formed between ligands and divalent metal ions (M^{2+}) ; it was noted that the ligands forms more stable complexes
with metal ions in binary solvent mixture. Therefore, solid metal complexes were synthesized and analyzed by using electronic spectra, FT-IR spectra, and were also tested for their biological activities. The results of elemental analysis, morphology and magnetic measurements of the metal complexes of various ligands were presented in the result and discussion.

4.5. Characterization and biological activity of 2-Aminoethane sulfonic acid and its metal complexes

In general, 2-aminoethane sulfonic acid, an analogue of β-alanine forms weak stable complexes with metal ions. The basicity of 2-aminoethane sulfonic acid was comparatively less than its analogue β-alanine because of its weak sulfo group, the role of co-solvent DMF in this work compensates the basicity and forms moderately stable complexes. Because, the co-solvent impacts the equilibria in solution due to change in the dielectric constant of the medium that changes the relative contribution of electrostatic and non-electrostatic interactions which in turn increase the stability constant values [234].

The physical characteristics and elemental analysis of the complexes of 2-Aminoethane sulfonic acid were given in Table. 19.

All the metal complexes were well stable, hygroscopic and soluble in methanol, ethanol and acetonitrile and partially soluble in water. The elemental analysis data of the compounds indicates a 1:2 metal: ligand stoichiometry for all the complexes. The analytical data of the metal complexes are in consistent with the expected formulation.

**Table. 19** Physical characteristics and elemental analysis of transition metal taurate complexes
<table>
<thead>
<tr>
<th>Compound</th>
<th>m.p (°C)</th>
<th>Content %</th>
<th>Color</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn(L)$_2$(H$_2$O)$_2$].2H$_2$O</td>
<td>256</td>
<td>17.5 (18.26) 8.86 (9.64) 17.94 (18.24) 11.23 (11.64)</td>
<td>Dark brown</td>
<td>62</td>
</tr>
<tr>
<td>[Cu(L)$_2$ (H$_2$O)$_2$]. H$_2$O</td>
<td>336</td>
<td>18.84 (18.4) 9.64 (9.36) 21.26 (21.02) 10.63 (9.88)</td>
<td>Bluish green</td>
<td>68</td>
</tr>
<tr>
<td>[Zn(L)$_2$].H$_2$O</td>
<td>348</td>
<td>16.74 (15.42) 9.14 (9.06) 19.54 (18.68) 10.82 (9.65)</td>
<td>White</td>
<td>71</td>
</tr>
</tbody>
</table>

4.5.1. UV absorption spectra of Taurine (2-Aminoethane sulfonic acid) and its metal complexes

Taurine act as a weak UV absorbing drug [235]. The dissociation constant of taurine was high (pK$_a$ = 8.95 in water), which cannot be detected sensitively in UV spectra due to the lack of an ultraviolet chromophore [236]. In our present study, binary solvent mixture (DMF-water) was used for synthesizing the metal complexes. The key role of polar solvent like DMF in binary solvent mixture increases the π* value (π* is solvent dipolarity/polarizability) from the Kamlet and Taft equation, thereby the increase in π* values, the band represents bathochromic shift [237]. Hence, the polar solvent tends to shift the absorption of taurine to longer wavelength which lower the absolute values of the extinction coefficients. This observation was agreed with that of Ley and Arends [238]. Figure. 23 represented the electronic spectra of ligand taurine and its Mn(II), Cu(II) and Zn(II) binary complexes respectively.
UV spectra of the ligand taurine and its metal complexes were recorded in the range of 200-800 nm. Absorption spectrum of taurine was at 256 nm which may be attributed to $\pi$ - $\pi^*$ transition. On complexation with metal ions, various electronic transitions ($n\pi^*$ and $\pi\pi^*$) occurred which causes the absorption bands relocated to shorter or longer wavelengths compared to the free ligand. This possibly confirmed that the ligand interacted with the metal ion leading to the formation of different kinds of complexes. The formation of these new bands in the metal complexes at higher wavelength may be assigned due to d-d transition or LMCT. In case of Cu (II) complex, a broad low intensity band centered at 747 nm which has been assigned as d-d transition, suggesting a distorted octahedral environment [239]. The similar case was for Manganese (II) complex also. In Zinc (II) complex, Zinc (II) has completely filled
d-orbital which will not exhibit d-d electronic transition and show absorptions only in the higher wavelength region at 278, 335 and 365 nm which was associated to the ligand electron transitions and with the assistance of elemental analysis and additional spectroscopic techniques, it is considered that the complex has a coordination number four and are expected to be tetrahedral [240].

**4.5.2. FT-IR characterization Taurine (2-Aminoethane sulfonic acid) and its metal complexes**

The main characteristic absorption bands of 2-aminoethane sulfonic acid and its binary metal complexes along with their proposed assignments were given in Table. 20. The coordinating atoms were determined on the base of the comparison of the stretching frequencies of the ligand and its complexes (Figure. 24). The stretching bands of the complexes were sharp instead of distinct bands which might be due to the zwitterionic nature of the ligand in the crystalline nature [241]. Different metal complexes (Mn (II), Cu (II) and Zn (II)) showed a shift in the stretching frequency of sulfonic acid (SO$_3^{2-}$) of ligand from 1229 cm$^{-1}$ to higher frequencies such as 1245 cm$^{-1}$, 1290 cm$^{-1}$ and 1284 cm$^{-1}$, which confirms its involvement in co-ordination [242-243]. The N-H stretching vibration of ligand at 3172 cm$^{-1}$ was moved to higher stretching frequencies with the complexes, implying that the co-ordination of the metal complexes with ligand was through the nitrogen atom [244-245].
This was further supported by the shift of υ(NH$_2$) to lower frequencies when compared to the free ligand at 1619 cm$^{-1}$ due to the co-ordination of the ligand 2-aminoethane sulfonic acid (-NH$_2$ group) to the metal ions. The formation of new bands at 501 – 548 cm$^{-1}$ and 620 - 695 cm$^{-1}$ were attributed to (M-N) and (M-O) bond stretching band frequencies correspondingly and conveyed as further evidence of coordination of metal via the nitrogen atom of amine group and oxygen atom of sulfonic group of the ligand [246-247]. Conclusively, the study of assigning the water molecules related with the complex formation was troublesome as the vibrations of the

**Figure. 24** FT-IR spectra of Taurine (2-Aminoethane sulfonic acid) and its metal complexes
ligand meddle in this region. From, further thermal study, it will be clear to confirm the nature of the water molecules present in the lattice or coordinated to the metal.

**Table. 20** Functional group analysis of the ligand and the complexes

<table>
<thead>
<tr>
<th>Taurine (cm⁻¹)</th>
<th>Taurine-Mn complex (cm⁻¹)</th>
<th>Taurine-Cu complex (cm⁻¹)</th>
<th>Taurine-Zn complex (cm⁻¹)</th>
<th>Tentative assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3172</td>
<td>3333</td>
<td>3472</td>
<td>3240</td>
<td>assym str of N-H</td>
</tr>
<tr>
<td>1619</td>
<td>1516</td>
<td>1560</td>
<td>1578</td>
<td>NH₂ b</td>
</tr>
<tr>
<td>1229</td>
<td>1245</td>
<td>1290</td>
<td>1284</td>
<td>assym str of SO₃</td>
</tr>
<tr>
<td>1187.17, 1112.98</td>
<td>1089.78</td>
<td>1184</td>
<td>1016</td>
<td>C-N b</td>
</tr>
<tr>
<td>1035.62</td>
<td>1014</td>
<td>540</td>
<td>1016</td>
<td>sym str of SO₃</td>
</tr>
<tr>
<td>-</td>
<td>652</td>
<td>530</td>
<td>646</td>
<td>M-O</td>
</tr>
<tr>
<td>-</td>
<td>518</td>
<td>683</td>
<td>540</td>
<td>M-N</td>
</tr>
</tbody>
</table>

**4.5.3. Thermal study of the metal complexes**

Thermogravimetric analyses of metal complexes of 2-aminoethane sulfonic acid were conducted in the temperature range of 30°C-600°C under Nitrogen atmosphere at a heating rate of 10°C min⁻¹. The thermograms were explained in detail further below.

a) Manganese complex

For manganese complex, an initial weight loss of 7.3% was noted below 120°C and 194°C which was attributed to the release of free and co-ordinated water molecules (calc. 7.5%) (Figure. 25). Afterwards, the decomposition of the substructure occurred at 200°C interrelated to the loss of taurine ligands (Obsd. 49.5, Calc. 49.8%) in the
temperature range of 200°C - 300°C. Hence, it can be assumed that during this thermal decomposition metal derivatives were formed, which decompose in the temperature range of 300°C - 540°C. Beyond 540°C, there was no significant weight loss, the final residue most likely was MnO₂ (Obsd. 24.47, Calc. 24.76%). The result for the formation of metal oxide in the corresponding oxidation state was further supported by XPS data. The complex which have peaks for Mn 2p₃/₂ and Mn 2p₁/₂ where their difference in their binding energies was 11.62 eV which showed the presence of manganese in its oxide form as MnO₂ (S1).

![Graph](image)

**Figure. 25** TGA-DTG spectrum for a) taurine–Mn (II) metal complex

b) Copper complex

For copper taurate complex, dehydration of the complex was noticed because of the release of water molecules below 200°C showed both the free and the co-ordinated water molecules present (Obsd. 18.36%, Calc. 18.57%) (Figure. 26). Later, the thermal changes between 160°C-430°C were associated to the degradation of ligand group which was not a single peak. Exothermic peaks were observed at DTG values 325°C,
399°C and 402°C with a great mass loss of 44.14% (calc. 44.82 %) might be both the endothermic and exothermic reactions occurred concurrently. The ligand decomposed into gaseous products evolved such as SO₂, CO₂ and CO. Thus, the residue above 430°C assumed to be CuO (Obsd. 19.63%, Calc. 19.75%) [248] where their binding energy difference between Cu 2p₃/₂ and Cu 2p₁/₂ was greater than 20 eV (S2).

**Figure. 26** TGA-DTG spectrum for taurine–Cu (II) metal complex

c) Zinc complex

For zinc complex, a weight loss of 8.5% was observed at 141°C due to the water molecules present in the crystal lattice (Calc. 8.72%) (Figure. 27). Later, the next observed changes are the thermal decomposition of the complex between 180°C to 400°C owed to the degradation of ligand moiety (DTG at 338°C, 351°C and 389°C) with a great mass loss (Obsd. 52.66%, Calc. 52.47%) correspond to the decomposition of taurine, thus the final product above 450°C corresponds to metal residue ZnO (Obsd. 11.44%, Calc. 11.73%). The evidence for the formation of ZnO was supported from XPS data of the complex. The complex which have peaks for Zn 2p₃/₂ and Zn 2p₁/₂
where their difference in their binding energies was greater than 20 eV which correspond to peaks for ZnO (S3).

**Figure. 27** TGA-DTG spectrum for Zn (II) metal complex

### 4.5.4. XRD crystallographic data

The well embossed peaks of X-ray diffraction pattern of taurine metal complexes have been indexed, refined and analyzed by using computer program from Match software with reference COD inorganic database (Figure. 28) [249]. The d-spacing value and its corresponding intensities were calculated (Table. 21). The lattices parameters like $a$, $b$, $c$, $\alpha$, $\beta$, $\gamma$, and $V$ (volume) are shown in (Table. 22). The indexing was compared between observed and calculated ($2\theta$) values and then confirmed. The density ($d$) of the complex was determined by the flotation method in a saturated solution of Benzene, KBr, and NaCl separately.
Figure 28 Powder XRD pattern of (a) Mn(II), (b) Cu(II), and (c) Zn(II) complexes of Taurine

The crystal system of copper and zinc taurate complexes were found to be monoclinic, whereas for manganese was cubic. The Debye-Scherrer equation in X-ray diffraction and crystallography was a formula which relates the size of the crystallites in a solid to the broadening of a peak in a diffraction pattern. The Debye-Scherrer equation was

\[
D = \frac{k\lambda}{\beta \cos \theta}
\]

where \(D\) = crystallite size, \(\lambda\) = wavelength of X-ray radiation (CuK\(\alpha\) = 0.154060nm), \(k\) = constant taken as 0.94, \(\theta\) = diffraction angle (in radians), and \(\beta\) = full width at half
maximum height (FWHM) (2.52 nm). The average crystallite size Mn(II), Cu(II) and Zn(II) complexes were found to be 50.5 nm, 37.9 nm and 41.6 nm, respectively.

### Table. 21 X-ray diffraction data of taurine metal complexes

<table>
<thead>
<tr>
<th>Taurine- Mn(II) complex</th>
<th>Taurine- Cu(II) complex</th>
<th>Taurine- Zn(II) complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>d (Å)</td>
<td>Intensity (%)</td>
</tr>
<tr>
<td>13.59</td>
<td>6.510</td>
<td>51.2</td>
</tr>
<tr>
<td>18.14</td>
<td>4.886</td>
<td>14.3</td>
</tr>
<tr>
<td>21.01</td>
<td>4.225</td>
<td>20.5</td>
</tr>
<tr>
<td>22.6</td>
<td>3.931</td>
<td>39.9</td>
</tr>
<tr>
<td>23.92</td>
<td>3.717</td>
<td>30.5</td>
</tr>
<tr>
<td>25.75</td>
<td>3.457</td>
<td>15.8</td>
</tr>
<tr>
<td>31.82</td>
<td>2.810</td>
<td>100</td>
</tr>
<tr>
<td>36.11</td>
<td>2.485</td>
<td>16.8</td>
</tr>
<tr>
<td>41.53</td>
<td>2.173</td>
<td>43.9</td>
</tr>
<tr>
<td>45.49</td>
<td>1.992</td>
<td>50.6</td>
</tr>
<tr>
<td>56.59</td>
<td>1.625</td>
<td>21.1</td>
</tr>
<tr>
<td>66.11</td>
<td>1.412</td>
<td>10.1</td>
</tr>
<tr>
<td>75.41</td>
<td>1.259</td>
<td>13.3</td>
</tr>
</tbody>
</table>
Table. 22 Determination of lattice parameters from X-ray diffraction data

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Manganese (II) Taurate</th>
<th>Copper (II) Taurate</th>
<th>Zinc (II) Taurate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>298K</td>
<td>298K</td>
<td>298K</td>
</tr>
<tr>
<td>X-ray Radiation</td>
<td>CuK(_a1)</td>
<td>CuK(_a1)</td>
<td>CuK(_a1)</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>1.5406</td>
<td>1.5406</td>
<td>1.5406</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Cubic</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>F m-3m</td>
<td>C 12/ m1</td>
<td>P 121</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>(\beta)</td>
<td>90</td>
<td>105.42</td>
<td>106.36</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>(a) (Å)</td>
<td>5.638</td>
<td>13.646</td>
<td>10.443</td>
</tr>
<tr>
<td>(b) (Å)</td>
<td>5.638</td>
<td>4.046</td>
<td>6.326</td>
</tr>
<tr>
<td>(c) (Å)</td>
<td>5.638</td>
<td>18.625</td>
<td>7.924</td>
</tr>
<tr>
<td>Density (g/cm(^3)) calc</td>
<td>2.154</td>
<td>2.628</td>
<td>2.542</td>
</tr>
<tr>
<td>Volume (cm(^3))</td>
<td>179.21</td>
<td>1012.32</td>
<td>229.64</td>
</tr>
<tr>
<td>Average Crystallite size (nm)</td>
<td>68.3</td>
<td>50.5</td>
<td>41.6</td>
</tr>
</tbody>
</table>

4.5.5. Magnetic property of taurine metal complexes

The magnetic property of metal complexes were measured using SQUID (superconducting quantum interference device). Figure. 29 (a-b-c) showed M-H curves between magnetization versus applied field ((-5 Tesla ≤ H ≤ 5 Tesla) at different temperature 5K and 300K. The magnetic nature for the Mn (II) and Cu (II) complex were established to be paramagnetic and for Zinc it was diamagnetic. While, Figure. 27 (d-e-f) showed M-T curves between zero-field-cooled magnetization verses temperature (5 ≤ T ≤ 300K) at H=300 Oersted (Oe). The value of magnetization for the paramagnetic nature of the complex was given in the Table. 23.
Figure 29 Field dependent magnetization (M-H) curves of (a) Manganese taurate and (b) Copper taurate c) Zinc taurate at temperatures of 5k and 300k; and mass-corrected temperature-dependent magnetization curve (M-T) of (d) Manganese taurate and (e) Copper taurate f) Zinc taurate at H of 300 Oe
Table 23 Magnetization value for Manganese taurate and Copper taurate

<table>
<thead>
<tr>
<th>Sample</th>
<th>Magnetization (emu.g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5K</td>
</tr>
<tr>
<td>Mn(II)-Taurine complex</td>
<td>20.2</td>
</tr>
<tr>
<td>Cu(II)-Taurine complex</td>
<td>10.0</td>
</tr>
</tbody>
</table>

4.5.6. SEM Morphology for taurine metal complexes

The morphology of 2-aminoethane sulfonic acid metal complexes were photographed by FE-SEM and the elemental analysis (EDS) was used to identify the presence of metal present in the complex shown in Figure. 30.

The complexes are found to be crystalline in nature and they were agglomerated. The EDS analysis were used to identify the presence of metals which are co-ordinated to the ligand. The percentage composition of the elements were not identified accurately since they were aggregated poorly.
Figure. 30 SEM image (a,c,e) and EDS analysis (b,d,f) for the taurine Mn, Cu and Zn metal complexes
4.5.7. Cytotoxicity

The toxicity of the metal complexes was determined by MTT assay and compared with the ligand 2-aminoethane sulfonic acid (Figure. 31). 2-aminoethane sulfonic acid, a natural sulfur containing amino acid which was naturally present in body tissues in most of the animals have a wide role in physiological functions. The toxicity of 2-aminoethane sulfonic acid was well tolerated even at higher doses (4-6 g/kg) and protect the neurons against ethanol-induced apoptosis [250]. Figure. 31 showed survival of the cells treated with 2-aminoethane sulfonic acid were well survived. Metal complexes of 2-aminoethane sulfonic acid which exhibited better viability, minimal toxicity was observed when the concentration of the metal complexes increased. Higher cell viability was observed in the case of copper taurate complex when compared to other complexes. Studies showed that copper was observed to be an essential cofactor and necessary for the tumor angiogenesis processes [251-253], although other trace metals which were present in the body are not. Copper was not bounded by protein, hence it can be stored in cellular membranes [254]. As a result, copper-based drugs have high potential value in cancer treatments. Few studies showed that taurine derivative compounds inhibited the proteasomal activity (particularly, chymotrypsin-like activity), could strongly induce apoptosis in the cultured breast cancer and leukemia cells [255].
Figure 31 Cell viability of the ligand taurine and metal taurate complexes

4.5.8. Antioxidant activity

Taurine have a strong oxidant scavenging ability to protect the cells raised from oxidant-induced injuries by preventing changes in membrane permeability and cytotoxic agents [256-259]. Taurine, an effective oxidant, capable to delay the advancement of cancer by retarding the increase of ROS in tumors [260]. Taurine has also been described to improve the indications of metal-induced intoxication for Pb and Cd [261-263]. Lu et al. reported that taurine weakened the Mn induced neurotoxicity without altering the level of Mn in blood and thereby taurine progresses the impaired learning and memory ability due to the excessive exposure of Mn.
Figure 32 DPPH radical inhibition activity of taurine complexes obtained through absorbance at 517 nm

From the Figure 32, it has been further confirmed that the taurine possessed dominant antioxidant activity as compared to the metal complexes. Copper complex exhibited higher antioxidant activity than the other metal complexes. The calculated antioxidant activities (IC$_{50}$) of the ligand and their corresponding metal complexes were compared with the control (Table 24). The radical scavenging capacity of the ligand and metal complexes was directly dependent on its concentration. Several mechanisms were there for their antioxidant activity related to its specific applications [264-266]. For example, in case of metal (lead) induced oxidative stress, taurine enriched as antioxidant protection entity by means of suppressing the lipid peroxidation mechanism, thereby reducing the intake of GSH.
**Table. 24** Free radical scavenging activity of metal complexes of 2-aminoethane sulfonic acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ascorbic acid</th>
<th>*Ligand (Taurine)</th>
<th>Manganese taurate complex</th>
<th>Copper taurate complex</th>
<th>Zinc taurate complex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IC$_{50}$</strong></td>
<td>1.39 ± 0.76</td>
<td>2.02 ± 1.64</td>
<td>3.24 ± 0.54</td>
<td>1.99 ±0.74</td>
<td>2.09 ±0.93</td>
</tr>
<tr>
<td><strong>R$^2$</strong></td>
<td>0.965</td>
<td>0.978</td>
<td>0.942</td>
<td>0.973</td>
<td>0.943</td>
</tr>
</tbody>
</table>

**4.5.9. Antimicrobial study of the metal complexes**

Nowadays, Zwitterionic compounds have been extensively used in the biomedical field. In particular, the sulfonic acids and sulfonate compounds have significant role in biological process due to their excellent biocompatibility. The strong acid nature of sulfonic acid constituents which was in the zwitterionic form easily penetrate into the cell membrane and exhibited fungicidal, bactericidal, viricidal activities. From the Table. 25, the antimicrobial activity of taurine and its metal complexes were studied. The copper and zinc complexes showed higher activity when compared to the ligand taurine (Figure. 33).

Copper complexes or copper ions alone have been used commonly for disinfection of tissues for a long time. Various mechanisms have been proposed for its disinfection activity which comprised of membrane permeabilization in plasma, degrading the microbial proteins and suppress its cellular activity and membrane lipid peroxidation [267].

The mechanism for the microcidal activity of taurine complexes might be attributed to the enhancement of lipophilic characteristics of the complexes arisen from complex chelation. In general, chelation decreases the charge of the central metal atom because of its partial distribution of its positive charge with the donor groups of the
ligand. Additionally, chelation up surged the lipophilic nature of the metal, which later aided the transportation through the phospholipid layer of the cell membrane. The mechanism of the complexes could also be involved in the formation of weak hydrogen bonds, as well as the two amine groups present which illustrate the biological transformations occurred in the systems. Further, the activity was proportional to the concentration of the synthesized metal complexes. Hence, it was suggested that the complexes that have microcidal activity might be eradicating the microbes or by suppressing manifolding of the microbe by halting their active sites [268-270].

**Table. 25** Anti-bactericidal and anti-fungal activities of the ligand taurine and metal taurate complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition in diameter (mm)</th>
<th>Antibacterial*</th>
<th>Antifungal**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Taurine</td>
<td></td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Manganese taurate</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper taurate</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zinc myristate</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tetracycline*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketaconazole**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 33 Anti-microbial activity of Taurine and its metal complexes
4.6. Characterization and biological activity of Tetradecanoic acid and its metal complexes

The physical characteristics and elemental analysis of the complexes of myristic acid were given in Table. 26.

All the complexes of metals were well stable, hygroscopic and sparingly soluble in chloroform, methanol, ethanol and acetonitrile and insoluble in water. The elemental analysis data of the compounds indicates a 1:2 metal: ligand stoichiometry for Mn and Zn complexes whereas 1:4 for copper complex. The analytical data of the metal complexes were consistent with the expected formulation.

Table. 26 Physical characteristics and elemental analysis of transition metal myristate complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>m .p (°C)</th>
<th>Content % found (calcd.)</th>
<th>Color</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>O</td>
<td>M</td>
</tr>
<tr>
<td>[Mn(L)₂(H₂O)₂].2H₂O</td>
<td>256.8</td>
<td>61.97(61.23)</td>
<td>23.40(22.47)</td>
<td>14.63(13.66)</td>
</tr>
<tr>
<td>[Cu(L)₂(H₂O)₂].2H₂O</td>
<td>200.8</td>
<td>75.82(74.46)</td>
<td>9.67(9.36)</td>
<td>14.52(14.32)</td>
</tr>
<tr>
<td>[Zn(L)₂]</td>
<td>143</td>
<td>67.81(66.79)</td>
<td>17.46(17.21)</td>
<td>12.74(11.68)</td>
</tr>
</tbody>
</table>

4.6.1. Electronic absorption spectra of metal complexes

The UV spectra of myristic acid metal complexes were shown in Figure. 34. The absorption band at 259 nm assigned to the n-π* transition of ligand myristic acid (in heptane 210 nm) (Table. 27) [271]. The absorption bands for their corresponding metal myristate complexes were shifted to longer wavelength due to the coordination of the ligand to the metal ions. The broad band was observed at longer wavelength in case of Cu complex indicate d-d transition, presumed for a six co-ordinate species (d⁹), with octahedral geometry [272-273].
**Table. 27** UV absorption data of myristic acid and its metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength (nm)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid</td>
<td>259</td>
<td>n → π*</td>
</tr>
<tr>
<td>Manganese myristate</td>
<td>220,260</td>
<td>π → π*, n → π*</td>
</tr>
<tr>
<td>Copper myristate</td>
<td>281,646</td>
<td>L → Cu CT, d-d transition</td>
</tr>
<tr>
<td>Zinc myristate</td>
<td>228,289</td>
<td>n → π*, L → Zn CT</td>
</tr>
</tbody>
</table>

**Figure. 34** Electronic spectra of myristic acid and its metal complexes
4.6.2. **Functional group analysis of the metal myristate complexes by FT-IR spectra**

Specific analysis of the IR spectra of the complexes of metals and its comparison to the ligand was done and it gave information towards different transition metal ions. Therefore, a clear analysis for the IR spectrum of the ligand and the metal binding effect on the vibration was accomplished. The most significant IR spectral bands of all the systems and their assignments were obtained in Table. 28 and their spectra are given in Figure. 35.

**Table. 28** Functional group analysis of the ligand and the complexes

<table>
<thead>
<tr>
<th>Myristic acid (cm⁻¹)</th>
<th>Mn- Myristic acid complex (cm⁻¹)</th>
<th>Cu- Myristic acid complex (cm⁻¹)</th>
<th>Zn- Myristic acid complex (cm⁻¹)</th>
<th>Tentative assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3058.26</td>
<td>3464.94</td>
<td>3487, 3195.27</td>
<td>3466, 3190.26</td>
<td>O-H str broad peak</td>
</tr>
<tr>
<td>2917.59, 2849.43</td>
<td>3043.67, 2922.42</td>
<td>3041.74, 2997.84</td>
<td>3041.74, 2968.45</td>
<td>C-H str of methylene and methyl groups</td>
</tr>
<tr>
<td>1735.83, 1703.49</td>
<td>1612.49, 1506.41</td>
<td>1681.42, 1508.33</td>
<td>1614.42, 1506.33</td>
<td>C=O &amp; COO of ester</td>
</tr>
<tr>
<td>1466.86, 1405.23</td>
<td>1401.95,</td>
<td>1425.40, 1361.96</td>
<td>1403.88</td>
<td>C-O str of carboxylic acid</td>
</tr>
<tr>
<td>1122.25, 940.13</td>
<td>1136.07, 891.11</td>
<td>1164.65, 960.55</td>
<td>1168.86, 960.56</td>
<td>C-C str</td>
</tr>
<tr>
<td>740.13</td>
<td>732.95,</td>
<td>732.85,</td>
<td>734.88</td>
<td>C-H bending</td>
</tr>
<tr>
<td></td>
<td>456</td>
<td>610.28, 449</td>
<td>463</td>
<td>M-OH &amp; M-O</td>
</tr>
</tbody>
</table>

The IR spectra of all the metal complexes shows the M-O linkage around 449-463 cm⁻¹. The absorption bands in the range of 1688-1678 and 1394-1352 cm⁻¹
correlates the asymmetric $\nu_{as}$ COO$^-$ and symmetric $\nu_{sy}$ COO$^-$ stretching frequencies to the metal bound carboxylates [274-275]. The difference in $\Delta\nu$ COO$^-$ frequencies ($\Delta\nu < 230 \text{ cm}^{-1}$) for copper complex was indicative of bidentate coordination of both the carboxylate groups [276]. Also, for the Mn (II) and Zn (II) myristates the differences were lesser than expected suggesting the bidentate bonding in solid state.

Figure. 35 FT-IR spectra of myristic acid and its metal complexes
4.6.3. Morphology of transition metal myristate complexes

The morphology of myristic acid-metal complexes were analyzed by SEM shown in Figure. 36 (a-c-e). The Mn-myristic acid complexes formed thread-like structures, whereas the image of the Cu complex showed fine sharp and rod-shaped structure which indicates the semi crystalline nature. It was also observed from the figures that the Zn(II) complexes have plate-like structure [277]. The particle sizes of the Zn-myristic acid complexes comprised of the diameter range of a few microns. Still, particles with their size less than 1µm were also seen which combine to form larger size agglomerates. EDS analysis was performed to find the distribution of elements on the complex surface. The results obtained in Figure. 36 (b-d-f) proved that the synthesized complex composed of metal, C and O. The elemental analysis from SEM results for the complexes of metals were also in agreement with XPS composition analysis.

![Figure 36](image_url)

**Figure. 36** SEM image (a,c,e) and EDS analysis (b,d,f) for the myristic acid metal complexes

4.6.4. Thermal study of metal complexes
Thermo gravimetric (TG) analyses of metal complexes were conducted in the temperature range of 30°C-900°C. TG and DTG analyses were done to confirm the molecular structure of the complexes. The thermal stabilities with variation with temperature and their weight losses along with DTG peaks are listed in Table. 29.

**Table. 29** Thermal analysis data of myristic acid metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature range (°C)</th>
<th>DTG (°C)</th>
<th>Weight loss (%)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weight loss (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calc. Found</td>
<td></td>
</tr>
<tr>
<td>[Mn(L)₂(H₂O)₂].2H₂O</td>
<td>30-260</td>
<td>305</td>
<td>18.94</td>
<td>18.67</td>
</tr>
<tr>
<td></td>
<td>300-600</td>
<td>497</td>
<td>26.26</td>
<td>25.48</td>
</tr>
<tr>
<td>[Cu(L)₂(HL)₂].2H₂O</td>
<td>300-600</td>
<td>374</td>
<td>75.38</td>
<td>75.92</td>
</tr>
<tr>
<td>[Zn(L)₂]</td>
<td>300-600</td>
<td>374</td>
<td>24.62</td>
<td>23.76</td>
</tr>
</tbody>
</table>

Thermal analysis of the metal myristate complexes was carried out to study the degradation pattern, thermal stability, and purity.

a) [Mn(L)₂(H₂O)₂].2H₂O

The thermal degradation of this complex occurred at two major steps (Figure. 37). The first step of the degradation takes place in the range of 20°C -260°C which corresponds to the elimination of two co-ordinated and two unco-ordinated water molecules beside of organic moiety (C₂₄H₅₄O₂). The second degradation fall in the range of 300°C -600°C which was assigned to loss of organic moiety from the anhydrous complex with weight loss of 25.48%. The final residue could be interpreted as MnO₂ from MnCO₃. The binding energy difference Mn 2p₃/2 and Mn 2p₁/2 was 11.8eV which evidenced the formation MnO₂ (S4).
Figure. 37 TGA-DTG spectrum for myristic acid – Mn (II) metal complex

b) [Cu L₂ (HL)₂].2H₂O

The thermal degradation of [Cu L₂ (HL)₂].2H₂O complex proceeded with the formation of intermediates and final products (Figure. 38). The mass loss observed between 50°C-200°C corresponds to the dehydration with a loss of two molecules of water (4.2%). The major thermal decomposition step occurred between 200°C-500°C were observed at 309°C, 358°C and 454°C due to the decomposition of ligand moiety such as H₂O, CO₂ and CO. The final residual product was Cu₂O [278-279]. The binding energy difference Cu 2p₃/2 and Cu 2p₁/2 was lesser than 20eV which support the result for the formation of Cu₂O (S5).
Figure. 38 TGA-DTA spectrum for myristic acid – Cu (I) metal complex

c) [Zn(L)₂]

The thermal degradation of the [Zn(L)₂] complex take place in mainly one degradation step (Figure. 39). This degradation step occurred at a maximum temperature of 374°C and the weight loss was around 75.92% because of the loss of the organic moiety. Theoretically, the loss of the molecules corresponds to a weight loss of 75.38% [280]. The binding energy difference Zn 2p₃/2 and Zn 2p₁/2 was greater than 20eV which confirmed the metal oxide in the form of ZnO (S6 pg.no133).
4.6.5. X-ray diffraction study of metal complexes

Powder XRD patterns of Mn(II), Cu(II) and Zn(II) complexes were recorded in the range (2θ = 0–80°) and shown in Figure. 40(a–c). The patterns showed sharp crystalline peaks indicating their crystalline phase. Scherer’s formula was used to calculate the average crystallite size (d_{XRD}) of the complexes [281-282]. The Mn(II), Cu(II) and Zn(II) complexes have an average crystallite size of 76, 80 and 59 nm respectively.

The X-ray diffraction data of the metal complexes were given in Table. 30. The X-ray patterns were indexed by the computer software Philips Xpert Pro (PCPDFWIN v.2.1) and the application of an interactive trial and error method with the characteristics of the various symmetry systems kept in mind was done until a good fit was obtained by comparing the major corresponding peaks (JCPDS No 211467 for Zinc & 290879 for Manganese). The observed values for Mn(II) and Cu(I) complexes

---

Figure. 39 TGA-DTA spectrum for myristic acid –Zn (II) metal complex
were good fit for monoclinic and tetragonal system for Zn(II) complex to give their corresponding lattice constant (Table. 31) [283].

Figure. 40 Powder XRD pattern of (a) Mn(II), (b) Cu(I), and (c) Zn(II) complexes of Tetradecanoic acid

Table. 30 X-ray diffraction data of Myristic acid metal complexes

<table>
<thead>
<tr>
<th>Myristic acid- Mn(II) complex</th>
<th>Myristic acid- Cu(I) complex</th>
<th>Myristic acid- Zn(II) complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\theta$</td>
<td>$d$ (Å)</td>
<td>Intensity (%)</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>--------------</td>
</tr>
<tr>
<td>20.77</td>
<td>4.273</td>
<td>39.9</td>
</tr>
<tr>
<td>22.26</td>
<td>3.990</td>
<td>42.0</td>
</tr>
<tr>
<td>23.3</td>
<td>3.814</td>
<td>100</td>
</tr>
<tr>
<td>26.64</td>
<td>3.343</td>
<td>34.0</td>
</tr>
<tr>
<td>29.3</td>
<td>3.046</td>
<td>64.1</td>
</tr>
<tr>
<td>30.06</td>
<td>2.970</td>
<td>41.9</td>
</tr>
<tr>
<td>32.07</td>
<td>2.789</td>
<td>49.2</td>
</tr>
<tr>
<td>34.95</td>
<td>2.565</td>
<td>23.1</td>
</tr>
<tr>
<td>36.97</td>
<td>2.430</td>
<td>29.5</td>
</tr>
<tr>
<td>41.73</td>
<td>2.163</td>
<td>32.2</td>
</tr>
<tr>
<td>45.75</td>
<td>1.982</td>
<td>30.4</td>
</tr>
<tr>
<td>47.27</td>
<td>1.921</td>
<td>31.6</td>
</tr>
<tr>
<td>54.44</td>
<td>1.684</td>
<td>28.5</td>
</tr>
<tr>
<td>60.21</td>
<td>1.536</td>
<td>28.5</td>
</tr>
<tr>
<td>75.54</td>
<td>1.258</td>
<td>26.3</td>
</tr>
</tbody>
</table>

Table. 31 Determination of lattice parameters from X-ray diffraction data

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Manganese(II) myristate</th>
<th>Copper(I) myristate</th>
<th>Zinc(II) myristate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>298K</td>
<td>298K</td>
<td>298K</td>
</tr>
<tr>
<td>Radiation</td>
<td>CuKa1</td>
<td>CuKa1</td>
<td>CuKa1</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Wavelength(Å)</td>
<td>1.5405</td>
<td>1.5405</td>
<td>1.5405</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
<td>Tetragonal</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁/C</td>
<td>P2₁/N</td>
<td>P4</td>
</tr>
<tr>
<td>α</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>β</td>
<td>118.62</td>
<td>92</td>
<td>90</td>
</tr>
<tr>
<td>γ</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>a(Å)</td>
<td>11.100</td>
<td>11.24</td>
<td>12.49</td>
</tr>
<tr>
<td>b</td>
<td>17.510</td>
<td>4.68</td>
<td>12.49</td>
</tr>
<tr>
<td>c</td>
<td>9.090</td>
<td>10.24</td>
<td>18.066</td>
</tr>
</tbody>
</table>

4.6.6. Magnetic nature of the metal complexes

Figure. 41 showed the magnetic behavior of the metal complexes from the squid measurements. With an increase in applied magnetic field (H), magnetization with linear relationship was obtained based on the magnetic nature of the metal complexes either diamagnetic or paramagnetic. The slope value (x >0) for the manganese complex indicates that the complex nature was paramagnetic whereas copper and the zinc complexes (x <0) were diamagnetic [284].
Figure. 41 Field dependent M-H curves of metal complexes of myristic acid

4.6.7. Determination of cell survival of ligand and metal complexes by MTT assay

Percentage cell viability was calculated by measuring the absorbance of formazan crystals (purple color) formed from reduction of MTT solution by the presence of mitochondrial dehydrogenase in only viable cells (Figure. 42). The cell viability of ligand myristic acid was found to be around 80%. But, the cytotoxic effect were less in the case of all the metal complexes of myristic acid which indicated that the metal complexes were more compatible for biological applications than the ligand [287-288].
Concentration of ligand myristic acid = 50 µM

*Figure. 42* Cell viability of the ligand myristic acid and metal myristate complexes

4.6.8. Biological activity of metal myristates (Microbial growth inhibitory activity)

The result of antimicrobial activity of the ligand and the metal complexes were shown in Table. 32. The efficacy of the metal compounds and ligands could be concluded by knowing the zone of inhibition (mm) value (Figure. 43). The ligand showed the moderate antibacterial activity. The strongest bactericidal activity displayed in the case of Copper complex of myristic acid produced higher activity and Zinc complex showed the moderate activity than myristic acid.
Table. 32 Anti-bactericidal and anti-fungal activities of the ligand myristic acid and metal myristate complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition in diameter (mm)</th>
<th>Antibacterial*</th>
<th>Antifungal**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Manganese myristate</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Copper myristate</td>
<td>-</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Zinc myristate</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline*</td>
<td>30</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>Ketaconazole**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These complexes were sensitive to bacteria, which functions against them and cure the disease. But, the manganese complex was resistant to bacteria which had no significant function in it. The anti-fungal activity of metal myristate complexes were tested against *Aspergillus niger* by agar well diffusion method and their activity compared with standard antibiotic ketoconazole. However, copper and zinc complexes of myristic acid showed moderate activity against fungi. These differences could be due to the nature and level of the antimicrobial agents for different metal ions and their mechanism.

The better microcidal activity was exhibited by copper myristates against gram-positive bacteria. This species variation of the fatty acid reactivity occurred to be firmly correlated with their structural difference of the various bacterial strain surfaces and their efficiency towards anti-microbial effect. The medium chain length fatty acid, like myristic acid enters into the cytoplasm for killing. The mechanistic action of the fatty acids depends on the molecular structure of the membrane present in the cytoplasm.
which might be reflected the fatty acid infused into the membrane of phospholipid bilayer, thereby enhancing the disruption in the membrane surface. In case of gram-negative bacteria, the charge of the outer membrane behaves as a passage barrier against medium chain length fatty acids, but the gram-positive bacteria can take up and transport the fatty acids from the cell wall to its inner membrane [285-286].

**Figure. 43** Anti-microbial activity of Myristic acid metal complexes
4.7. Characterization and biological activity of Acetylsalicylic acid and its metal complexes

The metal complexes of acetylsalicylic acid are more stable in air. They are soluble in THF, partially soluble in alcohol, but they are insoluble in water. The analytical data (Table. 33) shows that the metal to ligand ratio is 1:2 in all the complex systems and it can be represented as [ML₂(H₂O)₂], where L= acetylsalicylic acid ;M= Mn(II), Cu(II) and Zn(II).

Table. 33 Physical characteristics and elemental analysis of acetylsalicylic acid metal complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>m.p. (°C)</th>
<th>Color</th>
<th>C (%)</th>
<th>M (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylsalicylic acid (ASA)</td>
<td>136</td>
<td>white</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[Mn(ASA)₂ (H₂O)₂]. H₂O</td>
<td>123</td>
<td>white</td>
<td>72.4 (71.6)</td>
<td>16.1 (15.4)</td>
<td>66</td>
</tr>
<tr>
<td>[Cu(ASA)₂ (H₂O)₂] H₂O</td>
<td>126</td>
<td>blue</td>
<td>71.5 (70.6)</td>
<td>18.3 (18.1)</td>
<td>62</td>
</tr>
<tr>
<td>[Zn(ASA)₂ (H₂O)₂]</td>
<td>119</td>
<td>white</td>
<td>68.6 (67.8)</td>
<td>16.8 (16.2)</td>
<td>57</td>
</tr>
</tbody>
</table>

4.7.1. UV-Visible spectra of aspirin metal complexes

The electronic spectra for acetylsalicylic acid and its metal complexes was presented in Figure. 44. The absorption bands for acetylsalicylic acid were at 243 nm and 275 nm [288]. The former peaks could be attributed to π → π* transition of the aromatic ring, while the later corresponds to n → π* electronic transition. In contrast, the metal complexed with acetylsalicylic acid, bands are shifted indicating the formation of the product molecule. In addition, d-d transition band was also observed in
the complexes in the range of 300-750 nm indicating the charge transfer reaction occurred.

![UV-Vis spectrum of acetylsalicylic acid metal complexes](image)

**Figure. 44** UV-Vis spectrum of acetylsalicylic acid metal complexes

4.7.2. FT-IR spectra of aspirin metal complexes

The FT-IR characterization of acetylsalicylic acid metal complexes were presented in Figure. 45. The characteristic fundamental vibrations of acetylsalicylic acid are 3476.3 cm\(^{-1}\) (O-H), 1757 cm\(^{-1}\) (C=O of ester), 1675.6 cm\(^{-1}\) (C=O of COOH), 1374.7 cm\(^{-1}\) & 1308 cm\(^{-1}\) (C-O str of carboxylic acid), 1119.2 cm\(^{-1}\) & 1016.1 cm\(^{-1}\) (C-O str of ester) respectively. But, all metal complexes have shown hypsochromic shift in stretching bands correlated with hydroxyl and carbonyl groups of acetylsalicylic acid. Although, the strong absorption spectral band centered at 500-660 cm\(^{-1}\) corresponds to the metal complexes (M-OH & M-O=C). The characteristic absorption band at 1675.6 cm\(^{-1}\) was attributed to the symmetric stretching vibration of COO\(^-\) moiety, which was shifted to longer wavelength in case of metal complexes suggesting
the weakening of C=O bond and the stronger formation of M-O bond. These values are persistent with those detected in recent studies of acetylsalicylic acid metal complexes with other metal ions [288].

**Figure. 45** FT-IR spectra of acetylsalicylic acid and its metal complexes

### 4.7.3. Thermogravimetric studies of metal complexes of aspirin

In order to support the above mentioned spectral characterization, and to relate stability of the metal-ligand compounds, thermo gravimetric analysis study was performed at the heating rates of 10 °C/min starting from 30°C -800°C (Figure. 46-48).

The ligand acetylsalicylic acid decomposes at 136°C but the metal complexes decomposition takes place above 160°C indicating the formation of co-ordination complexes. Noticeably, the degradation pattern of these transition metal complexes occurred in four steps.
Figure. 46 Thermogram of Manganese complex of acetylsalicylic acid

Figure. 47 Thermogram of Copper complex of acetylsalicylic acid
For example, in Manganese (II) complex, the degradation takes place in the range of 0-200°C matches to the elimination of water molecules with an observed weight loss of 7.24% against the calculated weight loss of 7.32%, whereas the loss of ligand molecules degraded in the second and the third step in the temperature range of 130-230°C with a weight loss of 78.4% (calcd = 78.7%) and at the last step was the presence of metal-oxide (MnO₂) after the decomposition of the complex above 400°C. The formation of oxide MnO₂ was confirmed from XPS data where the difference in the binding energy between Mn 2p₃/2 and Mn 2p₁/2 was about 11.72 which was supported with other studies [289] (S7).

For copper complex water molecules present in the crystal lattice as well as the co-ordinated water molecules were lost below 200°C with a weight loss 12.14% against the calculated weight loss of 12.6%, whereas the loss of ligand moiety occurred in two steps in the temperature range of 210°C-700°C with an observed loss of 62.4% (calcd = 63.2%). then, the metal residue formed at 730°C (CuO). The binding energy difference between Cu 2p₃/2 and Cu 2p₁/2 was greater than 20eV which gives the
evidence for the CuO oxide formation (S8). Similarly for the zinc complex also the decomposition occurred with loss of co-ordinated water molecules 11.6% (calcd = 12.2%) below 200°C. The decomposition of ligand with a weight loss of 52.6% (calcd = 54.2%) in the temperature range between 210°C-750°C. Finally, the metal oxide formed above 750°C (ZnO). The difference in energy between Zn 2p\textsubscript{3/2} and Zn 2p\textsubscript{1/2} was 22.81eV which corresponded to the formation of ZnO (S9).

4.7.4. X-ray diffraction data of acetylsalicylic acid metal complexes

![XRD patterns](image.png)

**Figure. 49** Powder XRD pattern of (a) Mn(II), (b) Cu(II), and (c) Zn(II) complexes of Acetylsalicylic acid

X-ray diffraction data of acetylsalicylic acid metal complexes are presented in Figure. 49. The sharp intense peaks showed that the crystalline nature of the complexes prepared and the broad and poor resolution were due to some of the amorphous form of
the complexes. The arrangement of the crystals were well ordered with distinct peaks with their corresponding geometries mentioned in Table-34. The value of d-spacing were also calculated from Bragg’s equation \(2d\sin\theta = n\lambda\), where \(n=1\) and the corresponding wavelength for the measured radiation, \(\lambda = 1.5406\ \text{Å}\) and their intensities were shown in (Table. 35).

**Table. 34** Determination of lattice parameters from X-ray diffraction data

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Aspirin-Mn(II) complex</th>
<th>Aspirin-Cu(II) complex</th>
<th>Aspirin-Zn(II) complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>298K</td>
<td>298K</td>
<td>298K</td>
</tr>
<tr>
<td>X-ray Radiation</td>
<td>CuK(_{\alpha})</td>
<td>CuK(_{\alpha})</td>
<td>CuK(_{\alpha})</td>
</tr>
<tr>
<td>Wavelength (Å(^0))</td>
<td>1.5406</td>
<td>1.5406</td>
<td>1.5406</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
<td>Hexagonal</td>
</tr>
<tr>
<td>Space group</td>
<td>P 12(3)</td>
<td>P 12 /m 1</td>
<td>P3 (143)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>(\beta)</td>
<td>120.86</td>
<td>113.83</td>
<td>90</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>90</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>(a) (Å(^0))</td>
<td>10.332</td>
<td>9.966</td>
<td>13.04</td>
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<tr>
<td>(b) (Å(^0))</td>
<td>4.986</td>
<td>4.983</td>
<td>13.04</td>
</tr>
<tr>
<td>(c) (Å(^0))</td>
<td>9.238</td>
<td>9.223</td>
<td>9.417</td>
</tr>
<tr>
<td>Density (g/cm(^3)) calc</td>
<td>2.734</td>
<td>2.854</td>
<td>1.242</td>
</tr>
<tr>
<td>Volume (cm(^3))</td>
<td>473.92</td>
<td>306.35</td>
<td>1601.28</td>
</tr>
<tr>
<td>Average Crystallite size</td>
<td>19.2 nm</td>
<td>25.8 nm</td>
<td>21.2 nm</td>
</tr>
</tbody>
</table>

**Table. 35** X-ray diffraction data of Aspirin metal complexes

<table>
<thead>
<tr>
<th>Aspirin- Mn(II) complex</th>
<th>Aspirin- Cu(II) complex</th>
<th>Aspirin- Zn(II) complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 d (Å(^0))</td>
<td>Intensity</td>
<td>20 d (Å(^0))</td>
</tr>
</tbody>
</table>
4.7.5. SEM morphology of metal complexes

The SEM micrographs of the Mn(II), Cu(II) and Zn(II) complexes are shown in Figure. 50 (a–c) respectively.

![SEM micrographs of the metal complexes of acetylsalicylic acid](image)

**Figure. 50** SEM micrographs of the metal complexes of acetylsalicylic acid
It was seen from the figure b) that Cu(II) complex shows plate-like structure, while Mn(II) and Zn(II) complexes exhibited the irregular shape agglomerated structures. The particle sizes of these metal complexes were in the diameter range of few microns. Hence, the synthesized metal complexes were therefore believed to have high potential to prevail as medicinal drugs. In order to test their pharmacological activity, anti-oxidant, antibacterial and antifungal activity have been checked. Further, EDS analysis showed the metal content along with carbon which suggested the formation of the metal complex Figure. 50 (d-f).

4.7.6. Magnetic nature of the metal complexes

The magnetic behavior for the metal complexes of acetylsalicylic acid were obtained from squid measurements. When the applied magnetic field (H) increased, magnetization was obtained in a linear manner which revealed the magnetic nature of the complexes either paramagnetic or diamagnetic. Figure. 51 showed the positive slope value for manganese and copper complex (x >0) indicated the paramagnetic nature of the complex whereas the slope was negative for zinc complex of acetylsalicylic acid (x <0) which showed the complex was diamagnetic.
Antioxidant activities of the synthesized metal compounds

Generation of reactive free radicals such as superoxide anion, hydroxyl radical and hydrogen peroxide during normal cellular process in the body system. These free radicals interact with lipids and proteins and nucleic acids which build varied chronic diseases. For this reason, to restrict the damages caused by the free radicals it is necessary to modify the drugs that may be enriched in antioxidant. The primary role of the antioxidant are mainly to scavenge free radicals, fixing the cellular damage and terminate continuous chain reactions. The ligand acetyl salicylic acid also acts an antioxidant, its main ability to scavenge the hydroxyl radicals (OH\(^-\)) radical than the oxygen (O\(^2-\)) and reduced the toxicity caused by hydrogen peroxide (H\(_2\)O\(_2\)). The scavenging rate of the ligand acetylsalicylic acid is faster and better performance than that of glutathione and cysteine. The ability of metal complexes for scavenging the free radicals.
radicals plays significant role for many biological applications [29-291]. The newly synthesized acetylsalicylic acid metal complexes were evaluated for their antioxidant properties by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method (Figure. 51). The metal complexes which have anti-oxidant property reacts with it and produces stable 1,1-diphenyl-2-picrylhydrazine. Consequently, the band intensity of DPPH decreases [292]. The evaluated antioxidant activities (IC$_{50}$) of the ligand and their corresponding metal complexes were compared with the standard Ascorbic acid (Table- 36). In concentration-dependent manner, the radical scavenging ability of the ligand and the metal complexes increased. The lesser the IC$_{50}$ value exhibited higher antioxidant activity [293-294]. Copper complex has better antioxidant effect than the ligand [295]. Hence, the IC$_{50}$ value of the compounds in descending order was Ascorbic acid > Cu complex > ASA (Ligand) > Mn complex > Zn complex. The oxidizing power of these metal complexes which performs its functions by breaking the free radicals through hydrogen atom donation. Consequently, the outcome data earned from this study contributes the connection to the use of these synthesized metal compound used in treatments originating from oxidative stress.
Figure. 52 Antioxidant activity of ligand acetylsalicylic acid (ASA) and its metal complexes

Table. 36 Free radical scavenging activity of metal complexes of acetylsalicylic acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ascorbic acid</th>
<th>*Ligand (ASA)</th>
<th>Mn-ASA complex</th>
<th>Cu-ASA complex</th>
<th>Zn-ASA complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50</td>
<td>1.33 ± 0.86</td>
<td>1.57 ± 1.04</td>
<td>1.94 ± 0.54</td>
<td>1.534 ±1.13</td>
<td>2.09 ±0.94</td>
</tr>
<tr>
<td>R²</td>
<td>0.938</td>
<td>0.9665</td>
<td>0.993</td>
<td>0.945</td>
<td>0.921</td>
</tr>
</tbody>
</table>

4.7.8. Cytotoxicity of acetylsalicylic acid metal complexes

Huang and Dietsch reported that acetylsalicylic acid was not toxic to cultured cells (10Mm) [296]. Figure. 52 displayed the cell survival of acetylsalicylic acid and its metal complexes against HeLa cell line. Acetylsalicylic acid did not exhibit any significant toxicity even at higher concentration. The metal complex also exhibit very less toxicity from lower to higher concentrations. The copper complex has better viability when compared to other metal complexes. Few Studies reported that regular
intake of aspirin reduces the risk of colon cancer (40%) and 66% reduction in prostate cancer risk [297-298].

* Concentration of ligand 100 µg/ml

**Figure. 53** Cytotoxicity of ligand acetylsalicylic acid (ASA) and its metal complexes

### 4.7.9 Pharmacological action of acetyl salicylic acid and its metal complexes

Staphylococcus epidermis, Streptococcus mutans, Pseudomonas aeruginosa, Escherichia coli and Aspergillus Niger were taken as bacterial and fungal strains. Tetracycline and Ketoconazole were referred as standards. The test solutions were made in DMSO solvent, also used as control. The ligand acetylsalicylic acid and its corresponding complexes results were equated with the standards and the inhibition zone diagram was shown in Figure. 53. In the present research, the results showed that the complexes portray more growth inhibition potential than the ligand. It was found that the metal complexes are biologically active and chelation increases their activity. Though, the zone of inhibition (mm) of ligand and its complexes varied with organisms.
as well as metal ions (Table 37). Metal complexes subdued the growth of gram negative and positive bacterial strains and also its fungal activities.

The evaluation for microcidal studies explained that the toxicity of metal complexes against various bacterial and fungal strains could be because of the chelation effect. The response of chelation which reduced the polarity of the transition or any metal ion mostly due to the partial acquisition of its position charge with the donor ligand groups and also likely due to the delocalization of π electrons over the chelate ring. Hence, the specific chelation process increased the lipophilic effect on the central transition metal atom which later aided its permeability through the lipid layer present in the cell membrane. Thereby, their capability of killing the microbes increased with their increase in concentration.

**Table. 37** Anti-bactericidal and anti-fungal activities of the ligand acetyl salicylic acid and its metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive bacteria</th>
<th></th>
<th></th>
<th>Fungal species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>S. epidermis</strong></td>
<td><strong>S. mucans</strong></td>
<td><strong>P. aeruginosa</strong></td>
<td><strong>E. coli</strong></td>
</tr>
<tr>
<td>Ligand</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ligand-Mn Complex</td>
<td>25</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Ligand-Cu Complex</td>
<td>18</td>
<td>-</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Ligand-Zn complex</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tetracycline*</td>
<td>31</td>
<td>25</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Ketaconazole**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*and **: and the tested compounds were made in DMSO and 500 µg/ml of concentration was used.

Anti-bacterial and anti-fungal activity were measured in diameter (mm) in agar plates.
Figure 54 Bactericidal activity against ligand acetylsalicylic acid (ASA) and its metal complexes