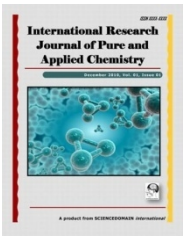

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# Stability Constant and Thermodynamic Parameters Determination of A Semi-synthetic Penicillin Derivative with Various Bivalent Metal Ions ($Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Sn^{2+}$, $Hg^{2+}$ and $Pb^{2+}$): A Potentiometric Study

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***Author's contribution***

*Anita Gupta is the sole author. Entire work including design of study, experimental work, calculations and interpretation of results is done by Anita Gupta only. The final manuscript is approved by author.*

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## ABSTRACT

This present work is a report on the stability constant values of a worthy antibiotic, Ampicillin Trihydrate; D[-]-α-aminobenzyl penicillin; ABP; a semi-synthetic derivative of penicillin with various biologically vital bivalent metal ions ($Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Sn^{2+}$, $Hg^{2+}$ and $Pb^{2+}$) recorded in water-ethanol medium 50% V/V at three different temperatures (298K, 308K, 318K)maintaining ionic strength at 0.1M $KNO_3$. The effect of temperature was also studied and the corresponding thermodynamic functions $\Delta G^o$, $\Delta H^o$ and $\Delta S^o$ were calculated and discussed. It is significant to study the stability constants of drugs with these metal ions as they may already be present in human body. The proton-ligand and metal-ligand stability constants were determined by Calvin-Bjerrum pH titration techniques as modified by Irving and Rossitti. This analysis was carried out in order to determine the $pk_1^H$ and log $k_1$, values at 298 K, 308 K and 318 K. The metal to ligand mole ratio was maintained at 1:5 in order to fulfil the maximum coordination number of metal ion. The titrations were done in a titration cell with three openings, kept immersed in a thermostated bath.

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The formation curves were plotted by taking value of $\bar{n}_A$ (the degree of formation of metal-ligand complexes) against pH of the system. These plots indicate that these metal ions form 1:1 complexes with ABP. The results show that the $pK_1^H$ values increases with an increase in temperature. The order of metal-ligand stability constants (log $k_1$) has been found to be $Cu^{2+} > Zn^{2+} > Cd^{2+} > Pb^{2+} > Sn^{2+} \approx Hg^{2+} > Co^{2+} > Ni^{2+}$.

*Keywords: Antibiotic; bivalent metal ions; stability constants; free energy; entropy.*

## 1. INTRODUCTION

Ampicillin Trihydrate; D [-]-α-aminobenzyl penicillin; ABP is a semi-synthetic derivative of penicillin, active as a broad-spectrum antibiotic. Against gram-positive bacteria, ampicillin has a similar mode of action as benzylpenicillin; against gram-negative bacteria, it has a mode of action similar to tetracyclines. Ampicillin should be stored in optimal conditions at lower pH otherwise it rapidly loses activity [1-3].

Ampicillin trihydrate is broad-spectrum semi-synthetic penicillin that inhibits cell wall synthesis [4] in E.coli, mainly effective in the treatment of gram-positive and gram-negative bacterial infections [5-8]. Ampicillin Trihydrate may cause some adverse effects like stomach upset, diarrhea, nausea, and vomiting during the first few days as the body adjusts to the medication. If an allergic reaction occurs while taking the medicine, symptoms include: difficulty breathing, skin rash and itching [9]. It is a widely used beta-lactam antibiotics, its toxicology and carcinogenesis studies were performed in rats and toxic lesions of stomach were observed in rats whereas no evidence for carcinogenic activity was found [10-12].

Also various metal ions play significant role in our biological system as almost one-third of the enzymes in our body involve metal ions in execution of different biological functions. Already extensive research work in the field of potentiometric studies of metal ion interactions with various drugs had been reported [13-16]. These metal ions serve to bind and orient substrates with respect to the functional groups in the active site so that redox reactions can take place by utilizing the property of variable valency of metal ions. In the absence of an appropriate metal ion, several biological reactions catalyzed by a particular metalloenzyme would be nearly unable to proceed or may proceed at negligible speed. The metal ions under investigation are positively charged and thus act as electrophiles, looking for sharing of electron pairs with other atoms that can form a bond or charge to charge interaction can take place. Metal ions have large ionic volume thus they can accommodate many ligands around them at the same time. On the other hand, the ligands donate electron density to the metal ions and act as nucleophiles. They are usually neutral or negatively charged.

In present study, the drug investigated Ampicillin Trihydrate; D[-]-α-aminobenzyl penicillin; (ABP) $C_{16}H_{19}N_3O_4S$. $3H_2O$ D-(-)-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (Fig. 1); M.W. 403.41; consumed by humans intermittently as per recommendation by the physicians. When the drug interacts with metal ions (already present in our body) it may have positive or negative health effect. Thus it is vital to study the stability constants of a drug with various biologically important metal ions. A lot of research is already done in this field but as per the review of literature, it is observed that the stability constants of this drug with various metal ions are not available anywhere.

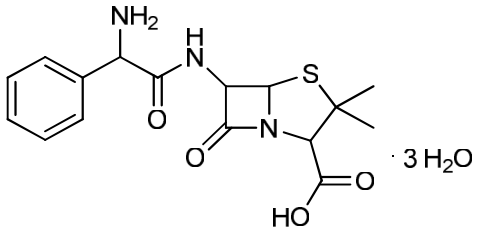


**Fig. 1. Chemical structure of ABP**

## 2. METHODOLOGY

ABP was obtained in extra pure form from a recognized chemical company. All other chemicals used were of AR grade. Double distilled conductivity water was used for solution preparation. The concentrations of free hydrogen ion was measured using a combined glass electrode attached to a EI pH meter having accuracy ± 0.001. These titrations were carried out in order to determine the $pk_1^H$ and log $k_1$, values at 298 K. The metal to ligand mole ratio was maintained at 1:5 in order to fulfil the maximum coordination number of the metal ion. The titrations were completed in a titration cell maintained at 298K, 308K and 318K. Nitrogen gas free from oxygen was bubbled through the solutions in cell in order to provide an inert atmosphere. The titration cell immersed in water bath for half an hour before the titration to attain the required constant temperature. The proton-ligand and metal-ligand stability constant was determined by Calvin-Bjerrum [17,18] pH titration techniques as modified by Irving and Rossitti [19]. This process involves the titration of acid, keeping concentration of both metal ion and ligand constant, with standard alkali. Each system consists of titration of the following three solutions prepared in 50% v/v ethanol-water mixture, total volume in each case being 50.0 mL: Free mineral acid, Free mineral acid + ligand and Free mineral acid + ligand + metal salt solution.

The above solutions were titrated against standard potassium hydroxide solution. After the addition of each portion of alkali, the highest steady reading was recorded in each case. The plot of pH versus the volume of KOH added represents three titration curves known as (i) blank or acid titration curve (ii) reagent or ligand titration curve and (iii) metal or complex titration curve. The difference in the volume of potassium hydroxide utilized for the ligand and metal titrations was a measure of extent of complexation. The complex titration curve was found to be well separated from the ligand titration curve indicating that the liberation of protons was due to chelation. A horizontal gridline at a particular pH value is drawn parallel to the volume axis in all the titration curves. The points of interaction of this line with the various curves (i) acid curve (ii) ligand curve and (iii) metal curve give the volumes V′, V″ and V‴ of the alkali used respectively at the particular pH as given in Table 1. Similarly other tables (Total 24 in number) were obtained for the eight different metal ions at three temperatures. Then the values of $\bar{n}_A$, $\bar{n}$ and pL were calculated using the standard expressions as given below:

$$\bar{n}_A = \left[ 1 + \frac{(V' - V'')(N + E^0)}{(V^0 + V')T_L^0} \right].$$

$$\bar{n} = \frac{(V''' - V'')(N + E^o)}{(V^o + V')\bar{n}_A T_M^o}$$

$$pL = log_{10}\left[\frac{1+{}^{P}K_{1}^{H}\left(1/anti\,log\,B\right)}{T_{L}^{0}-\bar{n}T_{M}^{0}}\;\frac{V^{0}+V'''}{V^{0}}\right]$$

Where N = 0.05, Eº = 0.0005 M, Vº = 50 mL, $T_L$º = 0.0025M, $T_M$º = 0.0005 M

N represents total concentration, Eº is initial mineral acid concentration, Vº is initial volume, $T_L$º is total concentration of ligand and $T_M$º is total concentration of metal ion.

**Table 1. Calculation of various parameters for stability constant determination of $Co^{2+}$ - Ampicillintrihydrate system at 298K**

| pH | V' | V'' | V''' | $\bar{n}_A$ | $\bar{n}$ | pL | $log\left(\frac{1-\bar{n}}{\bar{n}}\right)$ | pL- $log\left(\frac{1-\bar{n}}{\bar{n}}\right)$ |
|---|---|---|---|---|---|---|---|---|
| 4.7 | 0.23 | 0.85 | 0.96 | 0.7507 | 0.2933 | 2.6788 | 0.3819 | 2.2969 |
| 4.8 | 0.25 | 0.95 | 1.07 | 0.7167 | 0.3279 | 2.6877 | 0.3117 | 2.3760 |
| 4.9 | 0.25 | 1.01 | 1.08 | 0.6958 | 0.2051 | 2.6769 | 0.5884 | 2.0886 |
| 5.0 | 0.26 | 1.05 | 1.16 | 0.6829 | 0.3216 | 2.6920 | 0.3241 | 2.3679 |
| 5.1 | 0.27 | 1.08 | 1.18 | 0.6730 | 0.2926 | 2.6903 | 0.3835 | 2.3069 |
| 5.2 | 0.28 | 1.11 | 1.21 | 0.6658 | 0.3119 | 2.6938 | 0.3437 | 2.3501 |
| 5.3 | 0.28 | 1.14 | 1.26 | 0.6555 | 0.3705 | 2.7021 | 0.2303 | 2.4718 |
| 5.4 | 0.29 | 1.18 | 1.31 | 0.6454 | 0.4328 | 2.7108 | 0.1174 | 2.5934 |
| 5.5 | 0.30 | 1.22 | 1.37 | 0.6305 | 0.4627 | 2.7164 | 0.0649 | 2.6515 |
| 6.0 | 0.31 | 1.33 | 1.47 | 0.5934 | 0.4946 | 2.7252 | 0.0093 | 2.7158 |
| 6.5 | 0.32 | 1.42 | 1.58 | 0.5602 | 0.5628 | 2.7376 | -0.1097 | 2.8473 |
| 7.0 | 0.34 | 1.58 | 1.73 | 0.4998 | 0.5941 | 2.7490 | -0.1655 | 2.9145 |
| 7.5 | 0.35 | 1.63 | 1.79 | 0.4880 | 0.6742 | 2.7603 | -0.3158 | 3.0761 |
| 8.0 | 0.36 | 1.71 | 1.89 | 0.4589 | 0.8080 | 2.7801 | -0.6242 | 3.4044 |
| 8.5 | 0.37 | 1.81 | 1.98 | 0.4238 | 0.8080 | 2.7847 | -0.6241 | 3.4089 |

The ligand undertaken in present study contains a carboxylic group, as a result it can donate a proton and the remaining anion can act as a chelating agent. When any drug is mixed with metal salts in solution, there is a competition between the metal ion and the protons for capturing the ligand. It is obvious that there would be a change in the pH of the solution on the formation of complex. Subsequently, the complexation reaction can be followed potentiometrically. It was important to consider the possible hydrolysis of the reagents but hydrolysis during the titration of drugs has not been reported in literature or it can be ignored, because reagent solutions were used immediately after preparation and the titration time in each case was small.

The formation curves were obtained by plotting value of $\bar{n}_A$ against the pH value of the system. An exact value of practical proton-ligand stability constants were calculated by Bjerrum's[17] half integral method at 0.5 $\bar{n}_A$. The $pK_1^H$ values were also calculated by point wise method [19] and by interpolation at various $\bar{n}_A$ values, as shown in Table 2. When the maximum value of $\bar{n}_A$ obtained was less than one, the ligand is monobasic in nature. The metal-ligand complex stability constants were obtained by analysis of formation curves plotting $\bar{n}$ versus pL.

**Table 2. Stability constant data and thermodynamic parameters of metal complexes of Ampicillin Trihydrate; D[-]-α-aminobenzyl penicillin; (ABP) at three different temperatures**

| Cation | Stability Constant | Temperatures | | | -ΔG (KJ mol$^{-1}$) = 2.303 R T log k | | | ΔH (KJ mol$^{-1}$) | ΔS (Jmol$^{-1}$ deg$^{-1}$ ) |
|---|---|---|---|---|---|---|---|---|---|
| | | 298K | 308K | 318K | 298K | 308K | 318K | 308K | 308K |
| $H^+$ | $pK_1^H$ | 6.98 | 7.75 | 8.00 | - | - | - | - | - |
| $Co^{2+}$ | log $k_1$ | (i)2.72* | 2.68 | 2.65 | | | | | |
| | | (ii)2.60** | 2.62 | 2.66 | | | | | |
| | | (iii)2.73*** | 2.69 | 2.65 | | | | | |
| | Mean | 2.68 | 2.66 | 2.65 | 15.28 | 15.68 | 16.13 | -3.51 | 39.51 |
| $Ni^{2+}$ | log $k_1$ | (i)2.72 | 2.66 | 2.69 | | | | | |
| | | (ii)2.60 | 2.65 | 2.55 | | | | | |
| | | (iii)2.72 | 2.66 | 2.68 | | | | | |
| | Mean | 2.67 | 2.66 | 2.64 | 15.23 | 15.68 | 16.07 | -1.76 | 45.19 |
| $Cu^{2+}$ | log $k_1$ | (i)2.73 | 2.67 | 2.67 | | | | | |
| | | (ii)2.85 | 2.64 | 2.64 | | | | | |
| | | (iii)2.73 | 2.68 | 2.65 | | | | | |
| | Mean | 2.77 | 2.66 | 2.65 | 15.79 | 15.68 | 16.13 | -12.32 | 10.91 |
| $Zn^{2+}$ | log $k_1$ | (i) 2.70 | 2.69 | 2.69 | | | | | |
| | | (ii)2.83 | 2.50 | 2.48 | | | | | |
| | | (iii)2.71 | 2.68 | 2.68 | | | | | |
| | Mean | 2.74 | 2.62 | 2.61 | 15.63 | 15.44 | 15.88 | -8.03 | 24.06 |
| $Cd^{2+}$ | log $k_1$ | (i)2.71 | 2.68 | 2.65 | | | | | |
| | | (ii)2.75 | 2.56 | 2.57 | | | | | |
| | | (iii)2.71 | 2.69 | 2.71 | | | | | |
| | Mean | 2.72 | 2.64 | 2.63 | 15.51 | 15.56 | 16.01 | -14.05 | 4.90 |
| $Sn^{2+}$ | log $k_1$ | (i)2.71 | 2.69 | 2.67 | | | | | |
| | | (ii)2.68 | 2.65 | 2.56 | | | | | |
| | | (iii)2.73 | 2.70 | 2.68 | | | | | |
| | Mean | 2.70 | 2.68 | 2.64 | 15.40 | 15.79 | 16.07 | -3.51 | 39.90 |
| $Hg^{2+}$ | log $k_1$ | (i)2.70 | 2.68 | 2.65 | | | | | |
| | | (ii)2.72 | 2.65 | 2.58 | | | | | |
| | | (iii)2.70 | 2.69 | 2.65 | | | | | |
| | Mean | 2.71 | 2.67 | 2.63 | 15.46 | 15.76 | 16.01 | -7.02 | 28.31 |
| $Pb^{2+}$ | log $k_1$ | (i)2.72 | 2.68 | 2.69 | | | | | |
| | | (ii)2.73 | 2.63 | 2.64 | | | | | |
| | | (iii)2.72 | 2.68 | 2.70 | | | | | |
| | Mean | 2.72 | 2.66 | 2.68 | 15.51 | 15.68 | 16.31 | -10.54 | 16.69 |

**(i) Bjerrum half integral method; **(ii) Interpolation at various $\bar{n}$ values ***(iii) Graphical method*

These plots indicate that these metal ions form 1:1 complexes with ABP. Graphical treatment of data is met by plotting $log\left(\frac{1-\bar{n}}{\bar{n}}\right)$ against pL. This plot is a straight line and its intercept gives the value of log $k_1$ (Fig. 2).

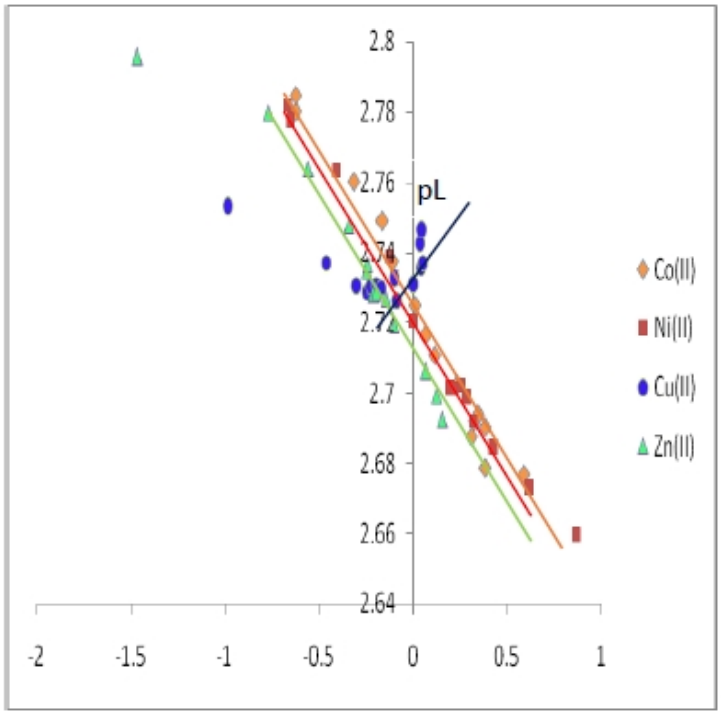


**Fig. 2. Plot of pL and $\log\left(\frac{1-\bar{n}}{\bar{n}}\right)$ of ampicillin trihydrate ; D[-]-α-aminobenzyl penicillin; (ABP) complexes with Co(II), Ni(II), Cu(II) and Zn(II) at 298 K**.

## 3. RESULTS AND DISCUSSION

The stoicheiometric metal-ligand complex stability constants were obtained by careful examination of formation curves plotted. This plot indicate that the value of $\bar{n}$ is of the order of one for the complexes of these drugs with all the metal ions, indicate the formation of 1:1 complex. Bjerrum's half integral method, interpolation of various $\bar{n}$ values and graphical method was used to calculate log $k_1$ values.

- Effect of pH: Only 1:1 complexes of all the metal ions $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Pb^{2+}$, $Sn^{2+}$, $Hg^{2+}$ and $Cd^{2+}$ with ABP are formed in the titration studies of these systems at 298K, 308K and 318 K. The complexation of different metal ions takes place almost in the same pH range at different temperatures.
- Effect of Temperature: A perusal of data (Tables 2) shows that the $pK_1^H$ values increases with an increase in temperature. Thus higher temperature is not favourable for the dissociation of proton from the carboxylic group of these compounds. The values of metal-ligand stability constant log $k_1$ decreases with an

increase in temperature. Thus rise in temperature is not favourable for the complex formation in these systems.

- Order of stability constant values: The metal-ligand complex stability constants (log $k_1$ values) has been found to be in the order: $Cu^{2+} > Zn^{2+} > Cd^{2+} > Pb^{2+} > Sn^{2+} \approx Hg^{2+} > Co^{2+} > Ni^{2+}$. Thus copper bivalent ion complexes have the highest value of stability constant with ABP. This order is found to be in good agreement with Irving-Williams order of stability. It is observed that cobalt bivalent ion complexes enjoy additional stabilization due to presence of Jahn–Teller distortion in geometry in comparison to nickel complexes. The stability of $Cu^{2+}$ complexes is much higher than what can be expected from its ionic radius, due to Jahn–Teller stabilization.
- Thermodynamic parameters: The values of free energies of formation of the complexes become more and more negative with rise in temperature. This indicates that the complex formation is a spontaneous process and spontaneity increases with temperature. The entropy changes are found to be positive for system under study and thus complexes of these metal ions with ABP are stabilized due to this factor only.

## 4. CONCLUSION

Stability constant values are used as an eminent tool by biochemists as it helps to determine the properties of metal-ligand reactions in aqueous medium over and above the actual biological system. Extremely low stability constant values (ranging from negative to 1) indicate that the metal-ligand complex is not only soluble in water but also readily dissociates into metal ion and ligand. For stability constants values above 6, less metal ions are released regardless of low/high pH and these compounds are not significant in biological systems as they consume more stomach acids to dissociate the metal ion from the complex. For various drugs to remain in biologically active form, the stability constants values should be in the range of 3 to 5.

In this study the stability constant values along with the corresponding thermodynamic parameters have been calculated for ampicillin trihydrate ; D[-]-α-aminobenzyl penicillin; (ABP) complexes with various biologically important bivalent metal ions. The stability constant values were found to be in biologically active range and highest value was found for $Cu^{2+}$ metal ion. These stability constant values could be quite informative for a biochemist during drug-design or drug discovery, is the major implication of present study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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